



SISEA

INTERNATIONAL NETWORK MEETING

HO CHI MINH CITY, VIETNAM

JUNE 8-11, 2009

HOSTED BY:  PASTEUR INSTITUT HCMC

FUNDED BY:  AGENCE FRANÇAISE DE DÉVELOPPEMENT

SISEA REGIONAL MEETING

SCIENTIFIC PROGRAM

June 8, 2009

<u>Time</u>	<u>Title</u>	<u>Speaker</u>
08:00 – 08:30	Registration	
08:30 – 09:00	Welcome and Introductory Remarks	M. Gerard Boivineau (French Consul General, Vietnam) Prof. Tran Ngoc Huu (Director, IPHCMC, Vietnam) Dr. Roberto Bruzzone (Scientific Coordinator, SISEA)
	Session I. Surveillance of Acute Respiratory Infections: from bed to bench	Chairs: Tran Ngoc Huu (Vietnam) and Charles Mayaud (France)
09:00 – 9:40	Keynote Lecture: Influenza viruses en route from animals to man	Hans-Dieter Klenk (Germany)
9:40 – 10:20	Aetiology of Severe Acute Respiratory Infections in Vietnam: Setting up the SISEA Project	Nguyen Tran Hien (NIHE, National SISEA Coordinator, Vietnam)
10:20 – 10:40	Laboratory capacity building for detection of SARI in Central Vietnam	Trinh Thi Xuan Mai (Institut Pasteur Nha Trang)
10:40 – 11:00	<i>Break / Poster session</i>	
11:00 – 11:25	Seroprevalence of anti-H5 antibody in rural Cambodia, 2007	Philippe Buchy (Institut Pasteur- Cambodia)
11:25 – 11:50	A Summary of Newly Established Laboratory- Based Influenza Surveillance in Laos	Thongchanh Sisouk (NCLE, Laos)
11:50 – 12:15	Surveillance of ALRI in Cambodia: results from 2 years of SISEA	Laurence Borand & Bertrand Guillard (Institut Pasteur- Cambodia)
12:15 – 14:00	<i>Lunch / Poster session</i>	

Session II. Surveillance, Investigations and Applications

Chairs: Jean-Louis Sarthou (Cambodia) and Vu Thi Que Huong (Vietnam)

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| 14:00 – 14:30 | Keynote: Surveillance of emerging diseases in South Vietnam | Tran Ngoc Huu (Institut Pasteur-Ho Chi Minh City) |
| 14:30 – 14:55 | 2B protein of EV71 functions as an ion channel and is a potential drug target | Bing Sun (Institut Pasteur-Shanghai & Chinese Academy of Sciences) |
| 14:55 – 15:20 | JEV and EV71 in South Vietnam | Phan Van Tu (Institut Pasteur-Ho Chi Minh City) |
| 15:20 – 15:45 | Antibody-dependent enhancement of SARS-CoV infection of human immune cells | Martial Jaume (HKU-PRC) |

15:45 – 16:15 *Break / Poster session*

Session III. Surveillance, Investigations and Applications

Jean-Jacques Bernatas (Laos) and Hans-Dieter Klenk (Germany)

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| 16:15 – 16:35 | Multiplex real-time RT-PCR detection assays for simultaneous identification of seasonal and novel swine influenza A virus subtypes and other influenza viruses | Wei Wang (Institut Pasteur-Shanghai & Chinese Academy of Sciences) |
| 16:35 – 16:55 | Revisiting influenza virus interaction with its receptor by STD NMR spectroscopy | Jimmy Lai (HKU-PRC) |
| 17:00 – 17:25 | Deciphering the Cellular Interactome of Influenza A Viruses | Beatrice Nal (HKU-PRC) |
| 17:25 – 17:50 | The use of high-throughput and high-content technologies in the search for new drugs against human parasites | Lucio Freitas-Junior (IP-Korea) |

19:00 – Late *Dinner*

June 9, 2009

<u>Time</u>	<u>Title</u>	<u>Speaker</u>
	Session IV. Interfacing with other regional programs: surveillance, research and training	Chairs: David Dennis (US-CDC, Vietnam) and Vincent Deubel (China)
09:00 – 09:30	Keynote: For an enhanced networking of professionals in communicable diseases in the Greater Mekong Subregion: the GMS CDC project's efforts	Stephane Rousseau (Asian Development Bank, Vietnam)
09:30 – 09:55	Novel generic platforms for virus identification and immune responses in respiratory and CNS viral infection: A brief introduction of Chinese key project on diagnostic platform in IPS	Ke Lan (Institut Pasteur-Shanghai & Chinese Academy of Sciences)
09:55 – 10:20	The Mekong Basin Disease Surveillance: A regional initiative	Moe Ko Oo (MBDS, Thailand)
10:20 – 10:50	A community based intervention to mitigate the spread of zoonotic diseases in poultry and humans: Pilot intervention in Laos and Cambodia with a potential scale-up for the region	Anne Conan (Institut Pasteur Cambodia)
	Risk based surveillance of zoonotic diseases in South East Asia	Flavie Goutard (Institut Pasteur-Cambodia & CIRAD)
10:50 – 11:10	<i>Break / Poster session</i>	
	Session V. Interfacing with other programs: surveillance, research and training	Chairs: Yves Champey (France) and Roberto Bruzzone (HKU-PRC)
11:10 – 11:35	Opportunities for collaborations in Vietnam between CDC and SISEA on influenza surveillance and pandemic preparedness	David Dennis (US-Center for Disease Control and Prevention, Vietnam)
11:35 – 12:00	Mission and activities of the Regional Emerging Diseases Intervention Center in Singapore	Philippe Cavaller (REDI, Singapore)
12:00 – 12:25	Infectious, vector-borne and food-borne diseases (MIVA/IVEFOOD): a new MSc international curriculum focusing on health in South-East Asia	Roger Frutos (CIRAD, France)
12:25 – 13:00	General discussion & closing remarks	Roberto Bruzzone (HKU-Pasteur Research Center)
13:00 – 14:30	<i>Lunch / Poster session</i>	

**Session VI. General discussion:
achievements and perspectives**

14:30 – 17:30 Poster Session/end (with tea, beer and more) *Parallel session*

14:30 – 17:30 Closed doors SAB meeting *Parallel session*

18:00 – 19:00 **General Discussion with SAB and
recommendations**

19:00 – *Dinner*

Regional Workshop on Acute Encephalitis

June 10, 2009

<u>Time</u>	<u>Topic</u>	<u>Name and Institution</u>
08:45 – 09:00	Welcome address	Vu Thi Que Huong (Institut Pasteur of HCMC)
09:00 – 11:30	Session I. Clinic, biology, pathogenesis and epidemiology	
	Introduction of infectious encephalitis workshop	Vincent Deubel (Institut Pasteur of Shanghai)
	Clinical pathology of encephalitis in Vietnam	Le Phuong Thao (Nhyan Dan Gia Dinh Hospital, HCMC)
	Major etiology of encephalitis	Philippe Buchy (Institut Pasteur of Cambodia)
	Pathophysiology of infectious encephalitis	Thong Kong Wong (University of Malaya, Kuala Lumpur)
	To be announced	Bridget Wills (Hospital for Tropical Diseases, OUCRU, HCMC)
11:30 – 11:50	<i>Break</i>	
11:50 – 13:20	Session II. Circulation of encephalitis viruses in Asia	
	Clinical manifestation of HEMPH and encephalitis caused by EV71	Truong Huu Khanh (Children hospital No. 1, HCMC)
	Japanese encephalitis and new viruses causing encephalitis in North Vietnam	Phan Thi Nga (NIHE, Hanoi)
	Japanese encephalitis surveillance in South Vietnam and JEV-MAC-ELISA test comparison	Huynh Thi Kim Loan (Institut Pasteur of HCMC)
13:30 – 14:30	<i>Lunch</i>	

14:30 – 18:00	Session III. Laboratory investigation of encephalitic viruses	Audrey Dubot-Pérès (IRD/Wellcome Trust, Mahosot Hospital, Vientiane)
	Protocols for investigation of infectious encephalitis	
	Multiplex PCR for enteroviruses	Nguyen Thi Thanh Thao (Institut Pasteur of HCMC)
	Multiplex for Herpes viruses	Nguyen Thi Thuong (NIHE, Hanoi)
16:00 – 16:20	<i>Break</i>	
	New approaches for the diagnosis of viral encephalitis	Wei Wang (Institut Pasteur of Shanghai)
	Experience in the laboratory for identification of viruses causing encephalitis	Vu Thi Que Huong (Institut Pasteur of HCMC)
	Rapid detection of Japanese encephalitis using NS1 capture-ELISA	Vincent Deubel (Institut Pasteur of Shanghai)
	General discussion and preparation of the parallel working groups	
19:00 –	<i>Dinner</i>	

June 11, 2009

<u>Time</u>	<u>Topic</u>	<u>Name and Institution</u>
09:00 – 12:30	Session IV. Laboratory investigation of encephalitic viruses <i>Parallel Working Groups</i> Clinic, Biology, Pathology, Epidemiology Diagnosis: Serological and virological tests	Chairpersons: Marc Sanson & TBD Chairpersons: Audrey Dubot-Pérès & TBD
13:00 – 14:30	<i>Lunch</i>	
14:30 – 17:30	Session V. Guidelines, priorities, milestones, training programs	
19:00 –	<i>Dinner</i>	

ABSTRACTS

- 1. Surveillance of ALRI in Cambodia: results from 2 years of SISEA.** L. Borand¹, P. Buchy², B. Guillard³, S. Hem³, S. Mardy², P. Cavailler¹, M. Chan¹, S. Goyet¹, S. Vong¹ (¹Epidemiology and Public Health Unit, ²Virology Unit, ³Clinical Pathology Unit, Institut Pasteur of Cambodia, Phnom Penh, Cambodia)
- 2. Capacity building for laboratory diagnosis of human respiratory viruses by Multiplex-PCRs in Central Vietnam.** Bui Trong Chien¹, Trinh Thi Xuan Mai¹, Vien Quang Mai¹, Doan Thi Thanh Thuy¹, Nguyen Bao Trieu¹, Nguyen Sanh², Nguyen Thi Ngoc Hue³, Ho Van Nam⁴ (¹Institut Pasteur Nha Trang, ²Preventive Medicine Centre of BinhDinh province, ³BinhDinh Provincial Hospital, ⁴PhuCat District Hospital)
- 3. Seroprevalence of anti-H5 antibody in rural Cambodia, 2007.** Philippe Cavailler^{1#}, Simon Chu¹, Sowath Ly¹, Jean-Michel Garcia², Isabel Bergeri³, Leakhann Som⁴, Sovann Ly⁴, Touch Sok⁴, Sirenda Vong¹, Philippe Buchy¹ (¹Institut Pasteur of Cambodia, Phnom Penh, Cambodia; ²HKU-Pasteur Research Center, Hong Kong SAR, China; ³World Health Organization, Phnom Penh, Cambodia; ⁴Ministry of Health, Communicable Disease Control Department, Phnom Penh, Cambodia; #Current affiliation: Regional Emerging Diseases Intervention Centre, Singapore)
- 4. Mission and activities of the Regional Emerging Diseases Intervention Center in Singapore.** Philippe Cavailler, Za Hussein Reed, Soo Sim Lee, Rohini Rao, Ai Li Quake, Rod Hoff (Regional Emerging Diseases Intervention Centre, Singapore)
- 5. H5 Pseudotyped Lentiviral Particles: A new tool for sero-diagnosis of influenza H5N1 infection.** Simon Chu¹, Mey Channa¹, Y Bunthin¹, Jean-Michel Garcia², Philippe Buchy¹ (¹Institut Pasteur of Cambodia, Phnom Penh, Cambodia; ²HKU-Pasteur Research Center, Hong Kong SAR, China)
- 6. ¹Institut Pasteur of Cambodia, Phnom Penh, Cambodia; ²HKU-Pasteur Research Center, Hong Kong SAR, China)**
- 7. A Community-based Intervention to mitigate the Spread of Zoonotic Diseases in Poultry and to Humans.** Anne Conan (Institut Pasteur of Cambodia, Phnom Penh, Cambodia)

