Scientific Conference
of the Institut Pasteur International Network

EMERGING AND RE-EMERGING VIRAL INFECTIONS

National Institute of Hygiene and Epidemiology
Hanoi
November 27th - 28th, 2006

Organizing committee

Dr. Nguyen Tran Hien (nthiennihe@vnn.vn)
Dr. Dang Duc Anh (ducanhnihe@hn.vnn.vn)
Dr. Le Thi Kim Tuyen (kimtuyen@hn.vnn.vn)

Dr. Paul Brey (pbrey@pasteur.fr)
Dr. Jean-Luc Guesdon (guesdon@pasteur.fr)

Secretaries
Mrs. Tran Mai Hung (nihe@hn.vnn.vn)
Mrs. Sylvie Garnero (sgarnero@pasteur.fr)

Scientific committee

Dr. Paul Brey
Dr. Vincent Deubel
Dr. Arnaud Fontanet
Dr. Jean-Claude Manuguerra
Dr. Nguyen Tran Hien
Prof. Hoang Thuy Long
Dr. Pham Ngoc Dinh
Dr. Dang Duc Anh

Program

Monday 27th November

8:30 **Welcome addresses**

Representative of Vietnamese Ministry of Health
His Excellency Jean-François Blarel, French Ambassador, Hanoi
Mrs Michèle Boccoz, Director of International Affairs, Institut Pasteur, Paris
Dr Nguyen Tran Hien, Director of National Institute of Hygiene and Epidemiology

Session 1	ACUTE RESPIRATORY INFECTIONS CAUSED BY VIRUSES

MORNING SESSION

Chairman: Dr Nguyen Tran Hien (NIHE, Hanoi, Vietnam)

9:00 **Arnaud Fontanet**, Institut Pasteur, Paris: ***SARS: from bats to humans***

9:30 **Béatrice Nal**, HKU – Pasteur Research Centre, Hong Kong: ***Identification of cellular factors which regulate early and/or late stages of SARS corona virus replication cycle***

10:00 **Bing Sun**, Institut Pasteur of Shanghai, Chinese Academy of Sciences: ***SARS-CoV 3a protein forms an ion channel and modulates virus release***

10:30 **Coffee Break**

11:00 **Malik Peiris**, Department of Microbiology; University of Hong Kong***: "Avian flu H5N1" A threat to human health"***

11:30 **Philippe Buchy**, Institut Pasteur du Cambodge, Phnom Penh, Cambodia: ***AI H5N1 detection and surveillance in Cambodia***

12:00 **Vong Sirenda**, Institut Pasteur du Cambodge, Phnom Penh, Cambodia: ***Avian-to-human transmission of H5N1 in Cambodia, 2005***

12:30 **Lunch**

Posters session: 12:30 - 14:00

AFTERNOON SESSION

14:00 **Mark Simmerman**, WHO, Hanoi Vietnam: ***The burden of Human Influenza in East and Southeast Asia: What does the literature tell us?***

Chairman: Dr Mark Simmerman (WHO, Hanoi Vietnam)

14:30 **Nancy Cox**, CDC Atlanta, USA: ***Molecular epidemiology of AI H5N1***

15:00 **Le Quynh Mai**, National Institute of Hygiene and Epidemiology, Hanoi, Vietnam: ***The risk factors of H5N1 in Thaibinh province, 2004.***

15:30 **Cao Bao Van**, Institut Pasteur Ho Chi Minh City, Vietnam: ***Molecular evolution of highly pathogenic H5N1 influenza viruses in Southern Vietnam.***

16:00 **Nguyen Tien Dzung**, Department of Virology, National Institute of Veterinary Research, Hanoi, Vietnam: ***The Role of Domestic Ducks for Maintenance and Spread of Avian Influenza H5N1 in Vietnam***

16:30 **Dongjiang Tang**, HKU – Pasteur Research Centre, Hong Kong: ***Lentiviruses pseudotyped with the hemagglutinnine of a H5N1 influenza virus enter the cell in a pH-dependent way***

17:00 **End of the first day**

18:00 **Departure from NIHE for the French Embassy's party**

Tuesday 28th November

Session 2	ACUTE VIRAL ENCEPHALITIS SYNDROMES

MORNING SESSION

Chairman: Dr Pham Ngoc Dinh

08:00 **Vu Duy Nghia**, National Institute of Hygiene and Epidemiology, Hanoi Vietnam: ***Epidemiology of Viral Encephalitis in Bac Giang Province, Vietnam***

08:30 **Phan Thi Nga**, National Institute of Hygiene and Epidemiology, Hanoi Vietnam: ***Emerging viruses associated with acute encephalitis syndrome in Vietnam***

09:00 **Jean Claude Manuguerra**, Institut Pasteur Paris, France: ***Implementation of new or recent molecular identification tools for undiagnosed viral diseases and for newly generated virus genotypes***

09:30 **Coffee break**

10:00 **Kouichi Morita**, Dept. of Virology, Institute of Tropical Medicine, Nagasaki University, Japan: ***Japanese encephalitis virus ecology in Asia implies possible rapid region-wide West Nile Virus expansion: Needs of Development of West Nile fever vaccines***

10:30 **Gabriela Nicolescu**, Institut Cantacuzene, Bucharest Romania: ***The surveillance of West Nile virus circulation in Romania (2001-2005)***

11:00 **Nicolai Tokarevich**, Institut Pasteur de Saint Petersbourg, Russie: ***Results of many-years monitoring over tick-born encephalitis in St. Petersburg***

11:30 **Vincent Deubel**, Institut Pasteur Shanghai – CAS, Shanghai China, ***A comprehensive approach towards Nipah virus infection, prevention and treatment***

12:00 **Lunch**

Posters session: 12:00 – 14:00

Session 3	VIRAL HEMORRHAGIC FEVERS

AFTERNOON SESSION

Chairman: Dr Vincent Deubel (Institut Pasteur Shanghai – CAS, Shanghai)

14:00 **Nguyen Thi Kim Tien**, Institut Pasteur Ho Chi Minh City, Vietnam: ***Epidemiology of dengue/ dengue hemorrhagic fever in Mekong Delta area, a potential site for dengue clinical vaccine trial***

14:30 **Vu Sinh Nam**, National Institute of Hygiene and Epidemiology and Ministry of Health, Hanoi Vietnam: ***Success in dengue vector control in Vietnam using Mesocyclops as bilogical agent and Community-Based Methods***

15:00 **Amadou Sall**, Institut Pasteur de Dakar, Senegal: ***Viral adaptation and dengue emergence***

15:30 **Nguyen Thi Phuong Lan**, Institut Pasteur Ho Chi Minh City, Vietnam: ***Genetic analysis for susceptible gene to Dengue hemorrhagic fever in Vietnam***

16:00 **Philippe Despres**, Institut Pasteur Paris, France: ***Genetic polymorphism of CD209 (DC-SIGN) associated to dengue disease***

16:30 **End of the second day**

18:00 Departure from NIHE to restaurant for NIHE reception

Lectures

ACUTE RESPIRATORY INFECTIONS CAUSED BY VIRUSES

L 01

SARS: FROM BATS TO HUMANS

Arnaud Fontanet

Unité d'Epidémiologie des Maladies Émergentes
Institut Pasteur, Paris

In the first few months of 2003, an unknown animal corona virus (CoV) has emerged from the wet markets of Southern China to threaten the public health systems worldwide. Populations anxiety has been high, in relation with this new agent transmitted via the respiratory route, attacking young adults, with a high mortality rate, and able to reach all parts of the world within hours. Overall, more than 8000 people were infected over five continents, and 774 died from this new atypical pneumonia. What do we know today about the circumstances which allowed this virus to spread in human populations? An insectivorous bat of the Rhino holus genera has been found infected with a CoV 92% identical to that found in humans, and might be the animal reservoir of SARS-like CoV. Transmission to humans took place through a small wild animal, the masked palmed civet (Paguma larvata), eaten as a delicacy in exotic restaurants of South-East China. How were civets infected? No one knows. However, transmission from civets to humans most likely took place in market places or restaurants. The presentation will detail the epidemiological and biological determinants of SARS-CoV transmission to humans.

L 02

IDENTIFICATION OF CELLULAR FACTORS WHICH REGULATE EARLY AND/OR LATE STAGES OF SARS CORONAVIRUS REPLICATION CYCLE

Nal Beatrice, Millet Jean, Siu Lewis, Altmeyer Ralf

HKU-Pasteur Research Centre, Dexter HC Man Building, 8 Sassoon Road, Pokfulam, HONG KONG

During million of years of co-evolution, viruses and host cells have adapted themselves to each-others. Viruses have developed amazing strategies to exploit cellular machineries like vesicular and protein trafficking, biosynthesis and sorting machineries. On the other hand cells have reacted to viral invasions by developing innate defence mechanisms and by expressing restriction factors for viral replication. The understanding of cross-talk processes between viruses and host cells is a must to identify new targets for future therapeutic approaches.

Our objective is to characterize cellular "Interactomes" for viral structural proteins. In that aim, we have performed a large-scale yeast-two-hybrid screening, choosing SARS CoV envelope proteins S, M, E C-terminal domains as baits. The usage of envelope proteins C-terminal domains as baits should lead to the identification of cellular interacting proteins which may play a role either in pre-budding steps or in fusion/post-fusion events. These cellular factors could regulate trafficking and assembly of viral envelope proteins or regulate viral penetration during the early stages of infection. Very interestingly, we have identified several cellular candidates which may function in regulating SARS CoV infection. Mainly, these candidates belong to cell cytoskeleton, cell adhesion and signal transduction systems.
This study should lead to a better understanding of dynamic mechanisms regulating virus / host cells interactions and participate to the identification of new targets for future therapies.

L 03

SARS-CoV 3a PROTEIN FORMS AN ION CHANNEL AND MODULATES VIRUS RELEASE

Wei Lu, Vincent Deubel and **Bing Sun**

Institut Pasteur of Shanghai, Chinese Academy of Sciences.

