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LECTURE PRESENTATIONS

L1

HIV/AIDS, more than 25 years later: which challenges remain?

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BMC Proceedings 2011, **5(Suppl 1):**L1

The discovery of HIV in 1983 originated from a collective adventure, which mobilized clinicians, researchers and patients altogether. This collaboration was crucial to rapidly expand the knowledge of the virus and develop the first diagnostic tests and antiretroviral therapy (ART).
More than 25 years after the discovery of the etiological agent responsible for AIDS, research priorities still remain care, treatment and prevention with the major objective of developing a preventive vaccine.
Today, we have gained significant insight into the virus pathogenesis. The evolution and progression of the disease caused by HIV is closely linked to a number of determinants of the virus itself and the host. We also know today that, very early after exposure to the virus, a massive depletion of CCR5+ CD4+ T memory cells associated with microbial translocation occurs in the gastrointestinal tract of HIV infected patients. HIV infection is clearly inducing an inflammation and a generalized and persistent T cell activation, which may play a role in the persistence of HIV infection, resulting from the establishment of permanent reservoirs into host cells and in different host compartments. The reduction of the size of these reservoirs is representing one of the main challenges for the development of future therapeutic strategies.
Among other challenges in therapy, we also need to better understand the mechanisms leading to the severe complications (cardiovascular diseases, accelerated aging, cancer...) observed in some patients on long-term ART. Again, whether the inflammatory response is contributing or not to these complications remains an opened question.
The early acute phase of HIV infection appears therefore to be crucial in determining disease progression. Given the importance of the innate immune responses in this very early phase following infection and in driving adaptive immunity, further research on innate immunity in HIV infection are certainly among priorities for elaborating future therapeutic and vaccine strategies.
New technologies are today available to address all these scientific challenges. But they will only be overcome with a multidisciplinary and translational research for the global benefit of humanity.

L2

Enteric vaccines for the developing world: challenges and prospects

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BMC Proceedings 2011, **5(Suppl 1):**L2

Diarrhea is one of the top disease killers in newborns and young infants of the developing world, yet the research funding for vaccine development of enteric diseases falls far behind the investment that has been made in preventing diseases with lower burden to the world's population. Advances in this field have been delayed not only due to poor funding, but also due to knowledge gaps in our understanding of the gut mucosal immune system, especially its development during the early life.
Modern biotechnology has yielded an abundance of vaccine candidates against enteric infections. If these candidates are to ultimately reach those in need in developing countries, several lessons from clinical and field research done in these settings must be considered. These lessons include the need to develop vaccines that can be administered without needles or sophisticated delivery devices. These vaccines must work in the most impoverished populations and must be able to contain epidemics following complex emergencies. An ideal vaccine should prevent entry and replication of pathogens in the gut epithelium and confer herd protection. Such vaccine must protect all age groups at high risk and be safe and effective in immunocompromised people.
Extensive research is being carried out to identify protective antigens, pertinent vaccination regimens including prime-boost strategies and alternate delivery routes, novel delivery systems, new vectors and safe adjuvants for mucosal immunization. We have now documented the effectiveness of an oral killed whole cholera bacteria vaccine in children from a cholera endemic area in one of the largest clinical efficacy trials ever conducted for an enteric vaccine. The vaccine can be produced at industrial scale, is affordable to the poorest, and confers direct and community protection for at least 3 years. A second vaccine against typhoid fever is at a late stage of development and has been shown to be highly immunogenic in children. A third vaccine is at an early stage of development and targets *Shigella* bacteria, the causative agent of one of the most severe forms of diarrheal disease, bacillary dysentery. The vaccine is based on a protein antigen conserved among all species and serotypes (>50) of *Shigella* co-administered with a novel mucosal adjuvant and has already been shown to confer protection against experimental shigellosis in 3 animal models. In partnership with PATH, the IVI is conducting a program entailing production process development and early clinical testing of this subunit vaccine anticipated to commence in 2012.

L3

One step closer to a universal influenza A vaccine?

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BMC Proceedings 2011, **5(Suppl 1):**L3

The continuous antigenic drifts and occasional antigenic shifts enable human influenza viruses to escape the human immune system. Moreover, the frequent occurrence of human H5N1-infected cases and the recent emergency of a novel swine-like human H1N1 influenza virus further reiterate the risk of the introduction of a new pandemic strain to humans through *in toto* transfer of animal influenza viruses. The discovery of neutralizing antibodies that are broadly reactive with multiple influenza subtypes is therefore extremely important for the influenza pandemic preparedness, for use either for therapeutic purposes or as the basis of vaccine development. On the other hand, recently studies also suggest that cell-mediated immunity might be critical for cross-subtype protection against influenza virus infection. Using vaccinia virus-based H5 vaccine, we also demonstrated this approach might also capable of inducing cross-subtype protection. Mice received this H5 vaccine had no detectable neutralization antibody against other HA subtypes, suggesting that cell-mediated immunity might be account for the subtype protection. Here, we would discuss our recent findings on these research topics.

L4

Vaccines for neglected diseases: challenges and opportunities

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BMC Proceedings 2011, **5(Suppl 1):**L4

Infectious diseases exert a major burden of disease in developing countries with 99% of the global burden of infectious diseases, as measured by DALYs, in low and middle income countries. While better use of existing vaccines would make an appreciable difference, the greatest burden is caused by diseases for which we currently have no vaccines. The picture, especially in children, is dominated by diarrheal and respiratory diseases. Paradoxically these diseases have relatively low priority for funding in absolute terms, and especially in relationship to the burden of disease. Thus, new vaccines for these neglected diseases need both innovative scientific solutions and innovative development schemes involving scientific institutes, public financing and industrial input. The industrial input is critical: not only will vaccine manufacture require an industrial partner, but the knowledge to efficiently undertake the technical and clinical development leading to vaccine production largely resides in industry. A potentially important development in this area has been the recent formation of Industry Linked Vaccine Institutes: For example, the Novartis Vaccines Institute for Global Health and the Hilleman Laboratories. These are an important conduit for applying industrial know how for developing commercial vaccines to the pressing need for vaccines for neglected diseases of developing countries.

L5

Buruli ulcer disease: challenges and opportunities for Institut Pasteur International Network

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BMC Proceedings 2011, **5(Suppl 1):**L5

Buruli ulcer is a neglected tropical disease caused by *Mycobacterium ulcerans*. It is the third most prevalent mycobacterial disease in the world. The disease has been reported in over 30 countries, mainly in tropical and subtropical regions of Africa and has often been associated with swampy areas. This flesh-eating disease imposes a harsh reality on its victims who endure prolonged periods of suffering. Major challenges to the prevention and control of Buruli ulcer disease are the lack of knowledge surrounding the reservoir and route of transmission of *M. ulcerans*, lack of diagnostic tests that can easily be performed in rural areas, and limited treatment options.

During this decade, the Institut Pasteur International Network developed interest on Buruli ulcer, bringing together scientists from around the network and other research institutes, and allocate funds. It is in this way that a multidisciplinary effort was undertaken in Centre Pasteur of Cameroon on epidemiological and environmental factors associated with *M. ulcerans* to understand the mode of transmission.

Our first research work focused on risk factors and outlined protective factors such as the use of bed nets and the proper care of skin lesions with antiseptic solutions. These results were then used to reinforce education in a basic public health measures by awareness raising campaign. Our more recent studies assessing the role of water bugs as *M. ulcerans* host and/or vector in environmental context provided a first full picture in terms of biodiversity and seasonal and regional dynamics link with variation in the insect tissue colonization rate.

Furthermore, the Centre Pasteur of Cameroon initiated an international course on Microbiology of *M. ulcerans*. This course brought together scientists from 11 African endemic countries and provided knowledge for PCR detection of *M. ulcerans*, the most sensitive and quickest tool available for diagnosis and in tracing environmental sources of *M. ulcerans*. The Centre Pasteur takes advantage of this competence to serve also at country level, as Reference Laboratory for the National BU control programme; it provides diagnostic support for cases confirmation and expertise in different Public health interventions.

In conclusion, within Institut Pasteur International Network there is interest on Buruli ulcer, but more could be done; there are many opportunities to contribute from scientific point of view.

L6

Phenomic technologies in drug discovery of neglected diseases

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BMC Proceedings 2011, **5(Suppl 1):**L6

Leishmaniasis, Chagas Disease and Dengue are examples of neglected infectious disease that, together with Malaria, are responsible for over 1 million deaths each year. In all cases, new treatments are badly needed. Malaria treatment, although available, is becoming problematic due to worldwide spread of parasite resistance to drugs. For Leishmaniasis, drugs are often ineffective due to the pathogens' resistance, and in some cases the drugs themselves are potentially deadly for patients. For Chagas, available treatment is often ineffective past the acute phase of the disease, and for Dengue there is no current treatment available at all.

IP-Korea develops Phenomic Screen™, image-based high-content/high-throughput screening assays to find new drugs against Leishmaniasis. Using a whole-cell-based approach, we designed assays that allow selection of compounds that have potent activity against pathogens and are not toxic for the human cells. These cellular assays were adapted to high-throughput for automated image acquisition. Using this technology we have screened 200,000 drugs that impair the growth of the most deadly species causing visceral Leishmaniasis, inside their natural host cell, the human macrophages.

We have also developed a phenotypic screen for Chagas disease and screened 150,000 compounds, in collaboration with Pfizer and the Drugs for Neglected Diseases *initiative* (DND*i*), for their ability to inhibit *Trypanosoma cruzi*, the parasite causing Chagas, inside human host cells. Also, we established visual screening procedure to screen human genome in high content imaging mode within 8h. Genome-wide RNAi screening to identify human host proteins that are required in Chagas disease infection has been finished. The identification of these factors provides potential new targets for anti-parasitic therapies.

Dengue is still in the early stage but is progressing towards the development of a cell-based high-throughput screening assay using different virus serotypes and clinical isolates.

For malaria, we are developing HCS/HTS assays that target the invasion of human red blood cells by the deadliest of all malaria parasites, *P. falciparum*. We have screened 80,000 compounds for their antimalarial activity, and the selected hits will be re-tested for their ability to interfere with the invasion pathway using an image based high-content assay. In parallel, we are developing a phenotypic assay to screen for drugs that interfere with *P. falciparum* infected red blood cells cytoadhesion, one the main pathogenic mechanism of this parasite.

L7

Predict: surveillance and prediction for emerging pathogens of wildlife

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BMC Proceedings 2011, **5(Suppl 1):**L7

Nowhere in the world are the health impacts from emerging diseases of wildlife more important than in developing countries, where daily work and livelihoods are highly dependent on natural resources. Many of these same countries have little to no capacity for detecting disease emergence in wildlife and domestic animals prior to spread to humans. While the linkages of human, animal, and environmental health is at the heart of the One Health approach, an increasingly important prism through which governments, NGOs, and practitioners view public health, we still have three critically important challenges facing us: 1) we need a much broader and deeper knowledge of what pathogens are waiting to emerge from the animal kingdom, 2) we need to better target our investigations to maximize available resources, and 3) we need better tools to predict or determine if an organism is a pathogen of significance for causing human disease. The PREDICT project of the USAID Emerging Pandemic Threats program is endeavoring to build capacity for surveillance of emerging diseases in wildlife in order to help to address these three challenges.

L8

Networking the *Leishmania* research community for the development of novel anti-leishmanial intervention strategies

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BMC Proceedings 2011, **5(Suppl 1):**L8

The EU-sponsored LEISHDRUG consortium uses a highly interdisciplinary approach to reveal *Leishmania* signaling molecules associated with virulence of the pathogenic amastigote stage. LEISHDRUG coordinates the efforts of 13 partners across eight nations, including four institutes of the Institut Pasteur international network, with the major aim to exploit the *Leishmania* kinome for anti-parasitic drug development.
The consortium is based on three clusters with each two interactive scientific work packages that together follow the major stages of the drug development process, including identification of hit compounds and target kinases, hit-to-lead validation and lead characterization. We use innovative drug screening concepts not applied previously on parasitic systems, including visual high-content screening to discover compounds capable to kill intracellular *Leishmania* amastigotes without deteriorating the host cell. This phenotype-based strategy relies on fluorescent parasites and macrophages as read-outs and will allow simultaneous assessment of anti-leishmanial activity and host cell toxicity under physiological conditions. We apply a target-based strategy utilizing recombinant *Leishmania* protein kinases for inhibitor identification and structure-guided drug design. The identification of appropriate target kinases, with only limited homology to their mammalian counterparts relies on in silico analysis by applying novel bioinformatic tools developed by consortium members, and in vitro assays based on their phospho-transferase activity towards recombinant *Leishmania* phospho-proteins.
The major objectives of our consortium are (i) to screen small molecule and peptide libraries for hit compounds with leishmanicidal activity using phenotype- and target-based strategies, (ii) to identify anti-parasitic lead compounds and assess their pharmacokinetic profiles using cell-culture and experimental infection models for leishmaniasis, and (iii) to initiate lead optimization by structure-based drug design.

L9

Clinical research and networks – a good marriage?

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The largest, most devastating outbreak of an infectious disease in modern history occurred in 1918, when a highly virulent influenza A H1N1 virus spread throughout the world and killed between 20 and 40 million people. This haunting memory has led to continued concern about the ongoing circulation of avian H5N1 influenza virus and sensitized the world to the potential of the recent H1N1 2009 pandemic. Considering the potential death toll of a 1918-like influenza pandemic, the still evolving HIV/AIDS epidemic, the spread of drug resistance, and the massive and unprecedented changes in the environment and societies throughout much of the developing world such collective global but more importantly regional investment must be a top priority. Investment in scientific and health care infrastructure and consideration of new paradigms for clinical science are required to address the global health challenges of the 21st Century.
International cooperation, collaboration and sharing of data is essential, but this will only happen if there is trust engendered between scientists by long term shared endeavour via an equitable scientific partnership between the north and the south. Such partnerships cannot be generated quickly and only when the rich world feels threatened by something that might potentially spread to them from the developing world. The information from such research and the benefits need to be shared and flow equitably in both directions. There has been a remarkable explosion in the molecular and other basic sciences over the last twenty years. There is a very real danger that as we continue to neglect (and make increasingly and unnecessarily bureaucratic and complicated) patient orientated research and public health that it will be these areas that will hold back the phenomenal opportunities that might accrue from the basic scientific revolution. From SNPs to cellular responses, from cytokines to arrays and through to proteomics and beyond we can now deliver a mass of scientific data in minutes. Unless we can rationalise that data and put it into the context of a human being, the environment and community in which they live we will not deliver the promised benefits of this remarkable scientific age to people who need it most. We have neglected the clinical and public health bit of clinical science for too long and we have failed to build sufficient long term equitable scientific partnerships between the north and the south.
There is now a great opportunity to reinvigorate health research fully integrated with the basic and social sciences and to build strong international collaborations with the centre of gravity firmly based where the need is greatest. The potential opportunities that can arise from such collaborations and networks are enormous but like marriage such endeavours need a great deal of work in order to be successful and they may not be the answer to everything!

L10

Global health research collaborations: lessons from the Fogarty International Center

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BMC Proceedings 2011, **5(Suppl 1):**L10

The Fogarty International Center at NIH has the mission of supporting training and research in global health with a focus on problems in low and middle income countries. Our programs provide research opportunities in both infectious and chronic diseases that link investigators and institutions in the United States with groups in the developing world. The value of these long term investments is evident by their ability to support cutting edge research while training local investigators to develop independent streams of funding and support. The presentation will provide examples of our activities that might have application within the network of the Institut Pasteur.

L11

The price of altruism, and the limits of scientific inquiry

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BMC Proceedings 2011, **5(Suppl 1):**L11

The origin of kindness is a mystery. Where do giving and altruism come from: were they inherited on the wings of natural selection – a gift

bestowed upon us via the inching, evolutionary march of sacrificial amoeba, selfless penguins, and charitable baboons? Or is altruism a unique refinement, a singular human triumph over 'nature bloody in tooth in claw'? Darwin called this his greatest single riddle and ever since thinkers have tried to crack it.

One hundred and fifty years of attempts to crack the mystery of altruism can teach us a lot about how prior ideological commitments find their way directly into the scientific enterprise. Beginning with the debate between "Darwin's bulldog", Thomas Henry Huxley, and the Russian anarchist prince Peter Kropotkin, the history of such attempts tells a story of direct contact, and mutual interplay, between social, political and scientific thought. Whether it is the Chicago ecologists of the 1920s through 1940s, arguing for group selection and the "superorganism" as Fascism tore through Europe, or John von Neumann and the progenitors of Game Theory touting a belief in the utter selfishness of the "maximizing agent" as they modeled games on their swivel chairs at RAND, theories of altruism and cooperation have been closely linked to scientists' own understanding of the moral good and its social consequences.

Neurogenetics and fMRI studies are showing today that kindness may be located in our genes and in particular parts of our brain. Coupled to animal behavior studies, brain damage studies, evolutionary psychology logic, and mathematical modeling, they are supposedly pointed in the direction of cracking the altruism mystery. But precisely for this reason, we need to remember the fate of George Price, who will be the focus of this presentation. The achievements and tragedy of this hitherto forgotten American genius chemist-turned-evolutionist-turned-homeless vagabond, I will argue, provide salutary lessons regarding our understanding of altruism, as well as the limits of scientific inquiry. "Even if all possible scientific questions be answered", the twentieth century philosopher Ludwig Wittgenstein wrote, "The problems of life have still not been touched at all. Of course there is then no question left, and just this is the answer". Price's incredible life story will be presented to argue this point.

L12

Altruism – the enigma of self-interest

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BMC Proceedings 2011, **5(Suppl 1):**L12

The definition of altruism will be presented, highlighting the enigma behind this phenomenon in today's highly competitive world of market economy.

The growing challenges of hunger and poverty plague the world even as we entered the new millennium. As the gap between the rich and the poor widens, there is also a trend showing the increase of charitable acts and a growing interest in altruistic activities.

Hong Kong, where the East meets the West, has developed into a community that is highly competitive and market-oriented. Yet its citizens, while famous for being hard-driven and target-oriented, have also nurtured a charitable spirit that is widespread and deep-rooted. Most, if not all, of her citizen regularly engage in charitable donations and sacrificial services. Each year, the community here donates an amount that, on a per capita basis, ranks amount the top of the world. Data from donor profiling by one of the larger fundraising charities provide clues as to why people give and how they choose their favorite charities.

While donating money was the starting point of charitable work, current trend showed that more and more people now choose to donate their time serving the needy, by volunteering and even working fulltime with charities of their choice.

The author recounts his 14-year experience of fulltime service with an international charitable organization in Mainland China and East Asia, and discusses what motivated many of his colleagues to serve in impoverished regions of Asia. Foregoing the comfort and luxuries of modern world, young professional people with high education background chose to use their professional skills to serve the needy rather than making money for themselves. The motivation behind their decisions is discussed.

Evidence point to the fact that instead of being selfless, most donors and workers choose to serve because of a conviction that serving others bring satisfaction and/or reward to themselves. This concept of rewards is of course not in monetary terms. Alternative worldviews are common among workers in charitable organizations. The basis of these worldviews will be discussed.

ORAL PRESENTATIONS

O1

Tuberculosis but not Immune Reconstitution Inflammatory Syndrome (IRIS) prevents early and late NK cell degranulation reconstitution in HIV/TB co-infected patients

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Background: This study aims to evaluate the reconstitution of NK cell functions in HIV/TB co-infected patients developing IRIS versus those not having IRIS after TB and anti-retroviral treatment initiation.

Methods: (i) 138 HIV+/TB+ patients enrolled in the CAMELIA trial (ANRS 1295-CIPRA/KH001-DAIDS-ES ID10425) in Cambodia and 36 HIV+ patients with $CD4^+$ T cells < 200/mm^3 included (ii) NK cells repertoire analysis by immuno-staining in whole blood and, (iii) NK cells degranulation activity and cytokine production by CD107a essay with and without stimulation. After starting of TB therapy in HIV+/TB+ patients, the time points were weeks 2, 8, 14 and 34. Half of the patients started HAART at week 2 and the other half at week 8. The time point of HIV+/TB- were week 0 and 8. The results obtained at baseline and following HAART are presented.

Results: 37/138 HIV+/TB+ patients developed IRIS. 33 IRIS, 67 non IRIS and 36 HIV+/TB- were available for CD107a degranulation and IFNγ production analysis and 32 IRIS, 78 non IRIS and 36 HIV+/TB- for NK cell repertoires analysis. At baseline, CD107a degranulation was lower in HIV+/TB+ (IRIS and non IRIS) than in HIV+/TB- (median 8.88 and 6.62 vs 12.81, p=0.008; p<0.0001). The IFNγ production was also lower in HIV+/TB+ (median 1.91 and 2.46 vs 6.55, p<0.001). From baseline to 6 weeks of HAART, CD107a degranulation in HIV+/TB+ (IRIS and non IRIS) were lower than 8 weeks treated HIV+/TB- (0.09 and 0.51 vs 7.53; p=0.04 and p=0.002, respectively), whereas the IFNγ production was not different (p>0.05). At baseline, CD69 positive NK cells in HIV+/TB+ (IRIS and non IRIS) was higher than HIV+/TB-. After 6/8 weeks of HAART, NK cells activation decreased and there were no difference in all groups while the expression of NKG2D, NKp30, and NKp46 among NK cells in non IRIS, but not in IRIS were higher than HIV+/TB- [(median +1.40 vs -0.95 , p=0.03) ; (median +1.39 vs -3.66, p=0.01) and (median -0.13 vs -3.53 , p=0.05) respectively]. Concerning levels of NK receptors, we observed several differences in particular NKG2C which was higher in IRIS patients compared to non IRIS patients at week 34.

Conclusion: Co infection with Tuberculosis in HIV infected patients prevents NK cell degranulation reconstitution after TB and HAART treatment.

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O2

NS1-mediated delay of immune activation contributes to influenza A virulence in ferrets

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Most influenza A viruses cause a mild to moderate disease of the upper respiratory tract with low mortality levels, but certain strains are associated with a severe disease involving the lung and high mortality. Among the proteins known to contribute to virulence, the nonstructural protein NS1 has been identified as the main viral immune interference *in vitro*, and viruses lacking NS1 are highly attenuated in different animal models. To investigate if NS1 proteins of virulent strains are more efficient in interfering with innate immune activation, we generated recombinant USSR/90/77 viruses expressing the NS1 protein of either the attenuated strain PR/8, or the highly pathogenic 1918 "Spanish flu", all

belonging to the H1N1 subtype. While all three NS1 proteins interfered with type I IFN-mediated signaling, assessed by a luciferase reporter gene assay, the 1918 NS1 protein was significantly more efficient. Moreover, when cells were infected with the recombinant viruses, we observed that the presence of PR/8 NS1 was associated with an earlier and overall stronger type I IFN induction. Infection of ferrets with the different viruses revealed that the virus with the 1918 NS1 protein caused a more severe disease with overall higher clinical scores and a higher fever peak. The presence of NS1 from virulent strains correlated with a delay in virus clearance from the upper respiratory tract and spread to the lungs. Moreover, these viruses caused more lung damage with partial loss of the bronchial epithelial layer and alveolar swelling that persisted for up to four days. In contrast, the recombinant virus expressing the PR/8 NS1 protein resulted in little histopathological damage but slightly more inflammation. To assess the impact of the different viruses on immune activation in the upper respiratory tract, IFN and cytokine mRNA induction levels in nasal wash cells were quantified. Consistent with the *in vitro* studies, the virus with the PR/8 NS1 protein induced significantly more IFN-β at days 1 and 3 post-infection, and more IFN-α at day 1 post-infection. In contrast, presence of the more virulent USSR and 1918 NS1 proteins resulted not only in inhibition of early type I IFN induction, but was also associated with a delayed expression of the pro-inflammatory mediators, TNF-α and IP-10. Taken together, these results demonstrate the importance of NS1-mediated immune interference constitutes for influenza A virus virulence.

O3

Genetic determinants of hepatitis B vaccine response

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Hepatitis B is a major public health problem. Approximately one-third of the world's population has serological evidence of infection with hepatitis B virus (HBV). 350 million of these are carriers who have chronic HBV infection, and about a million of these carriers die each year from chronic liver disease, including cirrhosis and liver cancer. Fortunately, HBV is a vaccine preventable disease, and the increasing adoption of this vaccine has led to dramatic reductions in the morbidity and mortality caused by this virus. However, as much as 10% of the population fails to mount a protective immune response after vaccination. Twin and other epidemiological studies have demonstrated an unusually high heritability to this trait, which suggests a high likelihood of identifying genetic variation influencing HBV vaccine response. In search of such variation, we performed a two stage Genome Wide Association Scan (GWAS) in participants of a vaccine efficacy trial from Batam, Indonesia. In Stage 1, we used the fixed content Illumina Infinium 550K SNP BeadChip to genotype 2000 vaccinees. Tests of association were performed to identify 7,000 SNPs to carry forward to a second stage of 2,300 vaccinees. Several independent regions attained genome-wide significance, including the HLA-DR and HLA-DP regions. Additional details on the study design and results will be discussed in the presentation.

O4

Asian network for molecular diagnosis of primary immunodeficiencies

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BMC Proceedings 2011, **5(Suppl 1):**O4

Primary immunodeficiency disorders (PIDs) are inborn errors of the immune system. There are over 150 types of PIDs and because of their rarity, multi-center collaboration for pooled data analysis and molecular studies is important to gain meaningful insights about the phenotypic and genetic diversities of PIDs. Since 2001, our unit established collaboration with 30 pediatric centers in China and Southeast Asia to provide e-consultation and free molecular diagnosis for PIDs. It is imperative to organize the data systematically to yield information on epidemiology of PIDs in the region.

By Aug 2010, we have performed genetic tests for 392 patients referred to us and 243 patients (62%) have their genetic mutations identified. 107 patients (27%) were from Hong Kong, 222 (57%) were from mainland China while the rest were from Taiwan, Singapore, Malaysia, Thailand, the Philippines and Australia. In addition, 200 carriers were identified from 331 potential carriers in these 49 families. X-linked agammaglobulinemia (n=90), Wiskott-Aldrich syndrome (n=49), X-linked chronic granulomatous disease (CGD, n=28), X-linked hyperIgM (n=17) and X-linked severe combined immunodeficiency (SCID, n=19) constituted majority of cases. We also identified mutations of rare PIDs, such as autosomal-recessive SCID (IL7R, JAK3, RAG2 and DCLRE1C), autosomal-recessive CGD (NCF1, CYBA), defects of IL12/IFN-gamma axis in patients susceptible to mycobacterial infections, FOXP3 mutation in immunodysregulation- polyendocrinopathy-X-linked (IPEX) syndrome, SH2D1A mutations in X-linked lymphoproliferative syndrome, TACI in common variable immunodeficiency, ITGB2 mutations in leucocyte adhesion deficiency, and AIRE gene mutation in autoimmune polyendocrinopathy with candidiasis and ectodermal dysplasia (APECED). However, mutations could not be identified in about 40% of patients despite distinct clinical and immunological phenotypes. This is the group of patients that would probably yield novel genes responsible for PIDs with appropriate strategic analysis, combining both functional and genomic analysis.

Establishment of PID data and referral network is an initial step to multi-center collaboration. This constitutes the foundation for PID research and documentation of prevalence, disease burden and outcome of patients with PIDs in Asia, as well as identifying new genes critical for immunohomeostasis.

O5

Characterization of immunomodulatory activity of eIF4A protein

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Leishmania LeIF antigen, homologous to eukaryotic initiation factor eIF4A, was originally described as a Th1-type natural adjuvant and as an antigen that induces an IL-12-mediated Th1 response in the peripheral blood mononuclear cells (PBMC) of leishmaniasis patients. We showed previously that the induction of cytokines in monocytes of healthy subjects is not unique to the *Leishmania* protein. Indeed, 5 homologous proteins DEAD box in mammals and yeast were also able to induce the secretion of cytokines in monocytes of healthy subjects. In this study we aimed to validate eIF4A protein as a natural adjuvant. To achieve this objective, LeIF, eIF4A from mouse (MeIF4A) and Yeast (YeIF4A) were expressed and purified. The purified proteins were assessed for their ability to induce maturation of bone marrow-derived dendritic cells (BMDC). Their effect on DCs and their monocytic precursors in the peritoneal cavity of mice were analyzed. Our data showed that eIF4A proteins were able to activate BMDC to express co-stimulatory molecules and to produce IL-12p40/p70 and iNOS *in vitro*. Furthermore, eIF4A proteins induced inflammation in the peritoneal cavity of BALB/c mice similar to the well known adjuvant Alum. Indeed, injection of eIF4A proteins in combination with OVA protein induced a rapid recruitment into the peritoneal cavity of $Ly6C^{high}/CD11b^{+}$ inflammatory monocytes, $Ly6G^{high}/CDb^{+}$ neutrophils and myeloid dendritic cells (MHC IIhigh-CD11c+). This study highlights the adjuvant activity of eIF4A protein and suggests that the immunomodulatory properties of eIF4A could be exploited in vaccination or immunotherapy protocols.

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O6

Novel strategy using live non-pathogenic *Leishmania* expressing selected parasite antigens as a candidate vaccine for leishmaniasis

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Parasites of the genus *Leishmania* are intracellular protozoa which are transmitted to their mammalian host by the bite of infected sand flies and cause a group of diseases known as Leishmaniasis. Despite attempting different vaccination strategies, no human vaccine is yet available against this disease. There is increasing evidence that presence of a small number of live parasites is necessary to maintain durable immunity, and the only way to meet this requirement is by using attenuated live vaccines [1]. The main obstacle about attenuated live strains is the risk of reversion of the organism to its virulent state. Another approach to reach this strategy is to use non-pathogenic *Leishmania* such as *L. tarentolae*. This parasite is lizard parasite and has never been found associated with any leishmaniasis in humans and is considered as non-pathogenic to humans. Previous studies have shown that *L. tarentolae* can be used as a live vaccine against *L. donovani* and elicit a protective Th1 immune response [2]. Recently, by comparative genomic analysis and expression profiles of well-characterized virulence factors such as GP63, CPB, LPG3, Amastin and A2 between pathogenic *Leishmania* species (e.g. *L. major*, *L. infantum* and *L. braziliensis*) and non pathogenic *L. tarentolae* revealed that only A2 is absent at the level of DNA [3]. A recombinant *L. tarentolae* expressing the A2 protein was generated and its potential as a live vaccine against *L. infantum* infection in BALB/c mice was examined [4]. The A2 expressing recombinant parasites showed higher macrophage infectivity in comparison to *L. tarentolae* used as a control. Immunization (i.v. and i.p.) of BALB/c mice with recombinant *L. tarentolae* A2 elicited a strong protective immunity against virulent *L. infantum* challenge, manifested by a dramatic decrease in parasite burdens in the liver and the spleen of immunized mice. IFN-g production upon antigen stimulation indicated that protection is associated with a Th1 cell-mediated immunity accompanied by reduced levels of IL-5 production (the Th2 type response). Interestingly, although IFN-g production is also induced in groups of mice immunized with wild type *L. tarentolae*, cytokine levels are increased in the group immunized with the recombinant *L. tarentolae* A2 and especially when the vaccine regimen is administered via the i.p. route [4]. In continuation of these promising results, we are expanded this idea against *L. major* infection as a novel vaccine regimen by including two immunogenic parasite proteins (cysteine proteinases A and B, CPA/CPB).

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O7

Trypanosoma cruzi produces a population of tRNA-derived small RNAs which are recruited to specific cytoplasmic granules and secreted to the extracellular medium

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In the last decade a new family of small regulatory RNAs (sRNAs) of 20-30 nt in length, were recognized as key players in the regulation of gene expression at the transcriptional and post-transcriptional levels. The enzymatic machinery associated with the biogenesis and effector functions of these "sRNAs" has been found in the majority of the organisms studied. The exceptions for this are some species of trypanosomatids including *Trypanosoma cruzi*. This accounts for the absence of RNA interference phenomena in these parasites. In the present work we have cloned and characterized the small RNA population from *T. cruzi*, in two of its life cycle forms: epimastigotes and metacyclic stages. Our results showed a highly represented population of small RNAs derived from de cleavage of mature tRNAs representing about 30% in epimastigotes and 40% in trypomastigotes of small RNA fraction which we dubbed mini-tRNAs. Surprisingly, more than 98% of mini-tRNAs derived from the 5' half of tRNA for Asp and Glu and localize to particular granular structures in the cytoplasm of *T.cruzi* at all stages of its life cycle. These tRNA halves seem to be related with an Argonaute protein distinctive of trypanosomatids which was recently cloned and sequenced in our laboratory. This Argonaute protein was differentially expressed through the life cycle of *T. cruzi* and its expression was accentuated by starvation. Whereas their biological significance is currently unknown this mini-tRNAs population it has been described in the last year in other organisms as *Giardia lamblia, Aspergillus fumigatus* and many mammalian cancer cells. These mini-tRNAs have also been found in the supernatant of culture of *T. cruzi* as forming part of the secretome of these organisms, which led us to speculate about a putative role of these molecules in intercellular communication. This could represent a new family of small RNAs with relevance in gene expression regulation in particular for these unicellular parasites where gene regulation is achieved principally by post-transcriptional mechanisms. The complete knowledge of gene expression regulatory pathways in this kind of parasites that concern human health could be a source of study for development of vaccines or anti-parasitic treatment in base new tools as the RNA vaccines or biotechnological drugs.