8. **Opportunities for collaborations in Vietnam between CDC and SISEA on influenza surveillance and pandemic preparedness.** David T. Dennis (Influenza Division, U.S. Centers for Disease Control and Prevention (CDC), Hanoi, Vietnam)
9. **Causes of central nervous system infections at Mahosot Hospital.** Audrey DUBOT-PERES (IRD, Mahosot Hospital – Wellcome Trust – Oxford University Tropical Medicine Infectious Disease Center, Vientiane, Lao PDR)
10. **Investigation of Antibody-Dependent Enhancement (ADE) of influenza infection and its role in pathogenesis of avian flu.** I. Dutry¹, P.H. Li¹, R. Bruzzone¹, J.S.M. Peiris^{1,2}, M. Jaume¹ (¹HKU-Pasteur Research Centre, and ²Department of Microbiology, The University of Hong Kong, Hong Kong SAR)
11. **The use of high-throughput and high-content technologies in the search for new drugs against human parasites.** Lucio H. Freitas-Junior (Systems Biology of Pathogens Group, Institut Pasteur Korea)
12. **Optimization and evaluation of an influenza A (H5) pseudotyped lentiviral article – based serological assay.** Jean-Michel Garcia¹, Nadege Lagarde¹, Edward SK Ma², Menno De Jong³, Philippe Buchy⁴, Malik Peiris^{1,2} (¹HKU-Pasteur Research Centre, and ²Department of Microbiology, The University of Hong Kong, Hong Kong SAR; ³Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam; ⁴Institut Pasteur of Cambodia, Phnom Penh, Cambodia)
13. **Definition of the cellular interactomes of SARS-CoV and H5N1 HPAIV: Identification of human regulators of viral entry, assembly and egress.** F Kien, DJ Tang, J Millet, KT Teoh, H Ma, M Peiris, R Bruzzone, B Nal (HKU-Pasteur Research Centre and Department of Microbiology, The University of Hong Kong, Hong Kong SAR)
14. **Deciphering the Cellular Interactome of Influenza A Viruses.** Francois Kien, Dongjiang Tang, Huailiang Ma, Leo Poon, Roberto Bruzzone, Malik Peiris, Beatrice Nal (HKU-Pasteur Research Centre, & Department of Microbiology, The University of Hong Kong, Hong Kong SAR, China)
15. **Mechanisms of pathogenicity and host adaptation of influenza viruses.** Hans Dieter Klenk, Institut für Virologie, Philipps-Universität, Marburg, Germany

16. **Revisiting influenza virus interaction with its receptor by STD NMR spectroscopy.** Jimmy C. Lai¹, T. Haselhorst⁴, J.M. Nicholls³, J.S. Peiris^{1,2}, M. von Itzstein⁴, J-M. Garcia² (¹HKU-Pasteur Research Centre and ²Departments of Microbiology and ³Pathology, The University of Hong Kong, Hong Kong SAR, China; ⁴Institute for Glycomics, Griffith University, Australia)
17. **Novel generic platforms for virus identification and immune responses in respiratory and CNS viral infection: A brief introduction of Chinese key project on diagnostic platform in IPS.** Ke Lan (Institut Pasteur of Shanghai (IPS), Chinese Academy of Sciences, Shanghai, China)
18. **Development of an immunocapture ELISA to detect NS1 protein in Japanese encephalitis virus infection.** Yize Li¹, Dorian Counor¹, Peng Lu¹, Guotong Liang², Jean-Marc Lavergne³, Marie Flamand⁴, Vincent Deubel¹ (¹Institut Pasteur of Shanghai, Chinese Academy of Sciences; ²China Center for Disease Control and Prevention, Beijing; ³Institut de Chimie et de Biologie des Proteines, CNRS, Lyon; ⁴Institut Pasteur Paris)
19. **Etiology of community-acquired pneumonia among hospitalized adult patients in New Caledonia.** Sylvain Mermond¹, Alain Berlioz-Arthaud¹, Francine Baumann¹, Maurice Estivals², Hervé Lévénès², Régis Goursaud¹, Paul Martin¹ (¹Institut Pasteur de Nouvelle-Calédonie, BP 61, 98845 Nouméa, New Caledonia; ²Service de Pneumologie, CHT Gaston Bourret, BPJ5, 98849, Nouméa, New-Caledonia)
20. **Etiology of lower respiratory tract infections in hospitalized children in New Caledonia, a new project.** Sylvain Mermond¹, Myrielle Dupont-Rouzeyrol¹, Virginie Zurawski¹, Isabelle Missotte², Laurent Besson-Leaud², Jennifer Moïsi³, Maria Knoll³, Orin Levine³, Paul Martin⁴, Suzanne Chanteau¹ (¹Institut Pasteur de Nouvelle-Calédonie, BP 61, 98845 Nouméa, New Caledonia; ²Service de Pédiatrie, CHT Gaston Bourret, BPJ5, 98849, Nouméa, New Caledonia; ³Johns Hopkins Bloomberg School of Public Health, Baltimore, USA; ⁴AFSSA, Paris, France)
21. **Detection of HSV DNA in non-JEV Encephalitis–meningitis CSF.** Thuong Nguyen THI¹, Hien Nguyen Tran¹, Hien Nguyen Duc², Minh-Lien Trinh Thil², Cap Do Xuan³, Thu Nguyen Hien¹ (¹National Institute of Hygiene and Epidemiology (NIHE), Hanoi, Vietnam; ²National Institute for Infectious and Tropical Diseases (NIITD), Hanoi, Vietnam; ³Preventive Medicine Center of Thaibinh, Thaibinh, Vietnam)
22. **Etiology of Severe Acute Respiratory Infections in Vietnam: Setting up the SISEA Project.** Tran Hien Nguyen (National Institute for Hygiene and Epidemiology, Hanoi, Vietnam)

23. **Multiplex RT-PCR assay for diagnosis of HEV71, CVA16 and Enteroviruses.** Nguyen Thi Thanh Thao¹, Nguyen Thi Kim Ngoc¹, Patchara Phuektes¹, Peter Charles McMinn² (Institut Pasteur of Ho Chi Minh City, Ho Chi Minh City, VietNam; ²The University of Sydney, Australia)
24. **Detection of HSV DNA in CSF of non-JEV Encephalitis – meningitis.** Thuong Nguyen Thi¹, Hien Nguyen Tran¹, Hien Nguyen Duc², Minh-Lien Trinh Thi², Cap Do Xuan³, Thu Nguyen Hien¹ (¹SISEA Laboratory, National Institute of Hygiene and Epidemiology; ²National Institute for Infectious and Tropical Diseases; ³Preventive Medicine Center of Thaibinh, Hanoi, Vietnam)
25. **The Mekong Basin Disease Surveillance (MBDS): A Regional Initiative.** Moe Ko Oo (Mekong Basin Disease Surveillance, Bangkok, Thailand)
26. **Assessment of the sensitivity of Cambodian H5N1 strains towards Oseltamivir Carboxylate and Zanamivir.** Monica J. Naughtin¹, Simon Chu¹, Sek Mardy¹, San Sorn², Jeff Dyason³, Mark von Itzstein³, Philippe Buchy¹ (¹Institut Pasteur of Cambodia, Virology Unit, Phnom Penh, Cambodia; ²National Veterinary Research Institute, Ministry of Agriculture, Fisheries and Forestry, Cambodia; ³Institute for Glycomics, Griffith University, Australia)
27. **Epidemiology of Zoonotic Diseases in Southeast Asia (EPIZOOSEA).** F. Roger¹, F. Goutard^{1,2}, S. Vong², P. Buchy², S. San³, H. Davun³ (¹CIRAD (French Agricultural Research Center for International Development) EMVT Department, Montpellier, France; ²Institut Pasteur of Cambodia, and ³National Animal Veterinary Research Institute, Phnom Penh, Cambodia)
28. **The Situation of Japanese Encephalitis and other New Viruses Causing Encephalitis in Vietnam.** Phan Thi Nga¹, Do Phuong Loan¹, Bui Minh Trang¹, Nguyen Thanh Thuy¹, Nguyen Viet Hoang¹, Nguyen Tran Hien¹, Kouichi Morita², Barry R. Miller³, Mary B. Crabtree³, Antoine Gessain⁴, Jean Claude Manugueera⁴, Paul Brey⁴ (¹National Institute of Hygiene and Epidemiology, Hanoi, Vietnam; ²Institute of Tropical Medicine, Nagasaki University, Japan; ³CDC Fort Collins, USA; ⁴Institut Pasteur Paris, France)
29. **For an enhanced networking of professionals in communicable diseases in the Greater Mekong Subregion: the GMS CDC project's efforts.** Stéphane P. Rousseau (Asian Development Bank Greater Mekong Subregion Communicable Diseases Control Project)

30. **2B protein of EV71 functions as an ion channel and is a potential drug target.** Bing Sun (Institut Pasteur of Shanghai, Chinese Academy of Sciences, Shanghai, China)
31. **Infectious, vector-borne and food-borne diseases (MIVA/IVEFOOD): a new MSc international curriculum focusing on health in South-East Asia.** Chanin Tirawattanawanich¹, Roger Frutos² (¹Faculty of Veterinary Medicine, Kasetsart University, Paholyothin Rd., Chatuchak, Bangkok 10900, Thailand; ²CIRAD (French Agricultural Research Center for International Development)-UM2, TA A17-G, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France)
32. **JEV and EV71 in South Vietnam.** Phan Van Tu (Institut Pasteur of Ho Chi Minh City, Ho Chi Minh City, Vietnam)
33. **Emerging disease surveillance in Southern Vietnam.** Tran Ngoc Huu (Institut Pasteur of Ho Chi Minh City, Ho Chi Minh City, Vietnam)
34. **A Summary of Newly Established Laboratory-Based Influenza Surveillance in Laos.** Phengta Vongphrachanh¹, James Mark Simmerman², Darouny Phonekeo¹, Vimatha Pansayavong¹, Thongchanh Sisouk¹, Somvay Ongkhamme¹, Gary Bryce³, Andrew Corwin², Juliet E Bryant^{1,4} (¹National Center for Laboratory and Epidemiology, ²US CDC Influenza Division, ³US Navy Medical Research Unit, Jakarta, Indonesia, ⁴Institut Pasteur, Paris)
35. **Development of new technologies for improving the diagnosis of viral encephalitis.** Wei Wang¹, Wei Liu², Peijun Ren¹, Jin Zhang¹, Shenyue Wang³, Guoping Zao³, Vincent Deubel¹ (¹Institut Pasteur of Shanghai, Chinese Academy of Sciences, Shanghai; ²Guangxi CDC, Nanning; ³Shanghai Genome Center, Shanghai, China)
36. **Design of One-Tube Multiplex Real-Time RT-PCR for Specific Identification of Novel Swine-Origin (H1N1) and Seasonal Influenza A viruses.** Wei Wang¹, Leo LL Poon², Peijun Ren¹, Joseph SM Peiris^{2,3}, Vincent Deubel¹ (¹Institut Pasteur of Shanghai, Chinese Academy of Sciences, Shanghai, PR China ²Department of Microbiology, The University of Hong Kong and ³HKU-Pasteur Research Center, Hong Kong SAR)
37. **Pathology of infectious encephalitis.** KT Wong (Department of Pathology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia)