Abstract
Fourteen open reading frames (ORFs) have been identified in the severe acute respiratory syndrome-associated coronavirus (SARS-CoV) genome. ORF 3a of SARS-CoV codes for a recently identified transmembrane protein, but its function remains unknown. In this study, we confirmed the 3a protein expression and investigated its localization at the surface of SARS-CoV-infected or 3a-cDNA-transfected cells. Our experiments showed that 3a protein can form homo-tetramer complex through inter-protein disulfide bridges, which is a hint for ion channel function. The putative ion-channel activity of this protein was assessed in 3a-cRNA-injected Xenopus oocytes by two-electrode voltage clamp. The results suggest that 3a protein forms a potassium sensitive channel, which can be efficiently inhibited by barium. After FRhK-4 cells transfected with a siRNA, which had been demonstrated to be able to suppress 3a expression, followed by infection of SARS-CoV, the released virus was significantly decreased, while the replication of the virus in the infected cells was not changed. Our observation suggests that SARS-CoV ORF 3a functions as an ion channel, which may promote the virus release. This new finding will help us to explain the high pathogenesis of SARS-CoV and to develop new strategy for treatment against SARS infection.

L 04

AVIAN FLU H5N1: A THREAT TO HUMAN HEALTH

JSM Peiris.

Department of Microbiology, The University of Hong Kong, University Pathology Building; Queen Mary Hospital; Hong Kong SAR.

The "bird flu" incident in Hong Kong in 1997 was the first time that severe human disease associated with a purely avian virus was documented with 18 known human cases and 6 deaths caused by a highly pathogenic avian influenza (HPAI) A subtype H5N1 virus. Since then, H5N1 virus has caused widespread disease in poultry across three continents and has repeatedly transmitted to human in 10 countries with presently over 240 human cases and over 140 deaths. Given the unprecedented geographical extent of the affected area, it is likely that H5N1 avian flu virus is going to pose a long- term threat to human and veterinary health in this region[1,2]. Human-to-human transmission has so far been infrequent and inefficient. However, the possible adaptation of this virus to more efficient human-to-human transmission continues to pose a significant pandemic threat. The disease presents as a severe viral pneumonia rapidly progressing to acute respiratory distress syndrome and death. Oseltamivir is currently the mainstay for treatment of human disease, but on some occasions emergence of resistance may have contributed to a lack of clinical response. The biological basis for the unusual severity of human disease associated with the H5N1 virus remains unknown. Possible contributors are virus dissemination, increased viral replication competence, differing viral tropism and immunopathogenesis. In comparison to human influenza viruses such as H1N1, the H5N1 viruses are potent hyper-inducers of cytokine responses in primary human macrophage and respiratory epithelial cell cultures in vitro[3,4]. For example, in macrophages, the levels of TNF-α induced by H5N1 and the more recent H5N1 viruses of 2004 are more akin to those induced by *E.coli* endotoxin. The differential hyper-induction of cytokines by H5N1 viruses appears to involve in part the p38 mitogen activated protein kinase pathway[5]. The hyperinduction of cytokines in vitro is paralleled by observations in patients with H5N1 disease. Other avian influenza viruses have also caused infection and disease in humans[6,7]. The reason for concern about H5n1 virus is not the inevitability of an H5N1 pandemic but the likely severity of disease associated with such an event.

References:

1. Li KS, *et al*. **Nature**. 2004; 430: 209-13.
2. Chen H, *et al*. **Nature.** 2005; 436:191-2.
3. Cheung CY, et al. **Lancet** 2002; 360: 1831-1837.
4. Chan MC, et al. **Respir Res.** 2005 Nov 11;6:135.
5. Lee DC, et al. **J Virol.** 2005; 79: 10147-54
6. Peiris JSM, et al. **Lancet** 2004; 363: 617-9.
7. De Jong M et al. Nature Medicine – on line Sept 2006

L 05

INFECTIONS PAR LE VIRUS H5N1 AU CAMBODGE
H5N1 INFECTIONS IN CAMBODIA

Mardy Sek (IP Cambodge), Sirenda Vong (IP Cambodge), Ly Sowath (IP Cambodge) Jean-Thierry Aubin (IP), Sylvie van der Werf (IP), **Philippe Buchy** (IP Cambodge).

Entre les mois de janvier 2005 et d'avril 2006, 6 cas humains et 7 foyers aviaires ont été confirmés positifs pour le virus H5N1.
Nous avons analysé les résultats virologiques des patients et des animaux contaminés et étudié les génomes des virus isolés.
Les analyses virologiques ont montré, en outre, une virémie très significative chez tous les patients ainsi qu'une excrétion fécale parfois importante, même en l'absence de troubles digestifs. Ces données, rarement rapportées sont caractéristiques des infections par le H5N1. Elles suggèrent également la possibilité d'une réplication active du virus dans le tractus digestif humain. Ceci soulève des interrogations physiopathologiques et incite à envisager tous les risques de contamination fécale manuportée.

Tous les virus isolés en 2005 appartenaient à la clade 1. Alors qu'en 2006 tous les virus détectés dans le monde appartenaient à la clade 2 ou à de nouvelles clades, les gènes HA et NA des virus cambodgiens n'ont pas significativement évolué entre 2005 et 2006 et conservent les caractéristiques de la clade 1. Aucun pays voisins n'a déclaré de cas de grippe aviaire humaine ou animale dans les mois précédent l'épidémie cambodgienne de 2006. Cette relative « endémicité » du virus H5N1 de clade 1 au Cambodge et le caractère géographiquement isolé des épidémies laisse supposé que le virus ait pu rester latent ou circuler à bas bruit dans le pays entre juin 2005 et janvier 2006 et qu'il n'ait pas été réintroduit par les échanges commerciaux avicoles avec les pays voisins (sauf défaut de déclaration des cas par ces derniers) ni par les oiseaux migrateurs venant en majorité de Chine et des pays de la région.
L'analyse des autres gènes est en cours et apportera des informations complémentaires sur le profil de résistance aux antiviraux ainsi que sur le potentiel réplicatif des isolats cambodgiens.

L 06

AVIAN-TO-HUMAN TRANSMISSION OF H5N1 IN CAMBODIA, 2005

Vong Sirenda (IPC), Coghlan B, Sek M (IPC), Holl D, Seng H, Ly S, Miller M, Buchy P (IPC), Froehlich Y, Dufourcq JB, Uyeki T, Lim W, Sok T.

Background: The recent spread of avian influenza A (H5N1) has affected Cambodia causing high poultry mortality and six human H5N1 cases. To better understand avian-to-human H5N1 transmission, we conducted a retrospective poultry mortality survey and a sero-epidemiologic investigation in a village where a 28yr old farmer died of H5N1 in March 2005.
Methods: Poultry surveys were conducted in March in the human H5N1 case's village and cloacal swabs were collected from sick/dead poultry for H5N1 testing by rRT-PCR. A household chicken flock was considered likely to have been infected with H5N1 during the previous six months if >60% of the flock died, case fatality rate was 100% and all age bird died

suddenly within 1-2 days. In June, the H5N1 case's neighbors were asked about exposures to poultry in the past year and tested for H5N1 antibodies by micro neutralization.
Findings: Ninety-five percent of 163 households surveyed raised chickens. Based on the risk definition for H5N1 among poultry, 42 households were infected during Jan-Mar 2005, including a cluster of 25 with a 7.9 relative risk of H5N1 (p=0.001) within 30 days of the survey. H5N1 virus was detected in two chickens from the household near that of the human case. Although half of the 351 participants from 93 households reported collecting dead/sick poultry and chicken faces (47%) or plucking dead poultry feathers (47%), none had neutralizing H5N1 antibodies.
Interpretation: Asymptomatic H5N1 infection seemed low in Cambodia, despite poultry outbreaks with high mortality suggestive of H5N1 and direct contact with poultry among survey participants.

L 07

THE BURDEN OF HUMAN INFLUENZA IN EAST AND SOUTHEAST ASIA: WHAT DOES THE LITERATURE TELL US?

Mark Simmerman,

Influenza Surveillance and Control, WHO, Hanoi Vietnam

While the H5N1 avian influenza epizoonotic continues to capture international headlines, just 241 cases have been identified with 141 deaths worldwide as of August 23, 2006. Meanwhile, the global burden of vaccine preventable human influenza infection receives much less attention despite hundreds of millions of infections, 3 to 5 million cases of severe illness, and an estimated 1 million deaths annually. An aggressive international research agenda on avian influenza is under way but studies to define the burden of human influenza in the region are uncommon. While influenza is not commonly thought to be an important cause of illness or death in tropical and subtropical countries in East and Southeast Asia, a small but growing body of evidence suggests otherwise. Published data from Singapore, Malaysia, Taiwan, Hong Kong, Indonesia, Korea and Thailand indicate that depending on the season, sample type and laboratory methodology used, between 4% and 23% of all outpatient febrile respiratory illness is caused by human influenza virus infection. Data from Hong Kong, Singapore, and Thailand reveal that influenza is a common cause of pneumonia requiring hospitalization with rates equalling or surpassing those found in the United States. Finally, recent papers on mortality from Hong Kong and Singapore indicate that influenza infection is associated with all-cause, underlying Pneumonia and Influenza (P & I), and Circulatory and Respiratory (C & R) deaths at rates similar to, or in excess of those reported by studies with similar methodologies in the United States. The majority of the influenza disease burden occurs in children and adults >60 and many of these cases and deaths could be averted through the use of safe and effective vaccines. However, influenza vaccination continues to be poorly utilized in most of East and Southeast Asia with the exception of Taiwan, Japan and South Korea. While strengthening laboratory surveillance is a priority and additional disease burden studies are needed, it is now becoming clear that influenza is an important cause of illness and death in tropical and subtropical regions of East and Southeast Asia. The rapid economic growth in the region suggests that influenza vaccination could become more widely utilized to better control the disease and to improve preparedness in case a new pandemic strain of influenza does emerge.

L 08

MOLECULAR EPIDEMIOLOGY OF AI H5N1

Nancy Cox,

CDC Atlanta, USA

L 09

THE RISK FACTORS OF H5N1 IN THAIBINH PROVINCE, 2004

Le Quynh Mai, *National Institute of Hygiene and Epidemiology, Hanoi, Vietnam*
Nguyen Tran Hien, Pham Ngoc Dinh, Nguyen van Diu, Nguyen Thuy Hoa, Le Quynh Mai, Nguyen Le Khanh Hang, Hoang Vu Mai Phuong, Nguyen Viet Hoang, Thanh Kim Dung, Le Hong Phong, Nguyen Huu Tam, Nguyen thi Dan et al.

