

Abstracts

Abstract for the 8th International Meeting on Molecular Epidemiology and  
Evolutionary Genetics in Infectious Diseases  
Royal River Hotel, Bangkok, Thailand, 30 November–2 December 2006

Available online 20 January 2008

**Plenary lectures**

**The molecular epidemiology of enteric protozoan infections—Emerging issues and paradigm shifts**

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In recent years a variety of issues have emerged concerning the epidemiology of zoonotic protozoan infections that result from the ingestion of environmentally resistant infective stages. They have many features in common regarding their transmission, which can be direct, or via water or food, and most exhibit low host specificity. Although they have been the subject of research for many years, recent studies have raised fundamental questions concerning our understanding of the epidemiology of infections with these parasites. *Giardia* and *Blastocystis* both have wide host ranges and are genetically very divergent yet how this variability is reflected in terms of zoonotic potential, clinical significance and virulence is not clear. With *Cryptosporidium*, many taxonomic and epidemiological questions have been resolved but recent studies have not only questioned *Cryptosporidium*'s phylogenetic affinities, but have also revealed new aspects about its life cycle and development. These findings will have a major impact on both surveillance and control. In the case of *Toxoplasma*, recent studies in domestic animals and wildlife have raised questions about how the parasite is maintained in nature. In particular, the role of vertical transmission in wildlife populations may have been underestimated. In the case of *Blastocystis*, *Entamoeba coli*, *Chilomastix* and *Dientamoeba*, they have been largely overlooked in terms of their impact on public health yet their common, and sometimes concurrent occurrence, has raised questions about their clinical and zoonotic potential.

These emerging issues will be discussed with emphasis on how molecular tools and epidemiological studies can help resolve these questions.

***Leishmania* and sand flies: Parasite–vector co-evolution or opportunism?**

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Leishmaniasis is an emerging and re-emerging disease in several parts of the world. One of the principle areas of investigation that helps us to understand a change in disease epidemiology are the key biological factors responsible for disease transmission. For vector-borne diseases like leishmaniasis an understanding of vector specificity and the mechanics of transmission are two such key factors. Recent work in several laboratories has shown that *Leishmania* and sand flies provide a range of examples from both ends of the spectrum with regard to vector specificity. For example, *Leishmania major* and *Phlebotomus papatasi* appear to be a very specific parasite–vector combination, and co-evolution has driven the molecular differentiation of a specific ligand on the surface of the parasite that binds to a corresponding galectin on the wall of the sand fly midgut. Thus *P. papatasi* is a representative member of a group that can be called the “restricted” vectors of leishmaniasis. At the other extreme lies *Lutzomyia longipalpis*, which transmits *L. infantum* in Central and South America. There is now strong evidence that this parasite has only very recently been introduced into the Americas from Europe in last few hundred years, probably when European colonists brought *L. infantum*-infected dogs to America. *Lutzomyia longipalpis* was already there and was adopted as a vector by the incoming parasites, taking over from *P. perniciosus* and *P. ariasi* found in Southern Europe. All of these vectors belong to a second group, the “permissive” vectors of leishmaniasis. Although in nature they usually only transmit one particular species of parasite, this

appears to be more due to ecological constraints rather than any intrinsic barrier, as under laboratory conditions they can support the development of many species of *Leishmania*. Thus this parasite–vector combination can be regarded as a case of evolutionary opportunism. Current work is being pursued to investigate the molecular basis of this opportunism. Once the parasite has established an infection in a particular sand fly it must then overcome the challenge of transmission by bite: how can the parasite travel against the flow of an incoming blood-meal? Recent work has shown that a gel-like material secreted by parasites in the sand fly gut plays a key role in promoting transmission. The so-called promastigote secretory gel (PSG) creates a “blocked fly” that cannot feed properly. This material must be egested by regurgitation before bloodfeeding can proceed, thereby egesting the infective parasites at the same time. This mechanism of transmission appears to be common amongst the *Leishmania* parasite–vector combinations examined so far. It may have evolved either before or after the specialisation of individual *Leishmania* species to a particular vector, thus representing either a conserved or convergent evolutionary response. What lies ahead for SE Asia? Leishmaniasis may remain a relatively rare disease, but the emergence of an epidemic of cutaneous leishmaniasis in Sri Lanka in the past 5 years, cases of visceral disease in Thailand, and reports of leishmaniasis in kangaroos in Australia all illustrate that complacency is dangerous. As the examples mentioned above show, both parasites and vectors have shown themselves capable of adapting to new circumstances, either by the spread of a well-established parasite–vector combination due to changes in ecology or the establishment of a novel parasite–vector partnership.

### Pharmacogenomics of HIV

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In post-genomics era, we have found the additive effect of individual genetic variations in loci encoding metabolic enzymes, drug transporters, cell surface markers, and cellular growth and differentiation factors may play a significant role in the variability of response and toxicity of a number of drugs. The “one-size-fits-all” regimen of antiretroviral treatments results in interpersonal variation in drug concentrations and differences in susceptibility to drug toxicity. Many of the antiretrovirals are metabolized by polymorphically expressed enzymes (cytochrome P450, CYP450; glucuronyl transferase, GT) and/or transported by drug transporters (ABC and SLC families). The nonnucleoside reverse transcriptase inhibitors (NNRTIs), nevirapine and efavirenz, are metabolized primarily by CYP2B6. The associations have been identified between a frequent CYP2B6 variant (G516T) and NNRTI pharmacokinetics. Greater plasma efavirenz exposure was predicted by CYP2B6 G516T and recent data suggest that G516T also predicts nevirapine exposure. Study the effect CYP2B6 polymorphism in mother-to-child HIV transmission of single-dose Nevirapine is currently studied in Thailand. The clearest asso-

ciation between genetic variants and response relates to the hypersensitivity reaction that occurs with abacavir. The identification that the major histocompatibility complex haplotype acts as a strong genetic predisposing factor which can be translated into a pharmacogenetic test. However, much more work needs to be done to define the genetic factors determining response to antiretroviral agents. In Thailand, pharmacogenomics project was established in 2003. Study of allele frequency and linkage disequilibrium of markers in drug related genes loci are relevant to the objective of this project. We genotyped 1536 haplotype tagging SNPs known polymorphic sites in 182 drug related genes in 280 unrelated healthy Thai samples, which comprises 70 samples from each of four geographical Thai populations: North, Northeastern, Central, and South. This data is crucial for pharmacogenomics case–control association studies with clinical records.

### Molecular epidemiology of important bacterial pathogens in India: Ancient origins, current diversity and future epidemics

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*Mycobacterium tuberculosis*, leptospira and *Helicobacter pylori* are some of the bacterial pathogens that trigger diseases with a complex interplay between infection dynamics, pathogen biology and host immune responses. The whole genome sequence determination has greatly facilitated our understanding of these pathogens. Tuberculosis is the disease with a highest morbidity and mortality worldwide. The disease haunts millions of people in India with a huge death rate. The genetic diversity and evolutionary history of the underlying *M. tuberculosis* strains are largely unknown in the context of this country that has earned dubious distinctions for tuberculosis prevalence. Our ongoing, large-scale analysis of hundreds of strains of tubercle bacilli highlighted a clear predominance of ancestral *M. tuberculosis* genotypes in the Indian subcontinent, compared to other regions of the world, and support the opinion that India is a historically ancient endemic focus of tuberculosis. It is hypothesized that such ‘ancient’ bacilli are relatively ‘docile’ than some of the highly ‘killer’ ones such as the highly disseminating Beijing types which harbor inherent propensity to acquire multiple drug resistance (MDR) and are spreading in India through major metropolitan cities. Beijing strains are likely to evade and replace ancestral reservoirs of *M. tuberculosis* in the country. If that happens, India will probably face large, institutional outbreaks involving hospital wards, prisons, schools, etc. This is perhaps a major issue that needs to be addressed in the post-genomic scenario, with the same magnitude of zeal that researchers have shown towards drug discovery and diagnostic or vaccine development. Leptospirosis is another major pestilence, a worldwide zoonosis caused by the spirochetes of the genus *Leptospira*. The leptospirae have been extremely diverse pathogens having more than three hundred different strains or serovars with specific geographic distribu-

tion. But this enormous inventory of serovars, based mainly on an ever-changing surface antigen repertoire, throws an artificial and unreliable scenario of strain diversity. It is therefore difficult to track strains whose molecular identity keeps changing according to the host and the environmental niches they inhabit and cross through. To address this problem, we have developed highly sophisticated genotyping systems based on integrated genome analysis approaches to correctly identify and track leptospiral strains. These approaches are expected to greatly facilitate epidemiology of leptospirosis apart from deciphering the origins and evolution of leptospire in a global sense. The human gastric pathogen *H. pylori* is presumed to be co-evolved with its human host and is again a very highly diverse and robust pathogen. Our 'geographic genomics' study tests the theory that *H. pylori* existed in humans as a benign bacterium for thousands of years until it acquired some virulence factors from the microorganisms abundant in the human societies of the neolithic period, after the domestication of agriculture and livestock. We found traces of East Asian ancestry in the gene pool of Native Peruvian strains (Amerindian?). This finding supports ancient human migration across the Bering-strait (20,000 years BP). We also attempted to support the idea that the major single virulence factor of the bacterium, the *cag* Pathogenicity Island (*cagPAI*) was acquired during different times, at different places in the world and from a 'local' microbial source. We followed this with theoretical approaches to find significant overlap among the *H. pylori* population expansion time and domestication of agriculture in the world. This study provides some new insights into the ancient origins and diversity of *H. pylori* and the significance of such diversity in the development of gastro-duodenal pathology. Why has this bacterium survived for this long time in humans? Does this association makes the colonization beneficial or of low biological cost? These are the questions that need to be answered in the near future.

**Viral population size is a key element in the risk assessment of the emergence in humans of a pandemic prone H5N1 avian influenza virus**

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The surge of the global avian influenza epizootic caused by genotype Z H5N1 highly pathogenic avian influenza viruses (HPAIVs) has posed numerous questions, in particular to risk managers and policy makers. Scientific knowledge is limited on many aspects of the ecology and environmental properties of HPAIVs, in particular H5N1. In addition to being an animal health issue with strong impact on human nutrition and socio-economics consequences, the current widespread epizootic has spilled over as a human health issue. Indeed, some 250 zoonotic cases of H5N1 infections have been reported worldwide since the end of 2003. Current H5N1 HPAIVs are however poorly transmissible from domestic birds to humans and need specific conditions to achieve this passage. Besides, virus transmission between humans is rare and extremely inefficient. This is due to

two probable main reasons: 1/in humans, H5N1 HPAIVs find their preferred receptor structures (terminal sialic acid moieties in  $\alpha$  2,3 bonds) in the lower respiratory tracts (particularly in alveolar cells), 2/their optimum temperature of replication is higher than the temperature of the upper parts of the human respiratory tract. These facts could make it difficult for the virus to reach its proper targets in humans during the contamination process and could confine the virus deep in the lungs without possibility of easy exit, necessary for virus transmission. In the past, new virus subtypes emerged in the human population either by reassortments between human/mammalian and avian influenza viruses, as probably happened around 1957 and again around 1968, or by accumulation of point mutations as probably occurred with the precursor of the Spanish influenza virus. Indeed, some residues have been pointed out as important for the adaptation to new hosts and their accumulation could pave the way to a virus adapted to humans: 1/amino acid (AA) 627 on PB2 is probably involved in temperature dependence, 2/AA 223 in the haemagglutinin is involved in binding to terminal sialic acid moieties, which vary from one host species to another and within a host species from one tissue to another. Other determinants, probably in the NP or NS genes may greatly contribute to viral adaptation to their hosts. Influenza viruses are present in the form of quasi-species, *i.e.* populations of viral genomes bearing point differences between them. Viral diversity increases the probability of a group of minority viral genomes to harbour a set of mutations directly involved in an increased viral capacity for human-to-human transmission. Viral diversity depends both on virus intrinsic variation capabilities and viral population size. Influenza virus polymerase complexes are error prone and generate frequent point mutations. When a virus succeeds in changing host, its mutation rates seems generally higher in the new host from a phylogenetic viewpoint. This is also true among birds when an avian influenza virus (AIV) jumps from a duck species to chickens or turkeys. In the past, the hypothesis has been raised according to which precursor viruses would pre-exist in their current host where they acquire the necessary set of mutations through a hypermutation mechanism. This would be due to a polymerase complex with an error rate higher than that of other viruses, following the acquisition of point mutation (mutator mutations) affecting the enzyme fidelity. *In vitro* studies using avian-like influenza A (H1N1) viruses introduced in the pig population in Germany during the early 1980s suggest that there is no such thing as mutator mutations. Their conclusion was that the increased viral diversity was linked more to the global size of the virus population rather than to an enhanced mutation capacity of the virus. Applying this to the H5N1 current situation, it is probable that the animal host demographic factor, especially in domestic flocks, is a critical factor in viral diversity. For example, the poultry population increased from 1.1 billions in 1980 to 4.9 billions in 2002 in China only, offering a possibility of vast virus populations present at any one time in domestic poultry. Virus global maintenance in nature is a key element to understand its population dynamics. Data from the literature on AIV worldwide and long-term cycles in birds and in the environment are rather limited and there is a lot more to understand. Using a virus population

approach and basing it on sequence variation data, it should be possible to estimate the risk of a set of mutations to occur and thus the risk of viral emergence by accumulation of point mutations.

### Genome scan study of clinical malaria in Senegal and Thailand

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Malaria has exerted considerable selective pressure on the human genome, most notably apparent in the prevalence of hemoglobin mutations in regions endemic for malaria. Epidemiological studies in regions of high malaria endemicity have consistently shown that the severity of disease considerably decreased after the first years of life, whereas parasite prevalence and incidence remain high throughout adolescence and only decrease slowly in adults. Knowledge of the relationship between parasite infection and disease remains one of the major enigmas of malaria epidemiology. No clear picture of the mechanisms underlying naturally acquired immunity to malaria or disease transmission have yet emerged. We carried out a human genetic study of two well-defined cohorts in whom malaria parameters were recorded longitudinally from two continents, Senegal and Thailand. The major difference apart from genetic background between the two cohorts is the presence of *Plasmodium vivax* in Thailand. We first estimated genetic effect for each phenotype as quantitative traits by mean of variance component. We found that number of clinical malaria attacks for the three species (*P. falciparum* (PF), *P. vivax* and *P. ovale*) and trophozoite density of PF are significantly under human genetic influence. In addition human genetic factors showed significant effect on gametocytogenesis of PF, which may influence transmission of the disease. We performed genome screening linkage analysis and tested the effect some known and candidate genes. We confirmed the previous finding of linkage on chromosome 5q31 (*Pfll1*) with parasite infection level. We found a new region on chromosome 5p15, which showed linkage to clinical PF attacks both in Senegal and Thailand. There are genes involved in complement activation, cytokines, etc. We planned to perform systematic screening of this region using information from the public database.

### Defining risk to pathogenic infections: Utilizing the HAP-MAP database and broad based screens to discover host susceptibility genes to infectious diseases

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**Background:** The susceptibility to infection and subsequent manifestations of disease is dependent upon the host and

pathogen. Pathogen-encoded proteins have been discovered that regulate specific features of pathogen behavior and affect its survival in the host. Host factors, which affect pathogen survival and can be manipulated to enhance or inhibit infection, have been more difficult to discover.