O8

Chikungunya, a new threat propagated by the cosmopolite *Aedes albopictus*

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The recent outbreaks of chikungunya (CHIK) were due to an East-Central-South African genotype harbouring a substitution from an Alanine (E1-226A) to a Valine (E1-226V) at the position 226 in the E1 gene. The new variant E1-226V is very efficiently transmitted by an unusual mosquito vector, *Aedes albopictus*. We found that (i) *Ae. albopictus* females ensure a high replication rate of E1-226V up to 10^9 viral particles per female, (ii) the midgut plays a key role in limiting viral dissemination of E1-226A in the mosquito, (iii) the virus is detectable in the saliva as soon as two days after the infectious blood-meal, and (iv) *Ae. albopictus* is able to deliver up to 3000 viral particles with its saliva. All these characteristics led to exacerbate CHIK transmission by *Ae. albopictus*. However, this species is affected by the viral infection. Indeed, CHIK infection reduces sharply the

survival of *Ae. albopictus*, females laying their eggs just before dying. Females did not die from an excess of viral replication but more likely in attempting to mount an immune antiviral response. Moreover, by removing the intracellular bacteria *Wolbachia* from *Ae. albopictus* through successive antibiotic treatments, we aimed to determine if *Wolbachia* interferes with CHIK replication in the mosquito. We found that *Ae. albopictus* cleaned of *Wolbachia* was not affected by CHIK infection. Thus, *Wolbachia* may regulate viral replication in *Ae. albopictus* with consequences on its survival. So, the response of a vector to a particular pathogen is also closely linked to the presence of other microorganisms. Finally, we showed that *Ae. albopictus* was able to be orally co-infected with CHIK and dengue (DEN) viruses and to deliver concomitantly infectious particles of both viruses in saliva. This finding is of particular concern as *Ae. albopictus* is still expanding its geographical range and as both CHIK and DEN viruses can co-circulate in the same geographical regions. Indeed, reports of co-infections in patients with both viruses are increasing.

O9

Viral and bacterial etiologies of community-acquired acute lower respiratory infections among hospitalized Cambodian patients

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Community-acquired acute lower respiratory infections (ALRI) remain a major public health problem, particularly in tropical and low/middle-income countries. There is still a paucity of data regarding viral and bacterial etiologies. Beginning in April 2007, we enrolled all-aged patients hospitalized with acute respiratory symptoms excluding known tuberculosis and positive HIV serology in Cambodia. Etiology was assigned based on laboratory data from direct sputum examination, blood and sputum cultures and nasopharyngeal swabs. Diagnosis of ALRI was determined from medical charts' reviews and interpreting chest radiographs by expert pulmonologists. Severe ALRI case (SARI) was defined on the basis of oxygen saturation, respiratory rates according to age and severe clinical symptoms.

During April 2007 - December 2009, 2,824 patients presented with ALRI on admission including 948 (34%) in the <5 year-age groups. Of the 948 ALRI patients aged <5 years, 43.7% were diagnosed with parenchymal involvement (PI), 0.9% with pleurisy alone and 57.2% bronchi involvement (BI). In the >5 year-olds, the proportions were different: 56.4% PI, 38.1% BI and 12.9% pleurisy.

A total of 821 (28.9%) SARI were identified of which 639 (77.8%) aged <5 years. SARI accounted for 62% of PI in the <5 year-olds and 12% in the >5 year-olds. SARI was also frequently found in BI (71%) among <5 year-old children and significantly less frequent in older children and adults (8%).

Specimens for bacteriology testing was only available in 1,004 patients including 14 who aged <5 years, 30 in the 5 – 17 year age group and 960 (95.6%) among adults. In the 14 <5 year-old children, 12 bacteria were identified including *Burkholderia pseudomallei*, 2 *Streptococcus pneumoniae*, 2 *Haemophilus influenzae,* 2 *Klebsiella pneumoniae* and 4 acid fast-bacilli (AFB); in the 5-17 year age group, there were 4 AFB, 4 *H. influenzae* and 4 *S. pneumoniae*. Of the 960 adults, etiology was found in 525 (64.7%) ones of which the most commonly identified bacteria were AFB (48.8%), *H. influenzae* (21.1%) and *S. pneumoniae* (9.1%) followed by *K. pneumoniae* (7.8%) and *B. pseudomallei* (5.6%). Of the 70 SARI cases for which specimens were available, the most frequently found bacteria were AFB (18.6%), *B. pseudomallei* (10.0%), *H. influenzae* (8.6%), and *K. pneumoniae* (7.1%). Viral identification was possible in 927 (30.1%) of the 3,083 patients' nasopharyngeal swabs (57.7% in <5 year-olds, 15.5% in adults). The three most frequently identified viruses were rhinoviruses (42.3%), RSV (29.1%), and seasonal Influenza A & B (7%).

This is the first report of the etiology of community-acquired ALRI in Cambodia – a tropical/low-income country. Our results indicated that the etiology profile in Cambodia was similar to that of other Southeast Asian countries.

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O10

The J-GRID as a new player of networking

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Infectious diseases heed no national borders. In order to enhance international research collaboration, the government of Japan launched a program in 2005, which is now named the Japan Initiative for Global Research Network on Infectious Diseases (J-GRID). This program includes establishing collaborative research centers in Asian and African countries on a reciprocal basis between Japanese universities/institutions and overseas partner universities/institutions and setting up its headquarters CRNID at RIKEN to connect those bilateral collaboration centers into a network, the J-GRID. The J-GRID now consists of 12 collaboration centers in 8 countries (6 in Asia and 2 in Africa). Here, we describe the mission of J-GRID and some of its research results just coming out. However, the J-GRID is still in its infancy and needs a great deal of effort to maximize its research capacity and to ensure its sustainability. J-GRID is eager to learn a lot from Institut Pasteur Network and other international networks that are proud of long history, high quality research and great contribution to the global public health. What a single network is able to cover is limited. In view of the geographical complementarities, particularly in Asia, close collaboration between different networks (networking of networks) will certainly give rise to a profound synergistic effect.

O11

Early (2 weeks) vs. late (8 weeks) initiation of highly active antiretroviral treatment (HAART) significantly enhance survival of severely immunosuppressed HIV-infected adults with newly diagnosed tuberculosis: results of the CAMELIA clinical trial

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Background: Tuberculosis (TB) remains the largest cause of death among people living with HIV/AIDS, especially among those with profound immunosuppression. Case-fatality among co-infected patients occurs mainly in the first months after the TB treatment initiation. Therefore, robust data regarding optimal timing of HAART initiation within this early period is critically needed.

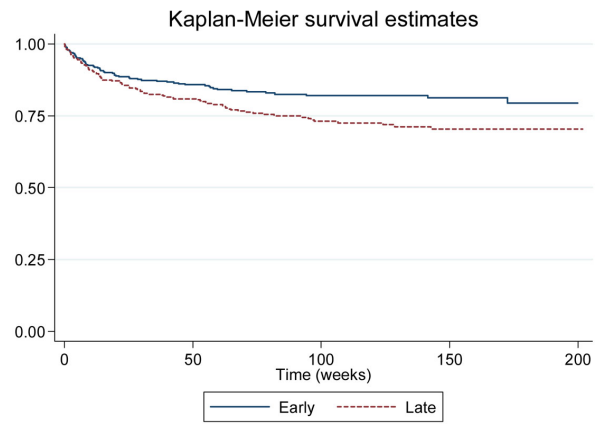

Figure 1 (abstract O11)

Methods: The CAMELIA (CAMbodian Early vs. Late Introduction of Antiretroviral drugs) clinical trial is an open-labelled randomized clinical trial designed to compare the impact upon mortality of early (2 weeks) vs. late (8 weeks) HAART initiation after TB treatment onset in treatment-naïve adults with newly diagnosed acid-fast bacilli (AFB) positive TB and CD4+ cell count ≤ 200 cells/mm^3. Patients received standard 6-month TB treatment plus stavudine, lamivudine and efavirenz in 5 hospitals in Cambodia, 2 in Phnom Penh and 3 in province. Patients were followed for 50 weeks after the last patient's enrollment. A log-rank test was used to compare Kaplan-Meier survival curves.
Results: 661 patients (early, n=332; late, n=329) were enrolled with a median age of 35 yrs, body mass index of 16.7 kg/m^2, CD4+ cell count of 25 cells/mm^3 and viral load of 5.64 log copies/ml. All AFB-positive samples including sputum in 538 (81.4%) patients, were cultured. As of May 13, 2010, 149 patients were known dead (59, early arm; 90, late arm). Enhanced survival was observed in the early arm (p=0.004, see figure). At week 50, median CD4+ gain was 114 cells/mm^3 and was not statistically different across arms (p=0.22); 96.5% of patients had an undetectable viral load and again no difference across arms was found (0.82). Figure 1.
Conclusion: Initiation of HAART 2 weeks after onset of TB treatment significantly improves survival in severely immunosuppressed HIV-infected adults with newly diagnosed tuberculosis.
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POSTER PRESENTATIONS

P1

Kinetics of neutralizing antibodies in patients naturally infected by H5N1 virus

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Background: Little is known about the kinetics of anti-H5 neutralizing antibodies in naturally H5N1-infected patients with severe clinical illness or asymptomatic infection. These data are essential for the design and interpretation of sero-epidemiological studies which are crucial to monitor the extent of asymptomatic or clinically mild H5N1 illness among humans or to identify risk factors associated with human contamination.
Methods: Using H5N1 microneutralisation (MN) and H5-pseudotype particle-based microneutralisation assays (H5pp) we analyzed sera sequentially obtained from 11 severely ill patients diagnosed by RT-PCR (follow-up range 1 – 139 weeks of disease onset) and 31 asymptomatically infected individuals detected in a sero-epidemiological study after exposure to H5N1 virus (follow-up range: 1-2 month – 11 months after exposure).
Results: Antibody kinetics measured by H5pp were similar to that from MN assay with a good correlation between the titers measured by the two methods (Spearman's correlation coefficient of 0.79, p<0.001). Of 44 sera from 11 patients with H5N1 disease, 70% tested positive by MN (antibody titre ≥80) after 2 weeks and 100% were positive by 3 weeks after disease onset. The geometric mean MN titers in severely ill patients were 540 at 1-2 months and 173 at 10-12 months and thus were higher than the titers from asymptomatic individuals (149 at 1-2 months, 62.2 at 10-12 months). Fractional polynomial regression analysis demonstrated that in all severely ill patients, positive titers persisted beyond 2 years of disease onset, while 10 of 23 sera collected 10-11 months after exposure in asymptomatically infected individuals tested negative.
Conclusion: We demonstrated a good correlation between the 2 tests used, hence confirming the validity of the H5pp test as a screening test in sero-epidemiological studies of H5N1 infection. Our data provide important novel insights into the dynamics of serological responses in patients with the full spectrum of clinical disease from severe though mild to asymptomatic H5N1 infection. Indeed, our results indicate that people with asymptomatic H5N1 infection have lower H5N1 antibody titers compared to those with severe illness and that in many asymptomatically infected patients the antibody titer decreased to levels below the threshold of positivity within one year. Hence delayed community sero-prevalence studies for H5N1 may underestimate the true burden of human infection.
Supported by the French Development Agency through the Surveillance and Investigation of Epidemic Situations in Southeast Asia (SISEA) project.

P2

Expression of a chimerical pcDNA encoding influenza virus M2 protein and HSP70 gene in eukaryotic cell lines

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The critical obstacle in developing of an effective human influenza vaccine is continuous antigenic variation of influenza A viruses occurred each year. Conserved antigens are important candidates for vaccines because it is not necessary to match the designed strains with circulating strains. M2 as an ion channel protein induce protective immunity. On the other hand, HSP70 is a molecular chaperon and immunostimulatory component, capable of eliciting effective humoral and cellular immunity against infectious disease. Genetically fusing antigens to HSPs leads to the enrichment of DNA vaccine potency.
In the present study, a chimerical DNA plasmid carrying influenza virus M2 protein and HSP70 Gene was constructed and the protein expression in eukaryotic cell line was evaluated. This construct could be used as a potent DNA vaccine. To gain this aim, Influenza A/New Caledonia/20/99 (H1N1) was inoculated into MDCK cell line. The supernatant was collected after 18 hours and total RNA was extracted using Easy-red (iNtRON) solution. Complementary DNA synthesis was carried out by RevertAid First Strand cDNA synthesis kit using uni-12 primer. Full length M2 gene (300bp) was amplified by polymerase chain reactions using designed primers. The PCR product was run on 2% agarose gel following by purification of specific band, cloned into pGEM-T Easy cloning vector (promega) and completely sequenced. The M2 gene was digested from T-vector and subcloned into the pcDNA3.1. *Leishmania amazonesis* heat shock protein (HSP70) gene was obtained by digestion of pGEM II- HSP70 and subcloned into pCDNA3.1. The digested M2 gene was then sub-cloned into the N-terminal of HSP70 in pcDNA3.1. Recombinant plasmids were transfected into COS-7 cells to evaluate protein expression.
The presence of M2 and HSP70 genes were confirmed by PCR, restriction enzyme analysis and electrophoresis. All the constructs were then verified by DNA sequencing. The vaccine candidates were transfected into COS-7

cells and protein expression was confirmed by indirect Immunofluorescense test, ELISA and western blotting. In ongoing project, the Immunomodulatory effect of HSP70 construct on DNA vaccine efficacy in animal models will be evaluated.

P3

Antibodies against human influenza viruses in *Galliformes* order

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BMC Proceedings 2011, **5(Suppl 1):**P3

Influenza A virus is always concerned as an infectious agent in birds and other mammalian such as humans. Although it is believed that barriers among various species restrict the transmission of influenza viruses, there are evidences implying that origin of all human influenza viruses is derived from avian influenza viruses. To determine the prevalence of human H1 and H3 viruses in the captive birds of *Galliformes* order, a serological surveillance was carried in Tehran Zoo, Saiee Park and Pardisan Park of Tehran, from November 2008 to February 2009. Sera samples were collected from 7 species including Chickens, Guinea fowls, Partridges, Pheasants, Turkeys and Quails and presence of antibodies was detected by haemagglutination inhibition assay. Sera of chickens immunized by human influenza vaccine were used as positive control in the assays. In total, 84.61% and 100% of sera samples had antibodies against human influenza H1 and H3 viruses, respectively. The Geometric Mean Titer (GMT) value for H1 antibodies was 30.33 whereas that related for H3 antibodies was 57.36. Significantly the highest GMT value and the greatest antibody titers were observed in chicken species. As HI assay is able to detect haemagglutination antibodies as soon as 2 weeks to 1 year post-infection, the results of this study indicate that seropositive captive birds were infected during recent year with H1 and H3 virus strain, closely related to human strains. Moreover, as the peak of influenza epidemic in human population in Tehran was at the same time with our sample collection, it could be concluded that influenza viruses' transmission between human and captive birds occurred.

P4

ImmunogenIcity of thE PandemRIX A (H1N1) 2009 Influenza vaccine in hemodialysed and renal transplanted patients

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Swine-origin pandemic human influenza A (H1N1) 2009 rapidly spread around the world since its initial reporting on the 25th of April 2009. To counter the virus, different vaccines were developed. In Belgium, the Pandemrix vaccine of GSK was used. Part of the Belgian population was vaccinated, especially those belonging to risk groups (immunodepressed patients, healthcare providers, patients with chronic disease, pregnant women….). In general, vaccine immunogenicity is less effective in immunodepressed patients than in immunocompetent patients.
In this study the immunogenicity of the Pandemrix vaccine was investigated by measuring neutralizing antibodies against A (H1N1) 2009 in sera collected from immunocompetent and immunodepressed patients such as hemodialysed and renal transplanted patients. In total 32 healthy volunteers (control group), 106 hemodialysed patients and 112 renal transplanted patients were recruited. Neutralizing antibodies were measured in sera collected before (day 0) and 1 month after a single shot vaccination (day 30) using a seroneutralisation (SN) assay followed by an immunoenzyme colorimetric reaction to detect influenza A (H1N1) 2009 antigen in infected cells. Geometric mean (GM) titers were determined at subject level by individual GM of quadruplicates at each time point. Individual and group level titer ratios (day 30/day 0) with 95% confidence intervals were determined as well.
The GM titer ratio for the healthy control group was 38 (23-62). For the dialysed patients, the GM titer ratio was 11 (8-17) and for the group of renal transplanted patients, the GM titer ratio was 5 (3-6). Differences in GM titer ratio between the healthy control group and each group of immunodepressed patients were significant (p<0,05; T test). Thirty out of 32 healthy participants (93%) had at least four-fold increases in SN titers between day 0 and day 30 (seroconversion). Among dialysed participants, 64 out of 106 (60%) had at least four-fold increases in SN titers between day 0 and day 30, and among renal transplanted patients, 49 out of 112 participants (44%) seroconverted between day 0 and day 30. Differences in seroconversion rates between the healthy control group and each group of immunodepressed patients were significant (p<0.05; fisher exact test).
These results suggest that only 60% of dialysed individuals developed sufficient neutralizing antibody titers after immunization with this vaccine. As for renal transplanted patients, only 44% of the patients developed sufficient neutralizing antibody titers after vaccination. Alternative vaccines, dosing, adjuvants or schedule strategies are needed to achieve effective immunization of these vulnerable populations.

P5

Construction of influenza virosome from influenza A H1N1 PR8

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Influenza is a major viral respiratory infection of humans, responsible for 300,000–500,000 annual deaths world-wide. The influenza viruses are competent of genetic variation, both by continuous, gradual mutation and by reassortment of genome segments between viruses. Antigenic drift is the gradual evolution of viral strains, due to frequent mutations of the surface glycoproteins hemagglutinin and neuraminidase. Novel and increasingly safer vaccines use well-characterized antigens. Conversely, these antigens are regularly too small to be highly immunogenic and would help from administration of a suitable adjuvant. The virosomes are reconstituted influenza virus envelopes devoid of central core and genetic in sequence. In this study we proposed to construct an influenza virosome structure from influenza A H1N1 PR8.
During the production process, MDCK cells were cultured then infected with influenza virus strain PR8 and finally the supernatant harvested and purified by ultracentrifugation and ultrafiltration. Purified influenza virus treated by DCPC as a detergent to resolve envelop of influenza virus. Then, RNP of influenza virus participated by using ultracentrifugation. The envelope of influenza virus was reconstituted by removing of DCPC by using overnight dialysis against HBS buffer. Finally, we observed empty influenza virus envelop by TEM, their called virosomes. The size of these particles was estimated between 50-150 nm.

P6

Influenza virus circulation in Cambodia

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Background: The Cambodian National Influenza Center (NIC) was established in August 2006 for the purpose of documenting the dynamics

of influenza disease and to virologically characterize the circulating strains. To continuously monitor influenza activity, a hospital-based sentinel surveillance system for ILI (influenza-like illness) with a weekly reporting and sampling scheme was initially established in five sites in 2006. In addition, hospital-based surveillance of acute lower respiratory infection (ALRI) cases was established in 2 sites.

Methods: The sentinel sites collected weekly epidemiological data from patients who fulfilled the ILI case definition, and took naso-pharyngeal specimens from 5 to 10 cases per week. Over 4100 respiratory samples were collected from hospitalized ALRI patients between 2007 and 2010. All samples were tested in the Virology Unit at the Institut Pasteur in Phnom Penh. Viral RNA was extracted and amplified by a multiplex RT-PCR detecting influenza A and influenza B virus simultaneously. Influenza A viruses were then subtyped and analyzed by hemagglutination inhibition assay. The susceptibility to neuraminidase inhibitor drugs (oseltamivir, and zanamivir) was determined using the NA-STAR kit (Applied Biosystems®). Genetic analyses targeting HA, NA, and M genes were conducted on strains selected randomly.

Results: We observed that 5.8% (30 of 516), 7.7% (96 of 1250), 15.3% (212 of 1382), 15.2% (909 of 6011) and 1.4% (25 of 1791) of overall clinical specimens were positive for influenza virus in the years 2006, 2007, 2008, 2009 and 2010 respectively. Until 2009, H3N2 virus was always the predominant influenza A sub-type detected: 100% in 2006, 65.9 % in 2007, 81.3% in 2008. No influenza B viruses were isolated in 2006 but accounted for 57.7% and 34% of all influenza strains in 2007 and 2008, respectively. Pandemic H1N1 (H1N1pdm) was first detected in Cambodia in August 2009 and subsequently replaced the seasonal H1N1 strain as the most frequently detected subtype. However, H3N2 continued to co-circulate in significant proportions through November 2009. Antigenic analyses show that seasonal H1N1 belonged to the groups A/New Caledonia/20/1999-like in 2007 and A/Brisbane/59/2007-like in 2008. H3N2 belonged to A/Wisconsin/67/2005-like in 2006 and 2007, and to A/Brisbane/10/2007-like in 2008 and 2009. The influenza B strains drifted from B/Malaysia/2506/2004-like in 2007 to B/Florida/4/2006-like in 2008 and then to B/Brisbane/60/2008-like in 2009. Sequences of the M gene obtained from representative 2007-2008 H1N1 and H3N2 strains contained the S31N mutation associated with adamantanes resistance. Except for a single H1N1 strain detected in 2007, no reduction in the susceptibility to neuraminidase inhibitors was observed among the influenza virus circulating from 2007 to 2010. Each year, the peak of influenza circulation was observed mainly from August to November. The proportion of specimens collected from patients with ILI which tested positive for Influenza virus varied between 0% (in May) and 51% (in October). In contrast, only 5 to 10% of the ALRI specimens were positive for influenza virus during peak of seasonal transmission. Since 2007, 4 new human cases of influenza A/H5N1 infection have been identified but by different surveillance systems (event-based surveillance, fever study)

Conclusion: Peak seasonal influenza activity in Cambodia occurred during the rainy season from August to November. Although Cambodia is a tropical country geographically located in the northern hemisphere, influenza activity has a southern hemisphere transmission pattern. Therefore, while the northern hemisphere vaccine may provide partial protection in Cambodia, the southern hemisphere vaccine is recommended. The drug susceptibility profile of Cambodian influenza strains revealed that neuraminidase inhibitors would be the drug of choice for influenza treatment and chemoprophylaxis in Cambodia, as adamantanes are no longer expected to be effective.

P7

Construction of a recombinant bacmid DNA in order to express Neuraminidase in insect cell line

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Non-replicating virus-like particles (VLPs) have been suggested as a promising platform for viral vaccines. Recently several studies have introduced VLPs produced in insect cells as a vaccine candidate against influenza infection. Neuraminidase (NA), one of the two membrane glycoproteins of influenza A viruses has been employed to construct such influenza VLPs to induce protective immunity.

In this study, the human influenza virus (A /New Caledonia 20/1999/(H1N1)) was propagated in MDCK cell culture. Viral RNA was extracted using RNX-plus solution. Complementary DNA synthesis was carried out using uni-12 primer. NA open reading frame (1413 bp) was amplified by RT-PCR using high fidelity Taq DNA polymerase. The amplicon was purified from the gel and cloned into pFastBac1 plasmid through SalI/XhoI sites. After verification of cloned NA by restriction analysis, it was subjected to automated sequencing bidirectionally. The recombinant pFastBacNA vector was transformed to E.coli DH10Bac cells which harbor bacmid DNA and helper plasmid to create NA recombinant bacmid. Restriction map analysis and sequencing results confirmed the fidelity of NA sequence. Homologous recombination between pFastBacNA and bacmid and creation of NA recombinant bacmid was verified by PCR using NA specific and M13 universal primers.

In an ongoing study, the cultured Sf9 (Spodoptera frugiperda) insect cell line will be transfected with NA recombinant bacmid to produce recombinant baculovirus expressing NA gene. This production is in its native highly glycosylated form appropriate to use in vaccine research projects. Recombinant baculovirus expressing NA gene can be also used with other individual recombinant baculoviruses expressing HA and M1 genes in production of influenza VLPs.

P8

Public knowledge, attitude and practice on influenxa pandemic (H1N1) 2009 prevention in Southern Vietnam

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Background: After quickly spreading since March 2009 in Mexico, influenza pandemic H1N1 has affected a large part of the world's population. Countries have made great efforts to contain the pandemic. An important key in containing community transmission and reducing the impacts of the pandemic influenza is to have local people educated to have good knowledge, attitude and practice toward influenza pandemic (H1N1). The aim of this study was to assess knowledge, attitute and practice of local people toward influenza pandemic (H1N1) prevention after launching education programs since early pandemic period (June 2009).

Methods: During the post-peak period of influenza pandemic (H1N1) (20 March – 8 April 2010) a cross – sectional survey was conducted in Cu Chi district of Ho Chi Minh City and Ninh Kieu district of Can Tho City. Among 304 individuals (32% male and 68% female) who were selected systematic randomly and representing for risk population as workers, students who living in the boarding houses, boarding schools. Outcome measures were perceived containment and prevention activities, received pandemic information. Knowledge, attitude and practice on personal and community prevention focused on hand washing, respiratory etiquette, avoidance of close contact with sick people, staying home if sick and house cleaning.

Results: 83.9% of respondents felt that the pandemic responses by the government were essential and timely. 87.5% have received the pandemic information through flyers, radio, TV spots, newspaper, and meetings. However, 63.3% felt satisfied with the provided information. Respondents rated face-mask and hand-washing as the most effective preventive measures (97%). The percentage of people who have good knowledge on pandemic influenza personal prevention (i.e. wash hands plus avoid close contact with sick people) was 74.7%, while only 36.8% had good knowledge on community prevention (cover nose and mouth when coughing or sneezing, stay home from work or school if sick, cleaning house). Female had better knowledge on house-cleaning than

male, however male had better practice than female with difference statistically significant.

Conclusion: Implementing education programs were successful and effective in raising public awareness about influenca pandemic. This proven that education measurement is one of important keys of pandemic containment strategies. Although the percentage of people having good knowledge and acceptable attitude were high, practice on community prevention among local people was still poor. Therefore, in the future education programs should focus on improving good practice for containing community transmission of influenza pandemic.

P9

New insights into clade 1 influenza A (H5N1) virus circulation in Cambodia and within the Southern Indochina peninsula

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BMC Proceedings 2011, **5(Suppl 1):**P9

Background: In Cambodia, the highly pathogenic avian influenza (HPAI) A subtype H5N1 virus was detected for the first time in January 2004. From 2004 to 2010, there have been 26 outbreaks in poultry and 10 human cases reported in the country but the origin of these epizootics remains unclear.

Methods: The phylogenetic relationships among the H5N1 strains were reconstructed by neighbour-joining and Bayesians methods. We analyzed the sequences of all 8 genomic segments of 40 H5N1 Cambodian viruses together with sequences from over 100 isolates from Southeast Asia including Vietnam, Thailand and Laos.

Results: All viruses isolated in Cambodia since 2004 belong to clade 1, genotype Z. Based on phylogenetic relationships, HPAI H5N1 virus was probably introduced from Thailand in 2004. In 2005 and 2006, several sublineages emerged in Cambodia and were probably the result of multiple introductions of H5N1 virus from Vietnam where similar strains were detected before outbreaks occurred in Cambodia. Interestingly, in 2006, we observed a north to south spread of the virus following a main road. A new sublineage appeared in summer 2006. Since then, all viruses isolated in Cambodia and South Vietnam clustered into this group, suggesting that this sublineage became endemic in the Southern Indochina peninsula. Other clades which have been imported to neighboring countries by migratory birds (i.e., clade 2.2) have not been detected in Cambodia.

Conclusion: Cambodia is essentially a poultry-importing country. The first poultry deaths were observed in semi-industrial farms that imported broiler and layer parental stocks from a sister company in Thailand, where concomitant outbreaks occurred. Then, multiple introductions of H5N1 viruses most likely occurred through illegal trading in poultry from Vietnam. Our data suggest that the clade 1 H5N1 virus is spreading essentially through poultry trading, particularly along road transportation routes. The role of the migratory birds appeared to be at most limited to local or regional transmission. The mechanisms described here would explain the maintenance and extension of the H5N1 virus within the Cambodia-South Vietnam regions for the last 6 years. This also highlights the persistent risk of H5N1 virus transmission in humans in the region while the new H1N1 pandemic virus that affects millions of humans is also frequently detected in pigs and shows a dangerous propensity to recombine.

P10

Comprehensive therapy for human H5N1

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Introduction: The mortality of human H5N1 (pneumonia) is very high. The cumulative number of confirmed human cases of avian influenza A (H5N1) reported is 504, and 59.3% of them have been dead (situation on 12, August 2010). Early intervention could improve the prognosis of this disease.

Methods: In order to initiate appropriate treatment earlier, we perform a three step strategy named "Comprehensive Therapy for human A (H5N1): CT-H5N1" in northern Vietnam since 2008. Upon step 1, residents are educated to visit healthcare facilities sooner when he/she gets sick after close contact with sick/dead poultry; on Step 2, medical staffs in provincial hospitals make a diagnosis using a rapid diagnostic test for A (H5N1) that our colleagues developed. If positive, the patient is initiated antiviral treatments immediately and is referred to Central Hospital (BMH), if he/she is in severe condition; and on Step 3, the patient in critical condition is given advanced and intensive treatments including blood purification therapy.

Results: Since December, 2008, we have been able to enroll 3 patients into our collaborative clinical research. All of them successfully survive.

Conclusion: CT-H5N1 would be effective; however enrollment of more patients is desirable.

P11

Transmissibility of pandemic H1N1 and genetically related swine influenza viruses in ferrets

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Zoonotic infections with swine influenza viruses have occurred sporadically in the past. However, sustained human-to-human transmission of a swine virus did not occur until the emergence of the 2009 pandemic H1N1 (H1N1pdm) virus. H1N1pdm possesses a unique gene combination with gene segments derived from the North America triple reassortant (PB2, PB1, PA, HA, NP, and NS) and the Eurasian avian-like (NA and M) swine influenza viruses.

To identify molecular determinants that enable sustained human-to-human transmission, we compared the direct contact and aerosol transmission efficiency of the pandemic viruses with related swine influenza viruses in ferrets. The transmission potential of seasonal H3N2 [A/Wuhan/359/95 (Wuhan95)], H1N1pdm [A/California/4/09 (CA04) and A/HK/415742/09 (HK415742)], and genetically related swine influenza viruses [A/sw/HK/4167/99 (H1N1) (swHK4167), A/sw/Arkansas/2976/ 02 (H1N2) (swAR2976), A/sw/HK/915/04 (H1N2) (swHK915) and A/sw/HK/201/10 (H1N1) (swHK201)] were studied. Ferrets were inoculated with 10^5 $TCID_{50}$ of the virus. Naïve direct contact and aerosol contact ferrets were introduced at 1day post-inoculation (dpi). Transmission was defined by detection of virus from nasal washes and/or seroconversion.

We observed direct contact transmission from inoculated donor ferrets to their cage-mates was observed for all viruses studied, albeit at different efficiency. Classical swine-like swHK4167 showed least efficient contact transmission as virus could be detected from all (3/3) direct contacts only at 6 dpi while viral shedding was detected at 4 dpi in other direct contact groups. Aerosol transmission was detected with human seasonal influenza virus Wuhan95 (2/3), H1N1pdm influenza virus CA04 (3/3), HK415742 (2/3), and swine precursor virus swHK915 (1/3). Transmission of Wuhan95 or CA04 to aerosol contacts was detected at 4 or 6 dpi, while transmission of swHK915 was detected later at 8 dpi. While the swine influenza viruses studied were able to transmit via the direct contact route, only swHK915 which shares a common genetic derivation for 7 genes with H1N1pdm possessed capacity for aerosol transmission, albeit of moderate efficiency. SwHK915 differed from swine triple reassortant viruses in the origins of its M gene. It is possible that the M gene derived from Eurasian avian-like swine viruses contributes to the aerosol transmissibility of H1N1pdm influenza viruses.

P12

Isolation and cloning of large subunit of Influenza virus A (H1N1) hemagglutinin gene into Bacmid vector to construct recombinant Baculovirus

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Influenza virus A (H1N1) is an important subtype of influenza virus that makes numerous consequences throughout the world. In the first step of viral attachment, main antigenic site of the HA1 domain from the globular head of hemagglutinin (HA) binds to human cell receptors, starting the disease process. In order to produce recombinant subunit protein vaccines, we focus on the nucleotide sequence of HA1 gene to generate the recombinant baculovirus shuttle vector (bacmid) to produce recombinant baculovirus in sf9 insect cells.