To elucidate the factors of H5N1 infection in Thaibinh province, 2004, we analyzed 356 specimens, which are collected from domestic avian (chicken, duck…), animal (dog, cat...) as well as environments thought molecular virology testing (conventional RT-PCR).
87 positive specimens had been identified in total (24%) , the different rate between different specimen groups (100% in waste water, 33% in swabs and feces of duck … and negative all in dog, rabbit , soil groups) suggest that : domestic avian are the main risk factors of H5N1 infection, however environmental factors (especially : waste water) can be the good condition for present , maintenance and transfer H5N1 virus to host species in Thaibinh province, 2004.

L 10

MOLECULAR EVOLUTION OF HIGHLY PATHOGENIC H5N1 INFLUENZA VIRUSES IN SOUTHERN VIETNAM

Cao Bao Van, *Institut Pasteur Ho Chi Minh City, Vietnam*
Van Cao, [1]* Hong Hai Vo Ho,[1] Le Ha Tam Duong, [1] Nguyen Thi Kim Tien, [1] Phan Van Tu, [1] Nguyen Thanh Long, 1 Ngo Thanh Long, [2] Dong Manh Hoa [2]

(1) Pasteur Institute in Ho Chi Minh City
(2) Regional Veterinary Center of Ho Chi Minh City

The molecular evolution of highly pathogenic H5N1 influenza viruses was characterized by whole genome sequencing and genetic analyzing a total of 20 human and avian viruses isolated in southern Vietnam from January 2004 to April 2005. All isolates were of avian virus origin and no reassortment with human influenza virus was found. The viruses contain multiple basic

amino acids at the hemagglutinin cleavage site, which is associated with a highly pathogenic phenotype. The amino acid substitution E627K, a determinant of mammalian host and associated with increased virulence was found in PB2 protein of 2005 human H5N1 isolate. A number of new amino acid mutations of 2005 isolates were clustered around and within the receptor binding site of HA and may affect the binding to human cells. Two amantadines resistance markers, the known S31N and the newly appeared L26I were found in the M2 proteins of all isolates. NA sequencing revealed one human 2005 isolate which had a mixed population of amino acid residues 274H sensible and 274Y resistant to Oseltamivir. Phylogenetic analysis confirmed that all isolates were originated from a single A/Goose/Guangdong/1/1996-like clade and belonged to Z genotype. Our findings indicate that H5N1 viruses in Vietnam are accumulating mutations to be more adapted to human host. The combination of increased virulence and emergence of Tamiflu resistance of 2005 H5N1 viruses

L 11

THE ROLE OF DOMESTIC DUCKS FOR MAINTENANCE AND SPREAD OF AVIAN INFLUENZA H5N1 IN VIETNAM

Nguyen Tien Dzung, DVM, PhD, Dao Thanh Van, DVM, Bui Ngoc Anh, DVM, Nguyen The Vinh, DVM, Bui Nghia Vuong, DVM, and Ken Inui, DVM, MSc.

Department of Virology, National Institute of Veterinary Research Hanoi, Vietnam

Running title:
Role of Domestic Ducks for Avian Influenza in Vietnam

Keywords:
Influenza A, avian / transmission / epidemiology, ducks, chickens, Vietnam, virus shedding

The role of domestic ducks was studied in the epidemiology of highly pathogenic avian influenza H5N1 in Vietnam. Serological survey of 130 households indicated that raising ducks together with chickens increases the risk of H5 virus infection by eight times. A natural case of H5N1 infection was studied in a flock of 500 domestic ducks. H5N1 infection resulted in high mortality with neurological signs in domestic ducks. Serological, virological, and sentinel birds studies showed that the infection spread through this flock within 28 days, and that the infectious virus continued to be present in the flock for at least 34 days after initial case was observed. The present study demonstrated the important role of domestic ducks for the maintenance and the spread of H5N1 especially in Vietnam where it is common to raise a mixture of different avian species in backyard flocks.

L 12

LENTIVIRUSES PSEUDOTYPED WITH THE HEMAGGLUTINNINE OF A H5N1 INFLUENZA VIRUS ENTER THE CELL IN A PH-DEPENDENT WAY

Isabelle Nefkens[1], **Dongjiang Tang[1]**, Kid Chu[1], Malik Peiris[2], Philippe Buchy[3] and Ralf Altmeyer[1]

1HKU-Pasteur Research Center, Hong Kong, SAR China
2 University of Hong Kong, Hong Kong, SAR China
3 Pasteur Institute, Pnom Phen, Cambodia

The pandemic spreading of the avian flu in poultry farms and the endemic presence in Asia have great economic and social impacts on several countries. Sporadic human cases have been confirmed in such countries and proven to be highly pathogenic and lethal in about 50 % of the cases. Closely monitoring of the spreading of the disease is a prerequisite to contain infection herds. We have developed a pseudo typed lent viral system where the hemagglutinine (H5) of a confirmed H5N1 infected Cambodian patient is expressed on the envelope. Entry of the viruses is then detected by means of the functionality of the luciferase reporter. Infectious particles containing H5 and the p24 capsid protein are co-migrating on a 20 % to 60 % sucrose gradient showing that reporter gene expression is due to infection with pseudo typed virus. Experiments show that the entry of these particles faithfully mimics the entry of the influenza virus. First, pre-treatment of the target cells with neuraminidase aiming to remove the sialic acids on the cell surface, diminish the infection. Secondly, the entry is a pH-dependent process. We used our H5 pseudo typed viruses as a tool to screen different human and avian blood samples for the presence of H5 neutralizing antibodies. We identified 100 % of previously H5N1 confirmed cases (7 out of 7) in our test with titres varying from 40 to > 1280. None of these sera were neutralizing for VSV-G pseudo typed viruses. In the near future, we plan to check the specificity of the assay by testing different subtypes of influenza and isolates originating from 2 different clades. Our initial tests show the feasibility of our screening method under BSL2 conditions. Currently, our assay is being validated as a early and fast large scale screening method method for H5N1 infection and seroprevalence. Consolidation of this application will make it a valuable tool to study the spreading of the virus.

ACUTE VIRAL ENCEPHALITIS SYNDROMES

L 13

EPIDEMIOLOGY OF VIRAL ENCEPHALITIS IN BACGIANG PROVINCE, VIETNAM

Vu Duy Nghia, Nguyen Tran Hien**,** Phan Thi Nga and Pham Ngoc Dinh, et al,

National Institute of Hygiene and Epidemiology (NIHE)

After several years of implementing JE vaccine in the EPI program (launched in 1997), the proportion of non-JE cases has increased gradually, representing around 60% of all children hospitalized with AES in the 2000-2002 period . From 1999 until 2005, over 783 viral encephalitis cases were reported in Bac Giang Province, among them, 65 were died, making the overview case fatality rate of 8,3%. Yearly, outbreaks of acute encephalitis were at peak in May-July. Most of cases have been documented among children aged less than 10 years old. Only 18.2% of cases were positive with JE virus. In 2005, a new virus so called "Bac Giang virus" was found by the NIHE Encephalitis Laboratory, Vietnam. Numbers of children referred to the Provincial Hospital range from 50 to 100 per epidemic season, with an estimated case-fatality rate of 10-20%. We found that several cases have a more rapid onset, higher case-fatality rate, negative anti-JE IgM serology, and even that many children have already been immunized against JE. The highest numbers of cases were reported mainly from 3 districts named Luc Ngan, Luc Nam and Yen Dung. The emergence of this new viral disease together with the introduction of litchi plantations in the area (since 1999), and the epidemic season coinciding with the fruits seasons of the litchi trees (May-July), suggests that animals eating litchis such as bats may play a role as animal reservoirs for the virus. It is strongly believed that the new virus is transmitted primarily by the bite of infected mosquitoes that acquire the virus by feeding on infected birds or bats. The intensity of transmission to humans is dependent on abundance and feeding patterns of infected mosquitoes and on local ecology and behaviour that influence human exposure to mosquitoes. More epidemiological analytic studies are needed.

L 14

EMERGING VIRUSES ASSOCIATED WITH ACUTE ENCEPHALITIS SYNDROME IN VIETNAM

Phan Thi Nga*, Nguyen Thanh Thuy, Komichi Morita**, Jean Claude, Mannuguerra***, et al

**National Institute of Hygiene and Epidemiology, Hanoi, Vietnam, **Institute for Tropical Medicine, Nagasaki University, Japan, ***Institute Pasteur Paris, France*

In Vietnam, 2000 – 3000 of acute encephalitis syndrome (AES) cases have been reported annually. AES cases due to Japanese encephalitis (JE) virus were confirmed at about 50 % - 70 % before 1997. In recent years, owing to JE vaccination, AES cases due to JE virus were reduced and now range from 30 % to 50 % of all cases. Besides JE virus, other viruses such as enteroviruses, herpes simplex have been confirmed to be responsible for certain AES. However, the cause of a large protion of these AES cases remains unknown and is thought to be other viral infections. This fact underlines the importance to detect and investigate novel viruses, especially those transmitted by mosquito vectors.

In this study, Aedes albopictus clone C6/36 cells were used to isolate virus from cerebrospinal fluid of AES patients (non JE patients) during 2001 – 2005. All isolates were checked by PCR with primers for Alpha, Bunya, Flavi viruses but the results remained negative. Isolated viruses were temporary classified into two groups based on morphology and properties of viral growth on the cells. One class causes strong cytopathic effects (CPE) on the cells after 24 – 48 h, and was shown to be an enveloped virus around 50 nm in diameter. The other class does not cause CPE on the cells was shown to be around 40 nm in diameter. The virus coded 02VN 208 (isolated 2002) and 04VN109 (isolated 2004) were chosen as prototype for typing and named Nam Dinh (NDi) virus and Bac Giang virus according to the location of patients, respectively.