**Methods:** We have utilized a process of random insertional mutagenesis coupled with siRNA knockdown of gene expression to discover and validate cellular genes that play roles in various aspects of intracellular pathogen replication. We used the dbSNP database of NCBI to view the single nucleotide polymorphism (SNPs) in these genes, which we then categorized for potential function by virtue of predicted alteration of protein structure or transcript processing. The HAPMAP database provides an assessment of the major haplotypes (ancestral fragments of DNA harboring a specific series of alleles at variant positions) in a given region of the genome (and their frequencies) in a sample of Caucasian, Chinese, Japanese, or Yoruban populations.

**Results:** The human viruses, reovirus, Ebola and Marburg, were used for selection of mutant cells, which were resistant to lytic infection. HIV was selected as the virus of interest to validate whether the mutant gene had broad based association with virus replication. SNPs were found in validated genes that were predicted to affect the coding sequence or non-coding regions that may affect transcription or translational efficiencies. It was found that for some of the candidate genes, haplotype frequencies were notably different among Caucasians compared to peoples from Asia or Africa.

**Conclusions:** Mammalian genes were discovered that have roles in infection of Marburg and Ebola viruses, reovirus and HIV. These genes contain potentially functional genetic variation of varying frequency across major populations. Genetic variation of these candidate host genes may be subject to selective pressure by pathogens and may modify susceptibility and disease course following exposure to a potential pathogen. Further analysis will help to develop genetic profiles, which can be used to personalize medicine and target therapeutics to at risk populations.

### Today knowledge and future challenges on human fascioliasis in Asia: The who initiative

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Fascioliasis is an important disease caused by two trematode species: *Fasciola hepatica*, present in Europe, Africa, Asia, the Americas and Oceania, and *F. gigantica*, mainly distributed in Africa and Asia. Human fascioliasis was considered a secondary disease until the mid-1990s. This old disease has a great expansion power thanks to the large colonization capacities of its causal agents and vector species, and is at present emerging or re-emerging in many countries, including both prevalence and intensity increases and geographical expansion. WHO (Headquarters Geneva) decided to launch a worldwide initia-

tive against human fascioliasis including two main axes: (A) transmission and epidemiology studies; (B) control activities by mainly treatments with triclabendazole (Egaten<sup>®</sup>), a single-dose highly effective drug. Results obtained during the last years have furnished numerous hitherto-unknown aspects and new information which have given rise to a complete new general picture of this disease, explaining why human fascioliasis has recently been included within the list of important human parasitic diseases. Fascioliasis is the vector-borne disease presenting the widest latitudinal, longitudinal and altitudinal distribution known. Recent studies have shown it to be an important public health problem. Human cases have been increasing in 51 countries of the five continents. Recent papers estimate human infection up to 17 million people, or even higher depending from the hitherto unknown situations in many countries, mainly of Asia and Africa. Major health problems are known in Andean countries, the Caribbean, northern Africa, and western Europe. In Asia, the area of most concern is the region around the Caspian Sea (Iran and neighbouring countries). Moreover, data from the beginning of this new century indicate that south-east Asian countries may also be seriously affected, with around 500 cases in the 2002–2003 period and up to 2000 cases from the beginning of 2006 to nowadays in Vietnam. When comparing different human endemic areas, a large diversity of situations and environments appear. Fascioliasis in human hypo- to hyperendemic areas appear to present, in the different continents, a very wide spectrum of transmission and epidemiological patterns related to the very wide diversity of environments. This large diversity indicates that, once in a new area where the disease is emerging, studies must be performed from zero and shall comprise a multidisciplinary approach to assess which kind of epidemiological pattern are we dealing with. Within this multidisciplinary, molecular epidemiology studies become crucial. Molecular markers developed during recent years shall be applied to both liver flukes from humans and animals and to freshwater lymnaeid transmitting snails, in order to establish which combined haplotypes are involved in the disease transmission locally. In Asia, molecular epidemiology studies performed in the area around the Caspian Sea show that the transmission pattern may be very complicated due to the overlapping of both *F. hepatica* and *F. gigantica*, the appearance of intermediate fasciolid forms, and the participation of lymnaeid vector species belonging to different groups as *Radix*, *Galba*/*Fossaria*, stagnicolines and *Pseudosuccinea*. A similar situation may be expected throughout other Asian regions, as in Vietnam and neighbouring countries. The fluke-snail host species specificity factor plays a fundamental role, although the domestic animal fauna, mainly livestock (mainly sheep, cattle, buffaloes, goats, donkeys and pigs) but sometimes also sylvatic herbivorous mammals as lagomorphs and rodents, are also worth noting. Molecular techniques as DNA target sequencing, single nucleotide polymorphisms (SNPs) and microsatellites are of great help to assess the transmission patterns and origins of human contamination, in the way to establish the appropriate individual prophylaxis and general control measures.

## Human immune response gene polymorphism versus HIV-1 and dengue virus diversity in SE Asians

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The immune response to pathogens is dependent on the presentation of microbial peptides by human leukocyte antigens (HLA) to T cells and natural killer (NK) cells. The genes encoding HLA and killer immunoglobulin-like receptors (KIR) are highly polymorphic. This diversity has functional implications and is likely to be driven by microbial selection acting on HLA and KIR gene loci. Different ethnic groups vary in their HLA and KIR allele profiles, which in turn can act as highly informative correlates of ethnicity in anthropological studies. The analysis of HLA and KIR gene profiles in ethnic Thais, has revealed that this major ethnic group is highly representative of the overall gene pool within the large populations of mainland SE Asia. Thus, this geographic region is most suitable for large-scale population-based genetic epidemiological studies of emerging infectious diseases such as HIV-1 and dengue, which are of increasing public health concern. HLA and KIR association studies with HIV-1 have been performed in numerous ethnic groups. A variety of effects have been observed particularly with HLA-B57, -B27 and -A11 molecules. There is evidence that the diversity of HIV-1 clades and recombinants infecting different populations is being driven by immune responses controlled by polymorphic HLA molecules. Such an effect may well be responsible for the prevalence of HIV-1 clade E or the CRF01\_A/E recombinant in the ethnic Thai, Cambodian Khmer and Vietnamese Kinh populations, while HIV-1 clades B and C and recombinants thereof have seeded predominantly into the more northern Sino-Tibetan-Burman populations of this region. By contrast, all four of the major dengue virus serotypes are known to circulate in mainland SE Asian populations. There is evidence in ethnic Thais that the outcome of exposure to dengue virus in previously exposed and immunologically primed individuals, associates with HLA-A2, -B5 and -B15 molecules, depending on the dengue serotype responsible for secondary infections. Taken together, these studies are of relevance to the design, testing, and implementation of new vaccine control programmes in populations at risk of exposure to HIV-1 and dengue.

## Drug targets and drug resistance in malaria

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Malaria is one of the most life-threatening infectious diseases in tropical and sub-tropical countries. The disease claims approximately 3–5 millions people each year. The emergence of drug-resistance *Plasmodium falciparum* to almost all the currently available antimalarial drugs in many regions of the world has caused treatment of malaria increasing problematic and thus there is an immediate need to search and identify new targets,

develop new and effective antimalarial agents, and understand the molecular basis of drug resistance in malaria parasites. So far, resistance in malaria has been found to be associated with specific single nucleotide polymorphisms (SNPs) in the gene, i.e. *pfMDR1* (N86Y) and *pfCRT* (K76T) are associated with quinoline resistance, whereas *pfDHFR* (N51I, C59R, S108N and I164L) correlated with resistance of antifolate antimalarial drugs. The DHFR of *P. falciparum* (*pfDHFR*) represents one of the most well-defined drug targets in malaria. Research on *pfDHFR* including gene cloning, expression, generation of mutants resistant to inhibitors and structural studies during the past two decades has contributed tremendously towards the understanding of antifolate binding and molecular mechanism of antifolate resistance in malaria. Studies of malarial DHFR will be discussed with respect to the interactions to malarial thymidylate synthase (TS) domain. The results could provide insights into better understanding of how effective inhibitors could be developed in order to overcome malaria resistance.

#### Acknowledgements

Works in WS's Laboratory have been supported by TDR grants.

#### Environmental change, infectious disease emergence, and dengue

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Regional environmental change, driven largely by globalization and population growth, with associated increases in resource consumption and waste generation, plays a primary role in the emergence of infectious disease, especially in tropical developing regions. Associated land use and transformation of resource production (urbanization, agricultural expansion and intensification, and natural habitat alteration), have produced changes in ecological systems, notably in landscapes and, in turn, their natural communities and ultimately in their pathogen, animal host, and human populations. Thus, the altered "host–pathogen" dynamics facilitate novelty, including exchange of genetic material among pathogens, resulting in rapid adaptation by the pathogens and more frequent generation of novel pathogen variants. Some will be more virulent, infective, and/or capable of enhanced transmission, contributing to disease reemergence or emergence. Factors related to public health infrastructure and climate variability, and their interactions with regional environmental change, also contribute significantly to disease emergence. Dengue and dengue hemorrhagic fever (DHF) is possibly the clearest case of disease re-emergence and emergence associated with regional environmental change, specifically urbanization. Failure to effectively control of dengue/DHF in many regions argues for new approaches that integrate research on the vector, pathogen, human and environment within a defined ecosystem. Classical ecological concepts are key to understanding population, community, and ecosystem level dynamics influencing disease emergence. But more recent advances and research

tools in evolutionary ecology are also fundamental to understanding both vector and pathogen transmission dynamics that underlying emergence. Integrating this research with social ecological concepts represents a promising new, transdisciplinary approach to dengue control.

## Symposiums

### Symposium "Coevolution host pathogen 1"

#### (1) Peopling of South America and South Asia insights through *Helicobacter pylori* genomics

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The human gastric pathogen *Helicobacter pylori* is co-evolved with its host and therefore, origins and expansion of multiple populations and sub populations of *H. pylori* mirror ancient human migrations. Ancient origins of *H. pylori* in the New World and in India are debatable. It is not clear how different waves of human migrations in these large continents contributed to the evolution of strain diversity of *H. pylori*. We tried to address these issues through mapping genetic origins of *H. pylori* of native Peruvians (of Amerindian ancestry) and Indians and their genomic comparison with hundreds of isolates from different geographic regions. For this purpose, we attempted to dissect genetic identity of strains by fluorescent amplified fragment length polymorphism (FAFLP) analysis, multilocus sequence typing (MLST) of the seven housekeeping genes (*atpA*, *efp*, *ureI*, *ppa*, *mutY*, *trpC*, *yphC*) and the sequence analyses of the *babB* adhesin and *oipA* genes. The whole *cag* pathogenicity-island (*cagPAI*) from these strains was analyzed using PCR and the geographic type of *cagA* phosphorylation motif EPIYA was determined by gene sequencing. In case of South American *H. pylori* populations, we observed that while European genotype (hp-Europe) predominates in native Peruvian strains, approximately 20% of these strains represent a sub-population with an Amerindian ancestry (*hsp-Amerind*). All of these strains however, irrespective of their ancestral affiliation harbored a complete, 'western' type *cagPAI* and the motifs surrounding it. This indicates a possible acquisition of *cagPAI* by the *hsp-Amerind* strains from the European strains, during decades of co-colonization. Our observations therefore suggest presence of ancestral *H. pylori* (*hsp-Amerind*) in Peruvian Amerindians which possibly managed to survive and compete against the Spanish strains that arrived to the New World about 500 years ago. We suggest that this might have happened after native Peruvian *H. pylori* strains acquired *cagPAI* sequences, either by new acquisition in *cag*-negative strains or by recombination in *cag* positive Amerindian strains. In case of Indian strains, almost all the isolates analyzed revealed a European ancestry and belonged to MLST genogroup hp-Europe. The *cagPAI* harbored by Indian strains also revealed European features upon PCR based analysis and whole PAI sequencing. These observations therefore suggest that *H. pylori* in India have ancient origins in Europe. Highly similar MLST and *cagPAI* genotypes observed for ethnically and

linguistically diverse Indian people might argue for a European-Central Asian root of population expansion in the Indian subcontinent. Predominance of genogroup hp-Europe in India amidst non-existence of other genogroups such as hp-Africa and hp-East Asia, point to the fact that the strains of former type carried a special fitness advantage in Indian stomachs, possibly conferred by complete and intact 'western' type *cagPAIs* to out-compete endogenous strains, if any. These results also might potentiate speculations related to large-scale replacement of the ancient indigenous people of India by outsiders, bringing first Neolithic practices and languages from the Fertile Crescent and Central Asia.

### (2) A genomics approach to understanding host response during dengue infection

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Dengue infection results in a wide clinical spectrum, ranging from asymptomatic, through fever (DF), to the life threatening complications hemorrhagic fever (DHF) and shock syndrome (DSS). Although we now understand that factors such as repeat infections and the type or magnitude of the host response are important in determining severity, the mechanisms of these actions remain largely unknown. Understanding this host-pathogen interaction may enable outcome prediction and new therapy options. Developments in biology now allow a "systems approach" to be applied to this problem, utilizing whole genomes of both human and virus, *in vitro* and *in vivo* to enable a more complete picture of their interplay to be built up. We have developed a whole genome approach to viral sequencing, to increase efficiency and enable large numbers of genomes to be completed, together with a web-based interpretation tool. We have also applied human genome expression arrays to characterize the types of host response made to the different viruses and also investigate the role of host variation using human whole genome genetic association studies. These technologies have identified novel host pathways involved in viral replication, and also host immune responses, such as the interferon signaling pathway, that are influenced by viral sequence and thus viral evolution.

### (3) Taming of host innate response by a potential biothreat agent *Burkholderia pseudomallei*

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*Burkholderia pseudomallei* is a facultative intracellular Gram-negative bacillus classified by CDC as a category B agent. It not only has potential for bioterrorism, but also causes potentially fatal septicemia in humans and animals. We have previously shown that *B. pseudomallei* is able to survive and replicate in mouse macrophage cell lines, escape into the cell cytoplasm and induce cytoplasmic protrusion leading to direct cell-to-cell spreading. The bacteria induces cell fusion, resulting in multi-

nucleated giant cell formation and apoptotic cell death. The macrophages infected with *B. pseudomallei* exhibit reduced and delayed formation of TNF- $\alpha$  and fail to stimulate iNOS and NO production, thus allowing the bacteria to survive intracellularly. One of the mechanisms responsible for the depressed response is most likely associated with a failure to induce IFN- $\beta$  production required for phosphorylation of STAT1 and induction of IRF1. The latter is one of the transcription factors needed to turn on the iNOS gene. On the other hand, we can favorably modulate host cell response by using immunomodulating agents, e.g., CpG oligodeoxynucleotide, that can boost up its innate immunity by enhancing iNOS production, increasing uptake and intracellular killing capacity of the macrophages. *B. pseudomallei* may also produce negative regulator that in turn turns off a subsequent host cell response to these stimuli. We recently demonstrated that *B. pseudomallei* could readily induce the expression of negative regulators that interfere with host cell response to interferon- $\gamma$  stimulation, thus allowing the bacteria to escape killing by the activated phagocytes. Furthermore, we now have additional information from DNA microarray study using Affymetrix chips with human lung epithelial cell line infected with *B. pseudomallei*. There was a down regulation of IL-6, IL-8 and the adhesion molecule ICAM-1 when compared with cells infected with its avirulent counterpart. Altogether, the data strongly indicate that *B. pseudomallei* successfully modulate host innate response for its own survival inside the infected host.