38. Vaccine-elicited Anti-SARS-CoV Spike Antibodies Trigger Infection of Human Immune Cell via a pH- and Cathepsin L-independent FcγR Pathway. Ming S. Yip¹, Francois Kien¹, Ping H. Li¹, Chung Y. Cheung², Isabelle Dutry¹, Yiu W. Kam¹, Nicolas Escriou³, Marc Daeron⁴, Beatrice Nal¹, Roberto Bruzzone¹, Malik Peiris^{1,2}, Ralf Altmeyer⁵, Martial Jaume¹ (¹HKU-Pasteur Research Centre, Hong Kong SAR, China; ²Department of Microbiology, The University of Hong Kong, Hong Kong SAR, China; ³Department of Virology and ⁴Department of Immunology, Institut Pasteur, Paris, France; ⁵CombinatoRx-Singapore Pte Ltd., Singapore)

Surveillance of ALRI in Cambodia: results from 2 years of SISEA

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Surveillance of ALRI has been ongoing in Cambodia for 2 years. As of April 2009, 2,435 people were enrolled in the study including 885 children and 1,550 adults. Enrolled patients stem from two provincial hospitals (Takeo and Kompong Cham ones); they represent 94.8% and 95.7% respectively of the new admissions with ALRI. A version 2.0 of the protocol, an information sheet and an informed consent form were created in April 09. These documents were submitted with the up-dated version of the Case Report Form to the Cambodian Ethics Committee which approved them on 22nd of May 2009. Validation of the files is in progress and the final validation form has been created. The clinical experts have already validated 1,369 files. Through this study, training could be offered to hospital staff notably clinical/chest xray interpretation training of 13 Doctors in pulmonary wards –adult and paediatric- in Phnom Penh hospitals, such as bacteriology training of 2 Lab Technicians at the Institut Pasteur of Cambodia.

From April 2007 to April 2009, bacteria are detected in 35% of the patients sampled with sputum and a blood culture. *Hemophilus influenzae* is the most common isolated with 33% of the positive cultures, followed by *Streptococcus pneumoniae* with 18%. 10% of the samples are positive for *Burkholderia pseudomallei*.

Respiratory viruses are detected in 39% of the samples collected by SISEA. Respiratory Syncytial Virus (RSV) accounts for 4% of the ALRI etiologies, followed by rhinoviruses (3%), influenza A and B viruses (3%), parainfluenza viruses (2%). In 12% of cases, more than 1 virus are detected in the same specimen. The highest positivity rate is found in children, especially in the 18 months-4 years age group (61% of samples positive for virus detection by PCR). Influenza viruses reach a prevalence of over 14% in young children < 4 years and rhinoviruses can be detected in more than 60% of the children between 5 and 14 years. We observe a seasonality of influenza virus and RSV with a peak of transmission during the rainy season while rhinoviruses and parainfluenza viruses seem cause infections all year long.

Conclusion: In agreement with the department of communicable disease control, Ministry of Health, Surveillance data have been sent on a monthly basis so that they are reported onto the yet to be developed national bulletin of respiratory infections. With the assistance of clinical experts in ascertaining clinical cases, we are now committed to furthering our analyses of surveillance data so that better clinical and epidemiological characterisation of various bacterial and viral infections can be achieved. Bacterial and viral infections have been identified for the first time in Cambodia through the SISEA and some of them (i.e. melioidosis as a differential aetiology to TB, influenza, hMPV infections, rhinovirus infections, etc) are of major importance regionally and globally.

This work was supported in part by the French Agency for Development through the SISEA program.

Capacity building for laboratory diagnosis of human respiratory viruses by Multiplex-PCRs in Central Vietnam

Bui Trong Chien¹, Trinh Thi Xuan Mai¹, Vien Quang Mai¹, Doan Thi Thanh Thuy¹, Nguyen Bao Trieu¹, Nguyen Sanh², Nguyen Thi Ngoc Hue³, Ho Van Nam⁴

¹Institut Pasteur Nha Trang, ²Preventive Medecine Centre of BinhDinh province, ³BinhDinh Provincial Hospital, ⁴PhuCat District Hospital

In 2008, the Vietnam Ministry of Health initiated the project for “Surveillance and investigation of epidemic situation in South-East Asia region” from 2008-2010. This project aimed to improve the capacity of laboratories, establish a program for epidemiological surveillance, investigation and quick response to some emerging epidemics in the centre of Vietnam. Locations for project implementation are Pasteur Institute in Nha Trang and 2 sentinel sites (Phu Cat district and BinhDinh province).

Testing respiratory viruses by Multiplex-PCR, there were some difficulties in the beginning of testing application: smear of bands in Multipex-PCR 1, some positive control could not be separated in one tube and preparing positive controls for SARI detection. Now, these problems were resolved progressively.

From October 2008 to April 2009, we analyzed 283 specimens collected (most of them in January to April 2009) from patients admitted to the hospitals of Phu Cat district and BinhDinh province. Overall, respiratory viruses were detected in 143 (50.5%) of 283 tested specimens. Rhinovirus, influenza viruses, parainfluenza viruses and enterovirus were important causes of morbidity in that time. HRSV and human coronaviruses hCoV-OC43, hCoV-229E, hCoV-NL63 and SARS- Coronavirus were not detected. Detection of respiratory viruses was strongly associated with the clinical symptoms of fever, cough, shortness breath and chest in drawing. The detection rate of all viruses decreased with increasing age of the patients. Twenty four patients (8.5%) were infected with 2 viruses, mainly adenovirus, rhinovirus and enterovirus.

This work was supported in part by the French Agency for Development through the SISEA program.

Seroprevalence of anti-H5 antibody in rural Cambodia, 2007

Philippe Cavailler^{1#}, Simon Chu¹, Sowath Ly¹, Jean-Michel Garcia², Isabel Bergeri³, Leakhann Som⁴, Sovann Ly⁴, Touch Sok⁴, Sirenda Vong¹, Philippe Buchy¹

¹Institut Pasteur of Cambodia, Phnom Penh, Cambodia; ²HKU-Pasteur Research Center, Hong Kong SAR, China; ³World Health Organization, Phnom Penh, Cambodia; ⁴Ministry of Health, Communicable Disease Control Department, Phnom Penh, Cambodia; #Current affiliation: Regional Emerging Diseases Intervention Centre, Singapore

Background - In Cambodia we conducted a seroepidemiologic survey, in April 2007, 9 weeks after the identification of a fatal H5N1 case, to determine the frequency of and risk factors for H5N1 transmission.

Methods - All residents of the affected village were interviewed about potential H5N1 exposure and bled for H5N1 serological testing. All sera were initially screened using pseudotyped lentiviral vectors. Positive sera were then tested for confirmation using HI test and standard microneutralization assay.

Results - Eighteen (2.6%) of the 700 villagers were tested as seropositives for H5N1 antibodies. None of the sick-handling practices were associated with anti-H5N1 immune response. In multivariate analysis, seropositive were more likely than seronegatives to report bathing or swimming in the community pond (44 vs. 24%, adjusted odds ratio 2.9, $P = 0.04$).

Interpretation – The relatively high proportion of villagers tested as positive for H5N1 antibodies reinforce the overwhelming evidence that the virus continue to circulate widely. Our study provides additional evidence that the exposure to a potentially contaminated environment remains an important risk factor for human infection.

This work was supported in part by the French Agency for Development through the SISEA program.

Mission and activities of the Regional Emerging Diseases Intervention Center in Singapore

Philippe Cavailler, Za Hussein Reed, Soo Sim Lee, Rohini Rao, Ai Li Quake, Rod Hoff
Regional Emerging Diseases Intervention Centre, Singapore

Background – REDI Center was established through a bilateral agreement between Singapore and USA. The Center opened in May 2004. REDI receives core funding from Singapore MOH, and grant funding from US DHHS, CDC and USDA.

Missions – i/To improve regional capacity for diagnosis, management, surveillance, prevention and control of Emerging Infectious Diseases (EIDs); ii/ To build capacity to conduct research on EIDs; iii/ To develop partnerships that will strengthen EIDs preparedness and respond to critical infectious diseases threats; iv/ To serve as a regional resource for training.

Regional conferences and training – In 2009, REDI cosponsored symposia on dengue and Chikungunya, and a training workshop on influenza virus isolation. REDI Center is assisting the Singapore MOH to establish Field Epidemiology Training Program activities, in Singapore.

Technical assistance for hospital case management for Vietnam, Cambodia, Lao and Indonesia – Since 2007 REDI has been providing technical assistance for improving care, treatment and infection control in 100 AI referral hospitals, in Indonesia. In 2009 REDI has been helping Indonesia to update its national guidelines for care and treatment of AI and sponsored and organized training workshops, and a short course on AI Critical Care (Training of Indonesian Trainers) that was held in Paris.

Trilateral Pilot Project on AI Control in Tangerang Project, Baten Province, Indonesia-

- *Goals:* to assess and eliminate H5N1 in poultry and human populations and to strengthen the local capacity for integrated surveillance and pandemic preparedness.
- *Accomplishments:* i/ baseline GIS/KAP survey, ii/ community education (increasing awareness and prevention of AI), iii/ training of outbreak investigation and response teams and iv/ training of medical, nursing and laboratory staff of the District Hospital.
- *Upcoming activities in market places:* follow-up of cohort of poultry workers, cross-sectional sero-surveys (baseline and 12 months), regular specimen collection (animal and environment).
- *Upcoming activities in the community:* Sero-survey and close monitoring of poultry mortality in selected sentinel sites.

H5 Pseudotyped Lentiviral Particles: A new tool for sero-diagnosis of influenza H5N1 infection

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Highly pathogenic avian influenza (HPAI), H5N1, has spread globally in birds and has infected over 420 humans, with an apparently high mortality rate (>260). To date, there has been an 88% mortality rate for the 8 patients in Cambodia known to have been infected by H5N1. Serological studies to determine the extent of asymptomatic or paucisymptomatic H5N1 infection in humans and other mammals and to investigate the immunogenicity of current H5N1 vaccine candidates have been hampered by the biosafety requirements needed for the 'gold standard' H5N1 micro-neutralization test. We have introduced a new technique in collaboration with the HKU-Pasteur Research Centre, where we can now produce lentiviral pseudotype particles which express the H5 glycoprotein of HPAI (H5pp) for use in a sero-neutralization assay. The advantages of using H5pp are that they are capable of a single-round infection, but do not produce progeny virus. Further advantages of this system are that these particles can be used under lower biosafety requirements than using the wild-type virus, and are adapted to high throughput testing. We will (i) describe this technique in detail, and (ii) present results from a comparative study using this technique together with the standard microneutralization assay used in a sero-prevalence study of over 1300 people living in the areas where the human infections occurred in Cambodia. Our results show that 21/1376 people had neutralizing antibodies to H5 glycoprotein using this new method, which was confirmed using the microneutralization assay. We conclude that the use of H5pp pseudotype particle neutralization assay is an effective way of detecting neutralizing antibodies in sera from avian and human H5N1 cases with the advantage that it can be performed in a BSL-2 laboratory. The technique is also useful for future studies on correlation of the neutralizing antibody activity to prevalence of H5N1 in the population, correlates of immune protection, virus clearance and disease progression.

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A Community-based Intervention to mitigate the Spread of Zoonotic Diseases in Poultry and to Humans

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In South East Asia, H5N1 virus infection has widely affected the poultry population. In Cambodia eight human H5N1 cases have been detected since January 2005. In Laos since 2003, two human cases and a total of 18 H5N1 outbreaks in poultry have been reported. To date, most of the H5N1 laboratory confirmed human cases in the world are thought to have had recent direct contact with infected poultry although the specific nature of the contact was not recorded. Direct contact, particularly when combined with poor hand hygiene, has been described as the essential risk factor for animal to human transmission. Transmission of H5N1 from poultry to humans, even in circumstances in which human–poultry interactions are regular and intense has been limited; however, as the virus continue to circulate and evolve among poultry, bird-to-human transmission may increase. Moreover there is mounting evidence particularly from Cambodia that indirect contact could have played a major role of transmission from poultry to humans, more particularly from a contaminated environment to humans.