Both NDi virus and Bac Giang virus were confirmed to be human pathogens base on Protein A Gold Immunoelectron Microscopy technique. To date NDi virus was confirmed to be RNA virus and around 12 kb the genome of NDi virus was sequenced, and subsequent investigations are ongoing. The sequencing of NDi virus (ORF-1ab) gene is only around 45 % sequence identity with arterivirus, NDi virus was temporary grouped into arterivirus. Designing primers for PCR, preparing reagents for ELISA have been carried out in order to detect the distribution of NDi virus strains as well AES cases due to NDi virus in Vietnam.

Characterization of the genome of Bac Giang virus is ongoing.

L 15

IMPLEMENTATION OF NEW OR RECENT MOLECULAR IDENTIFICATION TOOLS FOR UNDIAGNOSED VIRAL DISEASES AND FOR NEWLY GENERATED VIRUS GENOTYPES.

Phan Thi NGA[1], Maurice DEMANOU[2,3], Dong WU[3], Christophe BATÉJAT[3], Kim Giao NGUYEN[1], Nicolas BERTHET[3], Gilberte CORALIE[3], Fréderic FICHENICK[3], Claudine ROUSSEAUX[3], Anita REINHARDT[3], Ana BURGUIÈRE[3], Marie-Christine PRÉVOST[3], Stewart COLE[3], Ian GOLD[3], Giulia KENNEDY[4], Renaud MAILLEUX[3], Paul BREY[3], Antoine GESSAIN[3] and **Jean-Claude MANUGUERRA[3].**

1: National Institute of Hygiene and Epidemiology, Hanoi, Vietnam; 2: Centre Pasteur du Cameroun, Yaoundé, Cameroon (current address); 3*: Institut Pasteur, Paris, France;* 4: *Affymetrix, San Francisco, California, USA*

The proportion of undiagnosed viral infections in humans is considerably high throughout the spectrum of diseases they cause and can reach up to 39% in the case of thorough investigations of Lower Acute Respiratory Infections or even amounts up to 90% in viral like encephalitis. Lack of aetiological diagnosis in viral diseases can be due to many reasons including the following:
1/ Not all possible main human pathogenic viruses are thoroughly searched;
2/ The variety of viruses known to be able to occasionally infect human beings is so wide that it is not feasible to develop (in one single laboratory or a handful of them) a specific RT-PCR for each one of them;
3/ The pathogen causing the disease has not been isolated or detected in humans or has never been described at all.
After exhausting all available diagnostic assays for medically important viruses possibly causing the observed syndrome, a research oriented approach is followed:
1/ Samples are inoculated to various cell line cultures including C6/36, a continuous cell line obtained from a *Diptera* species. Virus amplification is monitored by cytopathic effect (CPE) observation and by Electron Microscopy (EM).
2/ Using IntKey, a software build to query the data stored in the International Committee on Virus Taxonomy Database (ICTV Db) [3064 taxons and 1153 characters], with available data, the outcome consists in a list of possible genera and/or families as candidates.
3/ Using 3 protocoles of random RT-PCR for differential display, followed by cloning and sequencing, sequences of various sizes are obtained and submitted to a BLAST analysis. The outcome of the latter analysis is compared with that of the taxonomic study.
4/ After random RNA reverse transcription and DNA amplification, amplicons can also be hybridised on a resenquencing PathogenID chip, a DNA microarray, jointly developed by Affymetrix and Institut Pasteur. Viruses can be identified by cross hybridisation with sequences already spotted on the chip and the outcome of the latter analysis is compared with that of the taxonomic study.

A superacute viral-like encephalitis affecting young children every year at a given period has been occurring for many years now in Asia and its aetiology has not yet been elucidated. We applied the above methods to the identification of the culprit virus. However, because the strain has been lost through multiple passages in cell culture, confirmation of our first results has not been achieved as yet. Starting back from the initial cell supernatant containing VLP grown in NIHE, new results are expected sometime soon.

We are also implementing a syndromic and updated version of the panvirology DNA chip created by Joseph deRisi aimed at the detection of all known respiratory viruses, even the most recently discovered such as bocaviruses. In addition, we are developing a new DNA chip using glass technology to identify in one experiment not only the type and subtype of influenza viruses but also the internal genes composition without sequencing. This could therefore prove to be a critical tool for rapid detection of virus reassortments, should such a event occur between human type A and avian A (H5N1) viruses.

L 16

JAPANESE ENCEPHALITIS VIRUS ECOLOGY IN ASIA IMPLIES POSSIBLE RAPID REGION-WIDE WEST NILE VIRUS EXPANSION: NEEDS OF DEVELOPMENT OF WEST NILE FEVER VACCINES

Kouichi Morita

Department of Virology, Institute of Tropical Medicine, Nagasaki University

Recent studies on Japanese Encephalitis Virus (JEV) showed frequent transport of virus strains harboured in Southeast Asia to East Asia through a JEV "highway". West Nile virus, also a tropical mosquito-borne virus, caused epidemics in U.S.A. since 1999 and has since steadily continued to spread, penetrating Pan-America and Caribbean islands causing a serious public health problem across North America. At present, there are no signs that WNV has affected Japan or other parts of East Asia. However, if the virus reaches the Asia-Pacific Region, spread could be attributable to the JEV "highway". Hence, vaccine development of WNV needs acceleration to effectively manage the threat that poses Asian countries.
We have experimentally produced WN vaccines; 1) inactivated vaccine candidate using purified vision; and 2) live vaccine candidate produced by reverse genetics. Inactivated WN fever vaccine candidate was developed using tissue culture technique using NY99-35262-11 WNV strain. In animal experiments it achieved 100% protection against wild-type WNV challenge. The live attenuated WN fever vaccine candidate is chimeric Japanese encephalitis/West Nile virus constructed from cDNA templates encoding envelope protein (E) of WN-NY99 virus. West Nile (WN) portion is inserted into a backbone composed of attenuated JE virus strain, ML-17. ML-17/WN chimeric virus multiplied well in both mammalian cell (LLC-MK2, BHK-21 and Vero) and mosquito cell (C6/36) cultures. The chimera virus produced intermediate sized plaques compared with parental viruses. Chimera virus-immunized mice at minimum dose of 1 x 105 FFU through intraperitoneal (i.p.) route, were protected against WN-NY99 virus challenge at dose of 100 x LD50, administered through same route. Also, chimera virus-immunized mice were partially protected against JE virus challenge. Thus, both inactivated and live WN fever vaccine candidates appear useful in controlling WNV infection and presents a potential tool for WN fever outbreak control.

L 17

THE SURVEILLANCE OF WEST NILE VIRUS CIRCULATION IN ROMANIA (2001-2005)

Gabriela Nicolescu*, Valeria Purcarea-Ciulacu*, Diana Popescu**, Gabriela Dumitrescu**, Al. Vladimirescu*, L. Prioteasa*,Elena Claudia Coipan*, Elena Falcuta*, Lucia Ilina**, Simona Bordea**, Cornelia Ceianu* and Aurora Alexse*

**Cantacuzino Institute, Bucharest, ***Army Centre for Medical Research*

The circulation of West Nile flavivirus (WNV) takes place in cycles between mosquitoes and birds as main hosts, and the mammals and humans are tangential hosts. The confirmation of human cases and the ecological surveillance including the investigations on domestic and wild birds, horses and mosquitoes were performed.

A number of 46 human clinical cases of neurological infections with West Nile virus were confirmed by IgM capture ELISA (Mac ELISA) among 1763 hospitalized persons with acute viral neurological infections in Romania, including Bucharest. The detailed data about the distribution in space and time of cases, the age and sex of patients, the characteristics of the micro-focuses where the cases appeared are presented.
Seroprevalence of the antibodies against WNV in 3470 domestic birds from 73 rural and urban localities of 14 districts had a mean value of 7.1 % (0 - 35.0 %), and in 645 wild birds from three districts and Bucharest had 25.3 % (12.8 – 38.1 %). Seroprevalence in 3971 horses in 90 localities from 5 districts and Bucharest had a mean general value of 15.0 %, and its variation was between 9.0 % in Teleorman district and 32.1 % in Tulcea district (where the virus is introduced by migrating birds in the Danube Delta). The dominance of the potential vectors of WNV among 23 identified species by investigation on 52456 mosquitoes collected in 33 localities of 14 districts was detected. Two WNV strains were isolated from Culex pipiens collected in August 2002 in a focus of blocks of flats in Bucharest. The aspects of the intensive WNV circulation in Romania are discussed.
Work supported by funding from FP6 of EU (Contract No. 010284/2004) and from VIASAN Research Programme of the Ministry of Research, Romania (Contract No. 167 / 2002).

L 18

RESULTS OF MANY-YEARS MONITORING OVER TICK-BORN ENCEPHALITIS (TBE) IN ST. PETERSBURG

Tokarevich N.[1], Antykova L.[2], Stoyanova N.[1], Bychkova E.[2], Kudina M.[2]

1.St.Petersburg Pasteur Institute, 2.City Center for Epidemiological Surveillance, St. Petersburg, Russia

The forest zones of St.Petersburg, its suburbia and Leningrad province are densely populated by ticks, mainly, Ixodes persulcatus. The mean index for Ixodes persulcatus contamination with tick-borne encephalitis virus (TBE virus) in 1996-2005 in St.Petersburg was about 1%. In the said period 72451 persons in St.Petersburg took medical advice having been exposed to tick bite; including 63659 persons (87.9%) bitten by ticks in Leningrad province, and 8 792 (12.1%) in the city. As far as ticks are concerned, the city outskirts with new cottages and places for recreation are the most hazardous. 789 cases of TBE were registered in St.Petersburg in 1996-2005. The mean incidence of TBE was 1.8/100000, that is 2.7 times lower than for whole Russia over the same period. Such rather low TBE morbidity in St.Petersburg is due first of all to low TBE prevalence in ticks, but also to efficient vaccination of the most menaced contingent, and administration of specific and non-specific anti-TBE preparations to the most of victims. The alimentary pathway (unboiled goat's milk) of TBE infection was proved in 123 patients, including group cases. Within the analysed period 17 patients died of TBE in St.Petersburg (the average lethality index was 2.1%). Mixed TBE/Lyme Berreliosis infections were diagnosed in some patients (up to12.0% in some years). Seroprevalence studies detected TBE virus antibodies in 2.0% of healthy blood donors.
Conclusions: Intensive developing of St.Petersburg suburbia inhabited by Ixodes persulcatus provides real preconditions for expanding and activation of TBE natural foci close to the megapolis, and causes TBE infection rise in humans. TBE virus infection in humans occurs much more often then registered. Intake of unboiled goat's milk is an important pathway of infection in humans. High probability of simultaneous infection with several tick-borne

pathogens provides additional difficulties in diagnostics and efficient medical treatment of patients.