For this purpose, the human influenza virus A /New Caledonia 20/1999/ (H1N1) was propagated in MDCK cell culture and viral RNA was extracted using Easy-red (iNtRON) solution. Complementary DNA synthesis and HA1 amplification was carried out using uni-12 primer and HA1 specific primers respectively. Expected PCR product was evaluated through 1% agarose gel, confirmed by restriction enzyme analysis, cloned into pGEM-TEasy vector (Promega) and completely sequenced. The gene of interest was digested from cloning T-vector and subcloned into pfastBac HT donor plasmid, confirmed by PCR, digestion and sequencing. The recombinant donor plasmid was transformed into the E.coli DH10Bac competent cells for site-specific transposition of the HA1 from the donor plasmid to a bacmid DNA through lacZ gene disruption. The high-molecular-weight bacmid DNA was isolated from the overnight cultures and verified by electrophoresis on 0.5% agarose gel and PCR analysis using either M13/pUC or gene specific primers. We are going to transfect sf9 insect cells with this recombinant Bacmid to generate recombinant baculovirus and produce large amount of HA1 protein for future studies. This is the first study of recombinant HA1 production in eukaryotic system in Iran.

P13

Impact of Prevnar vaccination on nasopharyngeal carriage of *Streptococcus pneumoniae* in healthy children in New Caledonia

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We assessed the impact of the heptavalent pneumococcal conjugate vaccine (PCV7) on the nasopharyngeal carriage of *Streptococcus pneumoniae* in healthy children aged 2 to 24 months, four years after its implementation in New Caledonia. The data were compared with those obtained, before the introduction of the vaccine, in the same target population

From February to October 2008, 592 children were enrolled prospectively, regardless of their vaccinal status. Between 2002 and 2008, the prevalence of the overall pneumococcal carriage and of vaccine type carriage decreased significantly, respectively 52.3% to 42.1% ($p < 10^{-3}$) and 46.9% to 22.2% ($p < 10^{-3}$). This reduction was offset by an increase (20.8% to 29.0%, $p = 0.013$) of the carriage of non-vaccine type pneumococci with reduced susceptibility to penicillin (PRSP), notably the serotypes 15B ($p=0.027$) and 19A ($p=0.001$). This increase in PRSP carriage was marked in the Northern Province ($p = 0.005$) and among Melanesian children ($p = 0.009$). Surprisingly this increase was mainly attributed to the vaccine type 19F ($p < 10^{-3}$).

In conclusion, as expected, the PCV7 vaccine led to a decrease of the pneumococcal carriage and the replacement of vaccine strains by non vaccine strains, however increasingly resistant to penicillin. In the Northern Province, the increasing carriage of penicillin resistant 19F strains escaping the vaccine is of concern and justifies a further comprehensive analysis using MLST genotyping.

P14

Further evidence for malaria elimination failutre on the island of Sainte Marie (Madagascar)

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Since 2006, the new malaria elimination programme has been implemented on Sainte Marie Island – a district of approximately 20,000 inhabitants, on the eastern coast of Madagascar. Key malaria interventions include mass distribution of long lasting insecticide-treated nets (LLINs), intermittent preventive treatment using sulfadoxine-pyrimethamine in pregnant women (IPTp) and ACT for treating uncomplicated malaria cases. Over 20,000 LLINs were distributed in 2006. The implementation of this programme on Sainte Marie is expected to generate useful and usable information to inform malaria elimination strategies in the entire country.

As part of the routine monitoring at the health district level, active detection of malaria was carried out in primary school children in Sainte Marie during the rainy season from January 27 to March 3, 2009. Giemsa stained blood smears were examined for malaria parasite at the Institut Pasteur de Madagascar. Also, blood spots were collected. The presence/absence of *pfdhfr* S108N and S108T mutations in *Plasmodium falciparum* isolates was detected by PCR/RFLP method.

Asymptomatic and consenting 524 children participated in this survey. The mean age was 8.8 ± 2.1 years. The malaria prevalence was 20.2% (95%CI: 16.9 – 23.9%) with a predominance of *P. falciparum* malaria (105/106) and a single case of *P. vivax*. Of the 105 isolates *P. falciparum* isolates, two (1.9%) harboured the S108N mutation at position 108 in pfDHFR.

Our findings demonstrate that in three years following the malaria treatment policy change, mutant *P. falciparum* strains potentially resistant to sulfadoxine-pyrimethamine emerge in Sainte Marie. This is considered as an alarming situation given the importance of the IPTp to control malaria in pregnancy. Also, the prevalence of malaria among children above five is still high. This is a further proof of the malaria elimination failure. Overall, this situation resulted from weaknesses in malaria control measures. A single massive distribution round of LLINs in 2006 is not enough. Besides, cyclones hit Sainte Marie every year and most of the nets disappeared with broken houses. We believe that key approaches to achieve malaria elimination in Madagascar are (i) coupling malaria surveillance with interventions with additive innovative approaches such as treating asymptomatic malaria cases with ACT and (ii) on-going maintenance of malaria prevention including the unfailing renewal of LLINs coverage and in door spraying of insecticide.

P15

A high-throughput cell-based assay to screen for drugs affecting PfEMP1 transport to RBC surface

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Upon infection of human red blood cells (RBCs), the malaria parasite *Plasmodium falciparum* starts to modify its host cell by exporting parasite-encoded proteins to the RBC cytosol and membranes. One of these proteins, the *P. falciparum* erythrocyte membrane protein 1(PfEMP1) is a

major virulence factor. It is expressed on the infected RBC surface and mediates cytoadherence to several host endothelium receptors, enabling parasite escape from spleen clearance but giving rise to severe hemorrhagic complications of malaria.
Here we show the development of an assay to screen for drugs affecting either PfEMP1 synthesis or transport to RBC surface. In this assay, we incubate synchronized young parasites with compounds in 384-well plate. After 20 h incubation, parasites are transferred to another plate containing human placenta BeWo cells and are allowed to bind to BeWo cells for 1 h. Unbound RBCs are washed out and BeWo cells and remaining bound RBC are fixed and stained respectively by the use of a fluorescent anti-Glycophorin A antibody and the DNA dye Syto60. Images are acquired in an automated, high-throughput confocal microscope platform and analyzed by an algorithm specialized for this assay. We expect that our screening to result in the discovery of anti-malarial drugs inhibiting PfEMP1 transport or assembly at the RBC surface and thus disabling a major parasite escape mechanism.

P16

Barriers to treated bednet usage in Timor-Leste: an explaratory study

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Timor-Leste has some of the highest malaria rates in Asia- the WHO reports that 100% of the population is at year-round risk. A 2007 survey estimated that ITN usage (30 day) was only 28.8% in the under-5 population, and the MDG report also highlights several large disparities in ITN usage across the population- 69.6% urban and 45.5% rural; and 54% of males and only 46% of females, according to the Timor-Leste National Statistics Directorate (2007) and The Millennium Development Goals, Timor-Leste (2009). There have been many qualitative surveys about attitudes towards ITN usage in Sub-Saharan Africa, but far fewer from SE Asia [1].
To more fully understand the barriers to usage in Timor-Leste, a series of nine focus group discussions were organized in July, 2010. These discussions covered a range of peri-urban and rural areas, and were separated by sex, to allow exploration of intra-household decision making processes. A total of 53 women and 46 men participated, all of whom were heads of households or decision makers, and owned at least one bednet. A range of social, logistic and economic barriers emerged from these discussions, and could facilitate the creation of more targeted behavior-change materials.

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P17

A new anti-malarial drug against murine malaria

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Eosin B, a common laboratory dye has been earlier reported to have good anti protozoan properties *in vitro*. We studied this drug for its effect on a murine malaria strain, *Plasmodium berghei* (*Pb*) *in vivo* using different tests. Full suppressive 4 days Peter's Test was used in infected outbred and inbred mice, using both ip and oral routes. Secondary biological assessment was carried out using dose ranging, ED50 and ED90 values obtained. Eosin B anti malarial activity at 400 Ug/ml given in both the routes was similar to that of artemisine and mice survival rate was double that of control and 3 days more than artemisine. *Pb*GST activity was monitored and it was seen that eosin B lowered this enzyme activity. Eosin B seems to be a promising drug, exhibiting good anti malarial effects in the murine model of disease.

P18

Improving potency of *Chlamydia trachomatis* major outer membrane protein muli-epitope DNA vaccine by fusion with human papillomavirues 6b L1

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Effective adjuvants are needed to design effective vaccines against *Chlamydia trachomatis* (Ct). The aim of this study was to observe the immune response stimulated by inoculation of a DNA vaccine encoding a fusion protein comprising multiple Ct major outer membrane protein (MOMP) epitopes and human papillomavirus 6b L1 (HPV 6b L1) as the basis for designing a novel DNA vaccine against genital Chlamydia infections. The recombinant sequence encoding MOMP multi-epitopes was tandemly inserted and engaged downstream of HPV 6b L1 to construct a plasmid vaccine. COS-7 cells were transfected with pcDNA3.1 (+)/Ct MOMP 168 encoding the Ct MOMP multi-epitope gene and co-expressed with the nucleic vaccine plasmid pcDNA3.1(+)/HPV 6b L1/Ct MOMP 168, which contains both the HPV 6b L1 and Ct MOMP multi-epitope genes. In addition, BALB/ c mice were inoculated intramuscularly (i.m.) with pcDNA3.1(+)/HPV 6b L1/Ct MOMP 168 or pcDNA3.1(+)/Ct MOMP 168. Serum IgG and secretory IgA (sIgA) in vaginal washes were then measured. The expression of HPV 6b L1/Ct MOMP multi-epitope was confirmed by western blotting, confocal microscopy and RT-PCR. Mice vaccinated with pcDNA3.1(+)/HPV 6b L1/Ct MOMP 168 had significantly higher IgG and sIgA antibody titers than pcDNA3.1(+)/Ct MOMP 16 controls. The results show that genetic fusion of the molecular adjuvant HPV 6b L1 to Ct MOMP 168 significantly increases the antigen-specific antibody response induced by the Ct MOMP 168 DNA vaccine.

P19

Enhancement of potent immune responses to HPV16 E7 antigen by using different vaccine modalities

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Human papillomaviruses (HPVs) are responsible for an enormous global burden of genital disease. HPV is annually associated with 500,000 new cases of cervical cancer and 250,000 cervical cancer deaths worldwide. There are more than 130 HPV genotypes that have been recognized from various clinical lesions. HPV types 16 and 18 are found in the majority of cervical cancer cases. The association between HPV infection and cervical cancer indicates that HPV serves as an ideal target for development of preventive and therapeutic vaccines. HPV genome codes eight early or regulatory proteins (E1-E8) and two late or capsid proteins (L1/L2). Two HPV oncogenic proteins, E6 and E7, are consistently co-expressed in HPV-expressing cervical cancers and are important in the induction and maintenance of cellular transformation. Therefore, immunotherapy targeting E6 and/or E7 proteins provides an opportunity to prevent and treat HPV-associated cervical malignancies. Effective therapeutic HPV vaccines should generate strong E6/E7-specific T cell-mediated immune responses. Currently, we have focused on different vaccine modalities

including DNA vaccines, protein vaccines, live vaccines and the combined approaches (e.g., prime-boost vaccines) against HPV infections. In our studies, various strategies were applied to enhance DNA vaccine potency including the utilization of adjuvants especially heat shock proteins (e.g., GP96) and delivery systems such as polyethyleneimine (PEI), polymer-peptide hybrid (PEI600-Tat conjugate) and also electroporation. At first, the level of humoral and cellular immune responses were compared by using HPV16 E7 + Gp96 co-injection as DNA/DNA and prime-boost (DNA/protein) immunization strategies in C57BL/6 mice model. Assessment of cellular immune responses against E7 antigen indicated that co-delivery of naked DNA E7 + Gp96 plasmid is immunologically more effective than E7 alone and induces Th1 response [1,2].

Furthermore, in another study we demonstrated that PEI600-Tat conjugate is efficient to improve immune responses *in vivo*. Indeed, PEI600-Tat/E7DNA complex at certain ratio induced IFN-γ response slightly more than that GP96 adjuvant [3]. In our recent study, we are attempting to develop a novel, non-pathogenic, parasitic vector, *Leishmania tarentolae*, as a recombinant live HPV vaccine candidate expressing HPV16 E7 and evaluate its ability to elicit E7-specific T cell responses in mice model as compared to E7 DNA vaccine. Moreover, a preventive and therapeutic DNA vaccine has been generated using HPV16 E7 co-linked to N-terminal fragment of GP96 and chemokines delivered by chemical system (PEI600-Tat conjugate) and/or physical method (electroporation). Altogether, combining new and improved immunotherapeutic strategies are crucial for enhancing long-term immune responses in patients.

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P20

Hepatitis B & C epidemiology in Morocco

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Hepatitis B (HBV) and C (HCV) viruses are major public health problems worldwide and are a serious cause of liver disease that may silently progress toward cirrhosis and hepatocellular carcinoma.

The epidemiology of HBV and HCV infection in Morocco was studied. A large screening of HBsAg and HCVab was performed by third generation ELISA. Hepatitis B and C are parenterally transmitted with respective prevalence of about 1.79% and 1.5% of the general population studied. HCV RNA was detected in the fully automated Cobas Ampliprep/Cobas Amplicor V2.0 (Roche Diagnostics), HBV and HCV viral loads are measured by the CAP/CTM real-time PCR (Roche Diagnostics). HCV genotyping was tested by Versant LIPA HCV II (Siemens). We have shown that HCV genotypes 1 and 2 are the most prevalent in Morocco and HCV genotypes 3, 4 and 5 are were less common.

Sequencing of HCV NS5B region of 2a/2c and unclassified 2 genotype have permitted us to analyze 16 samples in order to confirm the accuracy of Inno-LiPa 5'NCR results, especially for 2a/2c genotype. Surprisingly, 15 samples were assigned to subtype 2i and one sample clustered with 2j/2k subtype. This finding suggests that subtype 2i is not only found in French patients as published, but also in Morocco with high prevalence.

In the other hand, HBV genotype D was predominant in our patients, as this is the major HBV genotype in Mediterranean countries. Furthermore, the wild type, mixed infection, BCP mutations and precore mutant were found in ten, thirty four, four and seven out of 55 HBV isolates, respectively. The A1762T/G1764A BCP dual mutation was not found in our isolates. Four samples presented single mutation in the BCP dual mutation region, whereas six showed a novel G1764T mutation.

In conclusion, HBV and HCV infections are prevalent in Morocco, HCV is parenterally transmitted and HBV is parenterally and also sexually transmitted in our country. Nosocomial transmission of those two viruses is important, especially in high risk groups (hemodialyzed patients & haemophiliacs). HBV genotype D predominates in Morocco as in Mediterranean countries, HBV genotypes A and F are quite rare; these are possibly acquired from other countries. High circulation of precore and basal core promoter mutants is common in chronic hepatitis B infection in Morocco.

P21

Transmission of HIV, HBV, HCV in health settings: an anthropological approach on hygiene in Cambodia

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Background: The modalities of HIV, HBV, HCV healthcare-associated infections and the underlying social and cultural logics contributing to this transmission are not precisely known, since hospital hygiene has mainly been studied from a biological point of view until now. However, hospital hygiene is shaped by norms and social-cultural representations, which increase or limit the transmission of infectious agents, always taking place within social relations. In 2006-2009, an anthropological research project (ANRS 12102) aimed at documenting those issues in various health settings in Cambodia. Practices related to hygiene were analyzed from a cultural point of view, especially since norms are interpreted at local level according to social and symbolic logics.

Methods: We collected qualitative data in formal and informal sectors of care, mainly in general hospital services, maternity wards, primary health centers and in traditional practitioners' private clinics. We interviewed many participants regarding hygiene practices and social relationships amongst the staff and between health care workers and patients. We also investigated the local representations of hygiene, their impact on the relationships between health care workers and patients and perceptions of transmission risks by health care workers.

Results: In a context were hygiene practices were limited by the lack of adequate materials and equipments, other factors were identified, which influence and distort hygiene practices. They include: (1) informal and formal social relationships in hospitals, (2) major infection control roles played by cleaners in absence of professional acknowledgment, (3) lack of consideration for hygiene by health professionals that rely on low-ranking staff for hygiene practices. Besides these issues, various questions emerged regarding social science theory. Indeed, doing research on infectious disease transmission led us to include investigations and interpretations related to anthropology of development, historical and social perspectives on public health institutions, and social organization in hospital settings. The social condition of working class (the workers), the legal and illegal systems of care, various aspects related to the politics of reproduction were issues at stake, which leads to more general issues on social changes in Cambodia. Moreover, hygiene issue may be seen as an encounter of the biological body and the social body, whose construction and effects are deeply inscribed in the historical and contemporary forms of social organization and power distribution in Cambodia.

Conclusion: Our anthropological findings illustrate the importance of comprehensive understanding of hygiene practices; they need to be considered when designing intervention to improve infection control practices in a Cambodian medical setting.

P22

Genetic variability of Hepatitis B virus in Morocco

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Background: Morocco is a medium-level epidemic country for Hepatitis B Virus (HBV). However, little is known about clinical, virologic and phylogenetic features of HBV infection. The aim of the present study is to determine the HBV genetic variability, and its association with clinical outcome and severity of disease in Moroccan HBV carriers.
Methods: The study included 250 chronic HBV infection patients at different stages of liver disease (156 male and 94 female). Serum samples were tested for serological markers and HBV DNA levels. The HBV Surface and core promoter/precore regions were amplified and directly sequenced. The clinical, virologic and phylogenetic characteristics were investigated.
Results: The mean age of patients was 44 ± 12.2. Most of them were HBeAg negative (90%). The mean HBV DNA was 475104 ± 160591 UI/mL. Phylogenetic analysis identified 90% isolates in genotype D and 10% in genotype A. Most genotypes D isolates belonged to subgenotype D7 (80%) followed by subgenotype D1 (25%) and one isolate belonged to subgenotype D2. All genotype A strains belonged to subgenotype A2 and specified subtype *adw2*. In genotypes D strains, subtypes *ayw2* (91.7%), *ayw3* (3.3%) and *ayw4* (3.3%) were identified. A significance prevalence of mutations in the Major Hydrophilic Region (MHR) of HBsAg was found with P120T/S the most frequent. In the core promoter region, the most frequent mutations are G1757A (48.9%), T1773C (42.8%), C1766GÂ /T (40.8%), T1753V (30.7%) and A1762T/G1764A (24.4%). In the precore region, the most common mutations are G1896A (55.1%) and G1899A (34.6%). Double mutation in the core promoter A1762T/G1764A was found more frequently in HCC patients than that in non HCC patients (66.6 % *vs* 16.2%; p<0.001). In addition, the prevalence of C1653T, T1753V, and G1862T mutations was significantly higher in HCC patients compared with non HCC. However, the prevalence of the G1896A precore mutation was not different between patients with HCC and HBV carriers without HCC (55.5% *vs* 55%; p>0.05).
Conclusion: We described for the first time that Subgenotype D7/ayw2 is the most predominant in Morocco. The high frequency of mutations in the core promoter in patients with HCC indicates their association with severity of infection.

P23

A transgenic mouse model for studying HBV infection in neonate

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The human hepatitis B virus (HBV) infection is always a worldwide problem, especially the high risk infection of the neonate by chronically HBV infected mother. McGrane et al. [1] have demonstrated that the gene which is driven by the promoter of phosphoenolpyruvate carboxykinase (PEPCK) is mainly expressed in the mouse liver and immediately appears at parturition. We have constructed a transgenic mouse by using PEPCK promoter to drive the pre-S2 and S domain of HBV envelope protein to imitate HBV transmission from mother to child. We want to see whether hepatitis B surface antigen (HBsAg) can persistently exist or be cleared by immune system from the newborn mice.

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P24

A case series to describe twelve fatal patients cause by rabies disease in central coast region, Vietnam in 2008

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Background: Human rabies fatal cases were related to animal rabies but it is reported that often lacks of animal rabies data, human case is underreported and rabies immune globulin and vaccine are not available so that these contributed to raise the big issues for rabies control and prevention measures in the developing countries. Recently in central coast region of Viet Nam human rabies fatal cases trend to increase from 1.5 to 2 folds in 2006 and 2007. This study described the clinical manifestations and epidemiological characteristics, post-exposure prophylaxis; and explored the risk factors associate with rabies fatality among twelve patients in central coast region in 2008.
Methods: A case series descriptive study was used to reviewed medical records, conducted field investigation and interviewed household members of fatal case; And a case-control study was applied to explore the risk factors of rabies fatality.
Results: Among 12 fatal cases, average age of fatal case was 20.9 years old and male is higher than female 2 times. Most of victims were pupil (66.7%). Average of incubation period is 97.4 days (20 - 332 days). 100% patient manifested typical symptom of furious rabies with aerophobia, hydrophobia, apprehension of light, excitability. Average time from furious spasm to death is 5.2 days (1 - 30 days). The majority of fatal cases received neither anti-sera (11/12 case) nor vaccination (10/12 cases). Domestic dog bite account for 100% of fatal case and the majority of them were unvaccinated (91.7%) and unleash (83.3%). 60% of local people did not know clean and flush wound with water and soap. Case-control study result showed that risk factors associated with rabies fatality are "Did not know vaccination for dogs is necessary"(OR= 33 (CI: 5.1 - 246.4) p<0.001); "Did not aware of signs and symptoms of animal rabies" (OR= 7.5, (CI: 1.1- 55), p<0.05); and "use suspected rabies animal for food" (OR=7.86; CI: 1.36-48.7; P<0.01).
Conclusion: Rabies fatality must\can be prevented if we address to (1) enhance the active surveillance for animal rabies and vaccination domestic dog; (2) educate the school pupil, dog owner and local people on community prevention; (3) enhance the post-exposure prophylaxis, and anti-sera and vaccine should be available with a reasonable price at the district clinic\hospital.

P25

VHH selected against the viral spike protein can protect mice against lethal rabies virus challenge

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VHH are polypeptides (15 kDa) derived from the variable domain of single heavy-chain antibodies of *Camelidae*. They represent the smallest antigen-binding fragment of an antibody (human IgG = 150 kDA). VHH are currently being explored for a number of applications.
Anti-rabies virus VHH were cloned from lymphocytes of vaccinated llamas and selected for their affinity with the viral spike glycoprotein G and neutralizing potency in a cellular infection assay. Linkage of two VHH allowed recognition of two identical or different epitopes and increased the neutralizing potency in cells more than a hundred-fold. Next, we examined the protection efficiency of these bimeric VHH *in vivo*.
Contrary to irrelevant control VHH, pre-incubation of the virus with anti-G VHH fully protected mice against disease and mortality upon inoculation of the virus-VHH mix in the nose, muscle or brain. Preventive administration of anti-G VHH in the nose, 24 hours prior to intranasal virus challenge, also

almost completely prevented disease and lethal infection. This suggests that VHH remain sufficiently active for at least 24 hours at the site of administration in the nose to neutralize a significant part of the invading virus. Post exposure prophylaxis (PEP) by injection of anti-G VHH in the left quadriceps muscle 10 minutes after virus challenge in the right quadriceps muscle reduced mortality by 50%. Treatment 24 hours after virus challenge was however no longer effective, most likely because the virus had already reached the central nervous system and was no longer exposed to locally administered VHH.
Our results show that anti-G VHH can neutralize rabies virus in an Fc-independent way. VHH probably hinder the recognition of cellular receptors or interfere with the fusion of viral and cellular membranes. The bimeric constructs proved protective in different challenge models, but that protection in the PEP model was weak. This might be due to the short half-life of the used VHH. Considering that prolongation of the half-life of VHH is feasible by different approaches, VHH technology may offer perspectives as an alternative to antibodies for PEP. VHH have a low production cost, not contaminated by blood-borne pathogenic agents and are less likely to evoke allergic or immunopathological reactions. They have good thermal stability, which is an advantage in developing countries, where the cold chain for distribution and preservation can not always be guaranteed.

P26

New challenges for polio eradication in Russia

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During the post-eradication era of Global Polio Eradication, the role of supplementary virological surveillance of groups at risk for the disease increases. The goal of all types of surveillance is to detect possible wild poliovirus importation and evaluate vaccine-derived polioviruses circulation. The large outbreak of poliomyelitis caused by the wild poliovirus of type 1 in Tadjikistan and registration of some cases of poliomyelitis among Tadjik children who have recently arrived in Russia requires the systematic virological surveillance of the children from Tadjik migrants' families.
In 2006-2009 the Sub-national Polio Laboratory investigated the stool samples from 272 children from migrants' families. The strains of polioviruses of different serotypes were isolated from 10 children including 5 children from Tadjikistan. All strains were classified as vaccine-like strains. The percentage of isolation of polioviruses was higher among the healthy children from migrants' families (3.7%) than among the patients with acute flaccid paralysis (AFP) and healthy children who had contacts with them (1.9%). More prolonged excretion of polioviruses after vaccination was also demonstrated by the children from migrants' families.
In 2010 the laboratory examined the stool samples from 112 children, including 86 children from Tadjik migrants' families. Nine polioviruses were isolated from 86 Tadjik children (10.5%). Three strains of polioviruses of type 1 isolated from three healthy Tadjik children were classified as non-Sabin-like according to the results of ELISA-test and PCR with specific primers. The genomic sequencing confirmed that the isolated viruses were wild polioviruses of type 1. The supplementary immunization targeting to avoid the wild poliovirus transmission was held at a nursery school.
The systematic virological surveillance of AFP patients and children from the groups at risk for the disease and adequate epidemiological measures are indispensable in order to prevent wild poliovirus transmission and indigenous circulation of wild polioviruses after its importation to polio free countries.

P27

Pre-existing heterologous immunity to poliovirus vaccination may mitigate severity of hand, food and mouth disease caused by EV71

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The hand, foot and mouth disease (HFMD) has emerged as a major infectious disease affecting children in China since March, 2008. Over 2.7 millions cases have been reported according to the proceeding of the Ministry of Health of China so far. Majority of patients have mild symptoms such as fever and blisters in the mouth and a skin rash, but some patients develop more severe neurologic symptoms and even die from pulmonary edema due to brainstem infection. Of note, severe cases and fatalities arise dramatically this year. However, what factors cause the current outbreaks and affect the clinical outcome of patients remains unclear. We carried out a retrospective case-control study on Fuyang HFMD outbreaks. We found that the proportions of children who had timely received poliovirus vaccine are 70.9%, 62.4% and 41.7% in health control, HFMD patients without pulmonary edema and HFMD patients with pulmonary edema, respectively. There was a significant difference in the proportion of children who had received the recommended OPV immunization between the group with edema and the controls. In addition, there was also a significant difference between the patients with edema and the one without edema, suggesting that the untimely poliovirus vaccination correlates to the HFMD severity, namely pulmonary edema. Our recent preliminary study in animal model suggests that pre-existing memory CD4 T cells specific for poliovirus can recognize EV71 and may help to mitigate the disease of EV71 infection.

P28

Coxsackievirus A16-like particles elicit neutralizing antibody responses in mice

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Coxackievirus A16 (CVA16) is one of the major causative agents of hand, foot, and mouth disease (HFMD) currently prevalent in many countries and regions in the Far East. However, no vaccine for HFMD is yet available. Here we reported the production of CVA16 virus-like particle (VLP) and its immunogenicity in mice. Co-expression of P1 and 3CD of CVA16 in a baculovirus/insect cell system resulted in correct cleavage of P1 to yield subunit proteins VP0, VP1 and VP3. These three proteins were found to co-sediment by sucrose gradient analysis and assemble into VLPs. Mice immunized with VLPs generated high-titer CVA16-specific antibodies which efficiently neutralize live CVA16 *in vitro*. Collectively, our results indicate that CVA16-VLP can elicit potent neutralizing antibody responses and is therefore a promising vaccine candidate against CVA16 infection.
Coxackievirus A16 (CVA16) is one of the major causative agents of hand, foot, and mouth disease (HFMD) currently prevalent in many countries and regions in the Far East. However, no vaccine for HFMD is yet available. Here we reported the production of CVA16 virus-like particle (VLP) and its immunogenicity in mice. Co-expression of P1 and 3CD of CVA16 in a baculovirus/insect cell system resulted in correct cleavage of P1 to yield subunit proteins VP0, VP1 and VP3. These three proteins were found to co-sediment by sucrose gradient analysis and assemble into VLPs. Mice immunized with VLPs generated high-titer CVA16-specific antibodies which efficiently neutralize live CVA16 *in vitro*. Collectively, our results indicate that CVA16-VLP can elicit potent neutralizing antibody responses and is therefore a promising vaccine candidate against CVA16 infection.

P29

Chimeric virus-like particles presenting common neutralizing epitopes of enterovirus 71

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To develop an effective vaccine against multiple genotypes of enterovirus 71 (EV71), we generated chimeric virus-like particles (VLPs) repetitively displaying the common neutralizing epitopes of EV71 and evaluated their

immunogenicities in mice. The two conserved epitopes, encompassing amino acids 163-177 and 208-222 of VP1 of EV71, were fused to hepatitis B core antigen (HBcAg) and expressed in *E.coli*. The resulting fusion proteins were found to assemble into chimeric VLPs. Both unmodified HBcAg and chimeric VLPs induced HBcAg-specific antibody responses in mice, however, only chimeric VLP-immunized sera possessed EV71 epitope-specific IgG antibodies and efficiently neutralized different EV71 strains. Collectively, our results indicate that the chimeric VLP is capable of eliciting broadly neutralizing antibody responses and is therefore a promising EV71 vaccine candidate.

P30

Environmental, genetic and viral risk factors of nasopharyngeal carcinoma in North Africa

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NPC (Nasopharyngeal carcinoma) is a tumour that arises in the epithelium surface of the posterior nasopharynx and shows a peculiar geographic and ethnic distribution. Intermediate incidence (8-12 cases/100,000/year) was reported in the North African population (7-10% of all cancers among men), where NPC is also the commonest tumour of the ear, nose and throat region.

In Asia, the majority of the cases occur in the fifth and sixth decades of life. In contrast, in North Africa the age distribution is bimodal with a minor peak in people aged between 10 and 25 years old. This juvenile form accounts for approximately 20% of the patients and has specific clinical and biological features. Geographical correlation was also observed between percentages of young cases and consanguinity rates over endemic and non-endemic populations, which may therefore explain the shape of the age curve in North Africa.

Regardless of its incidence and geographic distribution, NPC results from the contribution of environmental, genetic and viral factors. These factors might however combine differently for Asian and North African patients and also among North African patients of the two age groups.

Environmental factors: The increased risk of NPC was associated with the consumption of rancid butter and rancid sheep fat. Butyric acid contained in these products is considered as a potential Epstein-Barr virus (EBV) activator and a possible causative substance for NPC. In addition, Marijuana smoking was associated significantly to high NPC risk independently of cigarette smoking which suggests dissimilar carcinogenic mechanisms between cannabis and tobacco.

Genetic factors: Genetic traits play a significant role in the development of NPC. Specific human leukocyte antigen (HLA) haplotypes have been reported to be associated with high risk for NPC, namely HLA-B13 in Tunisians, HLA-A3, B5 and B15 in Algerians and HLA-B18 allele in Moroccans population. In contrast, HLA-Aw33, -B14 and A9 were associated to low risk of NPC in Tunisians, Algerians and Moroccans, respectively.

Viral factors: EBV is etiologically associated with NPC, and the distribution of Latent Membrane Protein (LMP)-1 variants of EBV in NPC tumors co-segregate with geographic regions. Moroccan NPC patients showed a high prevalence of the 30bp deletion variant of LMP-1; the del-LMP-1 variant share identical amino acid substitutions with the Med variant. Evidence indicates that NPC-derived LMP-1 variants carrying 30bp deletion and specific mutations in the 3C-terminal region confer high oncogenic potential and a weak immunogenicity.

However, although it is widely accepted that EBV is etiologically associated with NPC; it is proven that other co-factors might be involved in the carcinogenesis process, such as HPV (Human papilloma virus) factors. Our results report that 34% of EBV positive Moroccan NPC biopsies harbour HPVs.

P31

Identification and characterization of novel B-cell epitopes within EBV latent membrane protein 2 (LMP2)

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The purpose of this study was to screen and identify the linear B cell epitopes of Epstein-Barr virus (EBV) latent membrane protein 2 (LMP2). The secondary structure and the surface properties of EBV LMP2A protein were analyzed. Then, the peptides with good hydrophilicity, high accessibility and flexibility and strong antigenicity were chosen and average antigenicity index (AI) of epitope peptide was further investigated. Three peptides were selected as potential linear B cell epitopes. The location and the sequence of amino acid were 199-209 (RIEDPPFNSLL), 318-322 (TLNLT) and 381-391 (KSLSSTEFIPN), respectively. The genes encoding potential B cell epitope were cloned and expressed in *E. coli* system. The immune sera of above different purified fusion proteins were obtained from BLAB/c mice by subcutaneously immunization for three times. Western blot showed that these epitope recombinant proteins could be recognized by the serum antibodies against the whole LMP2 of EBV obtained from nasopharyngeal carcinoma (NPC). Indirect ELISA measured the reactivity of individual sera from 196 NPC patients, 44 infectious mononucleosis (IM) and 108 healthy individuals to these epitope-fused proteins indicated that NPC patients were significantly higher compared with IM and healthy individuals ($P<0.05$). In addition, all the immune sera of peptide-fused proteins could response to native LMP2A antigen obtained from the EBV prototype strain, B95-8 cells. IFA confirmed that the recognition of the specific antibodies induced by the immune sera of epitope peptide-fused proteins was intracellular regions of LMP2A. These results demonstrated that these three predictive epitopes not only were immunodominant B-cell epitopes of LMP2A, but also may be potential targets for application in the design of diagnostic tools.