Moreover other infectious diseases in animals are assumed to be facilitated by poor management practices with improper housing and inadequate hygiene. These diseases include Classical Swine Fever in pigs, Newcastle disease in poultry or Hemorrhagic Septicemia in cattle. They involve also a high mortality in backyard flocks. The OIE/FAO actually recommends fighting the spread of infectious diseases by changing smallholders' husbandry practices to improve bio-security of poultry production. By reducing infectious diseases spread in poultry, human exposure risks to zoonotic diseases would be lowered in the context of intense and inevitable interactions between humans and domestic animals.

In light of husbandry's practice in South East Asia and potential contamination from the environment we propose a pilot study on sanitary management measures in rural villages of Cambodia and Laos. While the classic management is based on personal protection against risky poultry handling practices, the proposed intervention will encourage farmers to keep the household setting clean, safely discard animal wastes, quarantine newly introduced animals etc. combine with a monitoring of effectiveness. Villagers will be trained using a cascade training approach. To evaluate effectiveness of this intervention participatory approach will be used to analyse consequences on animal health, socio-economical trends and acceptability and sustainability. In Cambodia in a research aim, a scientifically sound design will be proposed (cluster randomizes controlled trial) to relate intervention with effectiveness in mitigating diseases in domestic animals. The major outcome will be a reduction of mortality in poultry flocks and better health status in bovine and swine. Such reductions could lead to socioeconomic gain, subsequently ensuring acceptability and sustainability of the intervention measures. Funding for the trial in Cambodia has been secured. Funding will be requested from SISEA to set up interventions in Cambodia villages (matching funds) and to validate regionally (i.e. Laos and Vietnam).

Opportunities for collaborations in Vietnam between CDC and SISEA on influenza surveillance and pandemic preparedness

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Beginning in 2005, CDC has developed and funded several cooperative agreement projects with the Ministry of Health of Vietnam (MOH) in support of influenza surveillance and pandemic preparedness. To date, these projects have been limited to non-research, capacity strengthening public health practice activities. The principal surveillance project, with the National Institutes of Hygiene and Epidemiology (NIHE) as the lead agency, established a national sentinel system for monitoring influenza-like illness (ILI) and severe viral pneumonia (SVP). This system utilizes 15 clinical sites linked to respective regional public health institutes which provide epidemiologic and laboratory support. A second project, with the MOH General Department of Preventive Medicine and Environmental Health (GPMEH), strengthens the MOH capacity for pandemic preparedness, including communications, early detection, rapid response and containment. A third cooperative agreement, with CARE International, has supported the development of models for behavioral change communications, surveillance, prevention and control of highly pathogenic avian influenza and pandemic influenza at the commune level.

In 2008, CDC and NIHE established a partnership for research on influenza and other emerging infectious diseases of public health importance. Proposed research projects, currently under CDC funding review, include studies on the burden of influenza illness, including ILI/influenza and hospitalizations for acute lower respiratory tract disease; the development of improved diagnostics for influenza, including rapid, subtype-specific, point-of-care diagnostics; molecular studies of the co-evolution of animal and human influenza viruses in Vietnam; and, analytic epidemiologic studies of risk factors for influenza A/H5N1 infection and disease.

In response to the pandemic threat posed by the swine-origin novel influenza A/H1N1 virus, CDC has developed an investigation strategy and plan for rapid implementation of special studies in selected tropical and southern hemispheric countries. Vietnam is a priority country within the tropical region because of existing partnerships and funding mechanisms, its epidemiologic and virologic capacity and information base, including knowledge on year-round activity of seasonal influenza viruses, and the special concern posed by possible interactions with endemic influenza A/H5N1 or other animal viruses.

CDC and SISEA network have similar shared interests and sometimes overlapping project objectives and activities in Vietnam and the region. Sharing of information, leveraged partnerships and collaborations between CDC and SISEA network have the potential to increase public health productivity of both organizations and their Vietnam MOH partners.

Grant Support. The total budget for 2008-2009 cooperative agreement funded MOH activities was 1.875 million USD; the budget for 2009-2010 is expected to be about the same.

Causes of central nervous system infections at Mahosot Hospital

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Infections of the CNS can be due to a wide variety of organisms, including viruses, bacteria, fungi, and parasites. Herpes simplex viruses (HSV), varicella-zoster virus (VZV), mumps, measles, Enteroviruses and Japanese encephalitis virus are responsible for most cases of viral CNS infections in immuno-competent individuals. However, over 100 viruses are known to cause encephalitis / meningitis in humans. Some of them, although unusual and/or initially restricted to certain regions of the world, have now become important clinical issues (eg Japanese encephalitis in Asia, West Nile fever in Americas). Importantly, the aetiological diagnosis of CNS infections remains globally poorly known, even in developed countries (<10% of confirmed cases in the 1998-2000 study of the California Encephalitis Project, Glaser et al. 2003).

The exotic and emerging viral encephalitides are caused by animal or human viruses and characterised by sudden unexpected outbreaks of neurological disease, usually in tropical and sub-tropical regions. It is noticeable that, over the past decade, a number of such zoonotic and vectorborne viral diseases have emerged in Southeast Asia and that these viruses were frequently associated with encephalitic presentations (Mackenzie 2005).

There is no published information on the causes of meningoencephalitis in the Lao PDR (Laos) and there are no data to inform patient care or national health policy. Meningo-encephalitis is currently treated in Lao hospital with appropriate antibiotics if a bacterial aetiology is suspected and occasionally with acyclovir if HSV is suspected. JEV vaccination is currently not available, but WHO and the Government of the Lao PDR are considering its introduction. Considering the importance of raw meat and hunting in the rural Lao diet, the importance of pigs (cf JEV & Nipah), the broad range of ethnic groups and geographical isolation, and the high incidence of dengue it is highly likely that there is a considerable burden of undiagnosed meningoencephalitis of diverse aetiology across the country.

In 2003 the Wellcome Trust-Mahosot Hospital-Oxford University Tropical Medicine Research Collaboration started to investigate the causes of bacterial CNS infection with a conventional bacterial culture service and serology for rickettsial and leptospiral disease. Dengue and JE ELISA serology have been performed on serum and CSF since 2006. In October 2008 virology tools were set up at Mahosot Hospital and permit the testing, following a fully validated strategy based on real-time PCR for a panel of "classical" pathogens (HSV, VZV, CMV, Dengue, Enterovirus, JE, Mumps, Measles, Influenza, TBE, West Nile), of prospectively collected samples from 300 patients. Soon, CSF will be routinely inoculated on Vero cells to permit virus isolation. Stored CSF will also be inoculated on Vero cells and molecular techniques, such as PCR with degenerate primes and pyrosequencing, will be used to determine the infecting agent in positive cultures.

Our approach is to use diverse tools (serology, PCR, culture) to look for the widest range as possible of potential etiological agents on fresh samples collected at the Hospital from patients with encephalitis or meningitis. This will allow the creation of an inventory of known aetiological agents in Laos patients with meningitis/encephalitis. The results will permit the guidance of prevention and therapeutic strategies for meningitis/encephalitis in Laos for the first time. In addition we can expect to obtain new information on clinical and epidemiologic patterns associated with specific etiological agents in this region. Our 2nd objective is to search for newly emerging or re-emerging pathogenic agents.

Investigation of Antibody-Dependent Enhancement (ADE) of influenza infection and its role in pathogenesis of avian flu

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The efficient adaptation of an avian influenza virus to transmit from human-to-human raises fears worldwide about emergence of a deadly pandemic. Differential preference of binding of influenza viruses to sialylgalactosyl residues in humans versus birds is believed to restrict occurrence of zoonotic avian infection. In addition to interaction with viral receptor, viruses may also rely on antibodies to enter and replicate into host cells, a phenomenon known as Antibody Dependent Enhancement (ADE) of infection. Because such phenomenon has already been demonstrated during H1N1 and H3N2 infections, ADE of avian H5N1 infection could allow an opportunity and alternative mechanism to the virus to gain a foothold in the human respiratory tract besides adaptation of its hemagglutinin to human sialic acid.

We aim to investigate occurrence of ADE of H5N1 infection in presence of human serum elicited after infection/vaccination with human seasonal influenza virus (*i.e.* H1N1 and H3N2). Our hypothesis is that: a) human serum contains antibodies to H1N1 or H3N2 viruses that facilitate entry of H5N1 viruses into human hematopoietic cells; and b) antibody-mediated viral infection is a key player leading to particular hallmarks of the disease pathogenesis.

We have carried our preliminary experiments and observed that sera from individual immune to influenza viruses bear different abilities to neutralize [serum from a volunteer vaccinated against H5N1] versus enhanced [serum from healthy individuals having received or not flu shot(s)] infection by H5-pseudotyped viral particles. Further experiments aiming to identify molecular partners (*i.e.* Fc receptors, others) as well as highlight occurrence of ADE of live H5N1 are now ongoing.

The use of high-throughput and high-content technologies in the search for new drugs against human parasites

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Leishmaniasis, Chagas Disease and Malaria are responsible for 500,000 to 1 million human deaths each year. These diseases are all caused by protozoan parasites and, although chemotherapy is available, drugs are mostly ineffective due to parasites resistance, and in some cases the drugs themselves are potentially deadly for patients. In Institut Pasteur Korea (IPK), we develop high-content/high-throughput screening assays (HCS/HTS) for finding new drugs that impair the parasites ability to cause disease.

Using a whole cell-based approach, we have screened 200,000 drugs that impair the growth of the most deadly species causing visceral leishmaniasis, *Leishmania donovani*, inside human macrophages. We are also developing a whole-cell model assay for discovery of compounds active against *Trypanosoma cruzi*, the causative agent of Chagas disease. In parallel, we developed a high-content assay for kinetoplast-directed drug discovery for *Leishmania* and *T. cruzi* parasites. The kinetoplast is a single mitochondrion, exclusive to order Kinetoplastida (*Leishmania* and *Trypanosoma cruzi*, among other parasites) and contains a number of excellent chemotherapeutic targets that are very unlikely to be found in the human host. The hits found in the whole-cell assays for *T. cruzi* and *Leishmania* will be further tested in the kinetoplast assay for assessment of their potential kinetoplast-targeting mechanism of action.

We are also developing HCS/HTS that target the invasion of human red blood cells (RBCs) by *Plasmodium falciparum*, the deadliest of all malaria parasites. We have validated, by means of 4,000-compound screening, a quantitative enzymatic approach to assay for *P. falciparum* viability. This assay is being up-scaled to screen 80,000 compounds, and the hits emerging from this assay will be tested on a secondary image based high-content assay for their ability to interfere with the invasion pathway.

We also develop basic research on the molecular mechanisms behind parasite's pathogenesis. More specifically, we are investigating the human components involved in the infection process by *T. cruzi*. With this aim, we performed a genome-wide RNAi screening to identify human host factors required during *T. cruzi* infection. Presently, all hits are being carefully analyzed and organized in order to provide a comprehensive list of the human proteins that are important for each step of the infective process. The identification of these proteins can expose the necessities of the pathogen and hence indicate potential targets for anti-parasitic therapies. By combining the understanding of the diseases basic biology with an unbiased search for new compounds active on these pathogens, we can define better drug targets and thus better drugs.