L 19

A COMPREHENSIVE APPROACH TOWARDS NIPAH VIRUS INFECTION, PREVENTION AND TREATMENT

Vincent Deubel,

Institut Pasteur of Shanghai, Chinese Academy of Sciences, PR China

Nipah virus is a member of the Henipavirus genus in family Paramyxoviridae. The virus was initially isolated in 1999 during an outbreak of encephalitis and respiratory illness in Malaysia and Singapore. The outbreak killed over 105 people and led to the culling of more than 1.1 million pigs. Affecting primarily swine, the virus apparently crossed the species barrier from fruit bats to pigs and then infected humans, causing encephalitis and nearly 40% patient mortality. Five outbreaks have occurred in Bangladesh between 2001 and 2005, and one outbreak has been reported from West Bengal in India. These outbreaks have been characterized by very high fatality rates, by the lack of contact with either bats or a domestic spill over host, and by the probability of human-to-human transmission. It is therefore predicted that related viruses will emerge from anywhere within this range, and that these related viruses may differ in transmission and other properties from Nipah virus, and must be considered a potential threat to human and livestock health. There is clearly a need for better prevention, diagnostic and treatment interventions to reduce morbidity and mortality in animal and human populations and to limit economic loss within the agricultural community.

In an effort to identify potential treatments and vaccines against Nipah virus encephalitis, we have developed an animal model and various diagnostic and therapeutic tools. We have produced a golden hamster animal model to test viral protein immunogenicity, antiviral potential of compounds as well as the protection offered by neutralizing monoclonal antibodies. Using our model, we showed that both Nipah virus glycoproteins G and F, when expressed as vaccinia virus recombinants, induced an immune response which protected against a lethal challenge by Nipah virus. Similarly, passive transfer of antibody induced by either of the glycoproteins protected the animals. Finally, we have tested several drugs or inducer of innate immunity to protect animals against a lethal challenge. We are pursuing studies to monitor and efficiently respond to Nipah encephalitis and to other emerging diseases.

VIRAL HEMORRHAGIC FEVERS

L 20

EPIDEMIOLOGY OF DENGUE/ DENGUE HEMORRHAGIC FEVER IN MEKONG DELTA AREA, A POTENTIAL SITE FOR DENGUE CLINICAL VACCINE TRIAL

Nguyen Thi Kim Tien[1], Nguyen Trong Toan[1], Vu Thi Que Huong[1], Nguyen Ngoc Rang[2], Christine Luxemburger[3], Laurence Pollissard[3], Jean Lang[3].

(1) Pasteur Institute of Ho Chi Minh City, Vietnam. (2) An Giang Provincial Hospital, Vietnam. (3) Sanofi Pasteur, Lyon, France

A dynamic cohort of 3-14 year-olds has been established in a southern province of Vietnam to estimate the incidence of dengue disease and sub-clinical infection during 2003-2004. In December 2003, 2189 children of 3-10 year-olds were enrolled in 5 nursery and primary schools and serum was sampled to assess dengue antibody levels (ELISA). IgM and IgG were detectable in respectively 66 (3%) and 459 (21%) children. Prevalence of IgG increased at age 10. During school periods all absenteeism related to febrile illness were investigated, and during school holidays home visitors detected febrile episodes. Acute and convalescent sera were collected from children with suspected dengue or viral infection. In December 2004, 831 children of 11-13 year-olds were also enrolled in the cohort.

In the end of each year, 22% then 19% children had moved to other schools and had been replaced by other pupils, and 6% then 4% were lost to follow-up. The annual seroprevalence rate were determined in 13.8% and 11.4%, respectively. In two years, 125 cases (35%) of laboratory-confirmed dengue disease were detected, either by isolation of the virus (5 DEN-1; 37 DEN-2; 2 DEN-3; 2 DEN-4) (37%) or by presence of IgM (96%). In the first year, the annual incidence of laboratory-confirmed dengue disease was 36/1000 person-year with a ratio of mild: hospitalised cases of 1:2. The results obtained in the second year are being analysed.

L 21

SUCCESS IN DENGUE VECTOR CONTROL IN VIETNAM USING *MESOCYCLOPS* AS BILOGICAL AGENT AND COMMUNITY-BASED METHODS

Vu Sinh Nam [(1)], Brian Kay [(2)], Nguyen Thi Yen [(3)] and Nguyen Thi Kim Tien [(4)]

(1) Vietnam Administration of Preventive Medicine, Ministry of Health, 138-A Giang Vo St, Ha Noi, Viet Nam.
(2) Australian Centre for International & Tropical Health & Nutrition, Queensland Institute of Medical Research, PO Box Royal Brisbane Hospital, Australia 4029
(3) National Institute of Hygiene and Epidemiology, 1 Yersin, Hanoi, Vietnam
(4)Pasteur Institute of Ho Chi Minh City, 167 Pasteur, Ho Chi Minh City, Vietnam

Dengue fever/dengue haemorrhagic fever (DF/DHF) epidemics first began in Viet Nam in 1959. Rapid social and environmental changes have increased incidence of dengue. With an average of 75,693 recorded cases and 173 deaths per year, DF/DHF is now a major public health problem in Viet Nam. The container breeding mosquito, *Aedes aegypti*, is recognized as the major global vector of dengue viruses, causing approximately 50 million infections annually. We developed a mosquito control strategy which incorporates four elements; (i) a combined vertical and horizontal approach which depends on community understanding, (ii) prioritized control according to the larval productivity of major habitat types, (iii) the usage of predacious copepods of the genus *Mesocyclops* as a biological control agent, delivered by (iv) community activities of health volunteers, schools and the public. From 1998-2003, we reported that community based vector control in the northern provinces of Nam Dinh, Hung Yen and Haiphong (*n* = 49,647 people) and in the central provinces of Quang Nam, Quang Ngai and Khanh Hoa (*n* = 27,167 people) had resulted in *Ae. aegypti* elimination in six of nine communes, with only small numbers of larvae detected in the others. In this communication, we report elimination in a further two communes and as a result of local post-project expansion in the northern provinces, *Ae. aegypti* elimination from 32 of 37 communes (*n*=309,730 people), including five communes in urban Haiphong. As a result, no dengue cases have been detected in any commune since 2002, and for the 18 communes in the Xuan Truong district of Nam Dinh, since 2001. In the South, where highest incidence of dengue, *Aedes aegypti* larval population has reduced by 90 percent at two communes in Long An and Hau Giang provinces (n= 35,350 people) after two year (2004-2006) implementation of the model. This suggests that this strategy is sustainable in Vietnam and applicable elsewhere, where the major sources of *Ae. aegypti* are large water storage containers.

Key words: Dengue fever, mosquito, *Aedes aegypti*, control, biological control, communities, Vietnam

L 22

VIRAL ADAPTATION AND DENGUE EMERGENCE

O Faye[1], M Diallo[1] , J Velasco2, PMA Zanotto[2], O Faye[1], Y Ba[1], M Ndiaye[3], **A A Sall**[1]

Institut Pasteur de Dakar, Dakar (SENEGAL) 2. University of Sao Paulo, Sao Paulo (BRAZIL), 3. Université Cheikh Anta Diop de Dakar, Dakar (SENEGAL)

With an estimated burden of 100 millions dengue cases per year in tropical areas where more than 2.5 billion people are at risk, dengue (DEN) is the most important arboviral disease affecting humans. DEN viruses comprise four serotypes (DEN 1-4) and cause dengue fever (DF), a flu-like febrile illness that can lead for some patients to severe syndromes such as hemorrhagic fever (DHF) or shock syndrome (DSS).

Two distinct DEN transmission cycles exist: 1) endemic/epidemic DEN involving human hosts and peridomestic Aedes mosquitoes 2) a zoonotic/sylvatic cycle in forest habitats of West Africa and Malaysia, involving non-human primates and several different Aedes mosquitoes; The mechanisms involved in generation of the endemic/epidemic cycles and interactions between the 2 distinct are unknown.

To understand these underlying mechanisms, studies on west african DEN vectors susceptiblity and DEN2 virus genome evolution within Aedes Aegypti have been undertaken. Susceptibility experiments consist in experimental infections of vectors with sylvatic or epidemic DEN2 strains while DEN2 viral genome evolution were based on comparative sequences analysis of viral populations before and at several times points after mosquitoes infection.
Our results indicated that i) susceptibility studies of the endemic DENV vectors, Aedes aegypti and Ae. albopictus, support the hypothesis that adaptation of the sylvatic strains to more efficiently infect these mosquitoes was a critical mechanism of endemic DEN emergence ii) adaptation mechanism may be linked to changes in the glycosylation of envelope protein of DEN2 sylvatic, a key player for virus entry in cells iii) massive diversification of DEN2 viral population occurs rapidly within a mosquito.

Our results are discussed in the light of the mechanistic standpoint and public health implications of DEN emergence from sylvatic progenitors. Indeed, the promising DEN vaccines under development could lead to a major public heath achievement as the elimination of human DEN or be short lived if sylvatic DENV strains can readily re-emerge via evolutionary mechanisms from sylvatic strains. Information critical to assessing this possibility includes the amount of genetic change required for adaptation of the sylvatic strains to the peridomestic vectors, and the ability of humans to serve as reservoir hosts with or without further adaptation.

L 23

GENETIC ANALYSIS FOR SUSCEPTIBLE GENE TO DENGUE HEMORRHAGIC FEVER IN VIETNAM

Nguyen Thi Phuong Lan, M. Kikuchi, Vu T. Q. Huong, Vu T. T. Ngu, Vo D. Tham, Tran V. Dat, Do Q. Ha, K. Morita, K.Hirayama.