P32

Surveillance of serious adverse events following immunization in resource poor settings

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The development of vaccines is one of the most important public health achievements. However, as the incidence of vaccine-preventable diseases has decreased, the general public has become increasingly concerned about vaccine safety. Vaccine safety is evaluated extensively through animal safety studies, clinical trials, and post-licensure surveillance. Safety monitoring in post-licensure surveillance has relied mainly on passive reporting systems such as the Vaccine Adverse Event Reporting System in the United States and epidemiological studies.

Vaccine safety profiles cannot necessarily be generalized to developing countries, where the incidence, type and severity of serious adverse events may differ significantly because of local environmental and genetic influences [1]. With the recent introduction of newly developed vaccines in sub-Saharan Africa (e.g., pneumococcal and rotavirus vaccines), the extensive use of established vaccines in preventive mass vaccination campaigns (e.g., yellow fever vaccine), and the planned introduction of vaccines for use primarily in resource-poor settings (e.g., malaria vaccine), there is an increased need for effective surveillance of serious adverse events following immunization (AEFI). Compared to high-income countries, few low-income economies have functional national pharmacovigilance (PV) systems in place, due primarily to a lack of resources, infrastructure and local PV expertise. The importance of functional national PV systems has recently been underlined with the implementation of yellow fever (YF) mass campaigns as part of the Yellow Fever Initiative (YFI).

The recent and novel use of YF vaccine for preventative mass vaccination campaigns in sub-Saharan Africa has increased the urgency of establishing functional PV systems. Between 2006 and 2013, the YFI will distribute vaccines to residents of high-risk areas in 12 West and Central African countries. A condition of funding from the GAVI Alliance is the implementation of AEFI surveillance. To address this requirement, AMP and WHO have provided technical support, including the development of surveillance tools (e.g., operational guide, notification and investigation forms, standard operating procedures for taking and transporting biological samples), the introduction of active case finding methods, and the creation and training of national expert committees to review and classify suspected serious AEFIs. Reference laboratories, including the Institut Pasteur in Cameroon, and the Robert Koch Institute in Germany provided diagnostic test results to facilitate the YF AEFI classification process. Best practices have been shared between different countries and African-based consultants have been trained to become local experts. Their expertise is now being requested for AEFI surveillance of additional vaccines such as the serogroup A meningococcal conjugate vaccine.

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P33

Novel gene mutations underlying two new cases of ALPS 0 syndrome

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Autoimmune lymphoproliferative syndrome (ALPS) is classified among primary immune deficiencies. This prototypic disorder of impaired apoptosis in humans is characterized mainly by autoimmune features and lymphoproliferation. ALPS type 0, caused by homozygous null mutations of the CD95 gene (Fas) leading to a severe ALPS phenotype, is a very rare subgroup with only 3 published cases. Here, we describe two North African male infants (a Tunisian and a Libyan) with ALPS 0 syndrome. They are born to consanguineous parents and were both investigated at age 10 months for lymphadenopathy, splenomegaly and auto-immune hemolytic anemia. The two patients showed increased levels of serum immunoglobulins. Immunophenotyping showed a high percentage (15% and 11% respectively) of CD4-CD8-TCRαβ+ and a complete absence of Fas expression. As previously described, we found elevated levels of IL-10 protein (1934pg/ml and 771pg/ml respectively) and sFasLigand (1.7ng/ml and >5ng/ml respectively) in their plasma. RT-PCR analysis demonstrated the skipping of exon 6, coding for the Fas transmembrane domain, for both patients. Sequencing analysis of the Fas gene showed that one patient has a homozygous substitution 16 nucleotides upstream of the 3' acceptor splice site of intron 5 (c.506-16A>G). This mutation may result in profound defects of gene expression at the level of pre-mRNA splicing as it breaks a potential branch point sequence. The other patient has a homozygous substitution within exon 6 (c.514C>T) which alters a potential exonic regulatory splicing site. These predictions have been obtained using human splicing finder software. The parents DNA was available for one patient and both were healthy heterozygous. Furthermore, these mutations were not found in 30 healthy individuals excluding a potential polymorphism. The two identified, previously non described mutations result in absent surface expression of the Fas receptor, precluding binding of FasL and leading to severe clinical symptoms. They expand on the few previously reported ALPS 0 cases and provide further insights into Fas gene molecular defects.

P34

Molecular basis of primary immune deficiencies in a highly inbred population

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Primary immune deficiencies are a heterogeneous group of inherited disorders in which dysfunctions of the immune system cause an enhanced susceptibility to infections. Their frequency is higher in Tunisia and the Maghreb as compared to European and North American countries. Indeed, our population is characterized by a high frequency of consanguineous marriages and this may account for the higher incidence of autosomal recessive primary immune deficiencies observed.

During the last fifteen years, we have been able as a reference center in Tunisia to establish the diagnosis of primary immunodeficiency in 397 patients including 12 patients from Algeria and Libya. The immunological investigation including phenotypic analysis of peripheral blood cells, lymphocyte proliferation assays, phagocytic cells functions and measurement of immunoglobulins as well as complement allowed us to classify these cases in combined immune deficiencies (27%), predominantly antibody deficiencies (23%), phagocytic cells defects (23%), complement deficiencies (1%), other well-defined syndromes (24%) and other immunodeficiencies (2%). The study of the molecular basis of these diseases in our settings as compared to other European, North American and Asian studies shows a high incidence of autosomal recessive diseases including Ataxia-telangiectasia, Bare Lymphocyte syndrome, Mendelian susceptibility to mycobacteria and Leukocyte adhesion deficiency. Moreover, we have observed a higher frequency of autosomal recessive forms of classically X-linked immune deficiencies such as autosomal recessive agammaglobulinemia and chronic granulomatous disease. A founder effect in our population with a single gene mutation accounting for most cases has been observed for several of these primary immune deficiencies.

The establishment of an accurate diagnosis allowed the appropriate treatment of these patients according to the cellular and molecular basis of the disease by bone marrow transplantation, substitutive immunoglobulin therapy, IFNg therapy...etc. The genetic study was aimed: first, to allow an appropriate genetic counselling and a prenatal diagnosis in these consanguineous affected families; second, to help unravelling the immune system functioning since these particularly rare autosomal forms of immune deficiencies offer a unique physiopathological model to study specific host defense pathways in humans.

P35

Study of primary immunodeficiencies in Algeria

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Primary immunodeficiencies (PIDs) are heritable disorders of immune system function. These defects are rare, but seem to be more frequent in populations with high consanguinity. The defect may affect T cells, B cells, phagocyte cells or complement proteins. Patients have increased susceptibility to recurrent infections, and may suffer from, allergy, autoimmune disorders or cancers.

The assessment of 303 patients suspected to have a primary immunodeficiency during seven years (January 2003 to July 2010) allowed us to diagnose 75 PIDs aged between 3 months and 32 years: 51 (68%) males and 24 (32%) females; the sex ratio is 2.12. 40 % of the patients are the offspring of consanguineous marriages.

The immunological tests that we used include measurment of serum IgG, IgA, IgM, IgE levels and IgG subclass levels; tetrazolium nitroblue test; lymphocyte immunophenotyping and flow cytometry evaluation of surface proteins as: CD11, CD15, CD18, MHC class II, CD40, CD40 ligand, CD25.

We report in this study the results from the 75 patients: 13 have "severe combined immunodeficiency (SCID), 11: MHC II deficiency, 30: agammaglobulinaemia, 1: Hyper-IgM syndrome, 9: common variable immunodeficiency, 1: IgG2 deficiency, 9: phagocyte defects, and 1 ataxia-telangiectasia.

The molecular characterization of these deficiencies is important for diagnosis in some cases, for prenatal diagnosis and for potential

therapeutic intervention strategies. We plan to set up other tests for evaluation of the other PIDs.

P36

Leishmania infantum LeIF protein is an eIF4A-like RNA helicase that modulates interleukin IL-12p70, IL-10 and TNF-α production in human monocytes

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Leishmania LeIF antigen, homologous to eukaryotic initiation factor eIF4A, was originally described as a Th1-type natural adjuvant and as an antigen that induces an IL-12-mediated Th1 response in the peripheral blood mononuclear cells (PBMC) of leishmaniasis patients. We aimed at addressing the role of this protein and defining the minimum fragment necessary for inducing cytokine secretion. The study necessitated expression cloning of *LeIF* and 9 domains. Comparative biochemical and genetic analyses of LeIF and yeast eIF4A showed that LieIF is both an RNA-dependent ATPase and ATP-dependent RNA helicase in vitro and highlighted differences with yeast protein. *In vivo* experiments in yeast showed that *LeIF* cannot complement the deletion of the essential *TIF1* and *TIF2* genes in the yeast *Saccharomyces cerevisiae* that encode eIF4A. However, expression of LeIF results in a dominant negative phenotype, which is abolished by deletion of the most divergent 25 N-terminal residues. LeIF is able to interact with yeast eIF4G; this suggested a role in the translation machinery. The assays measuring production of cytokines IL-12p70, IL-10 and TNF-α by PBMC-derived monocytes of healthy donors exposed to the different proteins showed that LieIF was able to induce the secretion of these cytokines. Unlike previous reports on LeIF from *L. braziliensis* and *L. major*, both amino and carboxyl parts of the protein were shown to induce the secretion of cytokines at significant levels. Our results suggest that this activity could be primarily located in amino acids 1-129 and 261-403. Furthermore, the induction of cytokines in monocytes of healthy subjects is not unique to the *Leishmania* protein. Indeed, 5 homologous proteins DEAD box in mammals and yeast were also able to induce the secretion of cytokines. This study confirms the importance of LeIF protein as a vaccine target and underscores its potential as drug target.

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P37

Comparative analysis of macrophage transcriptome of four mice strains after *L. Major* infection

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Leishmaniasis is a parasitic disease caused by a protozoan parasite of the genus *Leishmania* (*L.*), using the macrophage as the main host cell where it can survive and replicate. The infection outcome depends on the balance between the ability of the host to activate the macrophage microbocidal mechanisms to kill the parasite, and the pathogen's ability to suppress the host immune response and survive in the harsh environment of phagolysosomes. This ability to circumvent the host immune response may be the result of pressure exerted by the parasite on macrophage gene expression in a way that promotes their survival and multiplication.
In this study, we compared the expression kinetics of 82 Bone-Marrow-Derived Macropahge (BMDM) gene transcripts, from susceptible (BALB/c and PWK) and resistant (C57BL/6 and MBT) mice strains, following *in vitro* infection with *L. major* parasites, the causative agent of human zoonotic cutaneous leishmaniasis. These genes belong to several functional families e.g., IFN? pathway, TLRs, chemokines, nitric oxid production pathway or involved in various metabolic pathways.
Our results showed a clear contrast between the different profiles according to the infection stage (early vs. middle or late) in the four mice strains: regardless to targeted transcripts, the BMDM gene expression profile reflects, at early stages (3h post-infection), the genetic mice background and their susceptibility to *Leishmania* infection; whereas there is no such correlation at later stages.
Indeed, and independently of the gene function, macrophages of susceptible BALB/c and PWK mice showed a general inhibited expression profile while the resistant C57BL/6 and MBT mice expression profile levels are higher compared to non-infected BMDM.
Strikingly, this observation was not seen at later times of infection (24h and 72h post-infection) and the expression level depends on each transcript; a direct relationship between the mouse background and the level of expression of a given gene being less obvious. Whether, this early strain-specific effect of parasites on BMDM is due to a general phenomenon that may be encountered with any phagocytosed particle or specific to *L. major* will be discussed. This study provides a strong argument that susceptibility *versus* resistance to *Leishmania* infection of mice with different genetic backgrounds, would be partly due to the innate immunity.
These results indicate that these differences in activation of innate immunity illustrate, at least in part, the differences in clinical expression of experimental leishmaniasis in the four mice strains.

P38

Shortening the drug discovery pipeline: small molecule high content screening for lead discovery in neglected disease

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There is a pressing, global need for new therapies for neglected diseases such as Tuberculosis, Leishmaniasis and Chagas. Traditional lead discovery approaches, while effective have not been widely applied to neglected diseases as they are expensive and time consuming. At Institut Pasteur Korea, we have developed a core technology that enables the high content screening of hundreds of thousands of small molecules against the disease of interest. In doing so, we utilize relevant disease models that allow us to interrogate the disease in the context of the cell and the host-pathogen factors required for invasion, replication, persistence and release. The application of this core technology against Tuberculosis, Leishmaniasis and Chagas disease has resulted in the identification of novel, active compounds in less time and at less cost than traditional drug discovery methods. As these active compounds were identified in a cellular context, they can provide a more relevant starting point for new therapies.

P39

Evaluation of deletion of nuclease genes cluster in *L. major*

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The nuclease is a surface enzyme unique to trypanosomatid parasites. These organisms lack the pathway for de novo purine biosynthesis and thus are entirely dependent upon their hosts to supply this nutrient for their survival, growth, and multiplication. There is a cluster on chromosome 30 which carries 2 copy of nuclease genes and 5 identical

nuclease like proteins in *L. major* which are in *cis* form with 700-800 bp intergenic regions which have more than 80% homology. These data shows that this enzyme might play an important role in facilitating the survival, growth, and development of this important human pathogen. In previous studies, have been shown that *L. major* 3'NT/NU which is expressed specifically in promastigotes is not the key molecules involved in host purine salvaging pathway and thus in better understanding parasite strategies adopted to survive in sandflies. Therefore, only deletion of nuclease genes followed by sandfly infections experiments will allow determining its precise role in purine salvaging and sandfly infection and specificity. We have developed nuclease cluster heterozygote and homozygote knockout mutants, with homologous recombination technique, to evaluate deletion effects on survival of parasite and infection.

P40

Leishmania donovani promastigotes evade the antimicrobial activity of neutrophil extracellular traps

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Upon their recruitment to a site of infection and their subsequent activation, neutrophils release DNA and a subset of their granule content to form filamentous structures, known as neutrophil extracellular traps, which capture and kill microorganisms. In this study, we show that *Leishmania* promastigotes induced the rapid release of neutrophil extracellular traps from human neutrophils and were trapped by these structures. The use of *Leishmania* mutants defective in the biosynthesis of either lipophosphoglycan or GP63 revealed that these two major surface promastigote virulence determinants were not responsible for inducing the release of neutrophil extracellular traps. We also demonstrate that this induction was independent of superoxide production by neutrophils. Finally, in contrast to wild type *L. donovani* promastigotes, mutants defective in lipophosphoglycan biosynthesis were highly susceptible to the antimicrobial activity of neutrophil extracellular traps. Altogether, our data suggest that neutrophil extracellular traps may contribute to the containment of *L. donovani* promastigotes at the site of inoculation, thereby facilitating their uptake by mononuclear phagocytes.

P41

LiEIF and its recombinant polypeptides enhance the maturation of mouse dendritic cells and the production of the protective IL-12 cytokine

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Dendritic cells (DCs) maturation is associated with upregulation of costimulatory molecules (CD80, CD86, CD40) and secretion of cytokines including IL-12 which is important for generation of effective T cells. Proteins of *Leishmania* parasite that stimulate the production of IL-12 could be of significant interest either as immunotherapeutic components for leishmaniasis or as adjuvants. LeIF (*Leishmania* eukaryotic initiation factor) belongs to this group of proteins since it induces the production of IL-12, IL-10 and TNF-α by human monocytes of healthy volunteers. In particular, the induction of cytokines appears to be located in the N-terminal part (1-226) of the protein. In the present study we evaluated the ability of the recombinant protein *L. infantum* eIF (LieIF) and its constructs to induce *in vitro* the maturation of myeloid DCs (mDCs) and the production of cytokines supporting the polarization of Th1 type immune response. For this purpose, we used five synthetic peptides (16-18 aa) belonging in the N-terminal region, eight overlapped recombinant polypeptides covering the full protein sequence and the full-length protein. Enriched mDCs were obtained by *ex vivo* expansion of bone marrow cells, from BALB/c mice, cultured with the hematopoietic factor GM-CSF. The incubation of mDCs with the recombinant polypeptides, led to their maturation since it was observed a significant augmentation of the percentage of mDCs that express the molecules CD40, CD80 and CD86. On the contrary, the synthetic peptides did not enhance CD40 and CD86 expression and drove only to significant augmentation of CD80. In addition, we evaluated the ability of LieIF and its polypeptides to induce the production of IL-12, IL-10 and the expression of iNOS by mouse mDCs. We determined an augmentation of the percentage of mDCs that produce IL-12 upon their incubation with LieIF as well as with all the recombinant polypeptides and with three synthetic peptides whereas negligible amount of IL-10 was obtained. Augmentation of iNOS was found after incubation with the entire molecule of LieIF and with the recombinant polypeptides. In conclusion, LieIF and some of its recombinant polypeptides seem to have immunomodulatory properties demonstrating their potential use as therapeutic and prophylactic vaccine antigens.

P42

Do scars caused by past history of *Leishmania major* infection may harbor persistent parasites?

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Based on the knowledge that a cured infection protects the individual from re-infection, the development of a vaccine to prevent leishmaniasis has been a goal for nearly a century. Indeed, it is generally believed that after healing of leishmaniasis, sterile cure is never achieved and that few residual living parasites will remain sequestered within some host cells that offer them a safe shelter and hence maintain anti-parasite immune memory. This statement is mainly supported by data from experimental leishmaniasis in mice of susceptible or resistant phenotype, in which, live parasites could be recovered from lesions even after healing, and in which disease reactivation can be obtained by immune manipulation even after apparent complete cure.
Whether maintenance of a long-term immune effector memory in humans will also require persistence of live parasites is presently unknown but is very important in the perspective of a vaccine development. Our aim herein was to address the issue of *Leishmania major* parasite persistence *vs.* sterile healing in zoonotic cutaneous leishmaniasis (ZCL) by analyzing biopsies of scars from healed volunteers.
Skin-punch scars' biopsies (n=59, range of scar age: 1–5 years) have been obtained from volunteers (18–55 years old) living in two ZCL endemic foci, who had a confirmed past history, are clinically cured of ZCL and who gave their written consent. The whole protocol was approved by the local IRB. The specimens were taken under sterile conditions and local anaesthesia. Each specimen was divided into three parts: (*i*) the first sample was processed for quantitative real time PCR, (*ii*) the second was cultured in vitro in enriched medium and (*iii*) the third was inoculated into the footpad of susceptible BALB/c mice which were kept under observation for five months.
For *in vitro* isolation and after microscopic observation for at least 8 weeks, all cultures were found negative. For *in vivo* isolation, biopsy-inoculated mice were killed five months later, and skin fragments, draining lymph nodes and spleen were inoculated into culture medium, observed carefully for at least 8 weeks before being also designated as negative. PCR results were found negative for the majority of biopsies. These results indicate that any potential persistent living parasites after cure are unlikely to be sequestered in ZCL scars. This issue is of

importance in strategies for control of leishmaniasis and requires further discussion.

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P43

Moroccan *Leishmania infantum*: genetic diversity and population structure as revealed by multi-locus microsatellite typing

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Leishmania infantum causes visceral and cutaneous leishmaniasis in northern Morocco. It predominantly affects children under 5 years with an incidence of 100 cases per year. Genetic variability and population structure has been investigated for 55 strains isolated from infected dogs and humans in Morocco. A multilocus microsatellite typing (MLMT) approach was used in which a MLM type based on size variation in 14 independent microsatellite markers was compiled for each strain. MLMT profiles of 10 Tunisian, 10 Algerian and 21 European strains which belonged to zymodeme MON-1and non-MON1 according to multilocus enzyme electrophoresis (MLEE) were included for comparison. A Bayesian model-based approach and phylogenetic analysis inferred two *L. infantum* sub-populations; Sub-population A consists of 25 Moroccan strains grouped with all European strains of MON-1 type; and sub-population B consists of 25 Moroccan strains grouped with the Tunisian and Algerian MON-1 strains. Theses sub-populations were significantly different from each other and from the Tunisian, Algerian and European non MON 1 strains which constructed one separate population. The presence of these two sub-populations co-existing in Moroccan endemics shed the light on the possible scenarios of multiple introduction of *L. infantum* from/to Morocco; (1) Introduction from/to the neighboring North African countries, (2) Introduction from/to the Europe. These scenarios are supported by the presence of sup-population B and sub-population A respectively. Gene flow was noticed between sup-populations A and B. Five strains showed mixed A/B genotypes indicating possible recombination between the two populations. MLMT has proven to be a powerful tool for eco-epidemiological and population genetic investigations in *Leishmania*.

P44

Disruption of the AKT/MTOR pathway by *Leishmania major* promastigotes

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Signaling through the Akt/mammalian target of rapamycin (mTOR) pathway plays a pivotal role in the regulation of multiple cellular processes, including proliferation, apoptosis, protein synthesis and autophagy. It is therefore a major target of microbial infections and tumors. Protozoa of the *Leishmania* genus cause a wide spectrum of diseases in humans, termed leishmaniases, with clinical manifestations ranging from self-healing skin ulcers to life-threatening visceral disease. These parasites primarily infect macrophages and are renowned for their ability to sabotage host-cell signal transduction pathways. Here, we report that infection of Balb/c bone marrow-derived macrophages with the promastigote stage of *Leishmania major* results in rapid, time-dependent degradation of key components of the Akt/mTOR axis, including Akt, mTOR and the tuberous sclerosis complex-2 (TSC-2). Disruption of the Akt/mTOR pathway by *L. major* is dependent on the surface metalloprotease gp63, an important virulence factor of the parasite, and appears to be strain- and species-specific. The consequences of the degradation of key intermediates in the Akt/mTOR pathway on downstream responses are currently being investigated. These studies highlight a novel mechanism by which *L. major* interferes with macrophage functions and responses and will provide a better understanding of *Leishmania* pathogenesis.

P45

Epidemiology and genetic evolution of dengue viruses in the French Pacific Territories

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During the last decades, whole chains of dengue epidemics emerged in the South Pacific region. In contrast with the situation in hyper endemic continental countries and in the Caribbean, the epidemiology of dengue in the South Pacific islands Countries (SPICs) is characterized by the non-persistent co-circulation of multiple serotypes and the long-term predominance, with local re-emergences, of a single genotype. Local specificities in the epidemiological profile of dengue can also been observed between the SPICs, probably related to differences in the geographical situation, the eco-biological context (climate, endemic mosquito species), the demography and the population flew. In the present study, by focusing on the past $DEN3_{1989\text{-}96}$ and the recent $DEN1_{2001\text{-}10}$ and $DEN4_{2007\text{-}10}$ circulation periods, we addressed the question of the circulation of dengue viruses between the French Pacific Territories and the impact of the "local context" on viral genetic evolution. Hundreds of viral strains collected during both epidemic and endemic periods in French Polynesia (FP), New Caledonia (NC) and Wallis & Futuna (WF) were sequenced on the complete E gene. The phylogenetic analysis corroborates the previous observations on the predominant circulation of a single genotype. Within each serotype/genotype, the viral strains collected in the SPICs formed a "Pacific clade". Within this clade, the strains from the French Pacific Territories formed a unique lineage during the early epidemic/endemic circulation periods but diverged in distinct lineages when the virus re-emerged. By analyzing the in time/in space fixations of genetic mutations on the E gene, we observed that some mutations are shared by the French Pacific Territories but differ from the other SPICs. Moreover, although the majority of the mutations acquired in FP are also found on NC and WF strains, some seem to be specific to the Territory. Our results support the hypothesis of an impact of both the regional and local contexts on the genetic evolution of dengue viruses.

P46

Development of a cell-based high throughput assay system and IN-house image analysis software for screening of active compounds against dengue virus

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Dengue virus (DV) is a mosquito-borne pathogen capable of infecting multiple target organs in the human host. Infection with any one of the four dengue serotypes can cause an acute febrile illness known as Dengue Fever (DF), while subsequent infection with a heterologous serotype or a highly virulent strain can result in the more severe and sometimes lethal Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). According to the World Health Organization, approximately 2.5 billion people live in dengue endemic countries, with

an estimated 50 million cases occurring annually. The lack of an effective dengue vaccine has prompted the need to discover compounds that can inhibit DV infection. In this study, a cell-based, high-throughput assay system that utilizes an image-based analysis algorithm for evaluating active compounds that target DV infection is described. Using representative strains of DV1, DV2, DV3 serotypes, a human-derived hepatoma cell line (Huh7.5) and *Aedes albopictus* mosquito-derived cell line (clone C6/36) were inoculated with a high titer of each virus in a 384-well plate culture system in the presence of varying concentration of known active compounds. After 1-2 rounds of viral replication (48~72 h), infection was arrested by para-formaldehyde fixation. DV-infected cells were visualized by probing with D1-4G2-4-15 mAb, a flavivirus group-specific monoclonal antibody that targets the E protein, and a mouse IgG-specific AlexaFluor488™ secondary antibody. Images of the DV-infected cell culture are captured with an automated confocal microscope (Evotec Opera™), and analyzed using IM v3.0, a custom-based image analysis software developed by IP-Korea's Image Mining Group. A 10-point dose-response curve was generated for each active compound and reproduced in several experiments. This newly developed HTA (High Throughput Assay) system for Dengue can be a useful tool to screen large compound libraries for active drugs that have inhibitory effects to DV infection.

P47

Variation of dengue NS1 antigen measured by commercial ELISA kit in various forms of dengue infections and assessment of the association between NS1 antigen level and disease severity

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Background: Dengue is a systemic infection with a wide clinical spectrum including asymptomatic, mild undifferentiated dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue with shock syndrome (DSS). Detection of dengue NS1 antigen (Ag) in acute infection is a valuable tool for early diagnosis. The purpose of this study was to evaluate the sensitivity of the Platelia NS1 Ag kit (BioRad®) for the various forms of DENV infection and assess the potential role of NS1 Ag as a marker of severity.
Methods: We conducted the study at the Kampong Cham hospital, Cambodia in 2006 and 2007. After obtaining informed consent, we randomly collected serum samples and clinical data from dengue clinically suspected patients and also samples (at day 1 and 7 or at the appearance of fever) among some patients' family members to identify asymptomatic dengue cases.
Results: Among 260 confirmed dengue patients, the overall sensitivity and specificity of NS1 kit were of 57.5% and 100%, respectively. NS1 Ag test combined (no significant difference in relation with other parameters) with an in-house MAC-ELISA test significantly increased the sensitivity to over 85% ($p<0.001$). NS1 Ag positivity rate was significantly higher when comparing (1) DF versus DHF/DSS ($p<0.001$); (2) primary versus secondary infection ($p=0.001$); (3) patient with a viremia > 5 log versus those with lower viremia ($p<0.001$); (4) patients infected with DENV-1 versus the other 3 serotypes ($p<0.05$). In asymptomatic dengue cases, the NS1 Ag positive rate was lower than in dengue patients with samples collected (within 3 days of fever onset, $p=0.002$ and all days merged, $p=0.053$). In multivariate analysis, DHF/DSS was significantly more frequent in secondary infection (adjusted OR=6.6, $p<0.01$) when controlled with age, sex, day of fever onset, DENV serotype and viremia. Interestingly, severity was associated with high NS1 antigen level or DENV-1 infection (adjusted ORs: 0.21 ($p=0.002$) and 0.083 ($p=0.006$) respectively).
Conclusion: The overall sensitivity of NS1 Ag detection kit appeared relatively low; however, sensitivity varied widely depending on virus serotypes, presence/absence of anti-dengue IgG, viremia level, disease severity or asymptomatic/symptomatic infections. Nevertheless, laboratory diagnosis improved when combining NS1 Ag and MAC-ELISA testing.

P48

Quantifying the emergence of dengue inCE OF DENGUE IN HANOI, 1998-2009

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Background: There was a large outbreak of dengue fever (DF)/ dengue hemorrhagic fever (DHF) with 16164 suspected cases in Hanoi in 2009 which was 5.2 fold higher than the same period of 2008. We aim to investigate if DF/DHF is really re-emerging in Hanoi since the last large outbreak in 1998 by observing the disease incidence and the average age of infection from 1998-2009 because the single bad year is not sufficient to conclude that dengue is re-emerging in Hanoi. Also, the standardized morbidity ratios (SMR) in 14 center districts of Hanoi in 2009 were calculated to identify the high-risk districts after correcting for demographic factors. We also look at the climate factors which can influence the dengue incidence in Hanoi.
Methods: We analyzed 28479 cases reported from Preventive Medicine Center (PMC) of Hanoi and general population data from General Statistic Office (GSO). By using regression linear, we compared the average age infection by year. Poisson regression was used with year as a continuous variable to observe the incidence trend of DF/DHF in Hanoi. We used ArcGIS 9.3 to present SMR of DF/DHF between 14 districts Hanoi in 2009. We also used a Poisson regression to study the relationship between the climate factors such as temperature, rainfall, humidity and wind velocity and the incidence of Dengue in Hanoi. Linear regression model showed a slight increase in average of infection by year. Meanwhile, there was a significant upward trend of DF/DHF incidence from1998-2009 even we excluded two 'bad years' 1998 and 2009. Among fourteen center districts in Hanoi, three districts were identified as the high risk areas of DF/DHF which had SMR>3 in comparison with the average incidence of Hanoi. The maximum temperature, wind velocity and humidity were found to be correlated with the incidence of DF in Hanoi ($p<0.05$).
Conclusions: The rising trend of DF/DHF incidence support the idea that dengue is emerging in Hanoi whereas the increase of age infection is not in favor of that idea. However, the age of infection have just increased slightly and did not show a clear trend so that we cannot exclude that dengue is re-emerging in Hanoi. The hot spots of DF/DHF in Hanoi were identified which located in the super-center districts with a very high density population.

P49

Epidemiology and burden of dengue in Cambodia

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Background: Dengue is endemic in Cambodia, affecting mainly children. We summarized our results of recent studies aiming to better understand the epidemiology of dengue and estimate its burden in Cambodia. We analyzed national surveillance data from 2002 thru 2008 to characterize temporal trends and space-time patterns of the disease. We conducted active community based surveillance of dengue during 2006-2010 among 0-19 year-olds to estimate dengue burden and understand the transmission dynamics of dengue in rural and urban areas in the largest province of Cambodia (census ~ 8,000 persons under surveillance each year). Comparing the two surveillance systems using capture-recapture

methods we estimated the degree of under-recognition of dengue cases by national surveillance. We finally estimated economic burden of dengue using true incidence estimates and data regarding costs of illness among hospitalized and ambulatory cases.
Results: During 2006 – 2008, overall incidence rates ranged from 1.3% - 5.7% among the 0-19 year-olds. Dengue is highly focal in its geographic distribution; incidence rates by village and year ranged widely from 0 – 13% in 2006 (one of 16 villages accounted for 50% of the cases), 1 – 22% in 2007-2008 (32% of villages had incidence rates >10%). More importantly, dengue affected rural areas to the same degree as urban areas or even higher in 2007 (7% vs. 1.7% in 2007 and 1.7% vs. 2.1% in 2006). The highest age-specific incidences lied among pre-school children (<8 year-olds). The degree of under-recognition from the national surveillance system varied widely from 4 to 29 fold from year to year. Exposure to flaviviruses was high at early ages: 85% of 3-4 year-old children had antibodies to flaviviruses in 2007 which may reflect intense transmission around the households. By using wavelet analysis to study the temporal lag between epidemics at different districts we show the existence of a recurrent annual pattern. The annual epidemic started in a limited number of rural districts. The propagation to the rest of the country from those few "sparks" three patterns of travelling waves along the 2 main roads and the Mekong River. The societal costs of dengue were high: US$4 – 16 million annually. Moreover, families support the highest share with ~78% of total costs and 67% incurred debts to pay for these costs.
Conclusion: Costs of disease based on national surveillance data are not accurate. Our findings will become important factors in the public-health decision making process for Cambodia particularly the burden among the poor when balancing the benefits of introducing a potentially safe and effective dengue vaccine.
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P50

Molecular analysis of dengue 3 viruses detected from dengue outbreak areas in southern Vietnam in 2010

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There are four dengue virus serotypes, based on molecular analysis. Each serotype is divided to several different genotypes, such as there are 5 genotypes in dengue virus serotype 3 (DENV-3), in which genotype II was confirmed to be the main etiology cause the big dengue fever/dengue hemorrhagic fever outbreak in Vietnam 1998.
Molecular epidemiological study was carried out on DENV-3, which causing dengue fever/dengue hemorrhagic fever in some epidemic areas with high morbidity and mortality from southern Vietnam in 2010. In this study, viral RNA of DENV-3 were extracted directly from dengue fever/dengue hemorrhagic fever patient sera, typing by RT-PCR (reverse transcriptase-polymerase chain reaction) with Lanciotti specific primers, sequencing the entire envelope (E) gene and comparative analysis with DENV-3 strain isolated from the big outbreak of dengue hemorrhagic fever in whole country in 1998. The phylogenetic analysis of DENV-3 from Vietnam as well as previously published strains, the results showed that: Vietnamese DENV-3 belong to genotype II among 5 genotypes classified for worldwide DENV-3 and have been closely related to Thailand isolates. Further researchs need to be carried out in order to confirm the evidence for local genetic evolution and conserved functional structure of E protein of DENV-3.