Optimization and evaluation of an influenza A (H5) pseudotyped lentiviral article – based serological assay

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Serological methods are important for investigating the health threat posed by highly pathogenic avian influenza (HPAI) H5N1 and provide an option for sero-diagnosis, sero-epidemiology and determining evidence of naturally acquired or vaccine induced immunity. Micro-neutralization (MN) tests are currently the gold-standard for serological studies of HPAI in mammalian species but require handling live-virus in a biosafety level (BSL) 3 environment. We have previously reported the use of H5pseudotyped lentiviruses (H5pp) as an alternative to micro-neutralization tests in a BSL-2 setting (Nefkens et al., J Clin Virol, 2007). We described the production of influenza pseudotyped lentiviral particles, their biochemical as well as functional characterizations. We also showed that these particles can be neutralized by sera from individuals infected or vaccinated with H5N1 and therefore, and that they can be used as substitute of wild type BSL3 agent H5N1 virus. Our objective was to optimize and evaluate this newly developed H5pp assay.

We have optimized and evaluated the diagnostic performance of the H5pp assay using well-characterized sera from humans with confirmed H5N1 disease or controls. We found that the H5pp assay is a useful serological method for the detection and quantification of neutralizing antibody to H5-viruses that can be performed in a BSL-2 environment. In conclusion, H5pp provide a reliable and safe alternative for sero-diagnosis and sero-epidemiology of H5N1 infections

Definition of the cellular interactomes of SARS-CoV and H5N1 HPAIV: Identification of human regulators of viral entry, assembly and egress

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Little is known about the molecular interactions between viral and cellular proteins during infection with human Coronaviruses such SARS-CoV or Highly Pathogenic Avian Influenza (HPAI) H5N1 virus. Our objective is to identify human cellular factors involved in the regulation of early and late stages of viral infection.

Our research strategy is based on large-scale Yeast-Two-Hybrid (Y2H) screenings using endo-domains of viral envelope proteins as baits. These C-terminal domains are exposed to the cytoplasm during key steps of the viral life cycle and using them as baits in the Y2H screening can reveal important virus-host interactions during early (entry, fusion) or late (assembly and egress) stages of infection.

We have performed three screening campaigns and identified cellular factors which bind the Spike (S), Envelope (E) and Membrane (M) proteins of SARS-CoV and other human coronaviruses (HKU1, OC43, NL63, 229E) and the Hemagglutinin and M2 proteins of HPAI H5N1.

We have found that the SARS-CoV E envelope protein binds Pals1, a key regulator of epithelium polarity. We have mapped the binding domains and shown that pals1 is recruited to the site of virus assembly in infected cells. Co-immunoprecipitation studies suggest that E interacts with the whole polarity complex when overexpressed in Vero E6 cells. Our working hypothesis is that the interaction of E with pals1 leads to the disruption of infected epithelia. We are now testing this hypothesis in functional assays on differentiated and polarized epithelial cells.

We have identified and confirmed the interaction between the SARS-CoV S protein and the ezrin actin-binding protein. Our findings using both SARS-CoV S-pseudotyped lentiviral particles and replicative SARS-CoV virus suggest that ezrin is a restricting factor of infection by the SARS-CoV.

We have also identified cellular factors involved in cellular trafficking as interactors of the H5 and M2 influenza proteins. Their role in early and late stages of influenza life cycle is under investigation.

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Deciphering the Cellular Interactome of Influenza A Viruses

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Influenza A viruses are a threat for human and animal health and, as a consequence, for stability of worldwide Economy. Available vaccines do not induce long-lasting immunity and resistant viral strains, which cannot be counteracted by classical antivirals, tend to appear. Moreover the constant emergence of new strains from antigenic drift or the potential recombination of viruses within the animal reservoirs, increase concerns.

The elucidation of the cellular Interactome (ie all cellular factors the virus interacts with and exploits for its own profit) is a pre-requisite for the design of new, effective antiviral strategies. Our objective is to understand which virus-host interactions are responsible for pathogenesis. We classify interactions into three categories: 1/ those which are crucial for progression of the virus through its replication cycle (entry, trafficking, budding), 2/ those which affect a cellular function (polarity, cell cycle, immune response), 3/ those which affect both the virus life cycle and the cell function.

On the one hand, we study the fate of influenza virus structural proteins in the host cell, particularly their trafficking and assembly properties, using the highly pathogenic H5N1 influenza virus as a model. On the other hand, we analyze interactions between these viral structural proteins and cellular factors identified using a genome-wide library yeast-two-hybrid screening assay. We will present our progress towards the definition of the influenza A Interactome.

This work has been funded by the Research Fund for Control of Infectious Diseases & Area of Excellence on Influenza (HK), and by the French Ministry of Health through the RESPARI program.

Mechanisms of pathogenicity and host adaptation of influenza viruses

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Wild aquatic birds are the natural hosts for a large variety of influenza A viruses. Occasionally viruses are transmitted from this reservoir to other species, such as chickens, pigs and men, and may then eventually cause devastating outbreaks in domestic poultry or give rise to human influenza pandemics. Host range and pathogenicity are polygenic traits depending on the interaction of different viral proteins with specific host factors. It has long been known that proteolytic activation and receptor specificity of the hemagglutinin are important determinants for pathogenicity and interspecies transmission, respectively. Evidence is increasing that the viral polymerase, an enzyme that has to enter into the nucleus of the infected cell in order to promote replication and transcription of the viral genome, is also a major determinant of host range. Thus, in a comparative study of an avian influenza strain and its mouse adapted variant we have previously shown that adaptation to mice depended exclusively on mutations in the polymerase proteins. These findings supported the concept that adaptation of the polymerase to host factors is an important mechanism underlying interspecies transmission. We have now identified importin $\alpha 1$, a component of the nuclear pore complex, as such a host factor. We show that adaptive mutations in polymerase subunits improve binding to importin $\alpha 1$ in mammalian, but not in avian cells. As a result, nuclear transport of these proteins and efficiency of replication are enhanced in mammalian cells. These observations demonstrate that the interaction of the viral polymerase with the nuclear import machinery is an important determinant of host range and they will be discussed in the light of the ongoing outbreaks of H5N1 and novel H1N1 viruses.

Revisiting influenza virus interaction with its receptor by STD NMR spectroscopy

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Sialic acids (Sia), usually linked to galactose on cell surface in an α 2-3 or α 2-6 configuration, are identified as receptors for influenza virus. It was suggested that a switching of receptor preference from α 2-3 Sia-Gal (the major form in the avian enteric tract) to α 2-6 Sia-Gal (the major form in the human upper respiratory tract) is critical for the avian virus to adapt and transmit efficiently in humans. However, human parainfluenza strictly bind α 2-3Sia and still replicates in the upper respiratory tract. Therefore, the dogma has to be revisited. We hypothesized that receptor binding preference is not only depend on α 2-3 or α 2-6 Sia-Gal linkage preference but also other components and lengths of the glycans. In order to understand the infection and transmission of influenza virus, as well as develop much needed novel anti-influenza drugs, investigation of these additional receptor components is necessary.

Here we present a new quantitative method to study host-cell interaction. By the use of noninfectious influenza hemagglutinin(HA)-containing virus-like particles (VLPs) and the state-of-the-art saturation transfer difference (STD) NMR spectroscopy, receptor preference of HA to different sialyl-ligands was investigated. We recently published the proof-of-concept of this approach by confirming the preference of HA from avian influenza H5N1 to α (2,3)-sialyllactose (3'SL) versus α (2,6)-sialyllactose (6'SL)[1]. We are showing here unpublished data demonstrating the double mutations at amino acids Ser196 and Gln227 in HA from H5N1 virus extend its binding from 3'SL to both 3'SL & 6'SL, which is similar to the receptor preference of human H3N2 virus. Once set-up, this technique should allow us to map at atomic level the part of the glycan involved in the interaction with HA; a first step toward rational design of influenza entry inhibitor. This knowledge, coupled with the systematic investigation of glycans present in lungs by mass spectrometry (data not shown, [2]), will also help us better understand the tropism of influenza infection as well as viral inter-species adaptation.

1. Haselhorst T *et al.* (2008) *Angew Chem Int Ed Engl* **47**:1910-1912.
2. Nicholls JM *et al.* (2009) *Antimicrob Agents Chemother* (Submitted).

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Novel generic platforms for virus identification and immune responses in respiratory and CNS viral infection: A brief introduction of Chinese key project on diagnostic platform in IPS

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Along with the globalization, new infectious diseases emerged and old infectious diseases reemerged, some of them are still the major factors threatening human beings. Development of new techniques for diagnosis of different pathogens is critical for the control of the infectious diseases. The researchers in Institut Pasteur of Shanghai have been working on this aspect and developed a series of state in the art platforms such as viromics platform to detect viral infection in China. To improve the ability of pathogen diagnosis, Chinese government launched key projects for developing new techniques of virus detection last year. The researchers of IPS successfully obtained a project focusing on the development of novel generic platforms for identification of viral infection and of immune responses in respiratory and central nervous systems. This project is going to focus on five aspects: 1) Research on clinical sample acquisition, isolation and preservation. Researchers will establish a large panel of cell lines and primary cells (including genetically engineered cell lines) to improve propagation and isolation of viruses from clinical samples. 2) Development of nucleic acid-based generic platforms for quick identification of viruses; 3) Development of antigen chips for serology; 4) Development of antibody chips for quick identification of viruses; 5) Development of pseudotype-based assay for measuring neutralizing antibody responses against high pathogenic avian influenza H5N1 viruses. Seven research units in IPS and researchers from 7 other institutions are involved in this project. Through synergistic work, these platforms will be generated for virus screening and will contribute to the control of infectious diseases in China.

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Development of an immunocapture ELISA to detect NS1 protein in Japanese encephalitis virus infection.

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During flavivirus infection, nonstructural protein NS1 is released in a major soluble form. *In vitro*, NS1 protein is secreted from infected cells as a unique hexameric species. We had previously studied NS1 protein released in dengue virus-infected hosts and several kits for dengue diagnosis are now available. However NS1 protein produced during Japanese encephalitis (JE) infection has not been studied yet. In order to analyze the biological relevance of NS1 secretion, *in vitro* and *in vivo*, we have developed a sensitive Enzyme-Linked Immunosorbent Assay (ELISA) to detect the protein in the sera and cerebrospinal fluid (CSF) of JE virus (JEV)-infected patients.

JEV NS1 protein was produced constitutively in recombinant drosophila S2 cells and purified from cell supernatant. Mouse monoclonal antibodies (MAbs) were obtained from B-cell fusion of immunized mice and their immunoreactivities were characterized. Two MAbs were chosen for their high affinity with the hexameric NS1 form of JEV, one flavivirus specific Mb for antigen immunocapture and one JEV-specific labeled MAb for detection, respectively. Using purified JEV NS1 as a protein standard, the sensitivity of the capture ELISA was less than 1 ng/ml. NS1 protein was detected in the blood of JEV-infected mice. A preliminary test performed on a panel of archival sera and CSF collected in China and showing anti JEV IgM antibodies detected NS1 in few sera. Additional tests will be carried out to assess the specificity, sensitivity and validity of the assay.

Etiology of community-acquired pneumonia among hospitalized adult patients in New Caledonia

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The aim of our study was to describe the etiology of community-acquired pneumonia (CAP) in hospitalized adult patients in New Caledonia. One hundred and thirty seven patients with confirmed CAP admitted to the Territory Hospital of Noumea from December 2006 to November 2007 were enrolled prospectively.

Microbiological testing methods included blood and respiratory specimen cultures, urinary antigen detection for *Streptococcus pneumoniae* and *Legionella pneumophila*, serology on paired sera and respiratory virus detection by immunofluorescence on nasal swabs. Molecular assays were used for parallel detection of bacteria responsible for atypical pneumonia (*Legionella pneumophila*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*) and also for influenza virus detection.