Dept. Molecular Immunogenetics, Dept.Virology Institute of Tropical Medicine, Nagasaki Univ. Lab. Arbovirus Pasteur Institute HCMC, Pediatric Hosp.No.2 HCMC, CPM Vinh Long province,Vietnam

Dengue fever is getting a serious public health problem in many regions of the world. Almost 1% of the patients with Dengue Fever (DF) develop Dengue Hemorrhagic Fever (DHF), or Dengue Shock Syndrome (DSS). Two factors are proposed to be important to produce DHF / DSS. One is viral virulence and the other is host genetic factor. In this study, we made an experimental design to identify the host gene(s) contributing to the development of DHF/DSS in Vietnamese by hospital-based case control study.
The patients with DF, DHF or DSS were clinically diagnosed by WHO criteria, and their peripheral blood samples were collected at the Centre for Preventive Medicine, Vinh Long Province (VL), and the Paediatric Hospital No.2, Ho Chi Minh City (NDII) in 2002 to 2005. The patient's age ranged between 10 months and 15 years. 200 age and sex matched control samples were collected in VL. The number of the patients with DF cases was 100, with DHF cases was 210, with DSS was 415 in total from two sites. DNA was extracted from each blood sample, then HLA class I (HLA-A, B), class II (DRB1) and TNF-_ promoter SNPs typing were performed.
There was no significant difference in TNF-_ promoter SNPs alleles. However, HLA-DRB1*0901 was significantly decrease in DSS (P for trend = 0.0001), and HLA-A*24 was significantly increase in DSS (P for trend = 0.0005). The DRB1*0901 allele might directly contribute to resistance to DSS and A*24 allele might directly contribute to susceptible to DSS in Vietnamese. These may have consequence for preventive strategies.

L 24

GENETIC POLYMORPHISM OF CD209 (DC-SIGN) ASSOCIATED TO DENGUE DISEASE

Philippe Desprès

Flavivirus-Host Molecular interactions, Virology Dpt, Institut Pasteur, Paris. France.
pdespres@pasteur.fr

Dengue is an immense global problem that is increasing despite decades of effort to combate this disease. This effort, including vaccine and drug development, has been by necessity empirical. It is increasingly recognised that improved understanding of disease epidemiology and pathogenesis are needed to inform rational approaches to dengue control. Important recent advances have been made in understanding the molecular basis of dengue virus replication, but

in order to understand human disease it is crucial also to study host factors that play a role in determining clinical outcome.
We have studied the association of genetic polymorphism in CD209 (DC-SIGN) gene with dengue susceptibility and severity in dengue patients from Thailand. The C-lectin DC-SIGN (CD209) has been identified as an essential molecule for productive dengue virus infection of interstitial myeloid dendritic cells at the anatomical site of mosquito bite. It is likely that DC-SIGN concentrates dengue virus particles at the cell surface to allow efficient interaction with a yet-unidentified entry factor that is ultimately responsible for dengue virus internalization and pH-dependent fusion into host cells. A promoter variant in CD209 gene promoter, DCSIGN1-336, has been strongly associated with the risk of dengue fever (compared to controls), and with the risk of dengue fever compared to dengue hemorrhagic fever in dengue patients recruited from Thailand. DCSIGN1-336 affects a Sp1 binding site and transcriptional activity in vitro. Given that CD209 variant DCSIGN1-336 may play a role in dengue disease orientation, host response to vaccine candidates based on live-attenuated dengue viruses in terms of efficiency and safety might depend on genotype with respect to CD209 polymorphisms, which should be taken into account to optimize the medical vaccine designs.

SAKUNTABHAI , C. TURBPAIBOO, I. CASADÉMONT, A. CHUANSUMRIT, T. LOWHNOO, A. KAJASTE-RUDNITSKI , S.M. KALAYANAROOJ , K. TANGNARARATCHAKIT, N. TANGTHAWORNCHAIKUL, S. VASANAWATHANA, W. CHAIYARATANA, P. YENCHITSOMANUS, P. SURIYAPHOL, P. AVIRUTNAN, K. CHOKEPHAIBULKIT, F. MATSUDA, S. YOKSAN, Y. JACOB, M. LATHROP, P. MALASIT, P. DESPRÈS, & C. JULIER (2005). A variant in the CD209 (DC-SIGN) promoter is associated with the severity of dengue disease. Nature Genetics 37: 507-13

POSTERS

ACUTE RESPIRATORY INFECTIONS CAUSED BY VIRUSES

P 01

RELEVENCE OF MOLECULAR DIAGNOSIS FOR COMPARATIVE STUDY OF VIRAL ACUTE RESPIRATORY INFECTION

Wang Wei, Jie Shao#, Yongjing Wang, Lili Hou, Vincent Deubel

Insitut Pasteur of Shanghai, Chinese Academy Sciences, # Ruijin Hospital, Shanghai, PR China

There are hundreds of viruses that are responsible for Acute Respiratory Infection (ARI). Some fatal emerging viral infections like Influenza and Severe-Acute Respiratory Syndrome (SARS) have become serious public health issues worldwide. Early diagnosis and subsequent treatment are therefore essential for fighting viral infection. Several methodologies have been developed to detect easily maximum of viral agents and establish the etiology of ARI, including multi-cell culture, RT-PCR, and micro arrays.
In this study, we have compared a modified multiplex hemi-nested RT-PCR (previously developed by F. Freymuth et al.) with cell cultures followed by immunofluorescence, and a multi-virus low density and high resolution DNA array comprising 1,114 70-mer probes for 35 viruses (modified from J. de Risi et al.). These techniques have been used for the detection of viruses from nasal and throat swabs of 105 children with ARI in Nanxiang hospital in Shanghai. Twenty eight (28) strains of RNA viruses (26.6%) were identified by multiplex RT-PCR, including corona viruses (4 HKU-1, 1 OC43, 2 NL-63), human influenza A (4) and B (5) viruses, Para influenza viruses (2), enter virus (1) and human respiratory syncitial viruses (9). These results, compared to cell culture and DNA micro array data confirm the high sensitivity of the multiplex RT-PCR assay for the detection of a broad spectrum of respiratory viruses. Genotyping of the viral isolates by sequencing of relevant genes and phylogenetic analyses will also be presented.

P 02

DEVELOPMENT OF SUBUNIT VACCINE AGAINST SARS-CoV USING TRUNCATED SOLUBLE S GLYCOPROTEIN WITH BALANCED TH1/TH2 IMMUNE RESPONSES.

Yiu Wing Kam[1], Francois Kien[1], Yan Chung Cheung[2], Kid Chu[1], Jane Tse[1], Malik Peiris[2], Beatrice Nal[1], and Ralf Altmeyer[1]

1 HKU-Pasteur Research Centre, 8 Sassoon Road, Hong Kong SAR, China
2 Department of Microbiology, The University of Hong Kong, Hong Kong SAR, China

Vaccine-induced humoral antibody responses can prevent or aggravate viral infections. Previous study has suggested that recombinant native full-length S-protein trimer (TriSpike) was able to induce neutralizing and protective immune response in vitro and in vivo. Here, we investigated whether two soluble forms of the S-protein ectodomain, Secd (aa1-1184) and S1 (aa1-757) could induce neutralizing antibody response in animals. When mice were immunized with TriSpike, Secd or S1 in Alum adjuvant SARS-CoV-specific IgG were detected in sera and nasal lavages and IgG and IgA could be detected in stool samples. Antibodies in serum were capable of neutralizing SARS-CoV infection of FRhk4 cells in vitro. TriSpike in Alum gave the strongest neutralizing response with titer (1:12800), S1 in Alum (1:8000) and Secd in Alum (1:1400). When we analyzed the IgG isotype profile from those vaccinated mice sera sample balanced Th1/Th2 immune response were detected in Secd immunized mice sera sample. In contrast, TriSpike and S1 immunized mice sera sample gave biased Th2 immune response. We propose recombinant trimeric S-protein in combination with two soluble S-protein ectodomain could be candidate for a vaccine against SARS-CoV.

P 03

STUDY OF VIRAL PROTEIN INTERACTIONS WITH NATURAL KILLER CELL RECEPTORS

Joanna WY Ho[1], Beatrice Nal1 Kid Chu[1], Angel Porgador[2], and Ralf Altmeyer[1]

1HKU-Pasteur Research Center, Hong Kong, SAR China
2 Faculty of Health Science and Cancer Research Center, Ben Gurion University of the Negev,Beer Sheva 84105, Israel

Natural killer (NK) cell is one of the potent effecter cells in the innate immune system for the clearance of virus-infected cells without the need for prior antigen stimulation. However, the precise molecular mechanisms involved are still largely unclear.
It is known that recognition of haemagglutinins (H1N1 stain) on virus-infected cells by the activating receptors on the NK cells could induce target cell lysis, therefore, we would like to extend such investigation to haemagglutinins from H5N1, as well as to find out if other envelop proteins from other viruses could also be recognized by the activating receptors of NK cells. Our preliminary result shows that binding could be detected for the purified recombinant viral envelop proteins of SARS-CoV, Dengue, and Influenza (H5N1), but not for HIV and HCV, to the purified human NK cell activating receptor NKp44, but not to NKp46 and NKp30. Our preliminary analysis shows that those bindings might be sialic acid dependent. Further experiments are underway to investigate if such recognition by NK cells could lead to target cell lysis.