P51

Identification of cellular enhancing and restricting factors of dengue virus egress

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Dengue has emerged as the most important life-threatening illness in the world, especially in Asian countries around Hong Kong, where the incidence of dengue hemorrhagic fever (DHF) is much greater than other continents. However, little is known about molecular and cellular processes sustaining egress of dengue virus in the host cell.
To better understand the viral and cellular determinants of dengue virus egress, we have established a dengue VLP producing stable cell line (HeLa-prME) and demonstrated that dengue VLP was able to mimic the budding and egress process of dengue viruses so that it constitutes a safe and convenient tool for the study of egress of dengue virus. Under the support of RFCID grant, HeLa-prME cells were used to screen a siRNA library that included 122 cellular membrane trafficking genes.
Our screen results revealed that knockdown of ADP-ribosylation factor (ARF) 1 and ARF6 had significant effects on VLP production by HeLa-prME cell. Experiments with other ARF proteins, which were not included in the siRNA library, showed that the ARF4/ARF5 double knockdown could inhibit VLP production but had no effect on secretion of other proteins such as soluble dengue E protein, suggesting the specificity of their involvement in VLP production. Further experiments using real virus showed that the depletion of ARF4/5 by siRNA could significantly reduced the replication of dengue 1 virus, dengue 4 virus and yellow fever virus, confirming the important role of ARF4 and ARF5 for not only dengue viruses but also other flaviviruses.
Our study uncovered the importance of class II ARFs in the egress of dengue virus. Results from this project provided information on mechanism of dengue virus assembly and its dependence on cellular machineries.

P52

Phylodynamics, vectorial competence and genetic diversity of West Nile virus in Africa: implications for global emergence of West Nile

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West Nile virus (WNV) is a flavivirus (Flaviviridae family) and its transmission cycle involves *Culex* spp. mosquitoes and birds as reservoirs host whereas humans and horses are dead-end hosts. Clinical symptoms of WN human infections range from asymptomatic or mild influenza like disease to severe neurological and meningoencephalitis syndromes.
West Nile is a neglected emerging disease with major breakthrough in 1999 with the introduction of WN virus (WNV) in New York City and the subsequent spread to whole northern America over the last decade causing massive human and animals infection leading to some fatal cases. In Eastern Europe, circulation of WNV with recurrent emergences impacting human and animal health since 1996 is similar to the situation in the USA. Strikingly, in Africa WN appears to have a minor effect despite regular isolations from mosquitoes and vertebrates hosts. In addition, WNV exhibited a great diversity with eight different lineages among which only one (lineage 1) is found worldwide and 4 are present in Africa.
In order to understand factors underlying the different patterns of transmission and processes involved in the emergence of WN in the different contexts, genetic diversity, phylodynamics and vectorial competence of WNV have been studied in Africa. Phylogenetic analysis based on partial and complete genome suggests an interconnection of zoonotic amplifications in Africa with emergence in Europe as well as replacement between lineages over time. Vectorial competence of lineages circulating in Africa for a domestic mosquitoes *Culex quinquefasciatus* showed significant differences between strains of various lineages tested for infections, dissemination and transmission rates. Indeed the different strains can be classified as low, intermediary and high infection profile. Analysis of the transmission patterns with sequences of the strains suggest that glysoylation of the envelope protein of WNV, a key player in the virus entry in the cell, may play an important role. The implications of our findings are discussed in the context of global emergence of WN.

P53

Hantaviruses and the dilution effect in Southeast Asia

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Background: Until recently hantaviruses were poorly studied in Southeast Asian countries. To better understand the ecology of hantaviruses in Southeast Asia, we conducted a large scale serological survey of rodent species in several locations in Cambodia, Laos and Thailand. Recent articles indicated that the dilution effect (whereby pathogen prevalence increases with decreasing biodiversity) applied to some American hantavirus species. Therefore we also analyzed our data to establish if any relationship existed between hantavirus seroprevalence and rodent species diversity.
Methods: Small mammals were live-trapped in different habitat types within a 20 km^2 radius at seven main locations. Morphological and genetic criteria were employed to identify rodent species. An indirect immuno-fluorescence test was used to detect anti-hantaviruses IgG antibodies in sera. Rodent communities were characterized by species richness estimators utilizing several different indices. Diversity was then estimated using the reciprocal Simpson index and relationships between rodent diversities and hantavirus prevalence was investigated with the Kendall's rank correlation.
Results: Seropositive rodents were detected at five sites, with prevalence varying from 0 to 5.13%. Antibodies were detected in several rodent species (*Rattus exulans, Rattus nitidius, Rattus norvegicus, Rattus tanezumi, Maxomys surifer, Bandicota indica, Bandicota savelei*, *Mus cookii* and *Mus caroli*). The species prevalence at each site varied between 0-50%. Seropositive animals were more commonly found at sites with lower rodent biodiversity.
Conclusion: This study further increases the knowledge regarding hantavirus presence in southeast Asia. Seroprevalence rates observed were comparable to those found in previous studies. Because hantavirus seroprevalence increases as rodent biodiversity decreases, the risk for human disease may also potentially increase in southeast Asian areas where habitats are undergoing large-scale modifications with subsequent reduction in natural biodiversity.

P54

From serological surveillance to the identification of native human cases of hantavirus pulmonary syndrome in French Guiana

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Hantaviruses are rodent-borne negative-sense RNA viruses belonging to the *Bunyaviridae* family, genus Hantavirus. These emerging viruses cause cardiopulmonary syndrome in North and South America, which is a respiratory illness following the inhalation of dust contaminated by infectious rodent feces or urine.
Until recently, no information was available related to the presence of hantavirus in French Guyana, a French department in South America. Nevertheless, the description of atypical pneumonia cases unrelated to any known etiological agent and the identification of hantavirus reservoirs in neighboring countries led us to conduct a serological study in a collection of sera from patients who had presented compatible symptoms: the prevalence of IgG antibodies to hantavirus in this population was 1.42%.
After those retroactive results, systematic hantavirus serology screening was implemented in every newcoming patient with suggestive etiology. This led us to identify a native case in French Guyana. After this first case, a second case was registered 1 year later in December 2009 (in a periurban area). Molecular analyses were conducted to characterize genetically these two strains of hantavirus. Complete sequences of the S segment were obtained and phylogenetic analyses confirmed that strains isolated in French Guyana and tentatively named *Maripa* virus belong to the Rio Mamore species.
Human hantavirus epidemics are associated with fluctuations of rodent populations, caused by climatic, ecological and environmental changes, or growing human activities associated with nature or agriculture. In Guyana, 90% of the land is still tropical rain forest, but economic development results in growing pressures on natural habitats. Continuous surveillance of the virus in the human population would be beneficial. Furthermore, surveys of potential reservoirs may help to understand hantavirus dispersion and to reduce the risk of viral emergence.

P55

Hantavirus infection in human and rodents in central highlands and southern Vietnam during 2006-2009

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From 2006 to 2009, a surveillance of the situation of hantavirus infection in Southern part and Highlands of Vietnam was carried out based on the tested results of serum samples from 1,066 rodents representing 6 species and 245 *Suncus murinus*.
The results of tested rodent sera samples by ELISA, IFA and confirmed by Western Blot showed that the prevalence antibody to hantavirus was 16.76% from *R.norvegicus*, and 13.1% from *S.murinus*. The serotyping result by FRNT revealed that hantaviruses which circulate in Southern Vietnam belong to Seoul and TPMV serotype. RNA of hantavirus was detected from 3 lung tissues of *Rattus norvegicus* samples which are coded as CSG5, CSG11 and 24D12, all collected in Ho Chi Minh City (Sai Gon Habor and District 12). The sequencing and phylogenetic analysis on detected genes (partial small and medium segments) demonstrated the close genetic relationship with SEOV representatives found in Japan, Indonesia Singapore and Northern Vietnam.
The serological analysis revealed the circulation of hantavirus including Seoul virus (SEOV) from *Rattus.sp*, and Thottopalayam virus from *S. murinus* in Southern and Highlands of Vietnam. Besides, the detection of specific IgM and the neutralizing antibody against SEOV in patient indicated the first evidence of the circulation and transmission of SEOV from rodent to human.

P56

Can we eradicate Cysticercosis?

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Man is the only known definitive host of the tapeworm *Taenia solium* and becomes a carrier by eating undercooked pork contaminated with "Cysticercus cellulosae" (cysticerci). Pigs act as intermediate host and acquire cysticercosis by ingestion of eggs or proglottids from human

feces, which develop into cysticerci within tissue mostly without causing clinical symptoms in the host. Cysticercosis occurs in man in a context of "Fecal peril" by ingestion of egg-contaminated soil, water or vegetation or by auto-infestation. In theory separation of swine from humans, good cooking practice and hygiene should lead straightforwardly to the eradication of the disease! However cysticercosis is still a major public health problem in endemic regions with more than 50 million infected people and is now a re-emerging disease in industrialized countries due to human migration. It is also the second cause of seizure in tropical countries. So what are the pitfalls in cysticercosis control and what can we do?

Cysticercosis affects free roaming pigs with access to sites contaminated with human feces. Development of good rearing practice guides will be of major impact. Only few tools are available for ante-mortem diagnosis of porcine cysticercosis and tongue palpation remains the most commonly used tool. Therefore, the development of a rapid diagnostic test, usable in villages, to test cattle will be the second weapon. However, this will need recombinant antigens. Diagnostic obstacles also affect human patients presenting with seizures. Scans and biological tests are not readily available leading to the repeated treatment of patients.

New target proteins are thus needed to develop these tests. With the sequencing of *T. solium* genome which will allow identification and production of recombinant protein a new step in the right direction was made. Now a large advocacy to raise funds in order to get this strategy on track is needed. Here we summarize the current state of the disease, practical issues linked to the organization of a feasible control system in developing countries and new data available all over the world and in particular in Madagascar to sustain this advocacy.

P57

Five years follow-up of schoolchildren infected with schistosomiasis in Niger: evidence of the benefit of a regular praziquantel administration on the reinfection

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Background: The WHO's objective regarding schistosomiasis control is to maintain a low burden by maintaining lower parasitic charges in endemic regions. The schistosomiasis and helminthiasis control program was launched in 2004 in Niger. Although yearly praziquantel treatment does not clear entirely schistosomiasis worms in people, it allows reducing parasitic loads and thus avoids serious renal complications. A study was carried out to estimate the annual prevalence of *Schistosoma haematobium* infection and its related morbidity among schoolchildren living in 5 endemic villages between 2004 and 2009.

Results: A longitudinal follow-up of school age children was undertaken with pre-treatment examination and follow-up each year prior to mass drug administration campaign. Prevalence of schistosomiasis and anaemia was assessed through interview, urine examination, ultrasound of the kidney and urinary tract, and measurement of haemoglobin. At baseline, *S. haematobium* infection prevalence was 76.8% among the 1017 enrolled schoolchildren. Among the infected, 30.2% excreted more than 50 eggs/10 ml of urine. After 4 and 5 years of follow-up, urinary schistosomiasis was respectively retrieved among 44.3% and 26.5% of the 680 and 581 children, respectively. A proportion of 23.0% of uninfected schoolchildren at baseline became infected at year 4. Conversely, 48% of schoolchildren with positive egg count at baseline were found negative at year 4 ($p<0.001$). These results remained significant taking into account the village. At baseline, anaemia was present in 63.1% of children, and its prevalence significantly decreases with increasing age. This prevalence was reduced to 51.8%, 40.5% and 25.9% at years 2, 3 and 5 of follow-up respectively.

Conclusion: The prevalence of ultrasound bladder abnormalities was 37.7% at baseline and this prevalence dropped to 2.6% at year 5 of follow-up. Regular praziquantel treatment had positive impact on the prevalence of schistosomiasis as well as on its intensity and on the reinfection rate. These results encourage proceeding with the national schistosomiasis and geohelminthiasis control programme.

P58

Lessons of 10 years experience on CCHF in Iran

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Background: Crimean-Congo Hemorrhagic Fever (CCHF) is a viral zoonotic disease with high mortality rate in humans caused by CCHF virus (CCHFV) belonging to the genus *Nairovirus,* family *Bunyaviridae*, and congaing a three segment single-stranded RNA genome. The CCHFV is transmitted to humans by bite of infected ticks, by direct contact with blood or tissues of infected livestock and nosocomially. After Chaharmahal-va-Bakhtiari outbreak in 1999 whose serum samples was sent to South Africa for diagnosis, Arboviruses and Viral Hemorrhagic Fevers Laboratory (As National Reference Lab) was established in 2000 to precise and on time laboratory diagnosis of CCHF in the country. The Lab along with CDC of Iran (national health regulator) and Veterinary organization (control program of tick populations and livestock monitoring) are members of National Expert Committee on Viral Hemorrhagic Fevers (NECVHFs) for surveillance and control of CCHF in Iran.

Methods: Since the establishment of the laboratory as National Ref. Lab, probable human sera, suspected livestock sera and tick samples were analyzed by serological (IgM and IgG ELISA) and molecular (Real-Time and Gel-Based RT-PCR) assays.

Results: As our result show, the mortality rate of CCHF in the country has been declined in the recent years compared with the early year. Regarding transmission route, most important route of transmission has been through close contact with blood and tissue of infected livestock, so the highest proportion of CCHF infection has been seen in high risk professions such as slaughterers, butchers, farmers. By considering geographical distribution, Sistan-va-Baluchistan not only ranked the most infected province in Iran, but also, the infection has been seen in all years. On the other hand, other most infected provinces included Isfahan, Fars, Khorasan and Yazd respectively, although CCHF infection has been seen in 23 out of 30 provinces of the country. Concerning sex distribution, males are infected with triple times as much as females. Also, in age distribution the disease has been prevalent in age range of 21-40 years old. The CCHFV genome was detected in tick populations collected from different high risk areas. With respecting genetic analysis, Our phylogenetic studies on CCHFV genome extracted from human and tick demonstrated that the Iranian strain have had close relationship with Pakistani (Matin) strain and the genome extracted from both hosts were very similar altogether.

Conclusion: With efficient trainings for high risk professions, the mortality rates can be decreased in high risk areas. In addition, control and prevention programs such as tick population control may address the decline of the CCHF disease in endemic regions and to block its prevalence to other regions of the country. Lastly, import of livestock to the country should be monitored and population of livestock and tick in high risk areas and endemic regions should be always surveyed with serological and molecular epidemiology. With different data on mortality rate in different endemic regions, more pathogenesis and phylogenetic analysis should be performed.

P59

Molecular evolution of Zika virus, an neglected emerging disease in Africa and Asia

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Zika virus (ZIKV) is an arbovirus transmitted by mosquitoes isolated for the first time in Zika forest, Uganda in 1947 and repeatedly isolated in sub-Saharan Africa and South East Asia. Until 2000, only few human cases were reported but in 2007, the first major human outbreak was notified

in Yap Island, Micronesia leading to 99 cases. Despite the widespread distribution of Zika virus, very limited information is available on the genetic relationship between the circulating strains. Therefore, we undertook a study on phylogeny and phylodynamics ZIKV in Africa and Asia. Partial and full length genome sequences of 38 strains from Senegal, Ivory Coast, Burkina Faso, Central African Republic and Malaysia were analysed. Phylogenetic reconstructions and datation were performed while recombination and viral population migrations were investigated. Phylogenetic analysis of the E, NS5 and NS5/3'NC gene showed two distinct ZIKV lineages circulating in Africa and a third lineage formed by the Micronesia and Malaysia strains. Besides, analysis of full length genome sequence allows identification of 5 recombinants isolates in Senegal and Ivory Coast. The 3 gene regions sequences evolved at a average rate of 7.74 x 10^{-4} nucleotide substitutions per site per year. Using the same analysis, we inferred that the most recent common ancestor of all ZIKV samples could be trace 325 years ago. The virus may have arrived in West Africa around 300 years before the present. And the migration rates showed considerable movements of ZIKV between Senegal to Ivory Coast and also to the other countries of East and central Africa. In conclusion, our study confirms previous observations showing divergences between Africa ZIKV isolate from Asia and the evidence of recombinants strains. Asian strains may represent a divergent lineage related to a common ancestor with spread throughout Southeast Asia and the Pacific from Africa.

P60

QuickVue Influenza A + B rapid test for influenza surveillance in community

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Background: Rapid diagnosis of influenza not only facilitates timely clinical management, time series test results can also serve for disease surveillance purpose. We evaluated the performance of a community based system using QuickVue Influenza A + B test (Quidel Corp., San Diego, California) for influenza surveillance in Hong Kong.
Methods: As part of a large community study, subjects older than 2 years reporting at least two symptoms of influenza-like-illness were recruited from 30 outpatient clinics in Hong Kong prospectively from February 2007 to July 2010 around the influenza seasons [1,2]. Each subject provided a pooled pair of nose and throat swabs to be tested by the QuickVue rapid test on site. Overall test positive rate were weekly aggregated and compared with a hospital laboratory surveillance system (Weekly influenza virus isolation rate from the Queen Mary Hospital, Hong Kong) and a pre-existing community influenza sentinel surveillance system (Weekly consultation rate of influenza-like illness reported by general practitioners in private practice from the Centre of Health Protection, Department of Health, HKSAR).
Results: A total of 5,824 subjects were recruited throughout the study. We define each study week as either high influenza activity or low influenza activity using fixed thresholds after removing extreme values two standard deviations above the mean. The sensitivity of QuickVue data for picking up high influenza activity was 78% when compare with the laboratory data and was 100% when comparing with the community data.
Conclusion: Although the sensitivity of QuickVue Influenza A+B test decreases with specimen viral load, in which may miss out mild influenza infection cases [3], its excellent specificity allows more accurate influenza surveillance when compare with other syndromic based systems. Its point-of-care usage and easy-to-use nature also increase the flexibility and popularity for disease surveillance, especially in resource limited setttings where laboratory facilities and expertise are not available.

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P61

High throughput quantitative proteomic analysis of cellular host response to influenza virus in primary human monocyte-derived macrophages

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Host response to infection with pathogens such as influenza viruses serves to primarily generate immune responses in an attempt to counter the invading pathogens. Consequently, how the host interacts with the virus not only has important influence on its pathogenesis, but also its transmission in a population and dissemination within the infected host. On the other hand, an overly active immune response may actually lead to excessive inflammation, which could be detrimental to the host as many lines of evidence have suggested for human H5N1 infection and the 1918 H1N1 pandemic virus. Macrophages are key orchestrator of the immune response and being one of the most abundant cell types in the respiratory system that can be infected with influenza viruses. Therefore, this study aims utilize high-throughput mass spectrometry to compare time series global proteomic profiles of primary human monocyte-derived macrophages infected with highly pathogenic H5N1 and seasonal H1N1 influenza viruses, in order to allow combinational analysis of proteomic datasets with existing body of transcriptomic datasets to provide further insight into the cellular and molecular host response to influenza virus infection. Global proteomic profiling of influenza virus infected macrophages revealed that many of the proteome changes could not be accounted for by transcriptomic profiling with microarrays. The low concordance of the global proteome profiling results with transcriptomic data indicates that high throughput quantitative proteomic analysis can provide a significant additional dimension to enable combinational analysis with existing datasets from studies using high-throughput genomic platforms. Results from this study suggest proteome changes in infected macrophages that were common to both viruses could be involved in processes that are similar, such as viral replication as both viruses replicate equally well in our macrophage model. On the other hand, pathways derived from analysis of differentially affected proteomes may contribute to the high pathogenic nature of H5N1 viruses. Therefore systematic integrative analysis of datasets from different sources could significantly contribute to detecting differentially expressed genes or pathways that are of relevance during virus infection and allows assessment of heterogeneity. This is likely to contribute to target identification for host-directed treatment of disease caused by influenza viruses.

P62

Antibody-Dependent Enhancement (ADE) of infection and its possible role in the pathogenesis of influenza

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Antibody-dependent enhancement (ADE) of viral replication has been documented for viruses, such as dengue virus, Ross river virus, other alpha and flaviviruses, HIV and also influenza virus. ADE occurs when non-neutralised virus-antibody complexes find alternative receptors and routes entry into the cell via the Fc-receptor pathway. ADE has been

demonstrated predominantly in macrophages or Fc-receptor bearing cells although other types of cells have also been occasionally implicated (1,2). Thus, viruses may find routes of entry to cells lacking the usual virus receptor. Alternatively, the innate immune signals triggered by virus infection via the FcR pathway may be different to those triggered by entry via the physiological virus receptor. Both these consequences may have implications for virus tropism and pathogenesis. Recently there have been reports of increased infection rates by pandemic influenza H1N1 virus following receipt of seasonal flu vaccination (3,4). ADE has been proposed as one mechanism. Here we illustrate the possible role played by humoral immunity in providing Influenza A viruses an opportunity to better infect immune cells.

Our results showed that, in some cases, prior addition of human serum to the inoculum triggered an enhanced infection of target cells as illustrated by a ~2-5 fold increase in Influenza M-gene copy numbers. Immunofluorescent microscopy revealed that serum-mediated pdmH1N1 infection led to a higher number of infected cells. As the fold increase of infected cells paralleled the fold change in viral gene copies, we conclude that ADE was acting by increasing the number of infected cells rather than solely increasing the viral load per cell.

Based on their ability to enhance pdmH1N1 infectivity, human sera could be divided into 3 distinct groups: some showing neutralization of infection, others increasing the yield of pdmH1N1-infected cells. The third group were those with no or marginal increase of virus infection, one which was not sustained over the tested range of serum dilutions.

Our results demonstrate that the newly emerged pandemic H1N1 Influenza A virus infection of cells of the hematopoietic lineage may be enhanced by the presence of some human sera. In the light of the recent studies that report possible associations between vaccination and increased susceptibility to influenza infection, it is of relevance to deepen our understanding of the biological significance and molecular mechanisms underlying serum-mediated ADE infection of influenza virus.

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P63

Amantadin resistant variants of influenza A virus from Flu-like infected suspects in a Children Infectious Research Center in Tehran, Iran

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The matrix protein 2 (M_2) blocker, Amantadin, is approved by FDA to treat and control of influenza A virus infections in children and adults. Recently, some substitutions at amino acid positions are causing the emergence of anti viral drug-resistant strains of influenza virus.

To investigate the frequency of Amantadin resistance among influenza A viruses isolated in children (up to 10 years old) referring to Children Infectious Research Center in Tehran – Iran during the 2008 – 2009 Flu season, 124 samples were collected. Forty cases from them were detected as influenza A virus by Reverse transcriptase polymerase chain reaction (RT-PCR). The Large subunit of hemagglutinin (HA1) and M_2 genes were amplified by RT-PCR followed by sequencing.

62.5% of positive cases were 0-3 years, 10% of them were 4-6 years, and 27.5% were 7- 10 years. The result shows 70% were male. 50% of positive cases affected sporadic. Clinical symptoms in these positive cases were as follows: Sudden onset 51%, fever 69.4%, headache 21.8%, coryza 82.5%, weakness 54.8%, pharingitis 41.1%, bronchodyspenia 4%, cough 11.3%, agitation 58%, sputum 59.7%, and muscle pain 8%, gastrointestinal problem, 23.4%, otitis 13%. 97.5% of these forty cases did not received influenza vaccine and 40% received antibiotic.

Our data show that Amantadin resistant A/H_3 N_2 is caused by a single amino acid mutation that makes a Ser 31 Asp substitution in M_2 protein. Since these children might not used Amantadin, the drug resistance determined in this project is not related to direct usage of Amantadin by the patient. Continued surveillance is required to elucidate full Amantadin-resistant pattern of influenza virus in Iranian children, especially in the recent pandemics.

P64

The Merit Release Birds: Buddhist ritual and implications in the H5N1 virus contamination cycle

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Background: The Highly Pathogenic Avian Influenza (HPAI) H5N1 virus has dramatically spread throughout Southeast Asia since its first detection in 1997. Merit Release Birds, such as the Eurasian-Tree sparrow, are believed to increase one's positive karma when kissed and released during Buddhist rituals. Since these birds are often in close contact with both poultry and humans, we investigated their potential role in the spread of H5N1 virus in Cambodia, a Buddhist country were H5N1 virus is endemic.

Methods: Specific Pathogen Free (SPF) chickens were exposed to Eurasian-Tree sparrows inoculated with HPAI H5N1 virus. In a second series of experiments, Eurasian-Tree sparrows were exposed to SPF ducks inoculated with HPAI H5N1 virus. Tracheal and fecal samples were collected daily from all animals. After 15 days, the surviving birds were euthanized and autopsied. Samples were tested for H5N1 virus by real-time RT-PCR and egg inoculation. All experiments were conducted under Biosafety level 3+ conditions.

Results: When directly inoculated, Eurasian-Tree sparrows were susceptible to the H5N1 viral infection, with a fatality rate approaching 100% by 5 days post-inoculation (dpi). However, they did not contaminate the chickens maintained in the same isolator. SPF ducks were also highly sensitive to the HPAI infection, with a fatality rate of 80 to 90% within 8 dpi. Twenty percent of the naïve Eurasian-Tree sparrows which were in direct contact with the infected ducks in the isolator died from H5N1 infection. Large quantities of H5N1 virus were detected in the sparrows, particularly in their feathers.

Conclusion: Our study indicates that under experimental conditions, Eurasian-Tree sparrows are susceptible to HPAI H5N1 infection, either by direct inoculation or by contact with infected poultry. Although the HPAI H5N1 virus was detected in sparrow trachea and faeces, we did not conclusively demonstrate a risk of poultry contamination by infected Sparrows. However, the presence of significant quantities of H5N1 virus on sparrow feathers would suggest that the Merit Release Bird ritual represents a risk for human contamination in countries were the avian influenza virus is circulating.

P65

Environmental contamination during influenza A (H5N1) outbreaks in Cambodia

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In response to confirmed cases of H5N1 infection in humans or poultry, we conducted investigations in households of index case's and in the surrounding vicinity. Environmental specimens such as mud, pond water, aquatic plants and animals, poultry carcasses, faeces, soil and dust were collected in 6 households from 3 Cambodian provinces between

April 2007 and February 2010. Two techniques to concentrate influenza virus from water were used. The first was based on the biological property of the virus to agglutinate chicken red blood cells (RBCs). The second was based on an adsorption step on glass wool, followed by an elution step with a beef extract solution at alkaline pH, in combination with a second concentration step with poly-ethylene glycol (PEG). For mud samples, we used a method based on an elution step followed by a PEG-precipitation step. The hemagglutinin (HA), neuraminidase (NA) and matrix (MA) genes was amplified using a real-time RT-PCR (qRT-PCR) method. All samples that tested positive by qRT-PCR were inoculated into specific pathogen free (SPF) 9 to 11-day-old embryonated chicken eggs. From a total of 175 samples, 42 (24%) tested positive for H5N1 by qRT-PCR. Viral RNA was frequently detected in farm soil (66%), pond and puddle water (10%), mud (7%), live poultry's cloacal or tracheal swabs (5%), feathers (2%), straw from poultry cages (5%), and poultry faeces (2%). The number of RNA copies was highest in the contaminated mud and straw collected during an outbreak in Takeo province in 2010 (about 4.5 x 10^5 RNA copies per gram). Of the 42 positive specimens by qRT-PCR which were then inoculated in eggs, viable H5N1 could only be amplified from 3 samples. The longest persistence of viral RNA observed in the environmental specimens was 12 days following the last poultry death. Our study demonstrates that H5N1 RNA was frequently present on various environmental surfaces in the households of H5N1-infected patients and in the surrounding environment. We successfully detected viral RNA from mud and dry soil. However, the presence of RNA does not necessarily imply that the virus is still infectious or that human contamination could occur. We were only able to isolate viable virus from 3 out of 42 samples, and there was no correlation between these samples and those in which the highest quantity of RNA was detected. Nevertheless, the results underscore the potential role of the environment in H5N1 human and animal contamination as well as the importance for regular surveillance and disinfection of the surrounding environment following avian influenza outbreaks.

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P66

Formation of virus-like particles from human cell lines exclusively expressing Influenza Neuraminidase

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Background: Neuraminidase (NA) is an important viral component of influenza viruses and the target of most effective anti-influenza drugs in the market. Most of the data on NA function obtained so far has come from using native virions (which imposes bio-safety issues) or from purified NA proteins which may not have the same properties as that on viral surfaces [1]. We have developed virus-like particles (VLPs) containing only the NA, functionally and morphologically similar to the native virions. NA-VLPs may be useful in influenza research such as the investigation of the assembly and budding steps in the virus life cycle as this process is still unclear [2-5].
Methods: In order to determine the minimal set of viral proteins essential for virus budding, 293T cells were transfected with plasmids encoding for the haemagglutinin (HA), NA and matrix (M1) proteins singly, or in combination. VLP released into the culture medium were collected by ultra-centrifugation and their protein composition analyzed by western blotting. Budding of the VLPs were visualized by electron microscopy. Kinetics of the production of VLP containing solely NA was monitored by enzymatic activity assays and western blotting. Physical and functional characterization of the NA-VLP were carried out using (i) sucrose gradient centrifugation, (ii) neuraminidase activity assay, (iii) NA oligomerization analysis, as well as (iv) lectin staining of sialic acid on cell surface. In addition, the effect of NA enzymatic activity on VLP production was investigated by using sialidase inhibitor or a point mutation (E262D) in NA that inactivates the catalytic site.
Results: VLP formation was detected from cells expressing HA and NA alone but not from cells solely expressing M1 showing that HA an NA each contributes to the driving force for virus budding whereas M1 only had a limited contribution. The sialidase inhibitor and E262D point mutation studies showed that the enzymatic activity of NA is not required in driving virus budding of NA VLPs. However, release of VLP containing HA was completely dependent on sialidase, either as co-expressed NA or added exogenously. NA-VLPs were morphologically similar to influenza virions by electron microscopy and NA on the VLP surface was chemically and functionally comparable to that on infectious virus particles.
Conclusion: NA plays a key role in virus budding and morphogenesis at least for the N1 subtype that we studied. These NA-VLPs mimic NA from native virions and represents a very useful tool in influenza research [6].

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P67

Identification of human annexin A6 as a novel cellular interactant of influenza A M2 protein: implications for influenza life cycle

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Background: During its replication, influenza virus utilizes the host cellular machineries for many aspects of its life cycle. Characterization of such virus-host protein-protein interactions is a must to identify determinants of pathogenesis. The M2 ion channel protein plays a crucial role during the entry and late stages of the viral life cycle where its C-terminal domain, well conserved among influenza A viruses, is accessible to cellular machineries after fusion with endosomal membrane and during its trafficking along the secretory pathway prior to assembly and budding. The aim of the study is to identify cellular interactants of M2 that play important regulatory roles during influenza infection.
Methods: To identify cellular partners of M2 we performed a genome-wide yeast-two-hybrid (Y2H) screening approach using the cytosolic domain of M2 as bait and a human placenta random primed cDNA library as prey and tested more than 60 million interactions.
Results: From the Y2H screening, an interesting interaction with the human annexin A6 (ANXA6) protein, a member of annexin family proteins that binds to phospholipds in a Ca^{2+}-dependent manner was identified. Co-immunoprecipitation of myc-tagged ANXA6 and viral M2 proteins co-expressed in HEK293T cells after transfection and infection confirmed the direct interaction between ANXA6 and M2. We further investigated whether this interaction had any functional significance with regards to influenza life cycle. Using a RNA interference strategy to silence the ANXA6 gene in human lung epithelial A549 cells, we observed increased progeny virus titers either in a single or multiple viral growth kinetics

study suggesting a negative regulatory role for ANAX6 during viral infection.

Conclusion: A novel interaction between M2 and ANXA6 was identified. More functional studies are in progress to define precisely the potential negative regulatory role of this interaction during viral infection. A systematic dissection of the viral life cycle will be performed to identify the step(s) affected by the ANXA6 cellular factor using specific assays such as real-time quantitative RT-PCR in a single or multiple viral growth kinetics study, cell transduction with HA- and M2-pseudotyped lentiviral particles, virion attachment and internalization assay, immunofluorescence staining of NP protein as a marker of viral ribonucleoproteins localization, viral polymerase activity measurement and viral budding observation by electron microscopy.