### (4) The human EMR1 gene is under strong balancing selection, and may have a role in susceptibility to pulmonary tuberculosis

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One third of humanity is infected by *Mycobacterium tuberculosis* and more than two million people die from the infection each year. And yet, despite this awful toll, only a tenth of the infected billions will ever succumb to or even exhibit symptoms of the disease. This bespeaks a major role for genetic variability in determining the outcome of Tb exposure and infection. Identifying the relevant genetic variation has stymied investigators for some time, with most progress to date arising from studies of severe Mendelian mycobacterial susceptibilities. In a preliminary analysis of two distinct data sets comprised of (1) ~10,000 SNPs distributed throughout the human genome and genotyped in 42 active Tuberculosis cases matched with 42 household and community controls and (2) ~110,000 SNPs genotyped in 111 active cases and 116 controls, we have seen statistically significant associations among numerous SNPs. One of the genes that has been strongly implicated in our study is EMR1. This unusual gene is a member of the EGF-TM7 family of receptors that are predominantly expressed by cells of the immune system. In the course of resequencing this gene to search for putatively functional variants that could be involved in the TB disease process, we observed patterns of genetic variation that were strongly suggestive of natural selection. The



gene displays an elevated level of nucleotide diversity that ranks among the very extremes of the empirical distributions for human genes, a skew in the allele frequency spectrum towards intermediate frequency alleles, positive Tajima's *D* values, an elevated nonsynonymous substitution rate within the human lineage, the presence of highly divergent intermediate haplotypes, and a level of population differentiation (*F*<sub>st</sub>) that is lower than the global average. These data suggest that the EMR1 gene does not evolve in a neutral fashion and is more likely to have experienced strong balancing selection. Because proteins containing EGF-like modules are typically involved in protein–protein interactions and the observation that 14 of the 20 nonsynonymous variations reside within the extracellular portion of the receptor, the target of selection is probably directed against the EGF-like domain, and may involve recognition of pathogen associated molecular patterns (PAMPs). Biological studies that seek to identify the interacting target(s) of the EMR1 receptor can shed further insight into the nature of the evolution of the gene, and its involvement (if any) in susceptibility to infections.

## Symposium “Medical entomology 1”

### (5) *Aedes aegypti*: Experimental data supports a genetic background for shape variation

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Shape variation of the wing of *Aedes aegypti* was examined in three isofemale lines A, B and C under controlled laboratory conditions during ten generations. The landmark coordinate data were collected from cross veins and junctions for morphometric analysis. To quantify shape variation, we used the “metric disparity” index, known to be independent of sample sizes. Statistical comparisons were performed by non-parametric tests (bootstraps). It was assumed that isofemale lines had been founded by parents having different genotypes, and that no new genotypes appeared during the 10 generations of follow-up. Metric disparity was scored across lines within a given generation and across different generations within a given line. It was shown that the metric disparity index behaved as expected for an indicator of genetic diversity: increasing when mixing different lines, not increasing when adding individuals of the same line. In addition, a simple classification tree of the total sample showed that even after 10 generations, the wings were clustered into three groups according to the initial founders. This study suggests a genetic basis for wing geometry of *A. aegypti*. The epidemiological interest of wing shape behaving as a genetic character would be to help in detecting natural patterns of population structuring at a low cost. In the same way three experimentally isolated lines were recognized by individual wing traits, it is expected that any isolated field population

could also be detected. Similar conclusions were obtained previously on various old laboratory lines of *A. aegypti*.

**Keywords:** *Aedes aegypti*; Isofemale line; Shape variation; Metric disparity

### (6) Population structure of main malaria vectors in Asia, members of *Anopheles* species complexes: Implications on vector control

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In Asia, the anopheline biodiversity is very rich and the main malaria vectors belong to complexes in which species are morphologically indistinguishable. Recent advances in systematics and molecular identifications have allowed a clarification of phylogenetic relationships and a simplification of species identification among and within the sibling species or *Anopheles* groups. This is of primordial importance for applying appropriate vector control programs. The presentation of the latest data on the main malaria vectors in southern Asia will highlight the importance of precisely identifying the species, assessing relationships among members of complex, and testing phylogenetic hypotheses involving closely related *Anopheles* species to conduct adequate and efficient vector control strategies. The Minimus Complex is widely distributed on the Asian Continent and is composed of two species considered as malaria vectors in SE Asia. This complex belongs to the Funestus Group which comprises 27 closely related species distributed in Africa and in Asia. Based on molecular and morphological characters and a complete phylogenetic work, a new systematic scheme was recently presented which reflects the evolutionary relationships within species of this group. The Sundaicus Complex is distributed along the coast of Asia and is known as one of the main malaria vector in southern Asia. Recent molecular works on this complex have allowed the recognition of at least three species for which phylogeographic evolutionary scenario will be presented along with the malaria risk linked to specific human activities. Other *Anopheles* complexes with major malaria vectors will be mentioned such as the Dirus, Fluviatilis, and Culicifacies in relation to systematics and malaria transmission.

### (7) From population structure to genetically engineered vectors: New ways to control vector-borne diseases?

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Epidemiological studies on ticks and the pathogens they can carry (such as Lyme Disease) are showing some correlations between infection rates and biodiversity highlighting the “dilution” effects on potential vectors while other studies comparing sympatric small rodent species demonstrated that rodent species transmitting more pathogens are parasitized by more ectoparasite species. Further studies on host dispersion also showed some impacts on genetic diversity in the ticks with some other comparisons between tick sex, location and genetic flows within these ectoparasite populations. However, other studies highlighted no evidence in gene flows in *Ornithodoros coriaceus* and a far more complex situation with *Ixodes uriae*. The ongoing sequencing of *Ixodes scapularis* (vector of the Lyme Disease spirochaete *Borrelia burgdorferi*, the zoonotic *Babesia microti* and the HGE agent now part of the *Anaplasma phagocytophilum* complex). Furthermore, complementing results in genetic improvement in mosquitoes (genetic markers, sexing, genetic sterilization and fail-safe systems) will also increase performance as it has already been shown in field applications in developing countries. Recent results have greatly improved the fitness of genetically modified insects compared to wild type populations with new approaches such as the post-integration elimination of transposon sequences, stabilising any insertion in genetically modified insects. Encouraging results using the Sterile Insect Technique (SIT) highlighted some metabolism manipulation to avoid the viability of offspring from released parent insect in the wild, if necessary. Recent studies on vector symbionts would also bring a new angle in vector control capabilities. These new potential approaches will improve the levels of control or even in some cases would eradicate vector species and consequently the vector-borne diseases they can transmit. This paper will review the work on different genetic approaches to understand host/pathogen interface in vectors and new genetically modified techniques used to control them.

**Keywords:** Ticks; Mosquitoes; Biodiversity; Genetic flow; Genetic manipulations

#### (8) A comparative study of genetic lineages of dengue vectors, *Aedes aegypti* and *Aedes albopictus*, from Thailand and France

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In order to compare the genetic diversity among populations of two main vectors of Dengue in Thailand, *Aedes aegypti* and *A. albopictus*, were collected from several sites including urban, rural and forested areas of Bangkok, Nakhonpathom, KhonKaen,

ChiangMai and Kanchanaburi Province. Various methods were used such as ovitrap, landing catch and/or aspirator. In addition, *A. albopictus* from France was also collected and manipulated compared with the Thai populations. All adult mosquitoes were species identified and kept at  $-20^{\circ}\text{C}$  until processed. DNA extraction was carried out using a classical extraction buffer as previously described (Collins, 1987). Molecular characterization and genetic lineages identification were done, among all collected sample populations, by using three genetic markers including the mitochondrial NADH dehydrogenase subunit 5 (ND5) fragment, the nuclear ribosomal DNA second internal transcribed spacer region (ITS2) and, the mitochondrial cytochrome oxidase I (COI). Aligned sequences of *A. aegypti* and *A. albopictus* genes from six localities were compared pairwise. The preliminary result showed marked differences in nucleotide composition among *Aedes* mosquito populations of Nakhonpathom Province as compared to the others. This study reveals information on divergence of dengue vector from endemic areas and will help to understand vector competence and efficiency in transmitting the virus. Furthermore, it will serve as an informative knowledge on the species dispersal modalities and mean for implementing control strategies.

**Keywords:** Genetics; *Aedes aegypti*; *Aedes albopictus*; Thailand; France

#### “Student symposium”

##### (9) Molecular characterization of Thai *Ehrlichia canis* and *Anaplasma platys* strains isolated from dogs

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Canine monocytic ehrlichiosis caused by *Ehrlichia canis* is veterinary importance worldwide. In Thailand, there has been little information available on *E. canis* and its phylogeny. The objective of this study was to characterize and establish molecular structure and phylogeny of Thai *Ehrlichia* and *Anaplasma* strains. *Ehrlichia*-positive blood samples of dogs were extracted for genomic DNAs. 1.5 kb PCR products of 16S rRNA gene were obtained using designed genus-specific primers for *Ehrlichia* and *Anaplasma*. Nearly complete sequences of the 16S rRNA gene were compared with other sequences available in the Genbank database. Percentage of similarity as well as secondary structure analysis of 16S rRNA sequences indicated that they are new *E. canis* and *A. platys* strains. Phylogenetic analysis revealed that two strains of Thai *E. canis* were closely related and formed a single cluster within the cluster amongst previously published *E. canis* from different countries. *A. platys* found in this study showed close relation-

ship with earlier report of *A. platys* in Thailand. This report represents the first molecular characterization of *E. canis* in dogs from Thailand.

**Keywords:** *Ehrlichia canis*; *Anaplasma platys*; PCR; Thailand; Dogs

#### (10) *Leishmania braziliensis*: Population structure and reproductive modes

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Leishmaniasis are severe diseases affecting humans and animals caused by protozoan parasites belonging to the *Leishmania* genus and transmitted by female sandflies' bites. These parasitoses are widespread over all continents, except Antarctica. Nowadays, leishmaniasis still pose considerable public health problems. At present, it is suggested and even admitted that *Leishmania* species present a basically clonal population structure associated to rare sexual recombination events. However, such a statement mostly relies on population genetics studies that may be criticised. The markers used were little adapted (lack of resolution or dominant markers) and clonality was inferred from the analysis of linkage disequilibria across loci that are far from ideal in that respect. *Leishmania braziliensis* is an important leishmaniasis agent in South America. The principal objective of our work was to study the population structure and reproductive mode of this species in Peru and Bolivia and, for the first time, using microsatellite markers. On the whole, 124 human isolates (68 from Peru and 56 from Bolivia) were genotyped on 12 microsatellite loci. Various population genetics tests were applied. The results obtained appear in contradiction with a simple clonal propagation. Indeed, strong homozygosities found at each locus, associated to strong linkage disequilibria across loci, advocated for an inbred reproductive strategy. Further analyses suggest that a significant part of the high heterozygote deficits observed in our samples is likely the consequence of a Wahlund effect, i.e. the coexistence of strongly differentiated genetic entities within each sample. This work brings key information concerning the biology of these organisms and opens new prospects on the study of this species and other members of the genus.

#### (11) Clonal strains of *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis

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Cystic fibrosis (CF) is the most common genetic condition among Caucasians. Eighty percent of people with CF are infected with *Pseudomonas aeruginosa* by adulthood and most die from complications arising from chronic lung infections. *P. aeruginosa* is widespread in various environments including hospitals. It was once generally accepted that individual CF patients acquired *P. aeruginosa* from their environments and thus each patient carried their own unrelated (or unique) strain. Recently however, clonal (or epidemic) strains have been reported in Europe and Australia. In this study, DNA restriction fragment length polymorphisms (RFLP) of *P. aeruginosa* isolates from 112 patients attending an adult CF clinic at Royal Prince Alfred Hospital in Sydney, Australia were analysed using pulsed-field gel electrophoresis (PFGE). Cluster analyses were performed using computer-aided software (GelCompar II). Isolates sharing a similarity greater than 85% defined by the Dice coefficient with a position tolerance of 1.0% and an optimisation of 1.0% were considered as a clone. The DNA fingerprint of each isolate from the same clone had up to a three-band difference, which confirmed their close relatedness. Two major clones, AES-1 and AES-2, were isolated from 38% and 5% of 112 patients, respectively. There is a 66% similarity between AES-1 and AES-2 clones. The AES-1 strain had an identical DNA banding pattern with a previously reported Melbourne epidemic strain. The AES-2 strain was also identical to an epidemic strain reported from Brisbane known as a Pulsotype II. AES-1 isolates were significantly more resistant to gentamycin, amikacin and Timentin<sup>®</sup> than non-clonal isolates reflecting treatment difficulties. These strains have not been detected from the environment suggesting that person-to-person transmission may play a role in such cases. These results have led us to implement a segregation policy in our clinic as well as emphasising the important role of molecular typing in infection control.

#### (12) Influenza: An idea model bridging epidemiological and evolutionary dynamics

Zhenggang Wang, Chung-Chau Hon, Tsan-Yuk Lam, Fanya Zeng, Frederick Chi-Ching Leung

Annual outbreaks of influenza cause substantial morbidity and mortality, and also cause heavy economic losses. In recent years the threat of a human influenza pandemic has increased considerably as humans have become susceptible to infection by the avian influenza virus H5N1. However, our current understanding of influenza and the ability to evaluate the threat are limited. Several important issues, including the influence of climate variability on influenza epidemic patterns and intraspecific and interspecific interactions between various circulating influenza types, subtypes and strains, have not yet been sufficiently studied. In this study, we explored the immunological dynamics and epidemiological dynamics of influenza using our host immune unit-virus-susceptible (HVS) model. By matching model output to epidemiological patterns identi-

fied in surveillance data collected from United States, we found that three types of interspecific competitions (between influenza A and B, influenza A subtypes H1 and H3, and new and circulating strains) are essential to depict phylogenetic patterns of influenza. The study therefore, illustrates the population dynamics of the emergence, circulation and elimination of new influenza variants (subtypes or strains).

**Keywords:** Influenza; Co-circulation; Predator–prey model; Epidemiological dynamics; Evolutionary dynamics

## Symposium “Phylogeny of pathogens”

### (13) The evolution of gene overlap in RNA viruses

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Two of the most striking attributes of RNA viruses are their small genome size (the average RNA virus is only 9 kb long) and their high mutation rate. It has been argued that these attributes are linked, with genome size being limited by the accumulation of deleterious mutations in a long stretch of RNA. Genome size is also linked to mutational robustness, which can be thought of as the local gradient of the adaptive landscape around its peak. Sharper peaks represent less robust genomes where mutations have a proportionately greater negative effect on fitness. Viruses with larger genomes should evolve to be more mutationally robust because on average they can expect to experience more mutations per round of replication. We investigate the relative mutational robustness of RNA viruses by measuring the amount of gene overlap. Most RNA viruses have some nucleotides that code for more than one protein by being in two overlapping reading frames. In such viruses, some mutations will therefore affect more than one gene and hence will have an increased negative effect on viral fitness. We analysed the sequences of 700 viral species, correcting for phylogenetic non-independence, and found that – as predicted – gene overlap is strongly negatively correlated to genome length. Furthermore, in the relative abundance of different frameshifts, we find evidence for two evolutionary processes having been at work: new genes being created in other frames within older genes, and creeping overlap between originally contiguous genes that happen to be in different frames. We propose two simple evolutionary models to explain these processes.