The etiology of CAP was determined in 59.8% of cases (82/137) and 117 pathogens were detected. *S. pneumoniae* was the most common pathogen (48 isolates [41%]) followed by influenza virus A (26 isolates [22.1%]), *Haemophilus influenzae* (12 isolates [10.2%]), *Branhamella catarrhalis* (6 isolates [5.1%]), *Mycoplasma pneumoniae* (6 isolates [5.1%]), *Klebsiella pneumoniae* (5 isolates [4.2%]). The rate of atypical pathogens was very low (6%) especially regarding *L. pneumophila* (0%). Two or more pathogens were detected in 31 patients (37.8%). The most frequent and significant coinfection ($p = 0.004$) was *S. pneumoniae* associated with influenza A virus.

Only 4 influenza infected patients (15.4%) exhibited positive results by immunofluorescence technique on nasal swabs while PCR assay on pulmonary specimen yielded 15 positive results (57.7%). The diagnosis rate of *S. pneumoniae* infections was multiplied by 2.1 by using urinary antigen detection (from 16.8% to 35%), highlighting the lack of sensitivity of traditional techniques for an exhaustive detection of pneumococcal CAPs.

In our study, there was no significant association between ethnicity or comorbidities and the etiology of CAP. Incidence of CAP was significantly higher during southern winter ($p = 0.007$) and this increase was linked with seasonal influenza circulation observed at the same period in the general population ($p = 0.002$).

In New Caledonia, *S. pneumoniae* is the leading cause of CAP. Mixed CAP involving *S. pneumoniae* and influenza virus is very common during the winter season. This population of adult patients hospitalized with CAP is obviously a relevant sentinel group for influenza surveillance but require appropriate specimen.

Etiology of lower respiratory tract infections in hospitalized children in New Caledonia, a new project

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Pneumonia etiology research for child health (PERCH) is an international project based at the Johns Hopkins Bloomberg School of Public Health in Baltimore and funded by the B & M Gates foundation. The main goal of the project is to reduce child pneumonia mortality in the developing world through guiding the development of new pneumonia vaccines and treatment algorithms for 2015 onwards. The major part of the project is based on a case-control study of hospitalized pneumonia in representative developing country settings that will involve 6 different sites between 2010 and 2013. Upstream, the first phase of the project will last for 18 months and will focus on four different objectives: a) select high-quality sites that can conduct a rigorous case-control study of hospitalized pneumonia b) develop models for analyzing and interpreting the etiology data c) assess the specificity of MassTAG-PCR to identify pneumonia pathogens d) establish a set of standardized clinical, laboratory and data management methods for use in the case-control study.

As part of phase I of the PERCH project, we plan to investigate the etiology of lower respiratory tract infection (LRTI) in hospitalized children in New Caledonia. This case-control study will take place between September 2009 and October 2010 and will include children less than 16 years of age hospitalized with community-acquired pneumonia or bronchiolitis. For case patients we will collect blood, urine and respiratory specimens, including nasopharyngeal aspirates and induced sputum (with forced expiratory flow practiced by a chest physiotherapist). Blood and nasopharyngeal aspirates only will be required for control patients. An aliquot of respiratory specimen will be preserved at ULT for complementary molecular investigations.

Bacteriological testing methods will include blood and respiratory specimen culture, urinary antigen detection for *L. pneumophila* and serum antimicrobial activity testing for detection of pre-hospital antibiotic use. Molecular techniques will include *C. pneumoniae*, *M. pneumoniae* and *B. pertussis* detection by real-time PCR. Respiratory virus detection will be done on respiratory samples by immunofluorescence, multiplex PCR and MassTAG-PCR. Serological testing will be performed on paired sera.

This study will provide information on the etiology of LRTI in children in New Caledonia in order to improve treatment and prevention. In conjunction with the PERCH project we will also assess the utility of induced sputum with forced expiratory flow for bacteriological and virological testing and evaluate new laboratory techniques such as multiplex-PCR and MassTAG-PCR for the diagnosis of LRTI in children.

Detection of HSV DNA in non-JEV Encephalitis–meningitis CSF

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Although the Japanese encephalitis vaccination has been introduced in Northern Vietnam since 2000, morbidity and mortality of Acute Encephalitis Syndrome (AES) is still high, and according to some estimations, causatives of about 80% of AES have not been identified.

A pilot study was conducted to detect HSV encephalitis for non-JEV encephalitis and/or meningitis CSF collected during 2002-2007. HSV-specific primers and a fluorescent probe directed to the most conserved area of DNA polymerase (UL30) were used for real-time PCR (Kessler et al.).

The results showed that of 162 CSF, nine (5.56%) were positives, mainly in adult encephalitis, but also did happen in child and caused meningitis. There was no difference in gender. In conclusion, HSV is a causative of Encephalitis – meningitis in Northern Vietnam, the early screening at sites is necessary for the antiviral treatment.

Etiology of Severe Acute Respiratory Infections in Vietnam: Setting up the SISEA Project

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Etiology of Severe Acute Respiratory Infections (SARI) is still unclear in Vietnam. A study for identifying epidemiological and virological characteristics of SARI was set up in Hai Duong province, in the context of SISEA project. During the first 5 months of 2009, one hundred and nineteen specimens from patients with SARI were collected in district and provincial hospitals. Specimens were tested following SISEA protocols to detect 17 respiratory agents.

Laboratory results showed that positivity was 72.3. The highest positivity was Rhinovirus (27.7%), followed by hBoV (10.7%), parainfluenza 2 (10.7%), hAdV (8.9%) and hMPV (8.9%). The parainfluenza viruses appeared only in the first 2 months. Seasonal Influenza A, B, and C were detected since February with respective figures of 4.5%, 8.0%, and 0.9%. Co-infection was 16,1 % for two viruses and 1,85% for three viruses. The presence of RNA of SARS, hCoV 229E, hCoV NL63, and parainfluenza 4 has not been detected. The study should be continued with the bacteria detection to have complete spectrum of pathogens for SARI in Vietnam.

This work was supported in part by the French Agency for Development through the SISEA program.

Multiplex RT-PCR assay for diagnosis of HEV71, CVA16 and Enteroviruses

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Coxsackievirus A16 (CVA16) and human enterovirus 71 (HEV71) are two major etiological agents of hand, foot and mouth disease (HFMD) in children. Recently there were several large outbreaks of HFMD in VietNam and the Asia-Pacific region. A reverse transcription – multiplex polymerase chain reaction (multiplex RT-PCR) assay that can detect enteroviruses, CVA16 and HEV71 was developed. Identification of these viruses was performed with a mixture of three pairs of specific primers: one pair of published primers (F1 and R1) for amplifying all known enterovirus genomes (440 bp) and two new sets of primer pairs specific for the VP1 genes of HEV71 and CVA16, with 264 and 550 bp amplicons, respectively. Enterovirus isolates (ECHO, CVA, CVB groups) and EV71 strains from patients with HFMD were examined with the multiplex RT-PCR to evaluate the specificity of the assay. The sensitivity of the multiplex RT-PCR for HEV71 and CVA16 was also assessed. Taken together, the findings clearly indicate that this multiplex RT-PCR is a rapid, sensitive, specific and cost-effective assay for laboratory diagnosis of HFMD and is also potentially useful in the investigation of the molecular epidemiology of HFMD due to HEV71 and CVA16. Comparison of the optimised multiplex RT-PCR assay with single RT-PCR assays using clinical specimens in uncomplicated and complicated HFMD cases identified at the hospital will be performed to validate the multiplex RT-PCR assay.

This study was supported by a grant from the Wellcome Trust. Dr Nguyen Thi Thanh Thao was supported by an AusAID Australian Leadership Award Fellowship.

Detection of HSV DNA in CSF of non-JEV Encephalitis – meningitis

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Although the Japanese encephalitis vaccination has been introduced in Northern Vietnam since 2000, morbidity and mortality of Acute Encephalitis Syndrome (AES) is still high, and according to some estimation, causatives of about 80% of AES have not been identified.

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This work was supported in part by the French Agency for Development through the SISEA program.

The Mekong Basin Disease Surveillance (MBDS): A Regional Initiative

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MBDS was started with a project and is initiated to: a) reduce morbidity and mortality from communicable diseases, particularly amongst marginalized people living in the Mekong region, by developing an integrated approach to disease surveillance and response across borders; and b) to establish partnerships with other existing cooperation mechanisms.

The project aims to strengthen national and sub-regional capabilities in disease surveillance of, and outbreak response to five priority diseases such as Dengue Infection, Malaria, Severe diarrhea (including Cholera), Vaccine Preventable Diseases, and Outbreak of diseases with sub-regional significance, in order that they can be rapidly and effectively controlled.

Apart from Pandemic influenza preparedness tabletop exercises (TTXs) conducted in each country during 2006 and Regional MBDS TTX conducted in March 2007 (Siem Reap, Cambodia) MBDS partners will focus on the implementation of seven inter-related core strategies identified by MBDS leadership over 2008-2013. The seven inter-related strategies include:

1. Maintain & expand cross-border cooperation
2. More effectively address human-animal interface and improve community surveillance
3. Strengthen epidemiology HR capacity
4. Strengthen ICT capacity
5. Strengthen laboratory capacity
6. Strengthen risk communications
7. Conduct and apply policy research

Assessment of the sensitivity of Cambodian H5N1 strains towards Oseltamivir Carboxylate and Zanamivir

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Since 2003, currently circulating H5N1 avian influenza strains have caused outbreaks in 12 countries, including most of South East Asia, and have caused over 260 human fatalities, including 8 in Cambodia.

We have isolated and characterized the molecular determinants of a large panel of H5N1 strains isolated from poultry and human species between 2004 and 2008. Several strains have molecular alterations which are predicted to affect sensitivity to NA inhibitors. The aim of this study is to assess the susceptibility of a panel of Cambodian H5N1 isolates to Oseltamivir (TamifluTM) and Zanamivir (RelenzaTM).

A chemoluminescence-based *in vitro* assay of NA activity, which utilizes the artificial NA substrate 1,2-dioxetane derivative of sialic acid (NA-STAR), was used to determine the concentration of drug required to inhibit 50% of NA enzyme activity (IC₅₀).

These results demonstrate that Cambodian H5N1 strains from 2005-2008 from human and avian species all exhibit IC₅₀ values in the highly sensitive range (0.1-1 nM) for both Oseltamivir and Zanamivir. Development of resistance to NA inhibitors is a concern in the wake of an influenza pandemic, as these drugs will be one of the first lines of defense to protect the community against the pandemic strain until a suitable vaccine can be developed. This study contributes to the WHO global surveillance program for monitoring NA inhibitor resistance amongst Influenza strains.

Epidemiology of Zoonotic Diseases in Southeast Asia (EPIZOOSEA)

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Recent events have shown just how important this cooperation can be in dealing with zoonotic diseases such as highly pathogenic avian influenza and more recently with the new Influenza A/H1N1swl. In all these cases, controlling the pathogen at its source in animals could help to avoid subsequent public health problems, which explains the importance of suitable budgetary allocations for disease prevention and the usefulness of national joint committees with the participation of the Veterinary Services and the Medical Services, aimed at establishing permanent consultation and cooperation, a situation that unfortunately does not exist at all in too many countries.

The SISEA project aims to contribute to improving detection and responses of epidemics caused by emerging pathogens in South East Asia (Cambodia, China, Laos and Vietnam). In the framework of SISEA project, we intend to develop a zoonotic or OWOH component or sub-project, linking medical and veterinary sectors, EPIZOOSEA Project.