P68

Neuraminidase inhibitors sensitivity in Cambodian H5N1 and H1N1 pandemic influenza viruses

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Background: Since 2003, H5N1 avian influenza strains have become endemic in many countries in Southeast Asia, including Cambodia. In Cambodia alone there have been 26 outbreaks in poultry flocks and 10 human cases with 9 deaths. We have analyzed the genome of a large panel of H5N1 strains isolated from poultry and human between 2004-2010. Several strains have molecular alterations which are predicted to affect sensitivity to neuraminidase inhibitors (NAI), the primary drugs of choice in the treatment of H5N1 infections. In June 2009 the first H1N1 pandemic (H1N1pdm) viruses were detected in Cambodia and have since been the main influenza strains circulating during the epidemic season. The aim of this study was primarily the surveillance of oseltamivir and zanamivir drug resistance in Cambodian H5N1 and H1N1pdm isolates.

Methods: A chemiluminescence-based *in vitro* assay of neuraminidase (NA) activity, which utilizes the artificial NA substrate 1,2-dioxetane derivative of sialic acid (NA-STAR, Applied Biosystems®), was used to determine the concentration of drug required to inhibit 50% of NA enzyme activity (IC_{50}).

Results: We have identified a small number of H5N1 outliers with reduced susceptibility to NAIs and have further characterized mutations predicted to affect drug resistance using computer modeling, and recombinant viruses containing these mutations generated by reverse genetics. We have also investigated several previously described resistance mutations in the context of the Cambodian H5N1 virus using reverse genetics. We found no evidence of NAI drug resistance in H1N1pdm viruses in Cambodia.

Conclusion: We have monitored NAI sensitivity of H5N1 and H1N1pdm viruses in Cambodia and in general have not found NAI resistant viruses with classical resistance mutations. However, we have identified naturally occurring mutations in H5N1 viruses which reduce the sensitivity to NAIs. The ongoing surveillance and understanding of novel drug resistance mutations is of great importance in the global efforts against influenza disease and pandemics.

P69

Mutational analysis of H5N1 hemagglutinins: identification of molecular determinants for efficient packaging into pseudotyped lentiviral particles

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Due to the high pathogenicity of H5N1 viruses, we have developed and characterized lentiviral particles pseudotyped with HA (H5pp) from a Cambodia H5N1 isolate, which can be used as a safe tool for high-throughput serological studies without the requirement of BSL-3 facilities. However, not all H5 HAs give rise to efficient production of H5pp. The main objective of this study is to understand the intrinsic properties of H5cam and H5anh (derived from A/Cambodia/2005/40808 and A/Anhui/2005/01 respectively) which exhibited a dramatic difference in their abilities to generate H5pp; and to identify molecular determinants that control the assembly and release of H5 pseudotyped lentiviral particles.

H5cam and H5anh both exhibited high level protein expression in 293T cells. Although the cleavage of H5cam appeared to be slightly better, the level of cleaved HA2 was comparable between H5cam and H5anh. Next, flow cytometry analysis was used to compare surface HA expression for H5cam and H5anh. Indeed surface expression of H5cam was significantly higher than that of H5anh.

A deletion of lysine residue was found at the cleavage site of H5anh when compared with H5cam. Swapping of HA2 domain (including the cleavage site) did not improve H5anh-pp production. Of notice, 8 AA residues were found different for H5cam and H5anh at the 130-loop and franking region of receptor binding domain (RBD). A series of H5anh mutants at the 130-loop region were generated for mutational analysis. Strikingly, all H5anh mutants with alanine to valine mutation at position 134, despite other sequence differences at 130-loop flanking region, largely restored the ability of H5anh to pseudotype lentiviral vector.

In conclusion, H5cam and H5anh showed similar level of protein expression in total cell lysates when transfected into 293T cells. However surface expression of H5cam was detected at a higher level than that of H5anh. This may partially explain the inability of H5anh to produce H5pp. Site-directed mutagenesis revealed that a single valine residue at position 134 of the 130-loop of RBD is critical for cell surface association of H5 HA and hence efficient H5pp production in 293T cells. It is likely that H5 HAs with alanine134 also confer higher receptor binding affinity; and consequently H5pp release from the producer cells is less efficient.

P70

Identification of a novel interaction between the M2 proton channel of influenza A virus and cyclin D3: consequences for cell cycle progression

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To define cellular factors involved in the life cycle of influenza virus, we have used a genome-wide yeast-two-hybrid screening approach. Interestingly, we have identified a novel interaction between the 54 amino-acid cytosolic tail of influenza A virus M2 proton channel protein and the cyclin D3 cellular protein, a key regulator of the cell cycle G1-S transition. Very little is known about crosstalks between influenza A virus and the cellular machineries that regulate the cell cycle.

We confirmed the physical interaction between M2 and Cyclin D3, by co-immunoprecipitation studies in human epithelial cells. We then conducted immunofluorescence assays to analyze the relative distribution of cyclin D3 and M2 in infected A549 lung epithelial cells. Interestingly, we observed that cyclin D3 is relocated to or retained at the Golgi apparatus, where M2 is concentrated, at 5 hours post infection. In contrast, cyclin D3 was mainly present in the nucleus, with occasional localization in the cytoplasm, in non-infected cells. More importantly, at 10 hours post infection cyclin D3 was detected in the cytoplasm and at 24 hours post infection expression levels were much reduced.

To further study the consequences of influenza virus infection on cyclin D3 expression levels, we infected A549 cells for increasing time points and MOI and analyzed protein levels by western blotting. We found that level of expressed cyclin D3 is decreased upon infection and that this effect is dependent on both time of infection and MOI used. Those data suggest that cyclin D3 is either degraded or its expression down regulated upon influenza virus infection. Interestingly, protein levels of downstream effectors, namely cyclin dependent kinases CDK4 and 6, retinoblastoma protein RB, and cyclin E and A, were also diminished upon infection. By contrast, levels of the CDK inhibitors P15 and P16, which are upstream regulators of the G1-S transition, were unchanged.

Together, our data strongly suggest that influenza virus infection alters normal cell cycle progression by interfering with expression levels of cell cycle regulators. Most likely, the newly identified interaction between influenza M2 proton channel and cyclin D3 is involved in this process.

We will further investigate the effects of infection on the cell cycle progression by flow cytometry and decipher the molecular mechanism responsible for the down-regulation of cell cycle regulators in influenza infected cells. We will determine whether alteration of cell cycle progression is a strategy used by influenza virus to better replicate in host cells.

P71

Molecular epidemiology of human metapneumovirus (hMPV) in Cambodia

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Background: First identified in 2001, human metapneumovirus (hMPV) is a novel pathogen and causative agent of acute respiratory tract infection. Re-infection with hMPV is common, and currently there is no available vaccine against this virus. Two genetic subgroups (A and B) have been identified, both of which can be divided further into at least two distinct sub-lineages. Here we report the results of the genetic variability of hMPV strains circulating within Cambodia.
Methods: A total of 3858 samples were collected, between 2007-2009, from hospitalized patients presenting with acute lower respiratory illness in the regional hospitals of Takeo and Kampong Cham, under the guidelines of the SISEA (Surveillance and Investigation of Epidemic Situations in Southeast Asia) project. Nasopharyngeal samples were tested for 18 respiratory viruses using multiplex PCR/RT-PCR at the Institut Pasteur in Cambodia. To investigate the genetic heterogeneity of hMPV strains in Cambodia, regions within the highly variable G gene, and highly conserved F gene of 65 strains were amplified. Neighbour-Joining trees were constructed using Mega 4.0 software.
Results: Most of hMPV cases occurred in 2008 (89%) with a peak in August (32%). Only a very low number of hMPV positive samples were detected in November and December 2007 and in 2009. Partial F gene sequences were obtained from 48 samples, collected from patients between 3 months and 58 years of age. Phylogenetic analysis revealed the co-circulation of B2 (91%) and A2 (9%) genotypes. Genotypes A1 and B1 were not detected. Twenty eight partial G gene sequences were generated. Phylogenetic analysis results were consistent between the F and G genes. We observed more than 10% diversity at the nucleotide level between G gene sequences clustering within the B2 genotype.
Conclusion: This is the first study to investigate the molecular epidemiology of hMPV in Cambodia, and the first study to report a predominance of circulating genotype B2 strains in Southeast Asia. Our findings contribute to those of recent studies, demonstrating the high variability of the global distribution of hMPV genotypes. This emphasizes the need for ongoing genetic characterization of circulating strains to aid generation of an effective vaccine.
Acknowledgements: Supported by the French Development Agency through the Surveillance and Investigation of Epidemic Situations in Southeast Asia (SISEA) project.

P72

Klebsiella pneumoniae related community-acquired acute lower respiratory infections in CAMBODIA: clinical characteristics and treatment

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In many Asian countries, *Klebsiella pneumoniae* is the second pathogen responsible for community-acquired pneumonia and, as other Gram negative bacilli, might produce extended spectrum β-lactamases (ESBL). Still, very little is known about *K. pneumoniae* implication in acute lower respiratory infections (ALRI) in Cambodia. Here we describe the clinical and radiological features of Cambodian patients presenting with community-acquired *K. pneumoniae* ALRI, for which antibiotics and relevant clinical outcomes were recorded. Through ALRI surveillance in 2 provincial hospitals, *K. pneumoniae* was identified on sputum and blood cultures and confirmed by API20E gallery from adult patients with respiratory symptoms≤14 days. Patients with known tuberculosis or immunodepression were excluded. Clinical, radiological and microbiological data were recorded and patient's outcome was investigated after hospital discharge. A multivariate analysis of risk factors compared *K. pneumoniae*-infected and *Haemophilus influenzae/Streptococcus pneumoniae*-infected patients, 2 of the main ALRI-related pathogens in Cambodia, adjusted for the following variables: sex, tobacco, alcohol intake, cardiovascular disease, chronic lung disease, diabetes, hepatopathy, no prior treatment, hemoptysis, severity.
During April 2007-December 2009, among 3545 patients enrolled in surveillance, 47 *K. pneumoniae* ALRI were diagnosed in sputum (97.8%) and blood (2.1%) cultures, representing 7.7% (n=47/608) of identified bacterial etiology. The median age was 55 years (25-79) and 68.1% were females, including 75% postmenopausal women. Of the 43 available X-rays, 30 showed pneumonia (10 were necrotizing), 2 pleurisies and 11 infections on pulmonary sequelae. Severity was determined in 5 patients, 4 having pneumonia. The main known risk factors were previous medication (42.5%), chronic lung diseases (23.4%) and tobacco (21.3%); 10 patients were co-infected with a virus and 5 with tuberculosis. Producing-ESBL strains were found in 17.0% (n=8/47) of the cases, including 4 in pneumonia cases; most of those being sensitive to ciprofloxacin (n=7/8). An appropriate antibiotherapy according to the antibiogram was given to 13 patients (28%). Overall mortality was 40% (7 lost of-follow-up), higher during hospitalization and within a month after discharge.
When compared with patients presenting with *S. pneumoniae* and *H. influenzae* ALRI, *K pneumoniae* related ALRI were associated with female gender, prior treatment and severity on admission. In conclusion, *K. pneumoniae* related ALRI in Cambodia are often fatal, affect mostly women, and must be considered in patients hospitalized with severity criteria. The frequency of ESBL strains is extremely high among our infected patients. This is alarming in the context of high antibiotics intake often inappropriate and in absence of microbiology capacity in most public-sector hospitals.
Acknowledgements: Supported by the French Development Agency through the Surveillance and Investigation of Epidemic Situations in Southeast Asia (SISEA) project.

P73

Pulmonary melioidosis in CAMBODIA: a prospective study

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Melioidosis is a disease caused by the soil-dwelling Gram-negative bacterium *Burkholderia pseudomallei*. It is endemic in South-East Asia but remains poorly documented in Cambodia where laboratory facilities are scarce. We report here a cohort of culture-confirmed cases of pulmonary melioidosis identified in two provincial hospitals in Cambodia, describing clinical and epidemiological characteristics.
Patients with melioidosis were identified through a laboratory based surveillance of acute lower respiratory infections (<14 days of illness) in two provincial hospitals from April 2007 to January 2010. *B. pseudomallei*

was detected in sputum or blood through 42 cultures and confirmed by API 20 NE gallery. We collected clinical, microbiological and radiological data and visited patients several weeks after hospital discharge to document long-term outcome.
Melioidosis was found in 39 patients. The median age was 46 years including three patients ≤2 years and 56.4% were males. A close contact with soil and water was identified in 30 patients (76.9%). Pneumonia was the main radiological feature (82.3%), but pleurisy was also described in 6 patients. Eleven patients were severe. A positive blood culture was significantly associated with severe cases (90.9% vs. 50.0%; $p<0.05$) and with higher fatality (87.5% vs. 20%; $p<0.01$). A total of 24 (61.5%) patients died within 3 days, 23 without receiving any active drug against *B. pseudomallei*. One year after discharge, 11 patients were still alive and considered as cured.
Melioidosis is an emerging public health issue in Cambodia that requires nationwide access to laboratory facilities and timely appropriate treatment.
Acknowledgements: Supported by the French Development Agency through the Surveillance and Investigation of Epidemic Situations in Southeast Asia (SISEA) project.

P74

Identification of viruses in Acute Lower Respiratory Infections (ALRI) in Lao People's Democratic Republic

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Background: Acute respiratory infections are a major cause of mortality and morbidity worldwide. Information on viral etiology in ALRI from Lao PDR is limited. The aim of the present study was to use Multiplex PCR/RT-PCR methods for the detection of the major respiratory viruses.
Methods: Nasal/throat swab specimens were collected from patients enrolled in the ALRI surveillance programme within 2 hospitals, one in Vientiane Capital (Setthathirat Hospital) and the second one in Luang Prabang. From each sample, viral RNA was extracted and amplified by using 5 multiplex PCR/RT-PCR targeting 18 respiratory viruses.
Results: Between 2009 and 2010, Multiplex PCR / RT-PCR detected respiratory viruses in 111 (54.7%) of 203 samples. Single virus was detected in 44.8% (91/203) and virus co-infections were observed in 9.9% (20/203). Rhinovirus (40.5%), human Respiratory Syncytial virus (hRSV; 27.9%), and Influenza A virus (9.0%) were the most frequently detected viruses. Adenovirus and human Metapneumovirus were detected in 8.1% and 6.3% of ALRI specimen, respectively. Influenza C virus and SARS-coronavirus were not detected during the study period. Children < 5 years represented 50% of patients identified.
Conclusion: Our results provide new documentation about etiology of respiratory virus diseases in Lao People's Democratic Republic. In this context, multiplex PCR/RT-PCR offers a sensitive and reasonably priced diagnostic method for common respiratory viruses.
Acknowledgements: Supported by the French Development Agency through the Surveillance and Investigation of Epidemic Situations in Southeast Asia (SISEA) project.

P75

3D high content imaging: high level phenotypic quantification new opportunity for drug discovery

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Advances in automated imaging microscopy allow fast acquisitions of multidimensional biological samples. Those microscopes open new possibilities for analyzing subcellular structures and spatial cellular arrangements. We present a 3D image analysis framework perfectly well suited for medium-throughput screening. Upon adaptive and regularized segmentation, followed by precise 3D reconstruction, we achieve automatic quantification of numerous relevant 3D descriptors related to the shape, texture, and fluorescence intensity of multiple stained subcellular structures. A global analysis of the 3D reconstructed scene shows additional possibilities to quantify the relative position of organelles. Implementing this methodology, we analyzed the subcellular reorganization of the nucleus, the Golgi apparatus and the centrioles occurring during the cell cycle. In addition, we quantified the effect of a genetic mutation associated with the early onset primary dystonia on the redistribution of torsinA from the bulk endoplasmic reticulum to the perinuclear space of the nuclear envelope. We show that the method enables the classification of various translocation levels of torsinA and opens the possibility for compound-based screening campaigns. Finally we present real applications of 3D cellular phenotype quantification in the screening context.

P76

High content cellular microarray for automated drug target deconvolution

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Despite the promising paradigm offered by high-content screening, the concrete execution of hundred of thousands of visual cell-based experiments has remained highly challenging in terms of both statistical robustness and speed. An efficient computational method for cellular microarrays was developed at Institut Pasteur-Korea that allow for high speed, high content genome-wide siRNA screening. Details of the method and examples of data from genome-wide analyses will be featured in this presentation. In particular, we will demonstrate that the sudden ability to dramatically increase the number of experiments has created the opportunity for automated identification of a drug's target.

P77

Cognitive virtual microscopy: a cognition-driven visual explorer for histopathology – the MICO ANR TecSan 2010 initiative

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Within the last decade, histopathology became widely accepted as a powerful exam for diagnosis and prognosis in mainstream diseases such as breast cancer. Currently, analysis of medical images in histopathology largely remains the work of human experts. For pathologists, this consists of hundreds of slides examined daily. Such a tedious manual work is often inconsistent and subjective. The recent cognitive microscope – MICO - ANR TecSan project aims at radically modifying the medical practices by proposing a new cognitive medical imaging environment able to improve reliability of decision-making and prognosis assistance in histopathology. Our goal is to design a generic, open-ended, semantic digital histology platform including a cognitive dimension. MICO combines visual perception, pervasive exploration of whole slide images, context (including uncertainties) modeling, cognitive vision and quality of experience to reinforce a visual diagnosis assistance following an approach centered on the user behavior.

P78

Molecular characterization of subcellular localization and nucleocytoplasmic shuttling of PRV UL54

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Pseudorabies virus (PRV) UL54 protein localized almost exclusively to the nucleolus. By constructing a series of mutants, the putative nuclear localization signal (NLS), nucleolar localization signal (NoLS) and nuclear export signal (NES) of UL54 were for the first time mapped to amino acids ^{45}RRRRGGRGGRAAR^{57}, ^{61}RQRRR^{65} and ^{240}LQNLRLKLGPFL^{251}, respectively. In addition, nuclear localization of UL54 was important for its transcriptional regulation of glycoprotein C promoter. The nuclear import of UL54 was abrogated by dominant negative RanGTP and importin β1, respectively, indicating that UL54 targeted to the nucleus by means of a classic Ran- and importin β-dependent nuclear import mechanism. Heterokaryon assays demonstrated that UL54 was a nucleocytoplasmic shuttling protein and this property could not be blocked by leptomycin B, an inhibitor of the chromosome region maintenance 1 (CRM1). However, ectopic expression of the mRNA export receptor TAP(NXF1) promoted the nuclear export of UL54 and interacted with UL54, suggesting that UL54 shuttles between the nucleus and the cytoplasm via a TAP(NXF1), but not CRM1, dependent nuclear export pathway. The present study demonstrated that UL54 is a nucleolar protein, adding UL54 to the growing list of transactivators which localize to the nucleolus and shuttle between the nucleus and the cytoplasm.

P79

The SARS coronavirus E protein interacts with the PALS1 and alters tight junction formation and epithelial morphogenesis

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Intercellular tight junctions define epithelial apicobasal polarity and form a physical fence which protects underlying tissues from pathogen invasions. PALS1, a tight junction-associated protein, is a member of the CRUMBS3-PALS1-PATJ polarity complex, which is crucial for the establishment and maintenance of epithelial polarity in mammals. Here we report that the carboxy-terminal domain of the SARS-CoV E small envelope protein (E) binds to human PALS1. Using co-immunoprecipitation and pull-down assays, we show that E interacts with PALS1 in mammalian cells and further demonstrate that the last four carboxy-terminal amino-acids of E form a novel PDZ-binding motif that binds to PALS1 PDZ domain. PALS1 redistributes to the virion assembly site, where E is enriched, in SARS-CoV-infected Vero E6 cells. Ectopic expression of E in MDCKII epithelial cells significantly alters cellular polarity and induces formation of cysts with multiple lumens. We show that E expression delays formation of tight junctions and affects the subcelullar distribution of the apical and tight junction markers GP135 and ZO-1, respectively. We speculate that hijacking of PALS1 by SARS-CoV E plays a determinant role in the disruption of the lung epithelium in SARS patients.

P80

Investigation of Antibody-Dependent Enhancement (ADE) of SARS coronavirus infection and its role in pathogenesis of SARS

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Antibody-dependent enhancement (ADE) is a mechanism by which viruses, such as dengue, HIV and Ebola, gain entry into some target cells through the use of host antiviral humoral immune responses [1]. Here, we studied the ability of severe acute respiratory syndrome coronavirus (SARS-CoV) [2] to use ADE mechanisms to enhance its infectivity towards cells of the hematopoietic lineage.

We found that heat-inactivated immune serum from rodents vaccinated with recombinant native full-length Spike protein trimers [3] triggered infection of human immune cells (monocytic and B cell lines) by SARS-CoV Spike pseudotyped particle (SARS-CoVpp). The occurrence of antibody-mediated infection of human Raji B cells was further investigated by using live SARS-CoV. Similarly to results obtained with the SARS-CoVpp, only anti-SARS-CoV Spike serum, but not mock immune-serum, induced a massive increase of SARS-CoV viral genes (ORF1b and Nucleocapsid) and viral proteins (Membrane and Nucleocapsid) in Raji B cells. As revealed by immunostaining, only a relatively low, however significant percentage of the Raji cells get infected by antibody-mediated infection and did not allow direct assessment of productive replication by conventional cytopathic assays and TCID50 titration.

Taken together, our data suggested that SARS-CoV is able to enter human immune cells via an antibody-mediated pathway and immunological consequences of such infection are under investigation (productive replication, cytokines secretion profile and cell death etc). Our data raise reasonable concerns regarding the use of SARS-CoV vaccine in humans and pave the way to further studies focusing on the role of immune-mediated infection phenomenon during SARS pathogenesis.

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P81

Highlighting the genetic and epidemiologic disparities of *Mycobacterium tuberculosis* epidemic in 12 Caribbean territories in a first global study

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Tuberculosis (TB) in the Caribbean remains a significant health issue with many countries exceeding the WHO target of 5 cases/100,000 populations. As a developing nation, many of these Caribbean countries face serious challenges in the diagnosis, treatment, care and management of patients with tuberculosis. In light of the current problems facing the tuberculosis programs in the Caribbean, there is a need for studies to be conducted so as to better understand the epidemiology of this disease in such a heterogeneous setting. This investigation describes a first global molecular epidemiological study on 480 clinical *M. tuberculosis* isolates from as many patients, collected in 12 territories of the Caribbean: Bahamas, Barbados, Belize, Dominica, Guyana, Jamaica, St. Kitts and Nevis, St. Lucia, St. Vincent and the Grenadines, Suriname, Trinidad and Tobago, Turks and Caicos. Analysis of "de-identified" patient data showed that TB cases more often concerned males (male to female sex-ratio, 3.1), and persons within age group 25-45 years. The rate of TB/HIV coinfection was unexpectedly high with rates ranging from 44.4% in Guyana, 42.9% in Bahamas, 30.6% in Trinidad and Tobago, 21.4% in Suriname, 14.3% in Barbados and 13.5% in Jamaica. The highest rate of drug-resistant TB was observed in Guyana (27.8%, among which 76% were multidrug-resistant). Spoligotyping generated a total of 104 distinct patterns for the 480 isolates studied; 49 patterns containing 425 isolates (88.5%) corresponded to clustered strains (2-93 isolates per cluster), while the remaining 55 patterns

corresponded to unclustered strains (11.5%). A comparison of the spoligotypes with the SITVIT2 global database showed that the isolates belonged to the following predominant genotypic lineages: the ill-defined T lineage (31.0%), East-African Indian (EAI, 19.0%), Latin American and Mediterranean (LAM, 10.4%), the X clade (8.3%), Haarlem (5.8%), and Beijing (3.5%). The diversity of strains circulating in the Caribbean essentially represented their colonial past (clades of European descent such as Haarlem, and X clades) as well as population movements (EAI, Beijing). Lineages characteristic of the Indian subcontinent (East- African-Indian, Central-Asian) were seen in Trinidad and Tobago, Guyana, and Suriname where there is a large population of East Indians brought during the indentureship period, after slavery was abolished. Lastly, a peculiar local evolution of *M. tuberculosis* strains in Trinidad and Tobago was evidenced with the exclusive local emergence of a specific TB clone (named SIT566, belonging most probably to the X clade), which resulted in 56% of all TB cases.

P82

Effect of LPS, dsRNA or interferons on the phagocytosis of dying cells or mycobacteria by macrophages

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Infection with intracellular pathogens can trigger a panel of innate immune responses including cell death. Coupled with phagocytosis this often leads to clearance of the invader. However, some pathogens use the process to disseminate and proliferate. During *Mycobacterium marinum* infection, dying infected macrophages recruit fresh ones to the site of granuloma formation. The recruited macrophages phagocytose infected cell remnants, get infected and die, thus ensuring efficient spread and multiplication of the pathogen. Maturation of granuloma, the characteristic lesions of tuberculosis, requires tumor necrosis factor (TNF) and interferon (IFN)-gamma and represents a stalemate between host and pathogen sufficient to arrest infection without eliminating the bacteria. Indeed, our macrophage / mycobacterial infection model demonstrates that virulent H37*RvMycobacterium tuberculosis* is more efficient in macrophage infection and killing than the attenuated *Mycobacterium bovis* BCG vaccine.
Today it is still unclear whether the signal promoting macrophage engagement in phagocytosis is originating from a pathogen or the infected host, and whether the effect is general or target specific. Therefore, we used a quantitative cell line based assay to study the effects of PAMPs or cellular alarm signals on the phagocytic engagement and capacity of macrophages to engulf virulent or attenuated mycobacteria and dying cells. Our results demonstrate that pretreatment of macrophage like cells with double stranded RNA, lipopolysaccharide, type I or II IFN but not with TNF, can significantly increase their capacity to phagocytose apoptotic and necrotic cells but has little effect on the phagocytosis of free mycobacteria. Although this macrophage activation process is probably an innate immune response reinforcing the capacity of the host to dispose of dying infected cells, pathogens may exploit it for their propagation.

P83

Tuberculosos pandemic and dissemination of drug resistant strains: a challenge for Bulgaria

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Background: Since early Neolithic, Europe as a whole and Balkans in particular were at the crossroads of human migrations thereby transmitting human pathogens across the continent. Bulgaria located near the Europe-Asia border was in the front of these migrations that left their imprint on the population structure of human pathogens circulating therein. A re-emergence and wide dissemination of multidrug-resistant tuberculosis (MDR-TB) threatens national control problems. The early detection of resistance to first line anti-TB drugs is essential for the efficient treatment and constitutes one of the priorities of TB control of MDR strains. The rate of the MDR-TB among newly diagnosed TB patients in Bulgaria was estimated to be 10.7% that is much higher than in the neighboring countries. Here we evaluated fast molecular methods to detect drug resistant TB and studied the distribution of resistant properties in different clonal lineages of *M. tuberculosis* in Bulgaria versus its neighbors.
Methods: Drug-resistant and susceptible *M. tuberculosis* strains from newly–diagnosed patients were studied by different typing methods (spoligo-, IS6110-RFLP and 24-loci MIRU-VNTR typing). Mutations in the major gene targets related to drug resistance (*rpoB* RRDR, *katG315, inhA -15, embB306*) were detected by PCR and microarrays.
Results: The population of *M. tuberculosis* in Bulgaria was found sufficiently heterogenous (24-VNTR based HGI=0.89). Mutation in *rpoB531* was detected in the remarkably high rate among RIF-resistant strains (65%). Mutations in *katG315* and *inhA* -15 were detected only in 50% of INH-resistant strains. The *embB306* mutation was found in 63% of EMB-resistant strains. Comparison with genotyping results did not identify any strain cluster linked to drug resistance.
Conclusion: *M. tuberculosis* population in Bulgaria features several global, Balkan- and Bulgaria- specific lineages. *rpoB* RRDR and *embB306* mutations may serve for rapid genotypic detection of the majority of RIF and EMB-resistant *M. tuberculosis* strains in Bulgaria. The results for INH resistance are complex and more genes should be studied. The very high rate of *rpoB* S531L mutation may correlate with some specific features of the national TB control program (quality of the drug used) or is hypothetically linked to another molecular mechanism of RIF resistance. A local circulation of the particular clones appears to be an important factor to take into consideration in the molecular epidemiological studies of tuberculosis in Bulgaria. Emergence and spread of drug-resistant and MDR-TB in Bulgaria are not associated with any particular spoligotype or MIRU-VNTR genotype.

P84

IdentifICATION OF E3 ubiquitin ligase STUB1 as a negative regulator of FOXP3

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The level and activity of the forkhead family transcription factor FOXP3 determine the immune function of FOXP3+Tregs. At the beginning of infectious processes, FOXP3+Tregs may regulate effector immune cell responses and lead to failure to control infection. FOXP3+Tregs may also help to limit collateral tissue damage when the antiviral immune responses are too vigorous. Understanding the regulation of FOXP3 and the dynamic ensemble of FOXP3 with enzymatic cofactors in Tregs will provide therapeutic applications for major human viral infectious diseases including HIV, hepatitis B and C viruses.
How FOXP3 protein is negatively regulated in CD4+ regulatory T cells during viral infection and inflammation is currently unknown. Here we report that a stress-signal activated E3 ubiquitin ligase STUB1 appears as a negative regulator of FOXP3. Reciprocal co-immunoprecipitation studies indicate that STUB1 interacts with FOXP3 in vivo. Overexpression of STUB1 specifically promotes the ubiquitination of FOXP3, but not other subfamily transcription factor FOXP1. MG132 treatment increased the ubiquitination level of FOXP3, and overexpression of STUB1 induced ubiquitin-mediated degradation of FOXP3. In contrast to the wild type STUB1, ectopic expression of H260Q mutant STUB1, which disrupts its interaction with E2 conjugation enzymes, didn't lead to FOXP3 degradation. Thus, FOXP3 degradation is mediated by

enzymatically active STUB1. Moreover, FOXP3 degradation by STUB1 is also depend on its chaperoned binding, since overexpression of the K30A mutant of STUB1, which is incapable of interacting with chaperone proteins, also fails to promote FOXP3 degradation. Knockdown of endogenous STUB1 by shRNA could increase FOXP3 level in FOXP3 expressing T cells. Functionally, ectopic expression of STUB1 dramatically relieves FOXP3 mediated transcriptional suppression. Our studies identified a novel signal pathway to downregulate FOXP3 activity at posttranslational level by ubiquitin mediated protein degradation.

P85

Characterization of undifferentiated human bone marrow and blood derived mesenchymal stem cells and their potential for chondrogenic differentiation

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In recent years, the potential of cartilage tissue engineering techniques employing mesenchymal stem cells (MSCs) to repair damaged human cartilage and defects has generated much interest. Traditionally, sources of MSCs included patient's own bone marrow. However, little have been reported on adult peripheral blood (PB) as a source of MSCs, which has been a subject of much debate amongst scientists owing to its extremely low density in PB and the difficulties associated with extracting MSCs from PB. The objectives of this study were to isolate MSCs derived from bone marrow and peripheral blood as a source and to assess their potential to undergo *in vitro* chondrogenesis using a biocompatible three-dimensional scaffold. In defining MSCs, the cells isolated from its source must meet these 3 criteria: (i) adherence to plastic when maintained in culture; (ii) positive expression of several antigens such as CD29, CD105, CD166; (iii) ability to differentiate into osteoblasts, adipocytes & chondrocytes under *in vitro* inductive conditions. PB samples (2 ml) were collected and mononuclear cells extracted and separated using Ficoll–Paque PLUS via centrifugation. Subsequently, suspended cells were removed after 5 days of culture, and adherent cells left to grow. Cells were detached upon reaching 80-90% confluence and sub-cultured up to 4 passages prior to further experiments. MSC antigens were recognized by monoclonal antibodies CD29, CD105 and CD166. To distinguish MSCs from hematopoietic stem cells, CD34 surface markers were used as negative controls. The characterized human blood-derived progenitor cells were cultured in three-dimensional alginate scaffolds using chondrogenic induction medium to promote chondrogenesis. Chondrogenesis was quantitated by sulphated glycosaminoglycan (S-GAG) production measured by 1,9-dimethylmethylene blue (DMMB) assay. Furthermore, chondrogenic-MSCs were examined and histologically compared using Safranin O staining to that of human chondrocytes as a means to determine chondrogenic transformation. Gene expression analysis was carried out by reverse transcriptase-polymerase chain reaction (RT-PCR) of differentiated human blood-derived progenitor cells using chondrocyte (cartilage cell)-specific phenotypic markers. The results showed that the cell derived in our processing technique share similar characteristics with adult MSCs and chondrocytes. Induction of chondrogenesis has been demonstrated in human blood-derived progenitor cells, which could provide a ready source of chondrocytes for engineering biological therapies. In the practical sense, PB isolation would prove to be less invasive, less expensive and less traumatic for patients to undergo therapy as compared to the 2-stage procedure of current available tissue engineering technique.