### (14) Phylogenetic and antigenic analysis of *Orientia tsutsugamushi* isolated from scrub typhus patients in Thailand

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*Orientia tsutsugamushi* is the causative agent of scrub typhus infection and is a major cause of human disease in rural areas of South East Asia. Seventeen in vitro isolates of *O. tsutsugamushi* from patients with scrub typhus disease in north-eastern and western Thailand between 2003 and 2005 were examined to determine phylo-temporal and phylo-geographic relationships between the samples. Implications for diagnosis were also investigated. Phylogenetic analysis of the entire 56 kDa-type-specific antigen gene (~1.6 kb) found that the majority (11/17; 64.7%) of the isolates clustered within Karp genotype, and 4 of 17 (23.5%) isolates within the Gilliam/Kawasaki cluster. Two isolates grouped with each of the historical Thai isolates TA763 (Karp-like) and TA716 (Kato-like). Two-dimensional cross-binding of patient antibody reactivity against *O. tsutsugamushi* isolate antigens demonstrated relationships similar to 56 kDa gene nucleotide sequence results with distinct differences between the binding of Gilliam/Kawasaki antibodies and Karp antigens. Results from 56 kDa genetic analysis demonstrates a Karp type strain dominance similar that reported in studies from 1960s and 1970s. There were no clear geographical associations from this study however more isolates are required to confirm this observation. The majority of scrub typhus vaccine candidates are based on the 56 kDa protein of Karp type strain and the results presented here demonstrate that Karp type strain should be a major component of a future vaccine however it is unclear what is the efficacy of such a vaccine with other type strains?

### (15) Sequence analysis of the C-terminal region of merozoite surface protein-1 of *Plasmodium falciparum* (PfMSP-119) and *P. vivax* (PvMSP-119) as vaccine candidate antigens among Iranian clinical isolates

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In this study we analyzed the sequence variation of the C-terminal region of merozoite surface protein-1 of *Plasmodium falciparum* (PfmSP-119) and *P. vivax* (PvmSP-119) genes as the most promising blood stage vaccine target antigens, in 70 *P. vivax* and 92 *P. falciparum* infected blood samples collected from areas with different malaria endemicity in Iran. The presence of polymorphism in this region may compromise its use as a vaccine candidate. All *P. vivax* samples have shown 100% conserved sequences among northern and southern isolates, however the MAD20 allele was found significantly among *P. falciparum* clinical isolates in south. Furthermore, MAD20 allelic type showed four different allelic forms, while the K1 allelic type

showed no polymorphism. These results are discussed with regards to evaluation of these vaccine antigens in both malaria species, and in compare with the studies that were conducted in other areas in Southeast Asia, and Africa. Such study would complement this information and would allow comparing the Iranian *P. falciparum* and *P. vivax* populations with those found in distinct and contrasting epidemiological settings.

#### (16) Sequence variation in the T-cell epitopes of the *Plasmodium falciparum* circumsporozoite protein in Iranian clinical isolates

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The first subunit malaria vaccine tested in humans was based on the *Plasmodium falciparum* circumsporozoite protein (PfCSP). The T-cell helper epitopes Th2R and Th3R found in the carboxyl-terminus region included in some vaccine formulations, showed sequence diversity which could be a potential problem for vaccine efficacy. The aim of this study was to define the nature and extend of *PfCSP* genetic polymorphism in isolates collected from patients in Iran. The data would complement information obtained in other endemic settings. A total of 21 complete and 69 partial *PfCSP* sequences were derived from isolates collected in the south-eastern hypoendemic area of Iran. Although nine different allelic forms were observed in the 21 complete sequences, they were mainly due to variation in the repeat units number and arrangement, whereas only two haplotypes were noted for the combined Th2R/Th3R epitopes, for each of which only two allelic variants were noted. Comparison of the 3'-end region of all 90 sequences revealed only one more Th2R variant, and a total of five combined haplotypes of which three were dominant, and two only found in a minority of samples collected from non-Iranian patients who acquired the infection abroad. Thus, the *PfCSP* gene of the parasites circulating in Iran displays a very low level of diversity. These results contrast with observations made in Africa, but are akin to those observed in other regions (Papua New Guinea, Thailand and Brazil).

#### Symposium "Coevolution host pathogen 2"

##### (17) The analysis of candidate genes and their influence on tuberculosis susceptibility in a Canadian Aboriginal population

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Despite the ability to treat tuberculosis and the continued decline of mortality rates, tuberculosis continues to have a detrimental impact on the quality of life for many Aboriginal people in northern Canada where morbidity rates remain high. In a small isolated community, the rate of tuberculosis is 60 times higher than that of the average Canadian Aboriginal population. Previous research demonstrated that significant differences exist in the frequency of cytokine gene polymorphisms maintained by distinct Aboriginal and Caucasian populations in Manitoba. The Dené are a discrete Aboriginal cultural group and recent analysis has shown that this group maintains a high frequency of cytokine gene polymorphisms (TNF $\alpha$ , IL-6, IFN $\gamma$ , IL-10, TGF $\beta$ ) related to an effective Th2 immune response but a less effective Th1 response to infectious diseases. In addition, the Dené have a high frequency of gene polymorphisms in the Vitamin D Receptor gene which may in part, contribute to their susceptibility to tuberculosis. This presentation will describe the analysis of a panel of purported tuberculosis-susceptibility genes (Vitamin D receptor and cytokine SNPs) from a northern Canadian Dené cohort. The Dené have a unique history and prehistory in relation to other northern Canadian Aboriginal populations and as a result they have preserved their cultural identity and along with that, their distinct immunogenetic profile that is well adapted to a specific pathogen environment.

##### (18) Malaria *Plasmodium* agent induces alteration in the head proteome of their *Anopheles* mosquito host

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Host behavioural changes induced by parasites that increase the likelihood of parasite transmission has long captured the interest of parasitologists and behavioural ecologists. For instance, in pathogens—insect vector systems, several studies support the idea that pathogen manipulates the behaviour of their vectors, such as feeding behaviour, in a way that increase the contact with the vertebrate host and hence favour the parasites' transmission. Despite increasing evidence of such behavioural changes, the underlying mechanisms causing infected vectors to act in ways that benefit pathogen transmission remain enigmatic in most cases. Here, 2D difference gel electrophoresis coupled with mass spectrometry were employed to analyse and compare the head proteome between malaria (*Plasmodium berghei*) infected mosquitoes and uninfected mosquitoes (*Anopheles gambiae*). This proteomics

approach detected 12 protein spots in two cohorts of mosquitoes with altered levels in the head of sporozoite infected individuals. These proteins were subsequently identified using mass spectrometry and functionally classified as metabolic, synaptic, molecular chaperone, signalling, and cytoskeletal proteins. Our results indicate an altered energy metabolism in the head of sporozoite infected mosquitoes. Some of the up/down regulated proteins identified such as synapse associated protein, 14-3-3 protein, and calmodulin have previously been shown to play critical roles in the central nervous system of invertebrates and vertebrates. Furthermore, a heat shock response (HSP 20) and a variation of cytoarchitecture (tropomyosins) have been evidenced. These proteins shed light on potential molecular mechanisms underlying behavioural modifications and offer new insights into the study of intimate interactions between *Plasmodium* and its *Anopheles* vector.

**Keywords:** Malaria; Mosquitoes; Host–parasite systems; 2D difference gel electrophoresis; Mass spectrometry

### (19) The ORF2 glycoprotein of hepatitis E virus retrotranslocate from the endoplasmic reticulum to the cytoplasm and down-regulates NF- $\kappa$ B activity in human hepatoma cells

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NF- $\kappa$ B is a key transcription factor that has been implicated to play a crucial role in host survival during infection by pathogens. Therefore, it has been a priority of many pathogens to manipulate the cellular NF- $\kappa$ B activity in order to create a favorable environment for their survival inside the host. In this report, we provide evidence for a novel mechanism of inhibition of NF- $\kappa$ B activity, which is mediated by the major capsid (ORF2) protein of the hepatitis E virus. Heterologous expression of the ORF2 protein in human hepatoma cells was found to inhibit I $\kappa$ B $\alpha$  ubiquitination by interfering with the assembly of the SCF <sup>$\beta$ TRCP</sup> complex, thus resulting in stabilization of the cellular I $\kappa$ B $\alpha$  pool, with a concomitant reduction in the activity of NF- $\kappa$ B and its downstream targets. NF- $\kappa$ B inhibitory activity exhibited by the ORF2 protein was found to depend on its ability to retrotranslocate from the endoplasmic reticulum (ER) to the cytoplasm, where it was observed to be stably present. Further, retrotranslocation of the ORF2 protein was dependent upon the glycosylation status of the protein, mediated in a p97 dependent pathway and independent of ubiquitination of the former. The ORF2 protein, therefore, exploits the ER associated degradation pathway to gain access to the cytoplasm, where it interferes with the I $\kappa$ B $\alpha$  ubiquitination machinery, leading to inhibition of host cell NF- $\kappa$ B activity.

**Keywords:** Retro-translocation; NF- $\kappa$ B; ER stress; ERAD pathway; I $\kappa$ B $\alpha$  ubiquitination; ORF2 protein of hepatitis E virus

### (20) Development of a novel immunome-based *Candida* vaccine

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*Candida albicans* is one of the most important opportunistic dimorphic fungi responsible for hospital acquired fungal infection in humans. Generally, candidiasis rarely occurs in healthy individuals but it is frequently associated with patients who receive immunosuppressive drug therapy or long-term catheterization and patients who suffer from AIDS (Navarro-Garcia et al., 2001). To date, there are neither effective vaccines nor therapeutic protocols to eradicate these fungal infections. We therefore utilized immunomics approach to assist in the identification of the fungal immunoprotective epitopes. First, a molecular database of *C. albicans* virulence factors called CandiVF was developed (URL <http://antigen.i2r.a-star.edu.sg/Templar/DB-CandiVF/>). The database contained 153 virulence proteins of *C. albicans*. It also provided a T-cell epitope predictive algorithm called *Hotspot Hunter* which was previously developed by our group. *Hotspot Hunter* facilitated the prediction of promiscuous peptides that can bind specifically to eight supertypes of HLA-DRB1 (DRB1\*0101, \*0301, \*0401, \*0701, \*0801, \*1101, \*1301, \*1501). In order to verify T-cell epitope prediction by *Hotspot Hunter*, secretory aspartyl proteinase 2 (Sap2) was selected as a study model. Sap2 was a *C. albicans* common antigen during infection and capable of inducing IgE-mediated allergic reaction in atopic individuals (Suenobu et al., 2002). Primary sequence analysis revealed that Sap2 contained two different sequences, groups 1 and 2. A total 40 conventional overlapping peptides of Sap2 (20-mer overlapped 10-mer in length) were then synthesized. All peptides were used to stimulate peripheral blood mononuclear cells from HLA-DRB1 specific blood donors to determine the proliferative response. Eleven of eighteen peptides within the prediction areas were able to induce PBMCs proliferation. However, when anti-IL-2 ELISpot assay was used to confirm the cell proliferation result, only two of eleven peptides stimulated significant T-cell activation. Outside the predictive areas, peptide 11 could induce proliferation of IL-2 producing clone in one donor of HLA-DRB1\*04/04. The use of immunomics can assist the identification of immunoprotective epitope and the development of a potential peptide-based vaccine.

**Keywords:** Immunome; Immunomics; T-cell epitope; Database and *Candida albicans*

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## Symposium “A revolutionary way to detect pathogens: Epidemiological and clinical Importance of ultra-sensitive detection of endemic and emerging diseases”

### (21) New revolutionary ultrasensitive technique to detect pathogens based on their capture and concentration by ApoH coated nano-magnetic beads

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Despite remarkable advances in medical research and treatments during the 20th century, infectious diseases remain among the leading causes of death worldwide. The main causes for this phenomenon are: (1) emergence of new infectious pathogens; (2) re-emergence of old infectious diseases have reappeared after a significant decline in incidence; and (3) persistence of intractable infectious pathogens. Indeed, the previous decades have been marked by several striking episodes of emerging and re-emerging pathogens such as HIV, Marburg virus, Hepatitis C virus, Hantavirus, Ebola virus, West-Nile virus, Dengue virus, Yellow Fever virus and more recently SARS coronavirus and avian flu. New infectious diseases continue to evolve and emerge. According to the Center for Disease Control and Prevention in Atlanta 70% of emerging infectious diseases in humans are zoonotic pathogens. In order to anticipate the measures to be taken to prevent or control future epidemics, considerable attention has recently been directed to emerging and re-emerging infections at national and international level (<http://euro.who.int/surveillance>).

To adopt the appropriate containment measures towards emergent pathogens, fast, sensitive and reliable diagnostics are key element. Nucleic acid amplification is widely used for the detection and identification of pathogens. One of the main problems for pathogens detection in clinical but also in environmental samples is that they generate false negative results. This problem is mainly due to three reasons: presence of inhibitors, absence of a universal extraction method, lack of a rapid and reliable pathogen concentration methodology. The above-mentioned disadvantages would be compensated by the use of a very sensitive method consisting of a matrix-bound APOH which has a particular property to fix a broad panel of pathogens. Interestingly, APOH strongly interacts with various viruses such as, HBV, HCV, orthopoxviruses, Dengue virus, Hantavirus, H5N1, West Nile which are either endemic or emerging diseases in South-East Asia.

### (22) Capture and concentration of orthopox virus using ApoH coated nanomagnetic beads for ultrasensitive detection

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Major obstacles for the detection of pathogens in clinical or environmental samples are false negative results. This is mainly due to the lack of a rapid and reliable pathogen concentration methodology that allows detection of highly diluted samples, and the inability of most of the currently used technologies to eliminate or neutralize interfering “natural inhibitors” that could be present in biological samples. In order to improve virus diagnostics we wanted to exploit the “non-self” recognition and binding properties of human apolipoprotein H (ApoH). ApoH binds and captures pathogens enabling their concentration from different kinds of biological samples. We have used magnetic beads coated with ApoH recombinant protein as a pre-treatment step for orthopox viruses to improve the detection threshold and to increase the sensitivity for diagnosis. With this approach virus was concentrated, DNA was extracted and subsequently detected and quantified by real-time PCR. After ApoH-treatment, Vaccinia Virus was detected from highly diluted samples where diagnosis had been negative with a standard DNA preparation protocol. At present the concentration and improved detection of other viruses with an ApoH-enhanced protocol is under investigation.