The objectives should be:

- To build up and reinforce surveillance systems at the national level ; support the countries by making their animal health situation transparent and setting up mechanisms for the early detection of disease outbreaks
- To propose recommendations to be made on ways of improving cooperation between the Veterinary Services and the Public Health Services
- To develop a regional platform/network to improve early warning on infectious zoonoses in Southeast Asia.

A steering committee will be defined in order to monitor scientific activities at the regional level and to define agendas during the duration of the project. Medical and veterinary sectors will be part of this steering committee and should include key-researchers of the following institutions: Cambodia: IPC and NaVRI; China: Institut Pasteur-Shanghai and HKU-Pasteur Research Centre; Laos: NCLE, Institut Pasteur of Laos; Thailand: DLD (Department of Livestock Development, Bangkok) and Kasetsart University (Bangkok); Vietnam: NIVR: National Institute of Veterinary Research (Hanoi) and Pasteur Institutes.

A researcher or a Public health-Animal health partnership should be in charge of the scientific follow-up at the regional level. The regional activities are the quantitative evaluation of the surveillance systems (in relation with the REVASIA project runs by CIRAD, see hereunder), the prioritisation of zoonoses, the risk analysis of introduction and spreading of EIDs and risk mapping taking into account human and animal diseases' drivers, which both need regional experts' opinion meetings, trans-boundary surveys and studies.

Moreover, a 'one health' workshop and training will be proposed on the common topics of interest like surveillance, risk analysis, joint human and animal epidemiological study on zoonotic diseases etc.

The Situation of Japanese Encephalitis and other New Viruses Causing Encephalitis in Vietnam.

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Japanese encephalitis (JE) virus is leading etiology cause encephalitis for children in Asia and Pacific regions. So far, JE cases have been controlled in several North Asian countries such as Japan, South Korea owing to the intensive of using JE vaccine for children. In Vietnam, JE vaccine has been introduce in EPI program to prevent children from JE disease since 1997, making the insignificant decrease of JE cases in Vietnam. The efficacy of JE vaccine has been recorded in several provinces such as Ha Tay, Thai Binh, Thanh Hoa and Bac Giang...However; viral encephalitis case is still recorded.

To date, among confirmed viral encephalitis cases, the proportion viral encephalitis case due JE virus is ranged from 20 to 45.5 % (during 1998 – 2007); enterovirus around 2 % (2003); Herpes Simplex virus type 1 and type 4 around 7 % (2003). There must exist other viruses cause encephalitis, but it is not yet detected in Vietnam so far. Thus, searching new viruses are etiology cause encephalitis need to be carried out. During 2002 – 2007, the detection of new virus in cerebroi spinal fluid samples of viral encephalitis case was carried by the isolation of virus, yielding expected results. New viruses have been found such as the detection of Nam Dinh virus – an Aterivirus in Nam Dinh province, Northern Vietnam (2002); Banna virus, the virus belonging to *Reoviridae* family in Gia Lai, Highlands (2005) and Acmong virus in Bac Giang province, Northern Vietnam (2004 – 2007).

Therefore, the development of diagnostic reagents need to be mentioned for further research on sero-diagnosis and sero epidemiological surveillance in order to supply the information about the situation of other new viruses cause acute encephalitis syndrome in Vietnam.

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For an enhanced networking of professionals in communicable diseases in the Greater Mekong Subregion: the GMS CDC project's efforts

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Asian Development Bank Greater Mekong Subregion Communicable Diseases Control Project

Communicable diseases control (CDC) professionals in the Greater Mekong Subregion (GMS) meet each others intermittently at various regional or international events; they also exchange information together on an ad hoc basis afterwards. This does not imply however that a vibrant regional network of professionals exists; despite existing efforts of various partners, much more can be done.

Not only does networking permit a better sharing of information (filtered and targeted) among professionals, it also enhances a much needed coordination among partners, hence preventing duplications and allowing for a more rational use of scarce resources; ultimately networking provides appropriate ways and means for Governments to devise more responsive strategies and policies. Moreover, a sound and well coordinated networking leads to the development of Communities of Practice (CoP) that improves performance of both individuals and organizations. While the belief has often been that *“the larger the network the better”*, experience shows that *“the more focused and passionate the network, the better it is”*.

Networks require a certain level of facilitation. Because networks have neither time nor space limits, it is often difficult to readily identify within them who would be the most legitimate “coordinators” of professional regional networks (WHO, ASEAN, MBDS, ACMECS, GMS, Pasteur, US CDC, etc?). Instead, efforts should rather focus on identifying Knowledge Management “champions” who can take the lead on specific topics and foci.

There are several ways to enhance professional networking, ranging from face-to-face events – regional workshops, forums, symposiums, etc – to distance sharing via electronic means, such as electronic forums, electronic newsletters, interactive websites, teleconferences, web-based voice-to-voice telecommunication (Skype™, Google Talk™, MSN™, etc). Face-to-face meetings add naturally the “human touch” to direct communication but they are costly – including opportunity costs -- and they are truly productive only when event organizers have indeed optimized the networking aspects. Electronic forums, on the other hand, are often at no or low cost but they require a minimum IT working knowledge and the relevant “IT culture”; two conditions which – without sufficient motivation and proper facilitation – act as deterrents to active electronic networking. “Interoperability”, or the ability to share across diverse existing electronic systems, becomes one of today's growing issues in developing electronic-based professional networking.

The ADB GMS CDC project, through its Regional Coordination Unit, strives to enhance networking of CDC professionals in the subregion in several ways; since late 2006, it has (i) organized 20 face-to-face regional events (forums, cross-border workshops, symposium, Scientists' meetings, Regional Project Managers' meetings, etc), (ii) developed Joint Regional Operational Studies, (iii) developed a “Directory of Professionals involved in CDC in the GMS”, (iv) opened 4 CDC-focused electronic forums, (v) launched a GMS CDC newsletter, (vi) developed a filtered and targeted information sharing mailing,

(vii) maintained a GMS CDC website, and (viii) proposed the Terms of Reference of a regional “Clearinghouse” to optimize networking and facilitate coordination of CDC project operations.

Some key challenges to networking are: (i) the frequent poor understanding of the benefits networking can bring to professionals, (ii) the lack of incentives to network – or worse, the deterrent of doing so – (iii) the funding of face-to-face meetings, (iv) the lack of expertise in nurturing CoPs, and (v) the overly-reported lack of time by the professionals to network.

Regional networking of CDC professionals needs to be understood first and foremost as an investment. It requires genuine commitments, technical expertise and financial support.

2B protein of EV71 functions as an ion channel and is a potential drug target

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Enteroviruses (e.g. poliovirus, coxsackievirus, ECHOvirus, Enteroviruses 71) belong to the family of Picornaviridae, a large family of nonenveloped, cytolytic viruses that contain a single-stranded RNA genome. Frank J. van Kuppeveld's lab demonstrated that CVB3 2B increases the efflux of Ca^{2+} from the stores by forming transmembrane pores, and they postulated that enterovirus 2B protein forms pores in ER and Golgi membranes and thereby disturbs intracellular Ca^{2+} homeostasis.

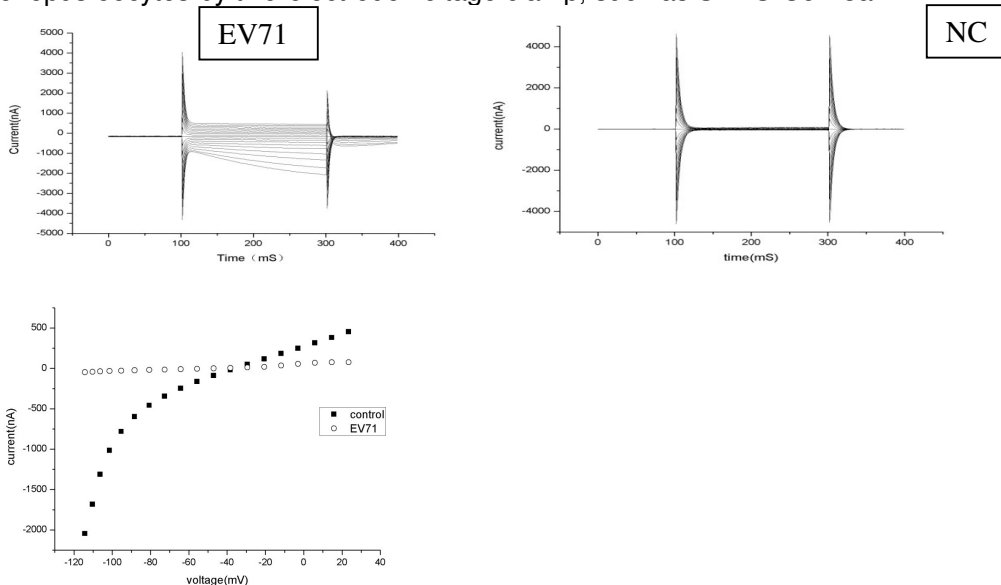
We checked 2B gene of enterovirus 71, and our data suggested that EV71 2B may have a similar function with coxsackievirus 2B protein.

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Score = 101 bits (251), Expect = 2e-20
Identities = 47/85 (55%), Positives = 62/85 (72%), Gaps = 0/85 (0%)

EV71 1  GDAFCMGFTDAVSREVEALKSHLIGSEGAVEKILKNLVKLISALVIVIRSDYDMVTLTAT 60
      G+AFG GFT+ + +V LK L+G + +EK LK LVK+ISALVIV+R+ D++T+TAT
CVB3 10 GNAFGSGFTNQICEQVNLKESLVGQDSILEKSLKALVKIISALVIVVRNHDDLITVTAT 69

EV71 61  LALICCHGSPWANVKSKTASILGIP 85
      LALIGC SPW W+K K + GIP
CVB3 70  LALIGCTSSPWRWLKQKVSQYYGIP 94
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However, our primary data shows that EV71 2B protein may form a cation channel in *Xenopus oocytes* by two-electrode voltage clamp, such as SARS-CoV 3a.



We will further confirm the ion channel activity of EV71 2B protein, study its role in viral life cycle, and screen a channel inhibitor to block its function, which may serve as a potential drug to against EV71 infection.

Infectious, vector-borne and food-borne diseases (MIVA/IVEFOOD): a new MSc international curriculum focusing on health in South-East Asia

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The International Network of Pasteur Institute, University Montpellier 2 (UM2), Kasetsart University (KU) and Cirad have launched together a new International Master curriculum entitled "Infectious, vector-borne and food-borne diseases". This MSc international curriculum is nicknamed MIVA in French and IVEFOOD in English. This curriculum is a double degree MSc, students getting a degree from both UM2 and KU.

This curriculum has started in March 2009 in Bangkok, Thailand, where all classes are taking place. A specific trait of this MSc curriculum is to be exclusively located in South-East Asia? It is opened to students and professionals from the whole region and from France and brings lecturers from international background coming from Thailand, Vietnam, Cambodia, Laos, Hong Kong and France.

The International network of Pasteur Institutes is a key partner in the curriculum participating to all the main aspects, i.e. student selection, teaching and laboratory training.

The MIVA/IVEFOOD curriculum is aiming at giving students scientific and technical knowledge which can meet the current societal demands. Students are given an integrated, multidisciplinary training over two years. Starting from the three main routes of contamination, direct infection, vector transmission and food contamination, the training addresses the biological, economical and societal aspects. The keystone of the program is "dynamics" and the training is addressing the mechanisms of pathogenesis, adaptation, evolution and, of course, emergence. More operational aspects such as diagnostic, nanotechnology, surveillance, alert, risk-assessment or quality assurance are also addressed. A specific session is devoted to legal and regulatory issues.