P86

Heterogeneous LPS of *Porphyromonas gingivalis* differentially modulate the innate immune response of human gingiva

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Objective: Porphyromonas gingivalis lipopolysaccharide (PgLPS) is a crucial virulence factor strongly involved in chronic periodontitis. PgLPS is known to contain both tetra- (Pg LPS1435) and penta-acylated (PgLPS 1690) lipid A structures with opposing effects. Present study aimed to examine the effect of two Pg LPS isoforms on human gingival epithelium.
Methods: Reconstituted human gingival epithelia (RHGE) were challenged with two isoforms of PgLPS together with *E. coli* LPS as the positive control. mRNA and proteins were harvested from tissues and culture supernatants were collected. Expression of pro-inflammatory and anti-inflammatory cytokines was evaluated by Q-PCR and ELISA. Involvement of pattern recognition receptors and signaling pathways were also analyzed by Q-PCR and western blot. Next, RHGE was blocked for CD14, TLR2, and TLR4 and followed by stimulation of PgLPS isoforms and effect was evaluated at cytokine level by Q-PCR and ELISA. Furthermore, we used "tissue proteomics" approach to study the differential proteomic expression profiles of gingival epithelium upon Pg LPS stimulation.
Results: It was shown that penta-acylated PgLPS1690 significantly upregulated the secretion of pro-inflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α in RHGE compared to tetra-acylated PgLPS1435. It seemed that regulation of pro-inflammatory cytokine by PgLPS1690 is mediated through both TLR2 and 4 and CD14/NF-kB axis for most of the cytokines. Proteomic studies indicated a differential protein profiles of RHGE induced with two isoforms.
Conclusion: *P. gingivalis* LPS heterogeneity differentially modulates the host innate immune response in human gingival epithelium, which may explain the niche-specific pathogenic mechanism of this periodontal pathogen.
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P87

Toll-Like Receptors are critical in controlling colonic inflammation and cancer

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Despite the presence of large number and diverse populations of commensal microbes, gut mucosa has evolved to maintain "microbial-tolerance", which is critically regulated by well-controlled Toll-like receptor (TLR) signaling. Deregulated TLR signaling has been linked to the pathogenesis of inflammatory bowel disease and colon cancer; however, the underlying mechanisms need to be further defined. In this study, we uncovered that lack of SIGIRR, a negative regulator for TLR and IL-1R signaling, led to increased genetic instability and LOH of *Apc*, resulting in spontaneous colonic polyposis in $Apc^{min/+}/Sigirr^{-/-}$ mice. Importantly, elevated colonic tumorigenesis in $Apc^{min/+}/Sigirr^{-/-}$ mice is dependent on the presence of commensal microbes in gut, implicating a critical role for TLR signaling in tumorigenesis. Furthermore, we demonstrated that SIGIRR-modulated TLR-mediated tumor initiation is mainly through the activation of the Akt-mTOR axis, which promotes cell cycle progression through its impact on posttranscriptional control of the key cell cycle regulators (Cyclins, c-Myc and cdk2). Moreover, abrogation of mTOR pathway by rapamycin prevented microadenoma and polyps formation in $Apc^{min/+}/Sigirr^{-/-}$ mice, providing new insights into treating human cancers. In addition, augmented production of proinflammatory cytokines, such as IL-6 and IL-23, further promoted tumor growth in $Apc^{min/+}/Sigirr^{-/-}$ mice. Epithelium specific re-expression of SIGIRR in $Apc^{min/+}/Sigirr^{-/-}$ mice ameliorated intestinal tumorigenesis. In summary, this study indicates that SIGIRR is a critical tumor suppressor that controls tumorigenesis by inhibiting TLR-induced mTOR and NFkB pathways in colonic epithelium.

P88

The 752delG26 mutation in the RFXANK gene associated with major histocompatibility complex class II deficiency: evidence for a founder effect in the Moroccan population

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Major histocompatibility complex class II plays a key role in the immune response, by presenting processed antigens to CD4+ lymphocytes. Major histocompatibility complex class II expression is controlled at the transcriptional level by at least four trans-acting genes: CIITA, RFXANK, RFX5 and RFXAP. Defects in these regulatory genes cause MHC class II immunodeficiency, which is frequent in North Africa. The aim of this study was to describe the immunological and molecular characteristics of ten unrelated Moroccan patients with MHC class II deficiency. Immunological examinations revealed a lack of expression of MHC class II molecules at the surface of peripheral blood mononuclear cells, low CD4+ T lymphocyte counts and variable serum immunoglobulin (IgG, IgM and IgA) levels. In addition, no MHC class II (HLA DR) expression was observed on lymphoblasts.
The molecular analysis identified the same homozygous 752delG26 mutation in the RFXANK genes of all patients. This finding confirms the association between the high frequency of the combined immunodeficiency and the defect in MHC class II expression and provides strong evidence for a founder effect of the 752delG26 mutation in the North African population. These findings should facilitate the establishment of molecular diagnosis and improve genetic counseling for affected Moroccan families.

P89

HIV drug resistance profile of HIV-1 CRF 01_AE protease and integrase coding regions in HIV infected Cambodian patients failing LPV-based 2nd line antiretroviral regimen

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Background: By the end of 2009, the number of HIV-1 infected patients on RTI-based 1st line and PI-based 2nd line ARV regimen in Cambodia reached 34,000 and 1,500, respectively. We already reported good virological and immunological responses after 1 to 4 years in cohorts of patients on 1st line and more recently, among an Esther cohort of 70 patients after 2 years on LPVr-based 2nd line regimen. However, emergence of LPV resistant associated mutations is becoming a major concern in low and middle income countries.
Objective: This study aimed to describe the resistance pattern of both the protease (PR) and integrase (IN) coding regions in HIV-1 CRF01-AE infected patients failing LPV-based 2nd line regimen in Cambodia.
Methods: Analysis of the Protease and Integrase drug resistance genotyping of 95 HIV-1 strains infected patients presenting detectable viral load on LPV/r-based 2nd line regimen in Cambodia.
Results: Lack of amplification in PR gene was observed for 18/95 presenting low viral load (median VL: 2.9Log_{10} copies/ml [IQR: 2.8-3.4]). The 77 other CRF01_AE strains, harbored polymorphism mutation in position M36, H69 and L89 conferring possibly resistance to TPV/r. Forty-nine (median VL: 5Log_{10} copies/ml [IQR: 4 - 5.5]) did not present any other PI associated resistance mutation. In contrast, 28 patients showed multiple resistances to PI. The median duration on LPV/r regimen was 34.5 months [IQR: 23.5 – 53.3] and the median VL was 5Log_{10} copies/ml [IQR: 4.3-5.6]. Twenty-five patients were resistant to LPV/r (7 possibly resistant). Twenty-seven were resistant to IDV, 21 and 19 to ATV/r and FPV/r, respectively. Twenty-five were resistant to NFV (10 possibly), 22 resistant to SQV/r (9 possibly). Seven showed resistance to DRV/r (5 possibly). Finally, excluding possible resistance, 21/28 (75%) was resistant for at least 3 PIs. Clinical investigation revealed that most of these 28 patients starting several RTIs and PIs early around 2000. All of them were sensitive to raltegravir, elvitegravir (integrase inhibitors), and etravirine (Non-Nucleoside reverse transcriptase inhibitorse).
Conclusion: This study indicates that 28/95 (29.5%) of Cambodian patients presenting detectable viral load on LPV/r–based 2nd line regimen developed resistance mutation for a large number of PIs. Most of them were not naïve for PI before LPV/r initiation. These results highlight an urgent need to evaluate the efficacy of LPV/r-based 2nd line regimen at the national levels, allowing to design of a next 3rd line ARV regiment in low and middle income countries.

P90

Identification of differential pharyngeal cytokine profiles during HIV infection

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Background: Significantly higher pharyngeal shedding of Epstein-Barr virus (EBV) is observed during HIV infection. Increased EBV shedding in pharynx is not affected even during highly active antiretroviral theyrapy (HAART). EBV positive monocyte populations have been shown to carry EBV to pharyngeal mucosa. Human cytokine profiles are often altered to facilitate herpes virus infection. Thus pharyngeal cytokine profiles may influence EBV reactivation and shedding during HIV infection. Our objective was to compare 37 pharyngeal cytokine profiles of HIV-seropositive patients who were or were not receiving HAART therapy.
Methods: 120 HIV positive volunteers under HAART and 72 HIV patients not undergoing antiviral therapy were investigated. All Volunteers with oral pathologies and smoking were excluded. Pharyngeal secretions were collected using standard procedures and analyzed for 37 cytokine profiles using human cytokine profiler array panels and ELISA. Cytokine interactome maps generated using Ingenuity database.
Results: Proteome profiler arrays demonstrated differential cytokine expression among HIV infected individual under HAART, HIV infected individuals without HAART and the healthy control. HIV group consisted of up regulated C5a, G-CSF, CXCL1, soluble ICAM1, IL-1α, IL-1β, IL1-Ra, IL-6, IL-8, IL-10, IL-12α, IL-13, IL-16, IL-25, IL-23α, IL-27, IL-32α, CXCL10, CXCL11, CCL-13, MIF, CCL3, CCL4, SERPIN-E1, CCL5, CXCL12, TNFα and soluble TREM1 and down regulated soluble sCD40, eGM-CSF, I-309, IFN-γ, IL-17A, IL-2, IL-4, IL-5. From these C5a, sTREM1, TNF alpha, CXCL12, CCL5, IL-17E, IL-23, IL-32α, IL-16, CCL3, IL-6 showed significantly higher expression levels in both HIV groups compared to healthy group ($P < 0.05$). TGF β1 levels in HIV patients undergoing HAART (0.5 +/-0.1 ng/ml) and without HAART (0.4 +/-0.1 ng/ml) were significantly increased when compared with the healthy control group (0.3 +/- 0.07 ng/ml) ($P = 0.0001$). TGF β1 levels had a significant positive correlation with higher CD4 counts in the group receiving HAART ($P = 0.006$). Cytokine interactome mapping revealed significantly increased immune cell trafficking in pharynx during HIV infection.
Conclusion: Pharyngeal TGF β1 levels are significantly increased during HIV infection. As TGF β1 is a known trigger of Epstein Barr viral lytic gene promoters, may influence pharyngeal reactivation of Epstein-Barr virus. Additionally, increased tendency for immune cell trafficking may facilitate EBV positive monocyte trafficking towards pharyngeal mucosa during HIV infection.

P91

Different susceptibility of mouse strains against Japanese encephalitis virus

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Japanese encephalitis is still the leading cause of viral encephalitis in Asia, where 30,000 to 50,000 cases are reported to WHO each year, resulting in an estimated 10,000 to 15,000 deaths annually. However, the majority of human infections with JEV are asymptomatic or very mild symptomatic and only about 1 in 250 infected persons develop clinical disease. Therefore, it is of importance to find out the cause of the different susceptibility with the comparison of different resistance towards JEV among various lab mice strain. We started with the observation of potential different organ tropism of JEV and distinct host immune response of JEV infection among resistant/susceptible (R/S) mice in vivo. Primary cells, including murine embryo fibroblast, bone marrow derived macrophage and bone marrow derived dendritic cells, were cultured in vitro to test specific host cell/virus interaction. We found that the virus levels in the bone marrow cells from R/S mice are significantly different.

P92

Phenotype detection of metallo-β-lactamase among the imipenem resistant *Psudomonas* and *Acinetobacter* in the tertiary care hospitals of Dhaka city

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Metallo beta lactamases (MBL) are enzymes that have wide spread of activity and they confer a high level of resistance to all β-lactams including carbapenem, except aztreonam [1]. MBLs require divalent zinc ion for their enzymatic activity which is not diminished by serine β lactamase inhibitors like salbactam, tazobactam, clavulinic acid etc but is inhibited by metal chelators like EDTA and thiol based compounds such as 2-mercaptopropionic acid (2-MPA), 10-phenanthroline, calcium dipicholinate etc [2]. They have constant and efficient carbapenemase activity. This MBL production is typically associated with resistance to aminoglycosides and fluoroquinolones further compromising therapeutic options [3].

There are no standard methods for the detection of MBL production in gram negative organism in routine Microbiology practice. The present study was undertaken to evaluate the screening tests like double disk synergy test (DDST) and disk potentiation test (DPT) using ceftazidime (CAZ) and imipenem (IPM) disks with chelating agents like EDTA and 2-MPA. A total of 132 *Pseudomonas* and 76 *Acinetobacter* isolates were obtained from Bangabandhu Sheikh Mujib Medical University (BSMMU), Bangladesh Institute of Research and Rehabilitation for Diabetes, and Endocrine and Metabolic disorders (BIRDEM) hospitals of Dhaka city. A total of 53 and 29 IPM resistant *Pseudomonas* and *Acinetobacter* isolates respectively were selected. EDTA- IPM micro dilution minimum inhibitory concentration (EDTA-IPM micro dilution MIC) method detected MBL in 44 (83%) IPM resistant *Pseudomonas* and 19(65.5%) *Acinetobacter* isolates. DDST with CAZ-0.1M EDTA and CAZ-2-MPA detected MBL in 73.6% and 67.9% of IPM resistant *Pseudomonas* and 55.2% and 48.3% of *Acinetobacter* isolates respectively. The detection rate was 67.9% and 66.1% in *Pseudomonas* and 51.7% and 44.8% in *Acinetobacter* isolates by EDTA-IPM and IPM-2-MPA methods respectively. In comparison to DDST, DPT with 0.1M EDTA showed higher sensitivity (89.7%) and specificity (100%) for detection of MBL in *Pseudomonas* and *Acinetobacter.* Isolates were also tested for AmpC β lactamase by DPT using chelating agent - aminophenyl boronic acid (APB) and it detected AmpC β lactamase in 6.9% and 21.1% MBL positive *Pseudomonas* and *Acinetobacter* respectively.

MBL producing *Pseudomonas* and *Acinetobacter* are emerging in our country. Rapid detection of these MBLs is necessary to institute appropriate treatment and effective infection control measures. Simple screening test like DPT using CAZ/IPM with 0.1M EDTA can be introduced into the routine clinical laboratories for their early detection.

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P93

Decreased virulence of a uropathogenic *Escherichiacoli pst* mutant is attributed to the repression of Type 1 fimbriae

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Extra-intestinal pathogenic *E. coli* cause urinary tract infections (UTIs), newborn meningitis, abdominal sepsis and septicemia. UTIs affect millions of women annually, and result in significant health care costs and morbidity worldwide. Uropathogenic *E. coli* (UPEC) is the predominant urinary tract pathogen, causing up to 85% of UTIs. Despite appropriate therapy, recurrent episode of UTI are common and bacterial strains are increasingly more resistant to many currently used antimicrobial agents. The *pstSCAB-phoU* operon encodes the phosphate specific transport system (Pst) and belongs to the Pho regulon, which is regulated by the two-component regulatory system (TCRS) PhoBR. Inactivation of the Pst system in *E. coli* and other bacteria leads to constitutive activation of the Pho regulon, perturbations in cellular adaptation, and decreased virulence. The role of the Pst system in uropathogenic *E. coli* (UPEC) was assessed by deleting the *pstSCA* genes in UPEC strain CFT073. In competitive (co-challenge) and single-strain infections, the *pst* mutant was attenuated for colonization of both the bladder and kidneys of CBA/J mice and was impaired for production of Type 1 fimbriae. Type 1 fimbriae are essential for UPEC virulence and their phase-variable expression is positively and negatively regulated by FimB and FimE, respectively. *In vitro*, in LB broth and human urine, repression of the *fim* structural gene *fimA* in the *pst* mutant correlated with increased orientation of the *fim* promoter in the OFF-position. *In vivo*, down-regulation of *fimA* in CFT073 Δ*pst* correlated with the up-regulation of *fimE*. To confirm the specific role of repression of *fim* expression by the *pst* mutant during UTI, *fim* phase locked-ON *pst* derivatives of the *pst* mutant and WT CFT073 strains were constructed. Compared to the *pst* mutant, the *fim* phase locked-ON *pst* derivative demonstrated a significant gain in colonization of the bladder, that was similar to that of CFT073 WT and CFT073 *fim* locked-ON strains. As Type 1 fimbriae are important for UPEC virulence, by promoting adhesion, our results suggest that the reduced bladder colonization by the *pst* mutant during UTI is predominantly attributed to down-regulation of these fimbriae. Since the Pho regulon is controlled by the TCRS PhoBR, molecules inducing the expression of the Pho regulon through inactivation of Pst or activation of PhoBR could potentially impair UPEC virulence by inhibiting colonization and the infection cycle, which is dependent on expression of type 1 fimbrial adhesins.

P94

Virulence and antimicrobial resistance characteristics of *Vibrio parahaemolyticus* isolated from environment, food and clinical samples in the south of Vietnam, 2010

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Background: *Vibrio parahaemolyticus* is a major cause of food poisoning in many countries. The rapid cases of this agent in recent years made it become an important etiology. However, the relationship between strains isolated from environment, food and clinical samples is still unclear. The presence of antimicrobial resistance and virulence factors such as tdh (thermostable direct hemolysin), trh (the TDH – related hemolysin) are proven to be candidate markers for studying on *Vibrio parahaemolyticus*.

In Vietnam, the existence of *Vibrio parahaemolyticus* is poorly understood. Our objective was to analyse the presence of *Vibrio parahaemolyticus* from different sources of samples as well as its virulent factors (tdh and trh genes) and antibiotic resistance characteristics.
Methods: 817 samples (food: 85, water: 299, stool of acute diarrhea: 433) were collected from May to July, 2010 at Ho Chi Minh City, Can Tho City, Ca Mau, Ben Tre, An Giang and Bac Lieu provinces from Southern of Vietnam. All samples were cultured and bacterial identified by API 20E. Besides, PCR for ToxR gene was performed for confirmation of *Vibrio parahaemolyticus*. The presence of either tdh or trh gene were done as described previously. Diffusion agar method and MIC were used for screening the antibiotic susceptibility of all identified strains.
Results: 15.91 % (130/817) *Vibrio parahaemolyticus* was isolated including 8.3% (36/434) from acute diarrheal patients, 40% (34/85) from foods and 20.1% (60/299) from environment samples. All strains had ToxR gene, but not for tdh and trh genes. In patient, just only 22.2% (8/36) strains had tdh gene and 19.4 % (7/36) strains got trh gene. All isolated strains from food were negative with both of tdh and trh gene. To trains isolated from environment, the presence of trh gene is 33.3% (20/60) and all of them got negative with tdh gene. To susceptibility test, most of strains was sensitive with tetracycline (90.77%, MIC ≥ 0.5 μg/ml), chloramphenicol (97, 69%, MIC ≥ 4 μg/ml), ciprofloxacin (100%, MIC ≥ 0.125 μg/ml), bactrim, doxycycline (93.08%) and resistance with ampicillin (34.62%, MIC ≥ 16 μg/ml), tetracycline and bactrim (6.92%), chloramphenicol, doxycycline (2.31% and 4.62%) respectively; reduced susceptibility was detected in *Vibrio parahaemolyticus* for ampicillin (45.38%), tetracycline, doxycycline (2.31%).
Conclusion: The circulation of *Vibrio parahaemolyticus* was quite high in the south of Vietnam. Virulence genes (tdh, trh) are not the only factors to cause diarrhea, we need to find another virulence factors. Most of isolated strains were sensitive with antibiotic except ampicillin.

P95

Investigation of genetic diversity of *Salmonella enterica* strains isolated from patients by Pulsed Field Gel Electrophoresis

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Salmonellosis is one of the world's most common zoonotic infections that cause a wide spectrum of different diseases in organisms. *Salmonella* infection in humans maybe occurs as acute enterocolitis, intestinal fever (typhoid or Paratyphoid) and bacteremia with or without clinical signs. There must be differences between *Salmonella* enterocolitis and all cases of acute diarrhea, including invasive bacteria such as *Campylobacter jejuni*, *Shigella* species, invasive *E. coli*, *Yersinia enterocolitica*, etc. There is a variety of microorganisms with different biochemical and serological characteristics in Salmonella's gender. These organisms are infective in many animals, in addition to humans. They can attack to the foreign tissue of the intestine and can cause intestinal fevers. Typhoid fever is the most important infections among the outside bowel disease. In epidemiological studies, genotypic methods such as PFGE can help us in distinguish correlation between strains within a species, discovering the source of infection and its prevalence ways and finally lead us to control the infections. Among the genotypic methods, PFGE method called the gold standard method, but it is not applicable in all laboratories due to the complexity and high cost of equipment and materials normally required.
PFGE is a golden standard for typing of many of bacteria such as *salmonella enterica* species. This method has advantages such as universality (all bacteria can be typed by this method), high ability of repetition and high differentiation power. Due to the occurrence of diarrheal disease in passengers and the occurrence of epidemics in various countries, especially in developing and developed countries, (The National Molecular Sub typing Network for Food borne Disease Surveillance) PULSE NET, provided standard protocols for typing of strains of bacteria that based on genotyping with Pulsed Field Gel Electrophoresis. We can determine the genetic relationship between strains of bacteria with this technique and let us to attempt to provide an appropriate treatment with knowing genetic variation based on the mutation and determine the antimicrobial resistance factors. With this technique we can also determine the genetic relationship between strains isolated from patients with isolates from other countries and internal strains that have been caused epidemics and Andmys. We studied the genetic relationship, genetic development, genetic diversity, antibiotic resistance and clonal relationship of *Salmonella* strains isolated from patients using PFGE alongside other techniques such as determination of antibiotic susceptibility profile and attempted to explore their the epidemiological importance and antibiotic susceptibility.

P96

Helicobacter Pylori stool antigens: post and pre-treatment evalutation of two methods performances in adult's strains in Algeria

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Diagnostic of *H.pylori* infection requires several methods, some of them are currently used in "Institut Pasteur "of Algeria. The aim of this study is to evaluate performances of two enzyme immunoassays used in detection of *H.pylori* using stool Ag before and after eradication, the first one "premier Platinium HpSA, Meridian Diagnostics" use a polyclonal Ac, the other one "IDEA HpStAR, Oxoid" use a monoclonal Ac. The reference test used in this study was UBT which is considered as a gold standard.
More than 1000 stools specimens were studied prospectively between 2000 and 2008, in adult subjects which are more than 16 years old. For each patient two Stools specimens are used, one before eradication and one minimum 04 weeks after treatment. 749 stools specimens were collected before eradication and only 265 ones after.
Our findings show that "IDEA HpStAR, Oxoid" results (specificity: 83%, sensibility: 91% before eradication, 83% after eradication) are better than "Premier Platinium HpSA®, Meridian Diagnostics" ones (specificity: 76%, sensibility: 64% before eradication, 56% after eradication). It seems evident that using monoclonal Ac gives more performances to the method, and allows detection of active infection with less of "false positive" also before than after eradication.

P97

New test for sensitivity evaluation of *Trichomonas vaginalis* to nitroimidazoles and nitrofurans *in vitro*

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Trichomonas vaginalis (Tv) is a causative agent of worldwide spread sexually transmitted disease, particularly affecting underdeveloped all segments of the population. Metronidazole is by far the most prescribed drug for the treatment of trichomoniasis. However, the appearance of drug resistant has led to the appearance of new drug alternatives. That is why the development of reliable and standard test for the evaluation clinical isolates sensitivity is necessary for routine laboratory practice. Traditionally used for this purpose the method of serial dilutions is laborious and time consuming.
A simple and practical method can be used for screening of sensitivity of Tv to anti-trichomonas drugs. This test contains of nutrient medium, special device for microscopic study and 8-well strip with drugs in fixed concentration which was previously determined. Using ten laboratory strains Tv it was found that minimal inhibitory concentrations (MIC's) were the following (μg/ml): metronidazole (5), secnidazole (2,5), tinidazole (2,5), nimorazole (2,5), ornidazole (2,5), clotrimazole (15),

nifuratel (1,25). Viability definition of Tv was performed using trypan blue stain and subculturing on a medium without any drugs. The scheme of assay was the following: poured into eight strip wells nutrient medium, inoculum cells trichomonads added and do microscopic examination after 48 hours of incubation under 37°C. The microscopic technique is based on the use of a special device consisting of eight micro chambers which allows examining eight probes at a one operation. 99 patients who had been confirmed of having chronical trichomoniasis were examined with this test. The test sensitivity to metronidazole, secnidazole, tinidazole, nimorazole, ornidazole, clotrimazole and nifuratel was performed as described earlier.

Results of sensitivity test with clinical samples were divided into three groups: resistance (R) - maintaining the viability of 50% or more trichomonads cells, sensitivity I (SI) - presence of 25% or less viable cells, sensitivity II (SII) – all cells lysis. The results showed the following sensitivity samples distribution: metronidazole - 23 (R), 47 (SI), 29 (SII), tinidazole - 58 (R), 30 (SI), 11 (SII), nimorazol - 71 (R), 19 (SI), 9 (SII), ornidazole - 51 (R), 41 (SI), 7 (SII), seknidazol - 57 (R), 28 (SI), 17 (SII), clotrimazole - 60 (R), 32 (SI), 7 (SII), nifuratel - 32 (R), 52 (SI), 15 (SII). One strain possessed multiple resistance.

Thus, the frequency of strains detection resistant to the action of drugs quite high, that argues the need to determine it "in vitro" for the choice of subsequent therapy.

P98

mechanistic Characterization of the nuclear import and export signals of VZV ORF9

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The ORF9 protein, a VZV-encoded late protein consisting of 302 amino acid (aa) residues, is a member of the highly conserved alphaherpesvirus UL49 gene family but shares limited identity with the UL49 prototype, HSV-1 VP22. As the orthologue of HSV-1 VP22, ORF9 is believed to be a major constituent of the VZV virion tegument. HSV-1 VP22 has been extensively studied; however, the functional properties of ORF9 are less well understood, despite the fact that its transcript is the most abundant viral message expressed during VZV lytic infection. As an important step toward understanding the detailed functions of ORF9 *in vivo* is to determine its precise subcellular localization, in the work presented here, to avoid the flaw of fixation protocol, living cells fluorescence microscopy technique, which is widely applied and developed in our group, was applied to deeply analyze the intracellular distribution of ORF9 protein and, to characterize its functional nuclear localization signal (NLS) and nuclear export signal (NES), as well as its transport mechanism in living cells.

Transient expression of ORF9 fused to enhanced yellow fluorescent protein (EYFP) in COS-7 cells showed the predominantly cytoplasmic localization in the absence of other viral proteins. Time-lapse examination of ORF9-EYFP demonstrated that the ORF9 was found to possess a pronounced cytoplasmic stage early post transfection, followed by translocation to the nucleus at a later time, which was consistent with the fact that VP22 is predominantly localized in the cytoplasm early in infection and accumulates in the nucleus late in infection, and the nuclear targeting of VP22 is independent of other viral factors.

By sequence analysis and constructing a series of deletion derivatives of ORF9 fused to EYFP and fluorescence microscopy analysis in live cells, a bona fide bipartite NLS of ORF9 was for the first time determined and mapped to amino acids (aa) 16 to 32 (RRKTTPSYSGQYRTARR), which containing two arginine-rich motifs RRK and RTARR.

Additionally, the NES was identified to locate between the leucine-rich region at amino acids 103 to 117 (LRHELVEDAVYENPL). Besides, ORF9 protein was demonstrated to target to the cytoplasm through the functional NES by Ran and chromosomal region maintenance 1 (CRM1)-dependent pathway, and to the nucleus through importin β-dependent mechanism that does not require importin α5.

P99

Varicella zoster virus immediate early protein 61 blocks the IFN-β pathway by degradation the activited IRF3

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Varicella zoster virus (VZV) open reading frame 61 (ORF61) is one of the four transcription regulated proteins, which is homologous to herpes simplex virus 1 (HSV-1) ICP0 and can partially complement the function of ICP0 in ICP0 deletion mutant HSV-1. Since ICP0 can inhibit the innate immunity in many levels such as IRF3 and PML, here we investigate the role of ORF61 in helping VZV evading IFN-β signal pathway. As the role of IFN-β in VZV infection has little been reported previously, our results demonstrated that IFN-β can limit VZV replication in Mewo cells and VZV infection can suppress the secretion of IFN-β in 293T cells and HeLa cells stimulated by SeV or poly(I:C). In addition, we try to explore the molecule mechanism by VZV to evade host innate immunity system especially the IFN-β pathway. We found that ORF61 can inhibit the activity of IFN-β promoter in 293T cells by reporter assays in the presence of SeV or poly I:C. The activity of ISRE (interferon-stimulated response elements, ISRE) promoter was also inhibited but not that of NF-κB promoter by ORF61, suggesting that ORF61 may interfere with the activity of IRF3. Ultimately, our results demonstrated that ORF61 degraded phosphorylation IRF3 via its E3 ubiquitin ligase activity. In one word, VZV ORF61 could inhibit IFN-β pathway and may play a critical role in VZV pathogenesis.

P100

Cloning of the Herpes smplex virus Type 1 genome as an novel luciferase infectious bacterial artificial chromosome

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Herpes simplex virus type 1 (HSV-1) is a ubiquitous human pathogen of skin and mucous membranes which associates with the infections of the mucocutaneous membranes, brain, and internal organs of infected neonates. As a member of the human herpesvirus family, HSV-1 contains a large DNA genome, encoding 84 unique open reading frames (ORFs), but the majority of its function is still elusive. In the present study, the genome of HSV-1 F strain was cloned as a stable and infectious BAC without any deletions of the viral genes. The BAC backbone sequences flanked by loxP sites were inserted into the intergenic region between UL37 and UL38. Cotransfection of the recombinant virus with a Cre recombinase plasmid resulted in the excision of the BAC sequences. Additionally, a firefly luciferase cassette was inserted to generate a novel luciferase HSV-1 BAC. Importantly, the resulting recombinant HSV-1 BAC_{Luc} behaved indistinguishably from the wild-type virus in vero cells, and the resulting luciferase activity could be quantified in vitro expediently. The recombinant HSV-1 BAC_{Luc} will facilitate HSV-1 research and provide the opportunity to exploit the power of BAC technology for production of recombinant viral vaccines.

P101

Identification of proteins that interact with the UL24 protein of herpes simplex virus 1

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Infection with herpes simplex virus 1 (HSV-1) typically results in the formation of cold sores; however, in individuals with a compromised immune system the infection can be severe. The virus life cycle is characterized by two distinct phases: a lytic replication phase occurring in

mucous membranes, and a latent state that is established in neurons of the trigeminal ganglia.
The UL24 protein of HSV-1 is implicated in both the lytic and latent phases of infection. Infection with a UL24-deficient virus *in vitro* results in reduced viral yields and the formation of syncytial plaques, where the plasma membranes of infected cells fuse together. *In vivo*, the absence of UL24 results in reduced viral titers and a drastic reduction in viral reactivation *ex vivo*. Previously, we showed that UL24 is both necessary and sufficient to induce the redistribution of the nucleolar protein nucleolin. The UL24 protein contains 269 amino acids and is expressed late in the virus life cycle. A bioinformatics study identified an endonuclease motif within the conserved N-terminal portion of the protein; however, its function remains to be demonstrated. Although several roles have been attributed to UL24, the mechanisms involved are unknown.
We hypothesized that UL24 functions, at least in part, through protein-protein interactions. To investigate this possibility, we used a virus that expresses UL24 with a hemagglutinin (HA) tag fused to its N-terminus, vHA-UL24. Lysates from infected cells were harvested, and fractionated on glycerol gradients. We found that UL24 was present in fractions corresponding to a much higher molecular mass than would be expected for UL24 alone, suggesting an association of UL24 with cellular or viral proteins during infection. To isolate potential UL24-binding proteins, we performed immunoprecipitations on fractions containing HA-UL24, using antibodies directed against the HA epitope. Immunoprecipitated proteins were resolved by denaturing polyacrylamide gel electrophoresis. Silver staining of the gel enabled the detection of proteins that appeared to specifically co-precipitate with HA-UL24. Candidate binding proteins will be identified by mass spectrometry. The identification of UL24-interacting partners will contribute to a better understanding of the roles of UL24 in different steps of the virus life cycle.