### (23) ApOH-capture technology enhances Andes hantavirus detection allowing virus concentration from plasma and urine samples of patients with acute hantavirus cardiopulmonary syndrome

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**Background:** Hantavirus cardiopulmonary syndrome (HCPS) is an emerging disease caused by new world hantaviruses. Hantaviruses (HV) are segmented RNA viruses belonging to the genus *Hantavirus* in the family *Bunyaviridae*. Hantavirus (HV) infections are mainly transmitted to humans by inhalation of virus-contaminated aerosols of rodent excreta and secretions, however, sporadic person-to-person transmissions of the Andes hantavirus (ANDV) have been reported. Based on the knowledge that human apolipoprotein H (ApoH), a constituent of human plasma, interacts with viral proteins, we wished to assess a possible interaction between ApoH and ANDV, the major etiological agent HCPS in South America.

**Materials and methods:** Blood and urine samples from acute-HCPS patients were selected on the basis of their availability. Samples collected as part of the research initiative NIH/NIAID #AI 45452 were kindly supplied for this study. Donor patients met the clinical criteria for HCPS and harbored IgM antibodies reactive with hantavirus antigens. HV genomic RNA was confirmed in plasma by an in-house developed RT-PCR/hemi-nested PCR, using primers designed to partially encompass the S segment ORF of the Andes virus, strain CHI-7913. Samples used as negative control were collected among the laboratory staff. ApoH-coated magnetic beads and ApoH-coated ELISA plates used in this study were supplied by ApoH Technologies S.A. and used following their instructions.

**Results:** We report that ANDV interacts with ApoH, and that ApoH-coated magnetic beads or ApoH-coated ELISA plates can be used to capture and concentrate virus from serum and urine samples, allowing virus detection by both immunological and molecular approaches. We then developed an ANDV-high throughput screen assay and assessed ANDV in urine samples, from 50 patients with acute ANDV-HCPS, collected during 5 days following hospitalization. 45 patients showed detectable amounts of ANDV in urine in at least one tested sample.

**Conclusions:** ApoH capture assay increases the sensitivity of virus detection by both molecular and immunological methods. This apparent enhancement in sensitivity most probably stems from the fact that virus is being concentrated from a larger sample volume. Additionally, we demonstrate that ANDV can be shed in the urine of infected individuals. Although, our data do not necessarily predict the presence of infectious virus in urine, the fact that ANDV is readily detected in urine samples of acute-HCPS patients not only lends support to the possibility that urine is a route for person-to-person transmission of HCPS but also raises the intriguing prospect that virus might be present in other biological secretions.

#### Acknowledgments

This study was supported by the Jeunes Equipes Associées à l'IRD program to MLL, and by US PHS grant number AI 45452 to MF. MLL is member of the Núcleo Milenio en Inmunología e Inmunoterapia.

#### (24) Elsevier reviewer workshop

Michel Tibayrenc, Bas Straub

### Symposium “Epidemiology and genomics of HIV”

#### (25) Host genetic polymorphisms, HIV variability resistance to infection and disease progression

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Research on host and viral factors associated with susceptibility to HIV-1 infection, disease progression, and response to anti-retroviral therapy has proved critical for designing efficient interventions. HIV entry into cells results from complex interactions between env gp120 with CD4 and co-receptors. CCR5, the major receptor for cytokines, is also the main co-receptor for macrophage-tropic R5 strains. The CCR5-delta32 homozygous deletion and the CCR5-m303 variant, have been associated with resistance to HIV infection, and CCR5-delta32 heterozygosity with delayed disease progression. Also, the minor co-receptor CCR2 64I variant has been associated with delayed progression. The effect on progression of SDF1 polymorphisms, a ligand for the chemokine receptor CXCR4, remains controversial. With regards to innate or specific host immune response to HIV, while there are conflicting results on the role of neutralizing antibodies, studies have shown an association between HLA class I alleles and natural resistance to infection or disease progression. Discordance between maternal and infant HLA genotypes may have a protective effect. Response to antiretroviral drugs involves both host genetics and HIV variation. While the ability of HIV to mutate and escape drug pressure varies with each drug's specific mechanism of action, human polymorphisms have been associated with increased toxicity of some antiretroviral drugs. Until today, the challenge has been to demonstrate the clinical significance of identified polymorphism following a pathogenesis hypothesis driven approach (gene/pathway candidates). Following completion of the human genome sequencing, numerous SNPs have been identified whose biological significance remains unknown, and the challenge is to discover associations between such SNPs and characterized phenotypes. Understanding a polymorphism functional significance becomes the next step. Discordant couples, transmitting mothers/partners, rapid progressors under therapy or long term untreated non-progressors are phenotypes which have been widely used. In the rapidly evolving field of HIV medicine, defining in large/diverse populations stable/unambiguous phenotypes is increasingly difficult.

#### (26) Synonymous substitution rates predict HIV disease progression as a result of underlying replication dynamics

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To investigate HIV evolution in relationship to disease progression, we developed a new computational technique that can estimate changes in the absolute rates of synonymous and nonsynonymous divergence through time from molecular sequences. This allows separating changes in generation time



and mutation rate from changes in selective pressure and effective population size. Using this technique, we have identified a previously unknown association between the 'silent' evolutionary rate of HIV and the rate of disease progression in infected individuals. This finding demonstrates that cellular immune processes, which are already known to determine HIV pathogenesis, also determine viral replication rates and therefore impose important constraints on HIV evolution. Humoral immune responses, on the other hand, are the major determinant of nonsynonymous rate changes through time in the envelope gene, and our relaxed clock estimates support a decrease in selective pressure as a consequence of immune system collapse.

**(27) The presence of anti-R7V antibodies in HIV-1 infected patients: A novel efficient marker for the non-progression to AIDS**

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**Background:** Concerning HIV life cycles, it is well known that the HIV acquires a cellular antigen when the virus is released from the host infected cell. The epitope is defined as a peptide, seven amino acids (RTPKIQV; R7V). The previously study reported that the presence of antibodies directed to the peptide R7V in HIV infection correlated with the non-progression to AIDS.

**Objectives:** To determine the correlation between the anti R7V antibodies in Thai treated-naive HIV-1 infected patients and the clinical status.

**Methods:** A retrospective study was carried out in 124 normal population and 128 Thai treated-naive patients, infected with HIV-1 for more than 5 years. Presence of anti R7V antibody was detected by the anti R7V ELISA. OD was read at 450 nM.

**Results:** We found that treated-naive HIV-infected patients presented anti-R7V antibodies at higher level than in normal population (32.8% and 0.8%, respectively;  $P < 0.001$ ). Relative to clinical status of patients, the frequency of positive anti R7V antibodies level had significantly higher in non-progressors and moderate progressors (100% and 49%, respectively;  $P < 0.001$ ). We did not find anti R7V antibodies in rapid progressors. These results demonstrated a strong correlation between the presence of anti-R7V antibodies and a good prognostic status of Thai HIV-1 infected patients.

**Conclusion:** This study provides the strongest evidence to date for the presence of the anti R7V antibodies in non-progression of HIV infection.

**Keywords:** Progression markers; Anti-R7V antibody; Non-progression; HIV-1 infected patients; ELISA; AIDS

**(28) Origin of HIV-1**

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West central African chimpanzees (*Pan troglodytes troglodytes*) are now recognized as a natural reservoir of simian immunodeficiency virus (SIVcpz) and the immediate source of at least two human cross-species infections: (i) HIV-1 group M, the pandemic form of human immunodeficiency virus type 1, and (ii) HIV-1 group N, thus far identified only in a few AIDS patients from Cameroon. A third lineage, HIV-1 group O also falls within the SIVcpzPtt radiation, but the ape reservoir of this virus has not yet been identified. First, we report here, the geographic distribution and the genetic diversity of groups M- and N-like viruses in wild chimpanzee communities in southern Cameroon and secondly the detection and molecular characterization of SIVs closely related to HIV-1 group O in wild-living gorillas (*Gorilla gorilla*) in the same country. More than 1300 ape fecal samples were collected at 18 remote forest sites in Cameroon. Overall, 62% were from chimpanzees and 21% were from gorillas. The remainder were found to be degraded or from other primate/mammal species following mitochondrial DNA analysis. All were tested for HIV crossreactive antibodies using a commercial HIV-1/2 confirmatory assay. Thirty three different *Pan troglodytes troglodytes* apes were found to be SIV-infected and six samples, corresponding to three different gorillas (as determined by microsatellite analysis) contained antibodies reactive with the HIV-1 envelope glycoprotein (gp41). Fecal RNA was isolated and partial pol and/or gp41 sequences were amplified by RT-PCR. Phylogenetic analysis of these SIV sequences showed that the 33 newly identified SIVcpz and the 3 SIVgor strains fall within the HIV-1/SIVcpzPtt radiation. The identified SIVcpz strains were characterised by a high genetic diversity and a phylogeographic clustering. The latter allowed us to trace the origins of HIV-1 group M and group N to distinct chimpanzee communities in southern Cameroon. Phylogenetic analysis of the 3 SIVgor strains revealed a monophyletic lineage within the SIVcpzPtt radiation which was most closely related to HIV-1 group O. We also confirmed further absence of SIVcpz infection in 78 samples from *Pan troglodytes vellerosus*.

These findings showed that chimpanzees likely served as the primary reservoir of SIVs now found in chimpanzees, gorillas and humans. HIV-1 groups M and N clearly arose by transfer of viruses from chimpanzees to men, while the origin of HIV-1 group O is less clear. Chimpanzees could have transmitted group O-like viruses to gorillas and to humans independently, or they could have transmitted the virus first to gorillas, which in turn transmitted it to humans.

**Symposium "Epidemiology and evolution of malaria"**

**(29) Molecular detection of malaria parasite *Plasmodium falciparum* in a member of *Anopheles hyrcanus* group from northern Iran**

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Nested polymerase chain reaction which targets the conserved 18S small-subunit RNA genes of parasite, not only permits a malaria infection in *Anopheles* mosquitoes to be detected, but also allow each *Plasmodium* species present to be detected. Mosquitoes were collected from Guilan province in northern Iran. After morphological identification and dissection, head and thorax of 197 pools (985 individual mosquitoes) of *Anopheles maculipennis* complex, *Anopheles sacharovi* and *Anopheles hyrcanus* group were used for DNA extraction. PCR amplified a 205 bp fragment of *Plasmodium falciparum* in one pool of *An. hyrcanus* group specimens from Fooman district. The PCR method shows greater sensitivity and specificity and confirmed the existence of *P. falciparum* in *An. hyrcanus* group that so far has been considered as non-vector in Iran. This unexpected presence of *P. falciparum* in *Anopheles hyrcanus* population urges prompts investigation and standardization of control methods in Islamic Republic of Iran, Azerbaijan and Armenia.

**Keywords:** *An. hyrcanus* group; *P. falciparum*; Malaria; Guilan; Iran

### (30) Molecular epidemiology of *Plasmodium falciparum* resistance to antimalarial drugs in Iran

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As chloroquine (CQ) resistance spreads across Iran, the sulfadoxine/pyrimethamine (SP) combination therapy with CQ are being used as alternative first-line drugs for the treatment and prevention of *Plasmodium falciparum* malaria. We determined the genotype of three different genes, *P. falciparum* chloroquine resistance transporter (*pfcr*), dihydrofolate reductase (*dhfr*), and dihydropteroate synthase (*dhps*) in 208 clinical *P. falciparum* isolates (pre-treatment and post-treatment) from Iran, which were collected during 2003–2005. DNA was isolated and analyzed by using polymerase chain reaction and restriction fragment length polymorphism (PCR/RFLP) to detect polymorphisms previously shown to be associated with resistance. The results showed that 77% of field isolates carried parasites with double mutant alleles of *pfdhfr* (C59R + S108N), while

retaining a wild type mutation at position 51 and all *pfdhps* (S436F/A, A437G, K540E and A581E). 18.7% of field isolates carried parasites with double mutant alleles of *pfdhfr* (C59R + S108N) and single mutant alleles of *pfdhps* (A437G). Limited and stable polymorphism over the time particularly in *pfdhps* revealed that the SP is still effective as antimalaria drug in Iran. Furthermore the putative key codons of novel candidate gene for chloroquine resistance, *Pfcr* was determined and the high levels of CQ pressure have led to strong selection of the *pfcr*76T, 220S and 326S polymorphism among *P. falciparum* isolates in Iran. These results may have important implications for the future surveillance of both CQ and SP resistance by the use of molecular markers in Iran.

### (31) Epitope mapping and analysis of sequence variation in VAR2CSA DBL3X involved in *P. falciparum* placental sequestration

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Pregnancy-associated malaria (PAM) is a major health problem, which mainly affects primigravidae living in malaria endemic areas. The syndrome is precipitated by accumulation of infected erythrocytes in placental tissue through an interaction between chondroitin sulphate A (CSA) on syncytiotrophoblasts and a parasite encoded protein on the surface of infected erythrocytes, believed to be VAR2CSA. Women in endemic areas develop immunity to PAM and anti-VAR2CSA antibody levels correlate with protection. VAR2CSA is a polymorphic protein of approximately 3000 amino acids forming six Duffy-binding-like (DBL) domains. For vaccine development it is important to define the antigenic targets for protective antibodies and to characterize the consequences of sequence variation. In this study, we show that the VAR2CSA DBL3X domain mediates binding to CSA which makes it a leading vaccine candidate. We characterize sequence variation in the DBL3X domain, comprising single nucleotide polymorphisms, deletions and variable number of tandem repeats, using Bayesian inference of selection pressure and recombination rate analysis. Combination of these results with structural modelling, shows that sequence variation mainly occurs in regions under strong diversifying selection, predicted to form flexible surface loops. From peptide array data we show that these regions are main targets of naturally acquired IgG and accessible for antibodies reacting with native VAR2CSA on infected erythrocytes. Interestingly, surface reactive anti-VAR2CSA antibodies also target a conserved DBL3X region predicted

to form an  $\alpha$ -helix. Finally, we identify DBL3X sequence motifs that are more likely to occur in parasites isolated from primi- and multigravidae, respectively. These findings strengthen the vaccine candidacy of VAR2CSA and will be important for choosing epitopes and variants of DBL3X to be included in a vaccine protecting women against PAM.