P 04

FCγII-MEDIATED SARS-CoV ENTRY IN HUMAN B CELL LINES

Jaume, M.,* Kien, F.,* Kam, YW.,* Li, PH.,* Cheung, YC.,# Peiris, JSM.,# Daeron, M.,$ Nal-Rogier, B.,* Altmeyer, R.*

** Hong Kong University-Pasteur Research Centre, Pokfulam, Hong Kong*
 # Department of Microbiology, HKU, Pokfulam, Hong Kong
$ INSERM U760 Dpt of Immunology Pasteur Institute, Paris, Fr

Public health measures have successfully contained outbreaks of the severe acute respiratory syndrome coronavirus (SARS-CoV), which infected more than 8000 people and caused 774 deaths worldwide, 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 before with vaccines against corona virus (such as feline infectious peritonitis virus).We have developed a SARS vaccine candidate who elicited an in vivo neutralizing and protective immune response in rodents. By using SARS-CoV pseudo typed virus (SARSpp) we have analysed the capacity of anti-Spike antibodies to mediate antibody-dependent enhancement (ADE) of viral entry in vitro. The experiments exhibited opposite pattern according to cell types: while heat-inactivated sera from immunized animals or convalescent SARS patients still inhibit SARSpp entry in prototypic permissive cell line, these sera induced virus penetration in human B cell lines although they do not express SARS-CoV receptor (i.e. angiotensin-converting enzyme 2; ACE2). Entry into human B cell lines occurred in an FcgRII-dependent and ACE2-independent fashion indicating that ADE of virus entry is a novel cell entry mechanism of SARS-CoV. We are now investigating the biochemical pathways (such as pH-dependency and endosomal/lysosomal proteases involvement) as well as the FcγRII subfamilies required in FcγRII-mediated SARS-CoV entry. Production of different anti-Spike monoclonal antibodies will allow us to characterize SARS-CoV epitopes as well as antibody isotypes involved in ADE phenomenon if any. Finally, we expect to study antibody-dependent enhancement occurrence during wild type SARS-CoV infection of human primary cells.

P 05

A HIGH THROUGHPUT PLATEFORM FOR THE STUDY OF VIRAL ENTRY: APPLICATIONS TO DRUG DISCOVERY, SEROEPIDEMIOLOGY AND CELLULAR BIOLOGY RESEARCH

Garcia J.-M., Nefkens, I., Nal-Rogier B., Choi J., Millet J., Kudelko M., Altmeyer, R:

HKU-*Pasteur Research Centre Ltd*.

Viruses are obligatory intracellular parasites. Attachment of the virus to the cells and fusion of the viral and cellular membranes are the first steps of the viral life cycle. Here we describe novel tools that will lead to enhanced identification of cellular factors governing virus entry, the identification of virus entry inhibitors and the analysis of the sero-prevalence of virus in the general population.

We are using pseudo typed lentiviral vectors expressing different reporter genes to mimic viral entry. These pseudo type viruses have been generated for HIV, SARS, HCV, dengue and H5N1. The specificity is brought by the surface glycoprotein used to produce them. The reporter gene encapsidated in these particles allows the monitoring of their infection or inhibition of infection. With that system, we can study viral entry at different level: identification of inhibitor of viral entry within a chemical library (drug discovery), quick identification of antibodies against a given pathogen as sign of exposure during an epidemic (seroprevalence in seroepidemiology), or identification of cellular partner involved in the viral entry by specific inhibition of some gene using a library of SiRNA (cellular/molecular biology). All of these applications have in common that they require the assaying of large number of potential effectors of biological activity against targets. So, we developed a high throughput plateform to support these applications.
Furthermore, as most of the time the screened samples are in very limited amount, we want to get as much information as possible per sample tested. Therefore, we also develop high the content approach of screening. This allows for example to obtain cytotoxicity information at the same time as virus entry inhibition in drug discovery or to assess seroepidemiology for different subtypes of influenza virus.

P06

KNOWLEDGE, ATTITUDE AND PRACTICES IN POULTRY HANDLING IN RURAL AREAS OF CAMBODIA, 2006

LY Sowath (IP Cambodia), HOLL Davun (Ministry of Agriculture), FROEHLICH Yves (FAO Phnom Penh Office) and **VONG Sirenda** (IP Cambodia).

Cambodia has experienced A/H5N1 avian influenza outbreaks in poultry and humans since late 2003. We conducted a survey regarding poultry handling in rural Cambodia to estimate the extent of interactions between humans and animals. We surveyed a random sample of residents >16 years of age of 23 villages in Kampong Cham and Prey Veng provinces in January 2006 using cluster sampling methodology. A total of 459 respondents (40% male) of 269 households were interviewed. The participants reported raising chickens (97%), ducks (39%), pigs (48%) and cattle (73%). Chickens and ducks flocks were free ranged (chickens 100% and ducks 96%) and of small size (median per flock 10; range 1-110). Poultry mortality was experienced in 60% households during the previous 6 months. Although half of the respondents (50%) thought it was important to report poultry mortality, only 7% made a report. The two key persons whom participants reported to when poultry deaths occur were the village veterinarians (72%) and village chiefs (33%). When asked about reasons that may discourage reporting, participants reported that they didn't know where or whom to report to (43%) or were used to not reporting poultry death (32%).Most respondents (97%) have heard about avian influenza mainly through TV (81%) and radio (78%). They knew about AI and transmission sources (72%) and how to protect themselves (61%). However, many participants still eat sick poultry (45%) or wild birds (33%) and touch sick or dead poultry with bare hand (75%). Our findings showed high level of poultry ownership. Despite common poultry death occurrence, many are willing to report mortality but may not know how. In addition, general media reports about avian influenza appear to have been effective at reaching rural people. However, despite widespread knowledge on avian influenza and personal protection measures, most rural Cambodians still have high level of at-risk poultry handling.

P07

INFLUENZA VIRUS TYPING AND SUBTYPING BY MULTIPLEX RT-PCR

Mahsa Shahidi$, Mansoureh Tabatabaian*, Rouzbeh Bashar*, Samad Amini-Bavil-Olyaee&, Ramin Sarami Foroushani&, Masoud Hosaini$, Fereidoun Mahboudi&, Masoumeh **Tavassoti Kheiri**

** $Shahid Beheshti University; *Influenza Unit, Pasteur Institute of Iran; &Biotechnology Dept., Pasteur Institute of Iran*

Influenza is a viral infectious disease with frequent seasonal epidemics causing world-wide economical and social defects. There is not a long-lasting preventive vaccine because of antigenic shift and drift of the virus. WHO collects samples from all over the world to announce the vaccine strains separately for the Northern and Southern semisphere every year. Therefore it is necessary to determine the epidemic virus strains of the area. Influenza viruses are typed A, B, or C based on matrix (M) and nucleocapsid (NP) proteins of the virus. Influenza A viruses are subtyped based on their hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins. Multiplex PCR is a fast diagnostic method to determine more than a strain in one reaction using different specific primers. A two-step multiplex reverse transcriptase (RT)-polymerase chain reaction(PCR) assay was developed to type and subtype influenza virus using specific primers for NP, M, NS, H1, H3, N1, and N2. To design the primers it was considered to have different sizes of the RT-PCR products. In this study we have set up the multiplex RT-PCR to determine the heterogenisity of the virus from patients' samples collected last year from Shiraz –Iran.

ACUTE VIRAL ENCEPHALITIS SYNDROMES

P 08

SURVEILLANCE ET PRISE EN CHARGE DE LA RAGE HUMAINE DANS LE RESEAU DES INSTITUTS PASTEUR.
VERS UN RESEAU DES CENTRES DE TRAITEMENT ANTIRABIQUE ?

Yolande Rotivel* et Etienne Sevin**

**Centre National de Référence pour la Rage, Centre de Traitement Antirabique Institut Pasteur, ** Epiconcept*

La rage est encore en 2006 responsable de plus de 50 000 décès annuels dans le monde, plus particulièrement en Asie et en Afrique où sont localisés de nombreux Instituts Pasteur. La majorité des Institut Pasteur du Réseau sont impliqués dans le contrôle de la rage et la prise en charge des traitements post-exposition, si ce n'est des malades. Comme celle de nombreuses affections virales, l'épidémiologie de la rage suit l'évolution des activités humaines et se globalise. C'est ainsi que des lyssavirus africains ont été récemment importés sur le territoire de France métropolitaine. L'émergence de ces virus hors de leur territoire habituel pose des problèmes diagnostiques et thérapeutiques nouveaux et met en évidence le besoin de formation et d'accès rapide à des informations fiables.
Depuis 25 ans, le Centre National de Référence pour la Rage, situé à l'Institut Pasteur, à Paris, a pour mission de coordonner les Centres de Traitement Antirabiquefrançais disséminés pour la plupart dans les Centres Hospitalo-Universitaires. Un Bulletin sur l'Epidémiologie et la Prophylaxie de la Rage Humaine en France est chaque année édité à partir des données de tous les Centres collaborateurs. Un logiciel (Voozanoo*, Epiconcept) permettant la saisie directe sur Internet des données de chaque Centre, intégrant également une fonction d'alerte, a été mis au point récemment et est utilisé depuis 2005. La mise à disposition de Voozanoo*, à l'ensemble du RIIP, comme cela avait été proposé en 2002, permettrait la surveillance de la rage humaine au niveau local et l'amélioration de la prise en charge par des échanges rapides d'informations, ouvrant la voie à l'épidémiologie, la recherche clinique et la formation dans un domaine où les Instituts Pasteur ont une compétence historiquement reconnue.

P09

IDENTIFYING ECOLOGICAL AND EPIDEMIOLOGICAL KEY FACTORS FOR RABIES DYNAMICS AND CONTROL IN NORTH AFRICA AND IMPLICATIONS FOR RABIES STATUS IN SOUTH WEST EUROPE

Chokri Bahloul

Institut Pasteur de Tunis

Rabies is a serious public health concern in North Africa and its reintroduction to Western European countries presently free of rabies in non-flying animals represents a serious threat. This project will address a global multidisciplinary approach to draw a precise picture of the rabies epidemiology in North Africa by identifying and quantifying epidemiological, ecological, sociological and vaccinological key factors for rabies dynamics. Furthermore and despite that the Western European countries have almost completely eliminated canine and vulpine rabies, they continue to declare some human and animal cases mostly imported from North Africa in addition to the presence of the disease in bats with some spillover to humans. Therefore, the study of rabies in N.Afr. and West Europe will determine the possible overlapping of rabies epidemiological cycles between the Western European and North African shores. Therefore baseline epidemiological data on human and animal rabies will be collected and analyzed by GIS in 4 North African countries for the identification of rabies hyperactive areas. Factors which contribute to human post-exposure therapy failures will be identified. KAP surveys will be addressed in order to weigh out key ethologic factors of dogs and the impact of human socio-cultural perturbations and human behavior in rabies dynamics and vaccination efficiency. Phylogenetic analysis will be carried out on rabies isolates collected from North Africa or of imported cases recorded in 3 European countries bordering the Mediterranean sea as well as bat lyssaviruses. We will try to elucidate whether lyssaviruses (rabies and rabies-related viruses) are circulating in bats in North Africa. Circumstantial data indicate exchange of bats between Europe and Africa most likely harbouring lyssaviruses. Therefore, we propose an ecological study of bat species in North Africa and Italy. All work packages will lead to a coherent analysis of rabies epidemiology.