P102

Role of the viral protein UL24 in nucleolar modifications induced by herpes simplex virus 1

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Herpes simplex virus 1 (HSV-1) infection induces multiple modifications to nucleoli. We and others have observed the redistribution of nucleolar proteins such as nucleolin, fibrillarin, B23 (nucleophosmin), upstream binding factor (UBF) and RNA polymerase I (RNAPI) during HSV-1 infection. UL24 is one of a limited number of HSV-1 proteins that localizes to nucleoli. In a murine model of ocular HSV-1 infection, viruses that do not express the UL24 protein exhibit reduced viral titers in the eye and in trigeminal ganglia, and a reduction in clinical signs of disease. UL24 is conserved among *Herpesviridae*. Specifically, the N-terminal region of the protein contains several stretches of highly conserved residues. Bioinformatics studies have identified an endonuclease motif in the N-terminal portion of UL24, although as yet, no nuclease activity has been demonstrated for the protein. Previously, we discovered that UL24 transiently localizes to nucleoli during infection, and is both necessary and sufficient to induce the dispersal of nucleolin from nucleoli throughout the nucleus. In contrast, the redistribution of fibrillarin is a UL24-independent event. Moreover, the largest subunit of RNAPI and the transcription factor UBF are redistributed to viral replication compartments and these effects are also UL24-independent. We recently investigated whether UL24 was implicated in other HSV-1-induced nucleolar modifications. We discovered that UL24 was involved in the redistribution of B23 during infection. Furthermore, expression of UL24 in the absence of other viral proteins was sufficient to induce the relocalisation of B23. Similar to what we found for nucleolin, the conserved N-terminal portion of UL24 was important for its effect on B23 redistribution. The impact of the mutant vUL24-E99A/K101A, in which the endonuclease motif has been altered, and of vUL24-G121A, which harbours an amino acid substitution outside of this motif, were tested. While the G121A mutation had only a modest effect on the ability the virus to induce the relocalisation of B23, the E99A/K101A mutation appeared to abolish this function. Interestingly, the E99A/K101A mutation was previously shown to have a large impact on viral pathogenesis in mice. We found that the effect of HSV-1 on the redistribution of B23 was similar in non-immortalized cells such as human foreskin fibroblasts, as in immortalized cell lines such as vero cells. The similarities with regard to the impact of UL24 on the spatial distribution of nucleolin and B23 suggest that these effects may be related to the same function of UL24 during HSV-1 infection.

P103

Characterization of viral determinants of herpes simplex virus type 1 pathogenesis by bioimagery

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The human pathogen herpes simplex virus 1 (HSV-1) infects mucous membranes leading to cold sores, following which a latent infection is established in neurons of the trigeminal ganglia (TG). While the infection in healthy individuals is usually benign, viral encephalitis may sometimes occur. Furthermore, the infection can lead to severe illness in immunocompromised individuals, and can result in permanent neurological sequelae in newborns.
The UL24 protein of HSV-1 is highly conserved throughout the *Herpesviridae* family, and has been identified as a viral determinant of pathogenesis. In a murine model of ocular infection, a UL24-deficient virus exhibits a modest reduction in viral titers in the eye and a severe reduction of viral titers in the TG. A virus that does not express UL24 also exhibits defects in the establishment of latency and in the efficiency of viral reactivation from latency, as compared to a wild-type virus. Although UL24 is important for the pathogenesis of HSV-1, its exact role *in vivo* is unknown.
We hypothesized that UL24 is important for viral dissemination to the trigeminal ganglia following ocular infection. To investigate this possibility, we generated a recombinant strain of HSV-1 expressing a second generation red fluorescent protein (RFP), mCherry. The RFP expression cassette, driven by the eukaryotic CMV promoter, was inserted within the intergenic region between the viral genes *Us7* and *Us8*. This virus, v*Us7-8*mCherry, behaved similarly to the wild type virus (KOS) in cell culture as well as *in vivo*. We did not detect a loss of the RFP cassette over multiple rounds of viral replication in cell culture or following passage of the virus in mice. Following ocular infection, histological cross sections of eyes and TGs harvested three days post-infection were observed by confocal microscopy. Detection of mCherry enabled us to easily visualize infected cells in both the eye and in TG.
This virus will be a powerful tool to study the role of UL24 in viral dissemination. In the long term, results from this project will help us further our understanding of the molecular mechanisms involved in viral pathogenesis, and possibly lead to the development of new therapeutic strategies.

P104

Screening and identification of host cellular factors interaction with immediate-early protein ICP22 of herpes simplex virus type 1

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Herpes simplex virus type 1 (HSV-1) is a common and widely studied human pathogen that can replicate in epithelial cells and other cells of the host or alternatively can remain latent in peripheral neurons. ICP22 consists of 420 residues and is encoded by a spliced mRNA transcribed from the US1 gene. It is necessary for efficient HSV-1 growth in animal models of infection as well as for efficient *in vitro* growth in some, but not all, cultured cells. For example, ICP22 mutants grow well in African green monkey kidney (Vero) cells, but not in human embryonic lung (HEL) cells. ICP22 is extensively phosphorylated during infection, primarily by UL13 and another viral protein kinase, US3. In addition to inducing the modification of the host cell RNA Pol II, several other functions have been attributed to ICP22; these functions include the induction of certain viral L genes, the alteration of cell cycle-related proteins, and the determination of virion composition. It is clear that ICP22 is a multifunctional protein localized to the nucleus of infected cells, however, the host cellular factors

of ICP22 as well as the biological functions of their interactions are still little known. In the present study, an yeast two-hybrid system was applied to identify the host cellular factors of ICP22 and five target candidates were yielded: (1) TATA box binding protein -associated factor (TAF1); (2) TAO kinase 3 (TAOK3); (3) Alpha thalassemia/mental retardation syndrome X-linked (ATRX); (4) Cyclin-dependent kinase 9 (CDK9); (5) Ras association domain family member 9 (RASSF9); (6) occludin/ELL domain containing 1 (OCEL1). To confirm some of the interactions by co-localization in living cells, ICP22 and two candidate targets were tagged with enhanced cyan fluorescent protein (ECFP), enhanced yellow fluorescent protein (EYFP), respectively. Upon cotransfection of COS-7 cells, RASSF9-EYFP and OCEL1-EYFP both co-localized with ICP22-ECFP in distinct nuclear domains, indicating they are host cellular factors interaction with viral ICP22 under physiological conditions.

P105

Enhancing the use of evidence-based decision-making processes to establish immunization policies and programmes at national level: what should be the roe of Pasteur Institutes?

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In both developed and developing countries, the need for evidence-based decision-making in immunization programs has become crucial in light of multiple health priorities, limited human resources and logistical capacities, as well as the high cost of vaccines relative to limited public funds that are available. An important step that countries can take to encourage well-informed decision-making is to establish a group of national experts to advise the policy makers at the Ministry of Health and program managers for making decisions, including choices of new vaccines and needed adjustments to existing programs and schedules.
So far, many countries have already constituted such National Immunization Technical Advisory Groups (NITAGs) while other countries are currently working towards their establishment with the support of technical partners such as WHO and AMP (SIVAC Initiative). Based on WHO recommendations, the composition of the advisory group should include two categories of members: core and non-core members. All core members should be independent and credible experts who serve in their own capacity and who do not represent the interests of a particular group. Core members only, should participate in advising and deciding on the final set of recommendations. Non-core members hold key positions with important government entities they represent (e.g. Outbreaks Surveillance Department, National Drugs Regulatory Authorities, National Public Health Laboratory etc.) and represent various important professional societies, and key technical partners (e.g. WHO and UNICEF).
It is recommended that the committee be multidisciplinary and represent a broad range of expertise from the following disciplines: clinical medicine (pediatrics medicine, adult medicine, geriatrics), epidemiologists, infectious diseases specialists, microbiologists, public health, immunology, vaccinology. Pasteur Institutes have a crucial role to play in the NITAGs' activities. They can either be sought to participate as non-core member or to send experts as core members.
As non-core member, Pasteur Institutes provide data regarding burden of disease related to their laboratory surveillance activities. They will also provide specific information regarding immunology and safety issues related to vaccines. They can also provide information regarding emerging diseases and therefore influence the national research agenda by having the NITAG issuing specific recommendations. As core members, the experts from Pasteur Institutes should refrain from promoting the policies and views and products of the organization but this leads to an indirect recognition of the expertise of the institution.
As shown in other countries, it also may conduct to NITAG formal request regarding surveys and studies related to vaccines to the institution. Among other countries, the SIVAC Initiative is currently supporting Cote d'Ivoire and Viet Nam in establishing and strengthening NITAGs. In both countries, Pasteur Institutes already participate actively to the process and should be able to share their experience with other institutes of the Pasteur network.

P106

Socioeconomic impact relating to clinical condition on Pandemic (H1N1) Influenza

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Background: In April 2009, Pandemic (H1N1) 2009 has reported from Mexico and has spread so rapidly in the world-wide. However, the number of dead cases caused by this infection has a big difference among the counties and regions. We describe the considerable reasons why first Nations people have experienced such high rate of severe conditions from the socioeconomic point of view which are related to healthcare.
Methods: The four categorized population in Mexico by their healthcare insurance which related to their economic status and living environment were analyzed focusing on their health behavior in the first wave of Pandemic H1N1 2009 was occurred in Mexico. Also, we surveyed the socioeconomic factors for the dead cases who hospitalized in National Institute of Respiratory Diseases (INER), Mexico and their behaviors relating to Pandemic (H1N1) 2009. Based on those data, we evaluated factors which might influence to their clinical conditions. Those aspects were discussed with the comparison with Japan where the total number of dead case was small.
Results: Pandemic (H1N1) 2009 was first announced from Mexico where had insufficient provision preparedness under this circumstance. However, poverty, the large uninsured population (about 60% of population), and the low sanitary level were evaluated as the factors which influenced to the clinical conditions as well as difficulty in information transmission in the country that has 65 indigenous minority languages as "national languages, along with Spanish." The areas with the large number of dead cases by the first 3 months matched with the area of rural poverty in Mexico. On the other hand, Japan has matured universal national health insurance system and only 0.2% of uninsured population. In addition, faster access to healthcare is their tradition when people gets symptoms and they have high sanitary knowledge including using masks.
Conclusion: Self economic condition, health insurance status, limited sanitation, and lack of information lead to the less access to the healthcare which resulted in delayed of medical intervention including anti-viral treatment. It is difficult to eradicate poverty in Mexico and the health insurance situation in the short period of time. However, proposing the advantage of Japan such as prompt information, and sanitary behavior will help to increase their chance to get early medical intervention.

P107

Surveillance of acute lower respiratory infections in Cambodia: lessons from the field

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Background: Acute lower respiratory infections (ALRI) are a leading cause of morbidity and mortality in developing countries. With the threat of emerging respiratory diseases, surveillance of viral respiratory infections has been an international focus of the recent years. In June 2007, we implemented in Cambodia a surveillance study of ALRI in two provincial hospitals. After 2 years of study, we highlight caveats and difficulties in interpreting surveillance data as a lesson from the field.
Methods: Patients of all ages, hospitalized for acute lower respiratory infection (fever and cough and/or sore throat <14 days), were included in the surveillance study - excluding patients with known tuberculosis or positive HIV serology. For each patient, a medical exam and a chest – radiograph were systematically done. Blood, sputum, naso-pharyngeal swabs were collected on admission for culture molecular testing at Institut Pasteur- Cambodia. Diagnosis was performed by expert pulmonologists after reviewing patients' medical and biological data and chest radiographs.
Results: From April 2007 to December 2009, 3,566 subjects were enrolled. A diagnosis could be assigned by experts to 3,160 patients (88.6%). Among the 1,057 <5 year-olds, ALRI was confirmed for 961 (91%) patients (i.e. parenchymal, bronchial or pleural infection) and 96 (9%) presented with either upper respiratory infections (n=47) or extra-respiratory or non-infectious respiratory diseases (n=49). Among the 2,103 subjects aged 5 years and over, 1,882 (89.5%) had a confirmed ALRI, 10 (0.5%) presented with an upper respiratory infection, 211 (10%) an extra-respiratory or non-infectious respiratory disease. Among the <5 year-olds, a bacterial etiology could be determined in less than 1% of the ALRI cases - mainly due to the very low availability of sputum samples- while nasopharyngeal swabs were obtained in 99% of the children<5 year-olds. The virological positivity rates on nasopharyngeal swabs were higher in children <5y (57.3%) than in older children and adults (15.6%, p<0.001), but without any significant difference between patients with ALRI and patients with non-ALRI diseases, in both age-groups.
Conclusion: Virological results provide an epidemiological picture of circulating and emerging viruses in the study population but causal associations between positive virology testing and ALRI have to be determined cautiously, taking into account bacteriological, clinical and radiographic data. Clinical data must include severity data and additional information such as time interval between symptoms onset and presentation, history of recent hospitalization or antibiotic intake, clinical evolution on treatment and time from the onset of symptoms.
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P108

Lower respiratory infections (LRIs) etiologies in hospitalized children in New Caledonia: a PERCH pilot study

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Background: Worldwide, lower respiratory infections (LRIs) are the most frequent cause of death in children under 5 years. The Pneumonia Etiology Research for Child Health (PERCH) project is a large, multi-center case-control study of hospitalized pediatric patients with severe LRI to determine the etiology and risk factors associated with the syndrome. By applying modern tools with standardized methods, PERCH will contribute to new, precise information to guide the development of future vaccines and treatments. A pilot study aims to describe LRI etiologies in New Caledonia and to evaluate new diagnostic techniques.
Methods: We started a 1-year case-control study in children aged 1 month to 15 years. We collected induced sputum (IS) with forced expiratory flow, nasopharyngeal aspiration (NPA), urine and blood from cases hospitalized with pneumonia or bronchiolitis, and NPA and blood from controls without respiratory infection. Bacteriological tests consist of blood and respiratory specimen culture and urinary antigen detection for *L. pneumophila*. NPA and IS were tested for *B. pertussis*, *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* using PCR. Virus detection was performed using fluorescence and PCR (multiplex and mass-tag). Antibody detection (for *M./C. pneumoniae* and respiratory viruses) was performed on acute and 30-day convalescent sera.
Results: Within 6 months, 19 controls and 80 cases (56 bronchiolitis and 24 pneumonias) were enrolled. The median age was 9 months [range 1 month – 11 years]. At least one respiratory pathogen was found in 81% of cases and 33% of controls. Bacterial pathogens were found in 50% of cases with pneumonia. S.pneumoniae and H.influenzae were the most frequently found bacteria. Viruses were identified in 29% of cases among which 50% were RSV. Viral/bacterial co-infections occurred in 8% of cases. Among children with bronchiolitis, RSV was the most frequently found virus (90%) with an epidemic peak in April-May, the beginning of the cooler season.
Conclusion: Diagnostic testing methods enabled detection of possible etiology in most of the ARI case.

P109

Networking to implement diagnostic capacity and (re-) evaluate the public health importance of leptospirosis in the Institut Pasteur International Network

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Leptospirosis is a tropical neglected disease that is highly under-diagnosed and under-reported, mostly because of a lack of diagnosis capacity. The Institut Pasteur International Network includes researchers and physicians that have a recognized expertise in the field of leptospirosis epidemiology and diagnosis. Taking advantage of this international and widespread network of expertise, we currently aim at implementing the diagnosis of leptospirosis in institutes, countries or regions where it was not available. With the financial support of the Institut Pasteur International Network, biologists and researchers from French Guyana, Cameroon, Guadeloupe (West Indies), Cote d'Ivoire, Cambodia, New Caledonia and France currently work at implementing and inter-calibrating leptospirosis diagnosis in their respective institutes.
Because of very slow and delicate culture of *Leptospira* spp., the early diagnosis of leptospirosis increasingly relies on PCR. Using its own molecular platform and expertise, each institute implements the PCR or qPCR method of its choice for the detection of pathogenic *Leptospira* spp. Common *Leptospira* suspensions are shared to commonly evaluate the sensitivity of the PCR assays.
The serological reference method uses the Micro Agglutination Test (MAT), a method that requires continuous culture of live leptospires and experienced staff for interpretation. Therefore, commercially available serological tests (Elisa for the detection of anti-Leptospira IgM) are used as a first diagnosis approach. The French National Reference Center then validates the results and identifies the infecting serogroup using a reference MAT panel.
These diagnostic tools are then offered to patients hospitalized with predefined leptospirosis-like symptoms, as a way to (re-) evaluate the contribution of leptospirosis to non-malarial febrile illnesses in these countries and regions.
The global architecture and goals of our Lepto-network will be presented, together with epidemiological research perspectives using the tools and expertise developed through this network.

P110

Deciphering leptospirosis eco-epidemiology in New Caledonia

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Leptospirosis is a zoonosis of worldwide distribution with major incidence in the tropics. In New Caledonia, it has been studied for years and is regarded as a major public health concern. It is characterized by an endemic pattern with seasonal epidemics arising during rainy periods, notably during "la Niña" periods. Both the endemic and epidemics have a rural epidemiology and result from the infection by a variety of *Leptospira* strains. The Institute Pasteur in New Caledonia (IPNC) is the reference laboratory for human leptospirosis and has been studying this disease for years, notably developing and implementing efficient molecular diagnostic tools. At the same time, an expertise on this disease has led to numerous studies on the epidemiology and physiopathology of leptospirosis.

IPNC recently started a study aiming at identifying the *Leptospira* strains currently circulating in New Caledonia and their corresponding animal reservoirs. Because leptospirosis is a complex pathology involving reservoir hosts, environmental sources of infection and susceptible mammals including Man, the control of human leptospirosis has to take these animal reservoirs into account. In any region, a limited number of pathogenic *Leptospira* strains are circulating which can cause disease in humans. Every single *Leptospira* strain is supposed to be maintained by one or several reservoir animal host species. Current molecular methods allow identifying the infecting *Leptospira* strain at a sub-species level, possibly by directly using these molecular tools with DNA extracted from animal and human clinical specimens.

We evaluated the usefulness of the sequence polymorphism of diagnostic PCR products and used this molecular tool for studying the prevalence and identification of the different *Leptospira* strains in both rodent and non-rodent Mammals. Because rodents are known to play a pivotal role in the maintenance of major *Leptospira* strains, an ecological study of rodent dynamics and their *Leptospira* prevalence is conducted in an area of highest incidence, taking seasonal and meteorological variability into account. Preliminary results confirm their role as reservoirs for *L. interrogans* Icterohaemorragiae and *L. borgpetersenii* Ballum and highlight a joint effect of temperature and pluviometry on rodents' populations and their *Leptospira* carriage. Additionally, the introduced deer *Cervus timorensis russa* was shown to be a reservoir for *L. borgpetersenii* Hardjo.

P111

Regional Emerging Diseases Intervention (REDI) Centre

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Genesis: The Regional Emerging Diseases Intervention (REDI) Centre is a research and training organization, founded by Singapore and the United States to enhance the Asia-Pacific region's capability and capacity to deal with emerging and reemerging infectious diseases. REDI Centre is chartered in Singapore as a non-profit, intergovernmental Organization.

Missions: i/ To promote international exchange of information, knowledge and expertise through the organization of scientific conferences, ii/ to act as a regional resource for training and research, iii/ to support programs that will strengthen the response and the control to critical health threats, and iv/ to improve regional capacity to prevention, surveillance diagnosis and management of Emerging Infectious Diseases (EIDs).

Regional conferences recently organized: Forum on Hand Foot and Mouth Disease, Chikungunya Symposium and Fourth ASEAN Congress on Tropical Medicine and Parasitology.

Laboratory capacity: Together with Singapore and international partners (WHO, US-NIH …), REDI Centre organized several on-site lab training in Singapore public health laboratories as well as regional trainings on laboratory techniques and principles for EIDs diagnosis (biosafety train-the-trainer courses, Influenza PCR and virus isolation and characterization workshops, Asia-Pacific Dengue workshops). REDI collaborated with partners to form a regional network with the aim to improve laboratory EIDs diagnosis and surveillance (biosafety, quality insurance, development of guidelines for EID diagnosis).

Clinical capacity: In the area of hospital infection control, REDI Centre actively contributed to the development of state-of-the-art training modules and currently assists the MOHs of Vietnam (implementation of the National Infection Prevention and Control Master Plan), Indonesia (revision of the National Guideline for AI management and infection control) and Cambodia (surveillance of nosocomial infections in Kampong Cham Hospital).Together with Pasteur Network, WHO and other partners, organization of a regional course on Infection Control in Vientiane (with Pasteur Network) and development of clinical guidelines for the clinical management of Hand, Foot and Mouth Disease

Epidemiology capacity: REDI centre supports Singaporean and Indonesian FETPs and co-organized (with Pasteur Network and National University of Singapore) several short regional courses:"Data-management and Basic Statistics", "Outbreak Investigation". In Indonesia REDI Centre collaborates in the Trilateral Pilot Project on Surveillance and Control of Avian Influenza in Tangerang (with Indonesian Ministries of Health and Agriculture) and in the realization of a study aiming to assess the H5N1 seroprevalence in poultry workers and in poultry in live market facilities (with US-CDC and NIHRD).

P112

Building bridges among public health stakeholders in Asia and Europe: the Asia-Europe Foundation (ASEF) Network for Public Health

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The recently occurred pandemic H1N1 has given lessons on the significance of a multisectorial approach in developing mechanism for responses and preparedness against the spread of communicable diseases. This approach can expand beyond the administrative jurisdiction of a country when there is transboundary movement of diseases when measures taken require cross-border coordination. In this notion, regional integration appears to be a mechanism to address this challenge.

The Asia-Europe Foundation (ASEF) Network for Public Health launched in May 2009 was established as part of the wider initiative of Asia-Europe Meeting (ASEM) Foreign Ministers' Meeting in combating possible human influenza pandemic. Financially supported by the Government of Japan for a period of 5 years (2009-2013), the ASEF Network is part of the wider ASEM Initiative for the Rapid Containment of Pandemic Influenza which also includes a regional stockpile.

The paper will highlight the three thematic working groups of the ASEF Network and their activities for the past two years and how these activities have contributed in building bridges among public health stakeholders in Asia and Europe.

P113

Animal-Human Health Interface and community based surveillance in Vietnam-a strategy under Mekong Basin Disease Surveillance Cooperation (MBDS)

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In recent years, zoonoses have been increasing. 75 % of emerging diseases are zoonoses such as SARS, Influenza A/H5N1, Rabies, Streptococcus suis, Nipah virus, etc. in Vietnam, ministries of health and Agriculture ate not collaborating closely enough to control zoonotic disease, information sharing between two ministries does not currently occur regularly and is generally only done when requested. Community based surveillance has not been done systematically and effective.

MBDS cooperation (including Cambodia, China, Laos, Myanmar, Thailand and Vietnam) was established in 1999 with financial support from Rockefeller Foundation. Its goal is to strengthen national and subregional collaboration on the interface between human and animal health, i.e., between Ministries of Health and Agriculture, to rapidly and effectively detect and control communicable diseases that are spread by poultry and animals and may affect humans. The 3rd phase of MBDS (2008-2010) focuses on 7 strategies including cross-border collaboration, animal-human health interface, capacity building, information communication technology development, laboratory development, risk communication and policy research.
After two years of implementation MBDS Vietnam, we have achieved effective results. The animal-human health departments sit together to develop interministerial circulars, for information sharing and collaboration in the prevention and control of zoonotic outbreak. Every year, two workshops were organized for animal and health sectors at national and provincial and district levels to share information, experiences and develop the collaboration mechanism. Animal and human health sectors have conducted 4 joint outbreak investigations on A/H5N1 in 2008 and 2009. Cross-border meetings have been organized between provinces of Vietnam with provinces of Cambodia, Lao PDR and China. Two Table Top Exercises on A/H5N1 control between animal and health sectors have been implemented (in Lai Chau and An Giang provinces). We have developed the model of community based surveillance in Quang Tri and An Giang provinces. Every year, MBDS organized Regional Forum for member countries to share information, experiences in outbreak surveillance, investigation, prevention and control.

P114

Molecular monitoring of causative viruses in child acute respiratory infection in endemo-epidemic situations in Shanghai

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Background: Numerous viruses are responsible for respiratory infections; however, both their distribution and genetic diversity, in a limited area and a population subgroup, have been studied only rarely during a sustained period of time.
Methods: A 2-year surveillance program of children presenting with acute respiratory infections (ARIs) was carried out to characterize the viral etiology and to assess whether using gene amplification and sequencing could be a reliable approach to monitor virus introduction and spread in a population subgroup.
Results: Using multiplex RT-PCR, 15 different respiratory viruses were detected within the 486 nasopharyngeal positive samples collected among 817 children aged <9 years old who presented with ARI during October 2006 to September 2008. A single virus was detected in 373 patients (45.7%), and two to four viruses in 113 patients (13.8%). The most frequent causative viruses were respiratory syncytial virus (RSV) (24.7%), human bocavirus (24.5%), and human rhinovirus (HRV) (15%). RSV was more prevalent in winter and among young infants. Cases of seasonal influenza A and B viruses were reported mainly in January and August. An increase in adenovirus infection was observed during the spring of the second year of the study. Sequence analyses showed multiple introductions of different virus subtypes and identified a high prevalence of the newly defined HRV-C species. A higher viral incidence was observed during the winter of 2008, which was unusually cold.
Conclusion: This study supports the usefulness of multiplex RT-PCR for virus detection and co-infection, and for implementation of a molecular monitoring system for endemic and epidemic viral respiratory infections.
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P115

The viral agents of Acute Encephalitis Sydrome in Ben Tre province 2008-2010

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Since 2005, Arboviruses and Enteroviruses have been confirmed as some of pathogens causing acute meningo-encephalitis in children in the South of Vietnam. Therefore, they have been selected in priority into a sentinel surveillance and investigation system of emerging infectious diseases in Ben Tre province during 2008-2010. The surveillance system for viral Acute Encephalitis Syndrome (AES) was established in 02 sentinel hospitals in Ben Tre province, Vietnam. All AES cases with macroscopic appearance corresponding to viral AES were enrolled into the surveillance. Their specimens (CSF, paired sera and feces) were only collected after patients or their legal guarantees had signed in the written ICF. The second serum of paired sera was collected on the field in case patient had discharged. MAC-ELISA was applied for detecting Arboviruses (such as Japanese encephalitis virus and Dengue viruses), and RT-PCR was used for detecting Enteroviruses, especially Enterovirus type 71.
From August 2008 to June 2010, 138 AES cases were enrolled into the surveillance system with 138 CSF, 276 sera and 138 feces specimens. Out of 138 reported cases from the surveillance system, 60 (43.48%) AES cases had positive testing results with viral agents, including: 7 (5.07%) Japanese encephalitis virus, 6 (4.35%) Dengue viruses, and 47 (34.06%) Enteroviruses. No case of infecting Enterovirus type 71 was detected. Epidemiological analysis shown that Enteroviruses circulated all year round, Dengue viruses circulated from August to October in accordance to the Dengue epidemic season in the South of Vietnam and Japanese encephalitis virus circulated sporadically in a year. Moreover, distribution of viral agents specific by age groups shown that age group of 0 – 14 (11.76%) and over 60 (18.18%) were the most susceptible for infecting Japanese encephalitis virus.
The surveillance system has provided valuable information as a baseline for future studies on viral agents of AES and for implementing Japanese encephalitis vaccination campaigns in Ben Tre province. The implementation in reality also raised the needs of developing and applying diagnostic tests with high sensitivity and specificity such as NS1 antigen detection for Japanese encephalitis virus and multiplex RT-PCR for Enteroviruses.
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P116

The agents of Sever Acute Respiratory Infection cases in Ben Tre province 2008-2010

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Since 2003, Southern Vietnam has become the most serious affected area by various emerging diseases such as: A/H5N1, SARS, Rubella, etc. Therefore, the establishment of emerging disease surveillance is essential and necessary to provide scientific information for good understand on the emerging diseases and for effective outbreak response.
A specific surveillance system for Severe Acute Respiratory Infection (SARI) was established in 02 sentinel hospitals in Ben Tre province from August 2008 to June 2010. Patient's signature on written ICF had been required before the enrollment. All of the enrolled SARI cases were investigated and their throat swabs were collected for testing. Multiplex RT-PCR was applied to determine 17 viral agents. In addition, blood culture has been

performed since August 2009 in order to detect bacterial agents on these SARI cases.

Out of 1,842 SARI cases enrolled into the surveillance system, 969 (52.61%) cases had positive testing results, including: 913 (49.75%) cases had positive testing results with viral agents and 56 (9.89%) of 566 blood culture specimens had positive testing results with bacterial agents. Moreover, the proportion of co-infection between virus and bacteria was 0.98%. There were 14 viral agents had been detected including: Inf A/H1N1pdm (1.41%), Inf A (2.61%), Inf B (0.98%), Inf C (0.11%), hRV (18.1%), RSV (14.13%), Boca (3.32%), Adeno (1.68%), Entero (0.65%), Corona HKUI (0.11%), hMPV (2.28%), Parainfluenza type 1 (1.58%), Parainfluenza type 3 (2.23%) and Parainfluenza type 4 (0.33%) viruses. A remarkable attention is that hRV and RSV which were identified as the cause of some sudden deaths in infants in China, Philippines and respiratory outbreak in Cambodia in 2008 were 02 predominant agents.

The major concern is that Inf A virus in the South predominates in summer season and this is very different from other regions in Vietnam and even other parts of the world. In addition, viral agents were shifted by seasons: Boca virus (January to April), Inf A (May to July) and RSV (August to November). The results of the surveillance show that the viral pathogens of SARI are variety and most of them have never been detected in the South of Vietnam. In addition, all types of Influenza virus are detected on SARI cases and the viral pathogens vary by the seasons.

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P117

SISEA activities in Pasteur Institute in Nha Trang, Vietnam, during 2008–2009

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Background: In recent years, the situation of dangerous infectious disease has developed more complex. Some emerging infectious diseases such as Severe Acute Respiratory Syndrome (SARS), avian influenza tend to globalization. In Central Vietnam, a lot of infectious diseases circulating every year caused serious consequences such as SARI, VE, DF/DHF, etc. For SARI, except influenza virus which has monitored, the other respiratory pathogens have not been concerned yet. It's difficult to detect some agents cause disease in regional and provincial laboratory. In order to respond the complex development of emerging diseases such as SARS, avian influenza, severe acute respiratory infection (SARI), viral encephalitis (VE) and Dengue fever/Dengue haemorrhagic fever, Pasteur Institute Network in Southeast Asia has implemented the project "Surveillance and Investigation of epidemic situation in South East Asia (SISEA)".

Results: A specific surveillance system for Severe Acute Respiratory Infection (SARI) was established in 02 sentinel hospitals in Bin Dinh province from January 2008 to December 2009. Patient's signature on written ICF had been required before the enrollment and samples (nasopharyngeal swabs) were analyzed by RT-PCR multiplex. There were 1,155 cases of SARI enrolled, with 47.2% testing positive for one of the 17 respiratory viruses included in our panel. Rhinvoirus (24%) and respiratory syncitial virus (18%) accounted for almost 50% of the positive samples. Adenovirus, human metapneumovirus and NL63 coronavirus were present in 5-6% of the samples, whereas other respiratory viruses showed lower incidence. Of note, only few samples tested positive for pandemic H1N1 influenza. In addition, we carried out a number of training and supervision sessions for the personnel of the sentinel hospitals, both in field epidemiology and molecular laboratory techniques.

Conclusion: This project is strengthening laboratory capability as well as epidemiological surveillance system to enable rapid diagnosis and prevention of dangerous epidemics, thus helping to contain their spread in the region. This is really necessary and responds to current practical needs in Central Vietnam.

P118

Epidemiology and viral etiologies of Severe Acute Respiratory Infections (SARI) in the Northern Vietnam

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Background: Severe Acute Respiratory Infections (SARI) is the leading global cause of morbidity and mortality from infectious diseases. Since January 2009, a Sentinel Surveillance System for Severe Acute Respiratory Infections has been established based in a Northern Province of Vietnam (Hai Duong province). The purpose of this program is to describe the epidemiology and identify the viral etiologies of SARI in a province of the North of Vietnam for rapid diagnosis and response to epidemic outbreaks.

Methods: Patients from a provincial and district hospital of Hai Duong province, Northern Vietnam were enrolled, using standardized case definitions of SARI. Clinical and epidemiological data were collected using structured questionnaire. 18 viral etiologies of SARI were examined from throat swabs of eligible patients using multiplex-polymerase chain reaction (RT-PCR) in the framework of the SISEA project.

Results: From January 2009 to September 2010, 484 patients were enrolled in the study. The average age of subjects was 12.7 years, ranging from 0 to 90 years and 53.1% were male. Of 484 specimens, 283 (58.47%) were positive for viral agents. Of whom, 218 (77.03%) were mono-infection, 65 (22.97%) had mixed viral infection with at least one other virus. The most common viral etiologies of SARI were seasonal influenza A/H1N1 (16.32%) and rhino virus (15.5%). Pandemic influenza A/H1N1/09 was first detected in this surveillance system in week 42nd of 2009 and lasted for 4 weeks only. After that, there were only 2 cases detected. The proportion of specimens positive with pandemic influenza A/H1N1/09 increased from 50% (week 42nd) and became totally predominant (accounted for around 77-100 %) from week 43rd to week 45th, and suddenly decreased to 0 % in week 46th. Rhinovirus infection seems occurred seasonally with the majority of patients appearing from January to March. However, no seasonality was observed for other specific pathogens causing SARI. Interestingly, this study has detected the presence of a parainfluenza 4 outbreak and of sporadic human FluC cases in 2009. In addition, this study first identified the presence of human corona virus NL63 and HKU1 in Vietnam.

Conclusions: SARI infections are caused by variety of viruses. The system is sensitive to identify pandemic influenza A/H1N1/09 community transmission. It is necessary to enhance the severe acute respiratory infection surveillance for early detection and rapid response.