**Keywords:** Malaria; VAR2CSA; Pregnancy; Vaccine; PfEMP1; DBL; Selection; Recombination; pepScan; Epitope

### (32) Polymorphisms in the genes of interleukin 12 and its receptors in association with resistance to severe malarial anemia in children residing in western Kenya

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Of the more than 1 million Africans who die from *Plasmodium* infection each year, most are children under five years of age. The majority of these deaths are from severe malarial anemia. *Plasmodium falciparum* has been shown to drive selection of human genetic variants for conferring protection against severe forms of malaria, such as severe malarial anemia (SMA). Malarial anemia is characterized by the destruction of malaria infected red blood cells and suppression of erythropoiesis. Recent studies in murine models of malarial anemia have demonstrated that interleukin 12 (IL12) significantly boosts erythropoietic responses. Furthermore, several immunological studies conducted in Africa have shown that IL12 production was suppressed in children with SMA compared to asymptomatic children. For these reasons the genes encoding the two IL12 subunits, IL12A and IL12B, and its receptors, IL12RB1 and IL12RB2, are attractive candidate genes for studying SMA. In this study, a total of 75 tagging single nucleotide polymorphisms (tagSNPs) covering these four genes were examined. Genotyping was performed with the iPLEX MassARRAY technology (Sequenom) in a cohort of 940 children from the Asembo Bay region of western Kenya, an area with intense malaria transmission. Individuals possessing two copies of IL12A common allele (rs2243140) at 3'UTR showed increased susceptibility to SMA (Hb < 6 g/dl and the presence of *P. falciparum* > 10,000/ $\mu$ L) ( $p = 0.009$ , RR 1.85, 95%CI: 1.10–3.10). Individuals possessing two copies of a rare variant in IL12RB1 (rs429774) appeared to be strongly protected against SMA ( $p = 0.0002$ , RR 0.24, 95%CI: 0.08–0.72) and overall severe anemia ( $p = 0.002$ , RR 0.49, 95% CI: 0.29–0.82). Identification of genetic polymorphisms that influence human host susceptibility to malaria infection and severe disease outcomes may help us to better understand the immune response to malaria and design novel treatments against severe malarial anemia.

## Symposium “Coevolution host pathogen 3”

### (33) The development of functional apical—basolaterally differentiated midgut cell cultures for the study of arbovirus and *Plasmodium*- host interactions

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The intractability of studying host–pathogen interactions within the arthropod mid-gut remains a problem in arthropod vector–pathogen research. For example, neither currently available cell lines nor primary mid-gut cell cultures support *Plasmodium* ookinete invasion, penetration and differentiation to oocytes whilst the absence of apical-basal differentiation and thus the control (temporal and cellular route) of mature infective arbovirus virion export is not correctly mimicked and indeed may be biochemically somewhat artifactual and affect cellular defective interfering virion function and accumulation.

Using a biphasic culture system and nanofiber based lamina hydrogels, we have established apical—basal differentiated primary mid gut cell cultures from Anopheline and Culicine mosquitoes. Although heterogeneous in cell morphology, they are principally comprised of columnar epithelial cells including vATPase<sup>+</sup> cells with mid-gut specific gene expression, as determined by RT-PCR. These cultures support *Plasmodium* ookinete invasion, penetration and basal lamina differentiation to oocytes (ca. 20% of cells) and so potentially provide the first amenable cell culture system for the detailed proteomic and genomic analysis of *Plasmodium gallinaceum*, *P. falciparum* and *P. vivax* mid-gut invasion and subsequent differentiation. They may also prove to be a tractable model system for the screening of both potential resistance genes and allelic variances between parasite and host isolates, and the subsequent screening of the efficacy of transgenes prior to the labour intensive genetic modification of mosquitoes.

Moreover, following apical infection (ca. 90% of cells), Dengue and Japanese encephalitis viruses are replicated and baso-laterally secreted; suggesting these cell cultures may be amenable to arbovirus–host/symbiont interaction studies as well as virus–virus interaction within the same host tissue or cell. To illustrate this point we present preliminary results of inducible RNAi and defective interfering particle encoding phagemids upon dengue replication and baso-lateral secretion,

as well as interactions with natural symbionts, in these cell cultures.

#### (34) Susceptibility to hantavirus infection and MHC class II gene diversity in rodents

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Immunogenetics may provide key insight into epidemiology and transmission ecology. It may contribute to the understanding of the distribution of area of endemism and of the risk of emerging diseases in natural populations. One of the leading goals of immunogenetics has been to understand the associations of genetics to immune related diseases. In this context, the Major Histocompatibility Complex (MHC) has been extensively studied. It is a central component of the vertebrate immune system. There are multiple lines of evidence supporting the idea that this polymorphism is maintained by some form of balancing selection mediated by pathogens and parasites through frequency-dependent selection. Among MHC, Class II genes are known for their role in Puumala Hantavirus infections severity in humans. During this talk, we are going to analyse the genetic diversity of two class II MHC genes (DQA and DRB) at different evolutionary scales in rodents in relation with Hantaviruses distribution and phylogeny. First, we analysed the phylogenetic organisation of MHC allelic forms within vole and mice species in Europe and Southeast Asia (two area of endemism of human disease caused by Hantaviruses). This revealed the existence of trans-species polymorphism (TSP) among voles. Additional data on mice is needed to test whether TSP occur among mice and between vole and mice. TSP indicates that a balancing selection acts on these genes, probably through the mediated-selection exerted by shared pathogens. Second, we searched for associations between MHC haplotypes and the presence of Hantavirus in the bank vole (*Clethrionomys glareolus*), the reservoir of the Puumala Hantavirus responsible of Hemorrhagic Fever with Renal Syndrome in humans. Voles were serologically checked for antibodies. Associations between genetic parameters (haplotypes or heterozygosity) and infection status were explored using multivariate analyses. We detected significant associations between one MHC-haplotype and the susceptibility to Hantavirus infection. Similar studies are under progress among other rodent species in Europe and Southeast Asia. Our preliminary results highlights the potential importance of immunogenetics in understanding the emerging of rodent-born diseases like hantavirus infection.

#### (35) Rodents biodiversity and associated infections in Southeast Asia

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We are currently involved in a Franco-Thai program devoted to a multidisciplinary investigation of Muridae rodents (“mice and rats”), their parasites and the pathogens that they may carry and/or transmit to human, with a better understanding of diseases emergence as an ultimate goal. In such a context, pathogens circulation in the wild is a complex but pivotal phenomenon which requires a continuum of scientific approaches to be accurately apprehended. In particular, a rigorously comparative study is mandatory in order to take into account the interactions between parasites, wild and domestic hosts (uncluding human) and their environment. This is the reason why we rely on both concepts and techniqueq from a wide range of disciplines including taxonomy, cytogenetics, phylogenetics, phylogeography, population genetics, ecology, geography as well as modeling. With the following objectives.

**Objective 1:** To precisely identify and characterize the rodent species acting as reservoirs, and to document their ecology, geographic distribution as well as the genetic structure of their populations. To assess parasite and pathogen diversity in relation to their associated rodent hosts. To provide co-phylogenies and co-phylogeographies in order to enlight the evolutionary relationships of hosts and parasites.

**Objective 2:** To map and correlate the observed rodents distributions with their species-specific environmental landscapes in order to extrapolate their potential “real” range and to anticipate their future distributions in relation to landscape modifications.

**Objective 3:** Computing epidemiological databases. Cross-checking field data and GIS data. Definition and standardization of a risk-scale. Finalization of maps of risks and distributions. Atlas of Thai Muridae. Reference collection of Thai Muridae. Education and training of students.

Following the listing of the objectives, we expose the first results of our studies and we sketch future projects.

#### (36) Human macrophage variability of *in vitro* infection by *Leishmania donovani*: A new approach to dissect human susceptibility to visceral leishmaniasis

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In its human host, *Leishmania donovani* is an intracellular parasite of macrophages and parasitism display a wide spectrum of clinical manifestations ranging from asymptomatic infections to fatal visceral leishmaniasis (VL). A bulk of evidence from mice and man now indicate that host genetic factors play an important role in determining susceptibility to VL. Until now studies aimed at unravelling human genetic susceptibility to VL have focussed on the analysis of clinical phenotypes. These clinical phenotypes probably result from the interaction of both host and parasite genetic factors but also environmental factors, making these phenotypes complex for genetic dissection. We propose here a novel alternative approach based on the study of a cellular phenotype established *in vitro*. In this study we have established the *in vitro* macrophage infection status of 20 healthy blood donors and their response to IFN $\gamma$ . We demonstrate the existence of an important inter-individual variability of the macrophage permissive phenotype (parasitic index ranging from 20 to 100 amastigotes/100 macrophages) as well as important differences in the individual response to IFN $\gamma$  (parasitic index reduced in only half of the subjects upon IFN $\gamma$  activation). In a second step we attempted to correlate the observed cellular phenotypes with the expression of various macrophage genes implicated in *Leishmania* recognition (CR1, MSF1R), macrophage activation/deactivation (IL1, IL6, IL10, IL12, RANTES, MIP1 $\beta$ , TNF $\alpha$ ) and macrophage microbicide activity (NOS2, ARG1, NADPH p40phox). Significant correlations were found between the ratio of NOS2/ARG1 and the p40 phox unit of the NADPH oxidase but none of the measured variables were explicative of the capacity to respond to IFN $\gamma$ . These preliminary results indicate that this approach provide a valuable tool to analyse host-parasite interactions at the host cell level. Indeed studies in mouse derived macrophages or human monocytic cell line have shown that infection by *Leishmania* has a profound influence on the macrophage transcriptome. However studies are lacking that address specifically the question of the variability of macrophage responses to *Leishmania* infection in human and its relation with resistance/susceptibility to VL.

#### Symposium “Molecular biology and epidemiology of pathogens and vectors”

**(37) Detection of glutathione S-transferase e2 (*gste2*) gene in Iranian and Pakistani populations of main malaria vector *Anopheles stephensi***

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One of the most important genes involved with metabolic insecticide resistance (specially DDT resistance) in *Anopheles gambiae* is the epsilon class of the glutathione S-transferase super family. In current study, PCR analysis of *gste2* region have shown nucleotide variations within populations of *Anopheles stephensi*, the most important malaria vector in Iran and middle east. Specimens were collected from three different zones including; Chabahar, Sarbaz, Nikshahr, Iranshahr, Saravan and Khash districts in Sistan and Baluchistan province, Iran, which are under insecticide application for a long time; areas that has not been treated with insecticides for a long time (Kazeroon), Fars province, Iran; and *An. stephensi* population from Pakistan. The result revealed that Iranian strains collected from Sistan and Baluchistan province were 100% identical in GSTe2 DNA sequences except Saravan strain which has showed 100% identity with Pakistani strain, and 99% identity with others. Kazeroon strain was 99% identical with both Pakistani and Iranian strains with a C  $\rightarrow$  G transversion and A  $\rightarrow$  C transition in 105th and 174th nucleotides, respectively. Pakistani and Saravan strains showed A  $\rightarrow$  G transition in position 243 and C  $\rightarrow$  T transition in nucleotide number 351. The follow up study on further specimens from those areas has detected two types of nucleotide variation in Sarbaz samples; one type is identical to Saravnan and Pakistani samples and the other type is similar to other Sistan and Baluchisatn samples. However, in amino acid level, all the sequences were 100% identical proving that the nucleotide variation which was observed does not involve with the insecticide resistance. We will further discuss the cloning results related to *gste2* in Sarbaz populations of *An. stephensi*.

**Keywords:** *Anopheles stephensi*; *gste2*; Glutathione S-transferase; DDT resistance; Malaria vector

#### **(38) Detection of *Wolbachia* spp. in Iranian *Culicidae* species based on 16srDNA and *wsp* genes**

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*Wolbachia* are a group of cytoplasmically inherited bacteria that cause reproduction alterations, including parthenogenesis, cytoplasmic incompatibility, feminization of genetic males and male killing. Based on 16srDNA and protein-coding gene (*wsp*) sequences *wolbachia* can be typed into six different super groups or strains (A, B, C, D, E and F). Three A, B and E

types and member of F type are found in arthropods. In this study, we aimed to detect the *wolbachia* in Iranian *Culicidae* (*Anopheles*, *Aedes*, and *Culex* spp) specimens by molecular technique. Mosquitoes were collected from Guilan, Kerman, Sistan and Baluchistan, Khorasan, Azarbijan, and Hormozgan provinces in Iran. DNA extracted from 204 specimens was amplified by using specific diagnostic PCR of *wsp* and *16srDNA* genes that can differentiate between A and B groups of *wolbachia*. The length of amplified fragments by 16srDNA B and *wsp* B primers was 261 and 442 nucleotide in sequenced specimens, respectively. Within Iranian *Culicidae* only B type of *wolbachia* was detected, while there was no discrepancy in the amplified products of *16srDNA B* and *wsp B*. Therefore, we postulate that the similarity of 100% in 16srDNA and *wsp* genes could be a reason for close evolutionary relation in *Wolbachia pipientis* populations circulating in Iranian *Culicidae* species.

**Keywords:** *Culicidae*; *Wolbachia pipientis*; Group B; 16srDNA; *wsp* genes

### (39) Genetic variability of tick-borne bacterial pathogens in Central Europe, and the development of DNA chip.

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In the recent years due to the global warming, agricultural changes as well as other human impact on the environment, several tick-borne pathogenic organisms have (re)-emerged in Europe. A total of 991 ticks from two different geographical areas in Slovakia (Carpathians Mountain and Pannonian Plain) and one in the Czech Republic (Czech Massif) were tested by PCR analysis for the presence of *Borrelia burgdorferi* s.l. and the members of the family Anaplasmataceae. The overall borrelial prevalence varied between 17–36%. *B. afzelii*, *B. garinii*, *B. valaisiana*, *B. burgdorferi* s.s. were present in all three sites. Moreover, *B. lusitaniae* was present at the locality in North-Central sub-mountain area of Slovakia where 80% of all positive ticks belonged to this species. *B. garinii* (43%) was consistently the predominant species in Carpathian basin regions of Eastern Slovakia. The most common species in the Czech Republic was *B. afzelii* (52%). The sequencing of 5S–23S rDNA revealed high intraspecific variability within the detected species. The DNA of *I. ricinus* ticks was also tested for the presence of *Anaplasma/Ehrlichia* spp. by PCR-SSCP analysis and sequencing of a variable 247 bp fragment of 16S rDNA. *Anaplasma phagocytophilum* was detected in 4.3% of ticks. Moreover 2.1% ticks were infected with *Neoehrlichia mikurensis*, and 0.6% ticks carried an *Anaplasma*-like microorganism, recently detected in *I. ricinus* ticks from Northern Africa. Furthermore, we have developed a new detection

system—microarray-based assay of bacterial tick borne pathogens present in Central-Europe. Our oligo-chip is a sensitive and reliable for detection of following genera: *Borreliae*, *Rickettsiae*, *Anaplasmae* and *Ehrlichiae*. This method is based on detection of fluorescent signal after hybridization reaction by using laser scanner, and has a potential of use in the large-scale epidemiological studies as well as fast diagnostic method, since all relevant pathogens can be detected in one step.