P10

SURVEY FOR HENIPAVIRUSES AND TIOMAN VIRUS IN FRUGIVOROUS BATS FROM MADAGASCAR

C. Iehlé, G. Razafitrimo, J Razainirina, N Volasoa Andriaholinirina, S Goodman, MC Georges-Courbot, D Rousset, JM Reynes

Institut Pasteur de Madagascar,
Field Museum of Natural History, Chicago, ILL;
UBIVE, Institut Pasteur

Fruit bats, especially species belonging to the genus Pteropus, have been involved as natural hosts for emerging viruses like the deadly henipaviruses Nipah and Hendra or the morbillivirus Tioman in Australia, Malaysia, Singapore and Bangladesh. It has been hypothesized that the geographical distribution of these viruses overlaps with that of Pteropus species outside these countries. This hypothesis was confirmed recently with the evidence of the presence of NiV in Pteropus lylei in Cambodia and Thailand.
The present survey was conducted among fruit bats of Madagascar to assess the presence of Nipah, Hendra or Tioman (like) viruses. We collected 427 sera, 118 urine and 285 pharyngeal specimens from the three endemic Pteropodidae species: Pteropus rufus, Eilodon dupreanum and Rousettus madagascariensis. Even though no virus could have been isolated on Vero E6 cells from the urine and pharyngeal specimens, serum neutralization tests revealed the presence of neutralizing antibodies against Nipah, Hendra and Tioman viruses in three Eilodon dupreanum and one Pteropus rufus individuals. Because more bats were found seropositive by ELISA (n = 23) than by serum neutralization test, we suggest that in Madagascar yet unrecognized paramyxovirinae related viruses have circulated rather than the known Nipah, Hendra or Tioman strains.
Malagasy pteropodids are hunted for food and are found alive or dead on local markets and in some restaurants. Further, people eat fruits from trees where pteropodids have fed. Consequently, recommendations should be made to limit contact between humans and bats.

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SEROLOGICAL EVIDENCE OF PARAMYXOVIRUSES IN PTEROPUS BATS IN SOUTHERN VIETNAM

Vu Thi Que Huong[1], Cao Minh Thang[1], Luong C.Quang[1], Hoang T.N.Dao[1], Doan T.M.Tam[1], Truong H.Phong[2], Vu D.Thong[3], M.C. George-Courbot[4], J.M. Reynes[5].

(1) Pasteur Institute of HCM City, Vietnam. (2) Soc Trang Province,VN. (3) Ins. for Eco.& Biol. Resourses, Hanoi, VN. (4) Pasteur Institute, Lyon. (5) Pasteur Institute, Madagascar.

In the last decade, Paramyxoviruses were related to emerged respiratory and encephalitis diseases in human and animals. Four of these new viruses have been described in the Western Pacific region with the same reservoir, such as Pteropus gender fruit bats. These viruses belong in different genders. Menange and Tioman viruses belong in Morbillivirus and Rubulavirus genders, respectively. Nipah and Hendra viruses were classified in a new Henipavirus gender. And the geographical distribution of this fruit bat (including Vietnam) seems to overlap with one of these Paramyxoviruses. So, it's necessary to identify the natural reservoir of the virus for better understanding the mode of virus transmission in livestock and prevention of the future introduction in Viet nam.

From June 2004 to June 2005, 39 Pteropus spp. including Pteropus lylei and Pteropus vampyrus and 29 Scotophilus kuhlii bats were captured in Soc Trang province in southern Vietnam. Their sera were tested by ELISA using the Nipahvirus, Hendravirus and Tiomanvirus antigens. The anti-Nipah virus antibodies were detected by ELISA in Pteropus lylei and Pteropus vampyrus with the positive proportion of 24% and 100%, respectively, and confirmed by seroneutralization test in Pasteur Institute-Lyon, France. Moreover, the anti-Hendra and anti-Tioman antibodies were also found in Pteropus lylei by ELISA with the positive proportion of 16% and 30%, respectively. The data shows the serologic evidence of paramyxoviruses in Pteropus spp. bats in southern Vietnam. Then, the serological surveillance of paramyxovirus will be done in pig sera and human meningo-encephalitis cases' sera and cerebrospinal fluids.

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ACUTE VIRAL ENCEPHALITIS SYNDROME IN SOUTHERN VIETNAM

Vu Thi Que Huong[1], Huynh T.K.Loan[1], Nguyen T.T.Thao[1], Phan V.Tu[1], Tran H.Khanh[2], Nguyen B.Hue[2], Huynh P.Thao[1], Hoang T.N.Dao[1], Doan T.M.Tam[1], V. Deubel[3], K. Morita[4].

(1) Pasteur Institute HCM City, Vietnam. (2) Children Hospital HCMC, Vietnam. (3) Pasteur Institute, Paris, France (4) Nagasaki Univ., Japan.

In southern Viet Nam, within the scope of epidemiological surveillance system, human viral acute encephalitis syndrome (AES) cases and deaths were annually reported since 1976. These AES cases have occurred sporadically and throughout the year. In terms of human morbidity and mortality, the average of 600 cases is estimated to occur annually and the case fatality rate (CFR) is approximately 1.4% - 10.4%. The Japanese encephalitis (JE) virus was confirmed as an important pathogen in 30% of children viral AES cases. The JE virus was isolated from patient, mosquitoes and sentinel pig. Some of them were analysed and shown to belong the genotype I in molecular epidemiology study. The other viral causes need to be identified for better control and prevention strategy.

In 2005, more than 300 acute viral meningo-encephalitis syndrome cases under 15 years old were hospitalized in Children Hospital in Ho Chi Minh City with 9.5% of CFR and 16.3% of persistent neuropsychiatric sequelae. The cerebrospinal fluid, paired sera, throat swabs and stools were collected and tested by mosquito and mammalial cell cuture, IgM capture ELISA and RT-PCR. Some viral causes were confirmed as JE virus (21%); enteroviruses (19%) including echoviruses (23%), enterovirus type 71 (14%) and Coxsackie B virus (2%); and Dengue virus (3.4%). Only 22.5% of AES death were identified such as JE virus (13%), enterovirus type 71 (6.5%) but 53% of neuropsychiatric sequelae cases caused by JE virus. Other viruses will be tested.

VIRAL HEMORRHAGIC FEVERS

P 13

THE PROGESS OF DENGUE PSEUDOTYPES

Peigang Wang, Ralf Altmeyer

HKU-Pasteur Research Center, Hong Kong, SAR China

Dengue viruses are the pathogens of dengue fever, dengue hemorrhagic fever and dengue shock syndrome that cause more that 50 million cases every year. The aim of our project is to develop a dengue pseudotypes system which will be a safe and effective tool for the study of dengue entry mechanism and screen of drugs for dengue therapy. The modified HIV genome carrying luciferase reporter gene was used in the experiments. Our results showed that the Dengue/HIV pseudotyped particles could infect lots of cell lines and the infection was a pH-dependent process. The infection of pseudotypes could be neutralized to half by dengue patients' sera. Some further experiments are in progress to increase the titer of dengue pseudotypes for the practical application.

P 14

RÉSULTATS PRÉLIMINAIRES DE L'INVENTAIRE FAUNISTIQUE ET ÉTUDE SYSTÉMATIQUE DES PETITS MAMMIFÈRES RÉSERVOIRS DU VIRUS DE LASSA EN CÔTE D'IVOIRE

C. Akoua-Koffi[1], B. Allali[1], S.Kouassi Kan[1], V. Akran[1], B. Kouakou[1], E. Adjogoua[1], H. Kadjo[1], H. Dosso[2], C. Denys[3], J. ter Meulen[4], **M. Dosso**[1]

1 : Département des Virus Epidémiques, Institut Pasteur de Côte d'Ivoire
2 : Centre de Recherche en Ecologie, Université d'Abobo-Adjamé
3 : Muséum National d'Histoire naturelle, Paris – France
4- Institut de Virologie de Marburg, Allemagne

Les fièvres hémorragiques virales demeurent un problème majeur de santé publique en Côte d'Ivoire, pays d'endémie amarile où un cas de fièvre à virus Ebola avec isolement d'un virus Ebola, sérotype Côte d'Ivoire a été décrit et une séroprévalence anti-virus de Lassa de 26% a été obtenu chez des travailleurs forestiers des districts frontaliers de la Guinée et du Liberia, pays d'endémie de fièvre de Lassa..
Contrairement à la fièvre jaune, très peu d'informations sont disponibles sur la fièvre de Lassa. Afin de documenter cette pathologie, un projet de recherche a été mis en place pour déterminer le niveau de circulation du virus chez les réservoirs potentiels et renforcer la capacité diagnostique au plan humain. Le volet mammalogique a permis de réaliser, suivant deux axes établis (axe ouest de Divo à Duekoué et axe est d'Aboisso à Bondoukou) et la station de Lamto, 1503 captures de rongeurs dont 60 % de Mastomys, 8% de Nanomys, 6,7% de Croccidura, 3,7% de Lophuromys, 3,5% de Uranomys, 3,1% de Praomys principalement. Les carcasses et les échantillons d'organes et sanguins collectés par autopsie sont en cours d'analyse pour la caractérisation mammalogique compète et virologique. Ces résultats contribueront à une meilleure connaissance de la fièvre de Lassa.
Mots-clé : fièvre de Lassa, réservoir, virus de Lassa, Côte d'Ivoire

C. Akoua-Koffi
Chef du Département des Virus Epidémiques
Institut Pasteur de Côte d'Ivoire
01 BP 490 Abidjan
Tél. : 225 23 50 02 10 / Fax : 225 23 53 79 03
akouamc@yahoo.fr / polioci@globeaccess.net

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