For this first year the MIVA/IVEFOOD curriculum is hosting students from Vietnam, Laos, Cambodia and France, most of them being professionals and more precisely physicians. Engineers, veterinarians and true Post-graduate students are also present. The MIVA/IVEFOOD curriculum is directly opening the way to PhD programs either in France, Thailand or in any other south-Asian country based on co-supervision agreement.

JEV and EV71 in South Vietnam

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Emerging disease surveillance in Southern Vietnam

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Background

Since 2003, Southern Vietnam has become the most serious affected area by various kinds of viruses such as: A/H5N1, Enterovirus 71, Rubella, Chikungunya... Although the situation of emerging disease in the region is more and more complicated, a specific surveillance system on emerging diseases has not been established yet. With all the concerns, the establishment of emerging disease surveillance is essential and necessary to provide scientific information for good understand the emerging diseases and for effective outbreak response.

Objective

To establish an emerging disease surveillance and response system in Southern Vietnam.

Implementation

The project will be implemented from June 2007 to June 2010 at 1 sentinel province hospital and 1 sentinel district hospital in Southern Vietnam. The activities of surveillance system will be taken place in all administrative levels (commune, district, province and region). Any clinical cases diagnosed severe viral pneumonia or acute encephalitis syndrome will be recorded by a standard questionnaires and collected specimens (swabs, paired sera, feces, cerebrospinal fluid). These specimens will be tested by RT-PCR, multiplex RT-PCR, real-time RT-PCR, virus isolation in cell culture, Mac ELISA and IgM Indirect ELISA to determine the causatives. At the same time, field investigation will be conducted by task force team in order to investigate cases, survey exposed people who contact with patient and collect their specimens.

Based on the obtained result analysis, the outbreak intervention measures will be selected and implemented appropriately, timely and effectively.

This work was supported in part by the French Agency for Development through the SISEA program.

A Summary of Newly Established Laboratory-Based Influenza Surveillance in Laos

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The pandemic threat from avian influenza A/H5N1 and novel influenza A(H1N1)-2009 underscore the importance of establishing influenza surveillance in developing subtropical countries. Laboratory-based influenza surveillance was first established in the Lao People's Democratic Republic in 2007. Three hospitals in the capitol city of Vientiane began surveillance for influenza-like illness (ILI) in outpatients in 2007 and expanded to include hospitalized pneumonia patients in 2008. Nasal/throat swab specimens were collected and tested for influenza and other respiratory viruses by multiplex ID-Tag™ respiratory viral panel (RVP) assay on a Luminex® 100 xMAP IS instrument. Basic epidemiological and clinical data collection was begun in 2008.

During January 2007 to December 2008, 526 outpatients with ILI were tested and 287 (54.6%) were positive for at least one respiratory virus. Influenza type A was identified in 63/526 (12.0%) and influenza Type B in 92/526 (17.5%). In 2008, 79 specimens were received from hospitalized pneumonia patients and 5 (6.3%) tested positive for influenza type A and 4 (5.1%) were positive for influenza type B. Children less than 5 years of age represented 19% of all viral infections in outpatients and 38% of pneumonia inpatients. High levels of influenza B infection were observed in January through March of 2007 and May through September of 2008. Implementing laboratory-based influenza surveillance requires substantial investments in infrastructure and training but represents an important advance for public health.

Our early results suggest influenza is a common cause of outpatient respiratory illness and pneumonia requiring hospitalization. Expanded surveillance is planned to improve geographic representation and better describe the epidemiology of influenza in Laos.

Development of new technologies for improving the diagnosis of viral encephalitis

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Although many new biomolecular techniques were developed in the recent past years, the etiologic profile of viral encephalitis (VE) is still unclear in more than 60% of the cases. To better identify the responsible pathogens and to understand the pathogenesis of viral encephalitis, more modern multidisciplinary approaches should be established and applied to the diagnosis of this disease. In 2007, Institut Pasteur of Shanghai, in collaboration of Guangxi CDC, started the project of surveillance and control of children with viral encephalitis in Guangxi province, China, under the SISEA program. IPS is developing a platform for rapid virus identification using cell biology and molecular biology techniques including:

- Genome study using multiplex RT-PCR and real-time PCR
- High density DNA Microarray
- High throughput sequencing and genome analysis
- Electron microscopy
- Serology test

56 cerebrospinal fluid (CSF) and serum samples from children VE patients were collected in 2007 and 41 samples in 2008, in which 48 of 56 cases in 2007 were confirmed to be infected with Japanese encephalitis Virus (JEV) by serology test (serum JEV IgM positive, *Panbio ELISA Kit*). A panel of published RT-PCR specific to prevalent VE pathogens: JEV, HSV, alphavirus, flavivirus or enterovirus, was applied to detect RNA extracted from all patients CSF samples. No positive result was obtained. All 56 CSF samples in 2007 were inoculated to VeroE6 cell and one virus was isolated. The isolate was confirmed to be JEV (strain JEV/GX131/2007) by specific JEV RT-PCR and the large scale sequencing combining with random PCR, but the JEV IgM serology test of this patient was negative.

It was very difficult to identify virus directly from clinical samples from VE patients by using conventional techniques as specific RT-PCR and cell culture. New strategies with multiple approaches should be applied. Firstly, it is necessary to develop more sensitive multiplex RT-PCR assays and DNA microarray specific to common viruses to improve the molecular diagnosis, while using random RT-PCR combining large scale sequencing (for example, the pyrosequencing) to identify rare or new viruses. Secondly, more sensitive cell lines should be used to improve the sensitivity of virus culture. Finally, although the detection of specific anti-virus IgM is largely used for JEV diagnosis, it may miss acute infection. An antigen capture assay for JEV may be used for virus diagnosis in CSF or sera of patients with encephalitis in Asia.

Design of One-Tube Multiplex Real-Time RT-PCR for Specific Identification of Novel Swine-Origin (H1N1) and Seasonal Influenza A viruses

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Influenza A virus (IAV) is the major cause of human respiratory infection with high risk to trigger an influenza pandemic in vulnerable population. After the emergence in Hong Kong in 1997 of avian influenza H5N1 virus, the world is currently facing a new epidemic after the emergence in April 2009 in Mexico of a new swine-origin influenza virus (S-OIV) H1N1 different from the seasonal H1N1 influenza viruses. In the current situation, two antigenically unrelated H1N1 viruses are concomitantly circulating worldwide and a rapid and specific diagnosis for each of them is necessary.

Recently, we performed sequential real-time reverse transcription-PCR (rRT-PCR) assays for identification of IAV M and HA genes from six known IAV infecting pigs, birds and humans. IAV M gene-positive samples tested by single rRT-PCR and fluorogenic SYBR Green I detection system can be further processed for H5 subtype identification using two primer set multiplex and SYBR Green I rRT-PCR. Negative samples are then tested in either H1 and H3 or H2, H7 and H9 one-tube TaqMan multiplex rRT-PCR identification assays. However, the M1 and H1-HA oligonucleotide primers and probe that were designed in this test did not match entirely with the sequences of the M1 and H1-HA genes of the S-OIV H1N1 virus.

New primers and probe were designed corresponding to highly specific but conserved nucleotide sequences of each M2 and H1-HA genes from published swine H1N1 strains and the recent human A/California/4/2009 S-OIV H1N1 strain. The oligonucleotides were added to the test tubes containing the pool of primers and probes targeting M1 or HA genes of seasonal influenza viruses (3). RNA samples were extracted from titrated virus stocks and were 10-fold serially diluted.

The limit of detection of the multiplex reactions for each of the novel and seasonal H1N1 and H3N2 virus tested was 1 TCID₅₀ for the M1 genes of seasonal H1N1 and H3N2 viruses and for the M2 gene of A/California/4/2009, whereas it was of 0.1 TCID₅₀ for the HA genes of the three viruses. The limit of detection using monoplex reactions remained similar for the M gene, whereas it was of 0.01 TCID₅₀, 10 times more sensitive than the multiplex reaction targeting the HA genes. Similar results were obtained when one swine influenza virus (A/SW/HK/PHK1578/03) RNA was tested. No cross-reactivity of the new primers and probe designed for S-OIV H1N1 detection was observed with seasonal influenza viruses or with nine major viruses responsible of respiratory infection.

We have improved here the multiplex detection assay developed recently for identification of avian influenza virus subtypes by adding a set of primers in the tubes targeting M2 and HA genes of the novel human and swine H1N1 viruses. This multiplex assay performed in 3 hours can be used to differentiate the current S-OIV H1N1 influenza virus from the seasonal influenza viruses in one tube and in the field using the mobile Smartcycler[®] instrument.

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Pathology of infectious encephalitis

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In Asia, infectious diseases involving the central nervous system (CNS) continue to cause severe morbidity and mortality. In the last two decades there had been several large epidemics of acute viral encephalitides in Asia caused by different viruses. These included known viruses such as Japanese encephalitis virus (JeV) and Enterovirus 71 (EV71), and novel viruses such as the Nipah virus (NiV). JeV is still very prevalent in the Indian subcontinent and Southeast Asia and it is the most important mosquito-borne encephalitis.

Typical of viral encephalitis, the pathology is characterized by perivascular cuffing, microglial nodules and neuronophagia. EV71 has caused large epidemics of hand, foot and mouth disease in children in Asia and other parts of the world. It is transmitted by the faecal-oral route, and rarely, it is complicated by fatal encephalomyelitis. Direct neuronotropism and retrograde viral spread from peripheral nerves to the CNS play a role in neuropathogenesis. NiV is a novel paramyxovirus that jumped the species barrier from fruit bat to infect humans. It causes a systemic infection but the CNS appears to be most vulnerable. The acute NiV encephalitic syndrome is caused by a dual mechanism of vasculitis-induced microinfarction and neuronal infection.

Other endemic infections such rabies, cerebral malaria, bacterial meningoencephalitides also contribute significantly to mortality, and should be considered in the differential diagnosis of CNS infections.

Vaccine-elicited Anti-SARS-CoV Spike Antibodies Trigger Infection of Human Immune Cell via a pH- and Cathepsin L-independent FcγR Pathway

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Public health measures have successfully contained outbreaks of the severe acute respiratory syndrome coronavirus (SARS-CoV) (which infected more than 8000 people worldwide with a mortality rate of about 10%) but concerns remain over future recurrences. Therefore continuous efforts have been made to develop safe vaccine strategies against SARS-CoV. Caution has to be taken for the safety of SARS-CoV vaccines due to the possibility of immune system-mediated enhancement of the disease, a fact that has been observed already with vaccines against coronaviruses.

We have developed a SARS vaccine candidate based on recombinant native full-length Spike-protein trimers (the envelope glycoprotein involved in SARS-CoV entry into host cells). Our vaccine protocol elicited an *in vivo* neutralizing and protective immune response in rodents. By using SARS-CoV Spike-pseudotyped viral particle (SARS-CoVpp) we have analyzed the capacity of immune-sera to mediate antibody-dependent enhancement (ADE) of viral infection *in vitro*. The experiments exhibited opposite pattern according to cell types: while complement-inactivated sera from immunized animals still inhibit SARS-CoVpp entry in prototypic permissive cell line, these sera induced virus penetration in human monocytic and lymphoblastic (B lineage) cell lines. Entry into human hematopoietic cells occurred via FcγR-dependent and ACE2-, pH-, cysteine-protease-independent pathways illustrating that ADE of virus infection is a novel cell entry mechanism of SARS-CoV.

Finally by comparing neutralizing versus enhancing potency of different SARS vaccine candidates we highlighted distinct patterns of ADE despite highly similar abilities of the immune-sera from different vaccine protocols to neutralize SARS-CoV infection.

Our study, by illustrating the occurrence of immune-mediated enhanced infection of SARS-CoV, advocates reasonable safety concerns regarding the use of SARS-CoV vaccine in humans as well as opens a new way of investigation in the understanding of SARS pathogenesis.

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