**Keywords:** *Ixodes ricinus*; *Borrelia burgdorferi* sensu lato; *Anaplasma*; *Ehrlichia*; Oligo-chip; Genetic variability; Tick-borne pathogens

### (40) Molecular detection of feline hemoplasma in stray cats in Bangkok, Thailand

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The stray cat population in Thailand is a considerable problem because they are usually poorly cared for and thought to carry infectious agents. Such agents include the feline ‘haemoplasma’ species, *Mycoplasma haemofelis* and ‘*Candidatus M. haemominutum*’, together previously known as *Haemobartonella felis*. The larger species, *M. haemofelis*, is an etiologic agent of infectious hemolytic anemia in cats, while the less virulent *M. haemominutum* has only been associated with disease in immunosuppressed cats. These pleomorphic bacteria adhere to erythrocytes and can spread among cats by blood transfer. Although not yet confirmed, these apparently obligate prokaryotic parasites are thought to be naturally transmitted by arthropod vectors that are abundant in tropical climates including Thailand. Conventional detection of *M. haemofelis* and *M. haemominutum* involves light microscopy of stained blood smears, and these organisms can be difficult to detect and differentiate from each other and stain artifacts. The objective of this study was to investigate the distribution of these pathogens in stray cats from Bangkok. To accomplish this we utilized applied molecular methods to detect and differentiate *M. haemofelis* and *M. haemominutum*. Five hundred blood specimens randomly derived from stray cats, were tested with a PCR-RFLP assay. This technique was able to detect and distinguish these two feline haemoplasma species based on their 16S rRNA gene (16S rDNA) sequences. Digestion of amplicons with *Hind* III yielded 76 and 117 bp fragments for *M. haemominutum*, and a single 170 bp fragment for *M. haemofelis*. Of the total specimens tested, 7.8% (39/500) were positive for *M. haemominutum* and 5.4% (27/500) were positive for *M. haemofelis*. Sequences from 170 and 193 bp amplicons

were 98 and 99% identical to corresponding 16S rDNA reported for *M. haemofelis* and *M. haemominutum*, respectively. To our knowledge, this is the first report of both feline haemoplasma species in cats from Bangkok.

**Keywords:** *Mycoplasma haemofelis*; *Candidatus Mycoplasma haemominutum*; PCR; Bangkok; Stray cats

## Symposium “Modeling infectious diseases dynamics and diversity”

### (42) Mechanical transmission of pathogens by tabanids: Development of a mathematic model; consequences in epidemiology and population genetic

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It is known for long that a number of pathogens (trypanosomes, rickettsia, bacteria, viruses) circulating in the blood of their mammalian hosts can be mechanically transmitted from a host to another by biting insects (Krinsky, 1976; Foil, 1989). However, parameters of mechanical transmission are not well known, as well as medical, epidemiological, economic and genetic impacts. A series of experiments carried out in cattle allowed to demonstrate mechanical transmission of trypanosomes (*T. vivax* and *T. congolense*) by tabanids (*Atylotus agrestis* and *A. fuscipes*) (Desquesnes and Dia, 2003a,b, 2004). High transmission power of tabanids was demonstrated as well as pathogenicity of trypanosomes mechanically transmitted. *T. vivax* is more easily mechanically transmitted than *T. congolense* due to its high parasitaemia. Data collected daily during the experiments allowed to develop a mathematic model of the transmission which dynamic has important epidemiological applications. The epidemiology of cattle trypanosomosis due to *T. vivax* in the absence of tsetse (Latin America) is very different from that observed in the presence of cyclical vectors (Africa) (Desquesnes, 2004). Medical and economical impacts are also different. In mixed transmission areas, the relative impact of mechanical transmission is very difficult to establish. In the presence of tsetse, cattle trypanosomosis is most often highly endemic with prevalence regularly above 70%. In such conditions, addition of mechanical transmission to cyclical one has little epidemiological impact; however, it contributes to the predominance of *T. vivax* versus *T. congolense*. Coming to population genetic, mechanical transmission increases the circulation of *T. vivax* amongst cattle, which tends to homogenize the parasitic genetic material present in hosts. Transferring a very little quantity of blood, mechanical transmission tends to clone parasites; not only, it tends to select the most prolific parasitic sub-populations. Consequences on genetics of mechanically transferred pathogens should be studied.

**Keywords:** *Trypanosoma* sp.; Tabanids; Mechanical transmission; Mathematic model; Epidemiology; Population genetic

### (42) Ecological and immunological factors in tuberculosis transmission and control

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Humans are exposed to populations of environmental mycobacteria (EM) whose composition varies between regions. Human populations are subject to variable potentials for Mycobacterium tuberculosis (Mtb) transmission due to differences in demographic and socioeconomic backgrounds. The bacille Calmette-Guérin (BCG) vaccine is widely used worldwide but its efficacy has revealed great variability against pulmonary tuberculosis. We develop mathematical models that describe the transmission of Mtb under constraints that are imposed by host immunity elicited by previous exposures to EM, Mtb and BCG. We describe how levels of tuberculosis and vaccine efficacy depend on the hypothesized interactions among the three mycobacterial populations. We determine a threshold in Mtb transmission – the reinfection threshold – above which tuberculosis endemicity is high and insensitive to both EM and BCG. By contrast, variability rules below the reinfection threshold.

### (43) Of mice and men—Asymmetric interactions between *Bordetella* species

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*Bordetella pertussis* and *B. parapertussis* are two closely related human pathogens causing whooping cough. *B. parapertussis* has received limited interest because its symptoms are typically milder, and its incidence allegedly lower, than those of *B. pertussis*. However, some epidemiological studies suggest that the prevalence of the two pathogens may actually be similar, because of under-reporting of *B. parapertussis* cases. Sustained coexistence of these two competing species is surprising. Recently, experiments in mice have demonstrated that cross-immunity between *B. pertussis* and *B. parapertussis* is not symmetric: immunity induced by *B. parapertussis* infection efficiently protects against subsequent infections by either species, while immunity induced by *B. pertussis* infection does not efficiently protect against *B. parapertussis* infections. Using mathematical models, we explored the possible consequences of this asymmetry on the coexistence of the two pathogen species at the population level. In particular, we investigate the effects of anti-pertussis vaccination and fitness variation on both the short-term dynamics and the longer-term equilibrium of the system.

### (44) Reciprocity between modelling and experiment to meet the challenge of controlling antigenically diverse pathogens

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Previous models for antigenically diverse pathogens have predicted vaccine induced strain replacement under certain con-



ditions. To assess the risk of such an event, predictive models for the transmission dynamics of these pathogens must be developed. When modelling the transmission dynamics, there is a conflict between the level of detail required to capture the interactions between strains and the sparseness of the data available to parameterise the models. It is necessary therefore to make simplifying assumptions about individual immune responses to develop viable models. This presentation will demonstrate how such assumptions impact on the population level behaviour of antigenically diverse pathogens. I will highlight the need for further experimental investigation to develop and parameterise models to a point where they have predictive value. As an example I will present a case study of human respiratory syncytial virus in rural Kenya where models were involved in the design of a cohort study. This example will demonstrate the challenges and potential for the fusion of modelling and experiment to address this problem.”

## Symposium “Molecular epidemiology of tuberculosis and leprosy”

### (45) A bioinformatic analysis of tuberculosis cases in New York

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The systematic DNA fingerprinting of human pathogens has led to valuable insights into the dynamics of disease transmission, evolution of strain families, and global movement of pathogens. In the case of tuberculosis (TB), multiple laboratory methods of varying resolutions have been developed to differentiate strains of the *Mycobacterium tuberculosis* complex (MTC). The three most commonly employed methods today are spacer oligonucleotide typing (spoligotyping), mycobacterial interspersed repetitive unit (MIRU) analysis, and insertion sequence IS6110-based RFLP typing. Currently in New York an MTC isolate of every newly identified case of TB undergoes spoligotype, MIRU, and IS6110-based RFLP analyses. This system of universal TB genotyping has produced a large collection of diverse data. Bioinformatic tools are being developed which permit analysis of TB genotype and epidemiologic databases *in toto*. In this study we utilized two web-based software tool collections to analyze New York spoligotype data: SPOT-CLUST (Vitol et al., 2006) and SIT VIT (Brudey et al., 2006). SIT VIT was also used for examining MIRU data. By combining basic patient demographic data and MTC global family assignments we examine such questions such as the

degree of recent TB infection in non-US-born persons, differences in strain families versus age at TB diagnosis, and global MTC family evolution. In New York City epidemiologists identified a group of *M. bovis* infections in US-born children (Winters, 2005). This same group is observed by graphing age at diagnosis versus TB strain global family. Graphs plotting patient MTC strain family versus country of birth and time in the US suggests compartmentalization of some strain families from particular areas of the globe. US-born patients in New York are only rarely observed to be infected with East Asia/India (EAI) associated strains. Bioinformatics analyses of TB genotyping and epidemiologic data could play an important role developing TB control and prevention programs.

**Keywords:** Tuberculosis; Bioinformatics; Databases; Molecular epidemiology; Genotyping

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### (46) *Mycobacterium tuberculosis* isolates from South India belongs to an ancient lineage

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The magnitude of the tuberculosis problem worldwide and the global traffic requires the application of effective approaches to decipher the portable genotype patterns of a particular region in comparison with the global patterns of

disease transmission. The genetic information resulting from high prevalence areas such as ours would prove useful for defining phylogenetic links that exists with TB genomes and for constructing models of genome evolution. This will also help in the elucidation of the evolutionary history of ancient tubercle bacilli. We have used a number of genotypic tools like IS6110 RFLP, Spoligotyping, Deletion Microarray, and Deletion PCR to understand the lineage of *M. tuberculosis* in southern India. We used a standardized international protocol for IS6110 RFLP and spoligotyping was done according to the protocol supplied by (Isogen Bioscience BV, Manarssen) and instructions of the manufacturer. All of the spoligotype patterns were coded using the octal code system. We then referred to a standardized international database of spoligotype patterns, SpolDB3 available at <http://www.pasteur-guadeloupe.fr/tb/spol3> [www.pasteur-guadeloupe.fr/tb/spol3](http://www.pasteur-guadeloupe.fr/tb/spol3) to determine whether each pattern had been previously reported. We selected 25 isolates for comparative whole-genome hybridization using an Affymetrix DNA chip (Santa Clara, California, USA). We identified putative deletions in the experimental strains relative to the sequenced reference strain H37Rv using DelScan software (AbaSci, San Pablo, California, USA), and confirmed the putative deletions by direct sequencing. For the detection of LSPs by multiplex real-time PCR, we designed a series of assays based on different TaqMan primer/probe combinations. We also used previously published studies of genomic deletions to identify phylogenetically important genomic deletions and screen for them using PCR. Our results showed that the major clade present in Tiruvallur, S. India belong to the group which is designated as the most ancient lineage of *M. tuberculosis*.

**(47) The use of proteomics and bioinformatics to identify novel antigens of *Mycobacterium leprae* towards the development of a rapid diagnostic test for the early detection of leprosy**

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Comparative genomic analysis of the *M. leprae* genome has identified 1614 open reading frames, and up to 165 genes with no homologues in *M. tuberculosis*. Diagnosis of leprosy is a major obstacle to disease control, and has been compromised in the past by the lack of specific reagents. Understanding which antigens might be useful in either cell mediated or antibody response assays has been hampered by the fact that *M. leprae* is an uncultivable microorganism, capable of growth only in humans, armadillos, and immunocompromised nude mice. Nevertheless, using proteomic analysis, we have identified 256 proteins from the native subcellular fractions of *M. leprae*, which represents approximately 16% of the potential open reading frames thought to be coded by the genome. In addition, using an oligonucleotide chip, we have further identified up to 702 genes that are thought to be transcriptionally active. Of these, there were 41 genes that coded for proteins that are unique to *M. leprae*, and 205 genes that coded for proteins that are conserved hypotheticals, proteins which are limited to mycobacteria. Using the information from these studies, a picture of those antigens that might be both specific to *M. leprae* and important in evoking either cell mediated or antibody responses in leprosy patients is unfolding. In previous work, we found that a number of recombinant *M. leprae* proteins or peptides behaved reproducibly as T cell antigens, inducing stronger IFN- $\gamma$  responses from tuberculoid leprosy patients than tuberculosis patients or endemic controls. In addition, we are examining the potential of protein/peptide arrays to define the humoral immune response to identify disease-state-specific antigen profiles. Those antigens that provide specific responses in leprosy patients could be developed into a rapid diagnostic test for the early detection of leprosy and epidemiological surveys of the incidence of leprosy, of which little is known.

**(48) Differentiation of *Mycobacterium* species that cause tuberculosis by genome analysis**

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*Mycobacterium tuberculosis* is the principal etiologic agent of human tuberculosis (TB) in most regions of the world while *M. africanum* is frequent in certain regions in the Africa. *Mycobacterium bovis* is the principal cause of TB in cattle and causes TB in humans upon ingestion of unpasteurized milk and products or after prolonged contact with infectious cattle. The other species of the *M. tuberculosis* complex (MTC) *M. microti*, *M. pinnipedii* and *M. caprae* are less frequently observed and the classification within the MTBC of rare variants such as the dassie and oryx bacilli remains to be defined. The genetic differences between MTBC strains, besides being the cause of host preference, can be useful for diagnosis and better define epidemiology and appropriated treatment, help identifying potential virulence markers and fine-tune MTBC phylogeny. Genetic characteristics such as deletions, insertions, inversions, duplications and Single Nucleotide Polymorphism (SNPs) are nowadays used to help identifying MTBC isolates to the species level. In the present study, 47 strains isolated from different TB patients from Ghana were characterized using either PCR-based protocols for detection of genomic deletion or PCR-RFLP for detection of earlier or presently described SNPs. A total of 13 deletions (IS1561', *cpf32*, RD701, RD702, RD713 RD711, TbD1, TbD1, RD7, RD8, RD9 and RD10) and 16 SNPs (*hsp65*<sup>540</sup>, *rpoB*<sup>1049/116</sup>, *katG*<sup>203/463</sup>, *gyrB*<sup>1450</sup>, *gyrA*<sup>95</sup>, *Rv 1510*<sup>1129</sup>, *aroA*<sup>285</sup>, *PRO-narGHJI*<sup>215</sup>, *3'cpf32*<sup>311</sup>, *RD13*<sup>174</sup>, *PPE55*<sup>2154/2148</sup>, *nat*<sup>751</sup> and *RD711*<sup>390</sup>) were analyzed. Using this approach, it was possible to differentiate all *M. tuberculosis* (*n* = 12) from *M. africanum* (*n* = 29) strains, and to recognize *M. africanum* subtype Ia (*n* = 9) and *M. africanum* Ib (*n* = 20). In addition, the presence of the hereby described SNPs (*nat*<sup>751</sup> and *RD711*<sup>390</sup>) were analyzed in a well-characterized collection of MTBC isolates (Huard, 2006); *nat*<sup>751</sup> was only present in *M. africanum* Ia, while *RD711*<sup>390</sup> was only present in *M. africanum* Ib. This suggests that some species or sub-species within the MTBC can be differentiated looking at particular SNPs and that newly discovered SNPs could help fine tuning the MTBC to the species level.

### Symposium “Molecular Epidemiology of Parasites”

#### (49) Identification of trypanosomes using molecular methods from wild caught tsetse in Tanzania

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Tsetse flies transmit many species of trypanosomes in Africa, some of which are human and livestock pathogens of major medical and socio-economic impact. Identification of trypanosomes is essential to assess the disease risk posed by particular tsetse populations.

We have developed two tests to replace the multiple species-specific PCR tests used previously to identify the trypanosome species carried by individual tsetse flies.

1. Generic PCR test: interspecies size variation in the PCR product of the ITS-1 region of the ribosomal RNA repeat region enables species identification.
2. Fluorescent primers: size variation within the 18S rDNA region is used to identify species, products are sized with greater accuracy by the use of an automated DNA sequencer. This enables identification of species with increased precision and sensitivity.

Using the generic systems, we have been able to identify a new species of trypanosome. The 18s region of this trypanosome has been sequenced and we can confirm the new trypanosome is most closely related to *Trypanosoma godfreyi*, a suid trypanosome. The easy identification of this new trypanosome by PCR will facilitate studies of its epidemiology.

Both methods facilitate the identification of samples quickly and accurately, and have been used for large-scale field studies. The results of these studies and the respective advantages and disadvantages of the two methods will be discussed.

#### (50) The human African trypanosomiasis: Interactions between the tsetse fly, its secondary symbiont *Sodalis glossinidius*, and the parasite

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Tsetse flies transmit African trypanosomes, the causative agents of sleeping sickness in human and Nagana in animals. This disease affects many people with considerable impact on public health and economy in sub-Saharan Africa, while trypanosomes resistance to drugs is rising. *Sodalis glossinidius*, a symbiont of tsetse flies, is considered to be involved in vector competence. In a former study no direct correlation was found between the presence of *S. glossinidius* and the ability of the insect to acquire *Trypanosoma congolense*. However, *Glossina palpalis gambiense* and *Glossina morsitans morsitans* were shown to harbour genetically distinct populations of *S. glossinidius*, suggesting that vector competence for a given trypanosome species could be linked to the presence of specific genotypes of the symbiont rather than a mere presence /absence. In order to assess this hypothesis, *Glossina palpalis* individuals were fed on blood infected with either *Trypanosoma brucei gambiense* (T.b.g.) or *Trypanosoma brucei brucei* (T.b.b.) species, and the genetic diversity of *S. glossinidius* strains isolated from dissected flies was investigated using AFLP markers. Correspondence between

occurrence of these markers and parasite establishment was analysed using multivariate analysis. We demonstrated that the distribution of *S. glossinidius* strains from T.b.g.-infected flies differed strongly from those from T.b.b.-infected individuals. Some AFLP markers were shown to be significantly linked to the ability of T.b.g. or T.b.b. to establish in the *Glossina* midgut. This suggests the differential presence of *Sodalis* genotypes to influence parasite establishment and could explain variations in *Glossina* vector competence in the wild. The markers identified may be useful to assess prevalence of *Sodalis* genotype facilitating establishment of parasite species, and to develop novel risk-management strategies.

**(51) Microsatellite EmsB, a relevant tool to explore the genetic diversity of *Echinococcus multilocularis* at different geographical scales**

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The cestode *Echinococcus multilocularis* is widely known in the Northern hemisphere to infect mainly foxes and rodents and accidentally human. However human seems not to be equal facing the alveolar echinococcosis (AE) disease. Many factors such as environmental conditions, immunology or genetic susceptibility can explain the heterogeneity of infection in human. Few data existed concerning the influence of the *E. multilocularis* genetic diversity. Up to now, the genotyping of subspecies was impossible due to a lack of fast evolutionary markers. Recently, a tandem repeated multi-locus microsatellite, named EmsB, was discovered in the *E. multilocularis* genome. Our objective was to assess the limit of EmsB discriminatory power at three geographical scales: (i) at a world scale, by studying Alaskan, European and Asian endemic areas, with parasitic lesions from definitive and intermediate hosts, (ii) at a continental scale by studying adult worms circulating in so-called old and new endemic areas in eight European countries and (iii) at a local scale ( $n \times 100 \text{ km}^2$ ) in the French region of the Ardennes. The hierarchical clustering analysis of the results helped us to explore relevant questions as the spatial dynamic, the circulation of the parasite between different hosts, the emergence or the re-emergence of AE in human, to increase our knowledge on the parasite transmission and to enhance the public health management.

**Keywords:** *Echinococcus multilocularis*; Microsatellite; Different geographical scales; Transmission dynamic

**(52) Identification of the iron superoxide dismutase gene repertoire in *Trypanosoma brucei gambiense***

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Superoxide dismutase (SOD) forms part of the defense mechanism that helps to protect organisms from superoxide anions. This enzyme is one of the isoenzyme systems commonly used to differentiate T.b. gambiense from T.b. brucei and T.b. rhodesiense. To understand the genetic basis of the differences observed between SOD electrophoretic profiles of T. brucei sub-species, we undertook the identification and the characterization of SOD gene repertoire in T.b. gambiense. This study was performed on seven stocks (4 T.b. gambiense group 1 and 3 group 2) showing different SOD profiles. Four SOD genes (soda, sodb1, sodb2 and sodc) were identified in T.b. gambiense genome. These genes were cloned and their predicted amino acid sequences were deduced. Few differences were observed between nucleotide sequences of the four SOD genes of T.b. gambiense group 1 and 2 stocks. Even with T.b. brucei, few differences were observed. Several amino acids specific to FeSOD were found in the four SODs sequences of T.b. gambiense. Aligning the four T.b. gambiense protein sequences with those of others organisms, important differences were found with MnSOD and Cu/ZnSOD, but high similarity with FeSOD; indicating that the SODs of T.b. gambiense are FeSOD. High similarity exists between the proteins sequences of T.b. gambiense and T.b. brucei. Despite the differences observed in SOD electrophoretic profiles, there is a genetic stability of the SODs genes in *T. brucei* sub-species.

**Symposium “Emerging viral diseases: Avian flu and SARS”**

**(53) Can the evolution of avian influenza virus (AIV) be predicted?**

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Because the previous influenza pandemics were caused by either viruses of avian origin or reassortant viruses with some of their genomes derived from avian viruses, it is believed that the H5N1 AIV, which already causes limited outbreaks in human, may become the source of the next pandemic. Continuously evolving, the virus may adapt to the point that it can transmit efficiently in human population. An insight in the viral evolution may help us to estimate the risk, understand the cause, and prevent the emergence of a pandemic strain. Trying to understand the viral evolution, we need to look at the viral dynamics and selection pressures. While the evolution of human influenza viruses is driven mainly by immunological selection pressure, it is not clear what drives the evolution of AIV. The current H5N1 AIV has diverged into multiple sublineages. Because these sublineages or clades are antigenically distinct, it is likely that immunological

pressure has played a role in the divergence of H5N1 AIV. It is also likely that the immunological pressure was the result of massive immunization in poultry, especially in China. The recent emergence of the new Fijian-like strains further supports this notion. In general, genomes of most AIV are in evolutionary stasis because of they are in equilibrium with their natural host. Once transmitted to a new host species, the virus starts rapid evolution to adapt itself to the new host. Virus–host interaction is, therefore, considered an important factor in the evolution of H5N1 AIV. Genome analysis has revealed positive selection pressure on some parts of the viral genome. Some of these sites may eventually fix and become host-specific residues when the virus reaches the optimum in the new hosts. Although the avian-human inter-species barrier may involve several genetic determinants, the most important one may be the receptor binding preference. A few point mutations in the receptor-binding pocket of hemagglutinin have been shown to increase human-type receptor binding of H5. We have found such mutation in viral quasispecies from a human respiratory specimen. This suggests that there is a selection pressure driving the virus toward human-type receptor specificity in human infection. Viral genomes may be shaped not only by selection pressures but also mutational bias. Recent observation has shown that avian and human influenza viral genomes are different in their GC content. It was suggested that this indicates a difference in mutational bias between the two hosts. However, I would like to point it out that this may not be entirely correct. Genome composition may also be influenced by codon composition, which could be affected by selection pressure. We analyzed codon volatility, which represents the propensity to change non-synonymously, and found some differences in the genome volatility between the two virus groups. Higher codon volatility in human viruses is likely the result of higher degree of evolution and changes, and may explain at least part of the difference in genome composition. We also found that hemagglutinin and neuraminidase genes of H5N1 AIV have high codon volatility suggesting that the genes are rapidly evolving. Such analyses may help us to identify viral genome regions with high potential to change, and may help us to predict the pattern of viral evolution.

#### (54) Avian influenza H5N1 in Russia

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In July of 2005 in Suzdalka village (Novosibirsk Region, Russia) the mass mortality of poultry and wild birds was registered. Highly pathogenic influenza viruses of H5N1 subtype were isolated from different organs of three dead birds. The A/Turkey/Suzdalka/Nov-1/05 strain was isolated from turkeys' spleen, the A/Chicken/Suzdalka/Nov-11/05 and A/Chicken/Suzdalka/Nov-12/05 strains—from chickens' kidney. This was the first case of isolation of high pathogenic influenza viruses of H5N1 subtype in Russian Federation.

The phylogenetic analysis of hemagglutinin gene sequences showed that these strains form a cluster (support index is equal to

99) with H5N1 strains isolated from birds during the spring outbreak of the avian influenza virus in Qinghai Lake in China in 2005. The analysis of birds' migration ways indicates that virus was imported to Siberia during the spring migration in 2005. In summer and autumn of 2005 high pathogenic influenza viruses of H5N1 subtype spread apart all South part of Siberia, reached Caspian Sea, countries of Caucasian region and were registered in various countries of Black Sea basin. In winter of 2005–2006 H5N1 virus was isolated repeatedly in different European countries, in Turkey and in the Southern part of Russian Federation: in Dagestan and in Krasnodar Territory. In 2006 during the spring migration, H5N1 virus was isolated again in South of Western part of Siberia.

The phylogenetic analysis of sequences of genes that code hemagglutinin, neuraminidase, nonstructural protein (NS) and matrix protein showed that all the viruses isolated in period from July of 2005 till May of 2006 in territory of Russian Federation were genetically close to H5N1 virus isolated in 2005 in Qinghai lake (Northern China) and were the products of microevolution of this virus. The analysis of birds' migration ways and spreading of high pathogenic influenza viruses of H5N1 subtype in territory of Eurasia during 2005–2006 showed the important role of swamps and lakes of South of Western part of Siberia in expansion of H5N1 virus to European countries, countries of Near and Middle East and Northern Africa.

#### Acknowledgements

The authors show the sincere gratitude to everybody who helped authors to carry out this work, especially Dr. Robert G. Webster, Dr. Alexander Lipatov and Dr. Michael Callahan.

This work at the FRSI “Vector” was carried out under the auspices of the Government of the Russian Federation and financial support Russian Ministry of Public Health and BioIndustry Initiative, USA (grants BII/RUX2-20411-NO-04 and BII/RUB2-20429-NO-05).

#### (55) Genetic diversity of bat SARS-like coronavirus and its interaction with ACE2

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Bats were recently identified as natural reservoirs of SARS-like coronavirus (SL-CoV) or SARS coronavirus-like virus. These viruses, together with SARS coronaviruses (SARS-CoV) isolated from human and palm civet, form a distinctive cluster within the group 2 coronaviruses of the genus *Coronavirus*, tentatively named group 2b (G2b-CoV). The bat G2b-CoV isolates have an identical genome organization and share an overall genome sequence identity of 88 to 92% among

themselves, and between them and the human/civet isolates. The most variable regions are located in the genes coding for nsp3, ORF3a, spike protein and ORF8 when bat and human/civet G2b-CoV isolates are compared. Genetic analysis demonstrated that a diverse G2b-CoV population exist in bat habitat. The spike protein (S protein) of coronavirus is known responsible for receptor binding to the host cells. The receptor binding domain (RBD) of human/civet G2b-CoV and its receptor angiotensin-converting enzyme-2 (ACE2) were well characterized. However, two deletion sites (5 and 12 aa, respectively) are located in S protein of bat G2b-CoV compared with that of human G2b-CoV. Thus the interaction between bat G2b-CoV S protein and the ACE of human and bat was investigated.

### (56) Detection of avian influenza virus in lung tissues of naturally infected chickens in Thailand by *in situ* hybridization

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*In situ* hybridization (ISH) was developed to detect of avian influenza virus (AIV) infection in lung tissues of chickens in Thailand. Each of 30 samples of AIV-infected chicken and non-AIV-infected chicken lung tissues were used in this study. The nonradioactive digoxigenin (DIG)-labeled 604 base pairs (bp), 544 bp, and 274 bp cDNA probes for viral RNA encoding the matrix protein, hemagglutinin, and neuraminidase, respectively, of AIV type A H5N1 strain were generated by the reverse transcription polymerase chain reaction (RT-PCR). The hybrid formation was detected with anti-DIG conjugated alkaline phosphatase and nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) substrate. The strong positive signal typically was exhibited a dark purple reaction product in the nucleus and cytoplasm of the bronchial and bronchiolar epithelial cells with red background of the nuclear fat red-staining whereas the less intense signal was detected in the interstitial and alveolar macrophages. In contrast, all of non-AIV-infected chicken lung tissues were negative by ISH. Therefore, ISH developed in this study was useful for detection of AIV in tissues taken from naturally infected chickens.

**Keywords:** Avian influenza virus; Digoxigenin-labeled DNA probe; *In situ* hybridization; Chickens

### Symposium “Evolution of animal and human trypanosomes”

#### (57) Molecular detection of *Trypanosoma lewisi*-like infections in rodents of Thailand

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*Trypanosoma lewisi* is the type species for *Herpetosoma*, a homogenous subgenus of several dozen named species often described as morphologically indistinguishable, “*T. lewisi*-like” parasites. These parasites normally infect rodents and utilize fleas as vectors. Although this trypanosome subgenus is considered non-pathogenic to normal hosts, some of them are on rare occasion reported in association with human disease. Recently, a *T. lewisi*-like infection was detected in a sick Thai infant, thus the objective of this study was to investigate the prevalence of *T. lewisi* infections among different rodents indigenous to Thailand. Blood was collected from a total of 129 rodents trapped from urban and rural areas of three Thai provinces (Loei, Kalasin and Phrae) between 2005 and 2006. These samples were processed for DNA isolation and tested with a PCR assay universal for the genus *Trypanosoma*, followed by internal transcribed spacer 1 (ITS1) sequence analysis to identify infections in positive samples. Amplicons of approximately 623 bp, the size consistent with the stercorearian trypanosome group, were generated from 21.7% of all rodents tested. Further analysis suggested that ITS1 sequences from these amplicons were 98% identical to that reported for *T. lewisi* from an experimentally infected rat. Only two of six rodent genera tested PCR-positive for these parasites. The highest prevalence of *Herpetosoma* infections was found among rodents from Phrae province (26%). These results suggest that, in Thailand, humans exposed to certain rodents or their ectoparasites could be at risk of infection with *T. lewisi*-like parasites. More work is warranted to identify vectors of these trypanosomes and to compare the prevalence of these infections among rodent and human populations in Thailand.

**Keywords:** *Trypanosoma lewisi*; Rodents; Internal transcribed spacer 1 (ITS1); Thailand

#### (58) Impact of mixed infections on Benzimidazole treatment efficacy in BALB/c mice infected with *Trypanosoma cruzi* major genotypes

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In the present study the impact of *T. cruzi* dual-clone infections on benznidazol treatment efficacy compared with the respective monoclonal infections was investigated. For this, eight clonal stocks, two of each major genotypes 19 and 20 (*T. cruzi* I), hybrid genotype 39 and 32 (*T. cruzi* II) were combined into 24 different mixtures. BALB/c mice were inoculated by intraperitoneal route, with 5000 blood trypomastigotes of each clone and treated with oral doses of 100 mg benznidazol/kg/20 days. The cure control was performed by fresh blood examination, hemoculture, PCR, ELISA and detection of anti-live trypomastigotes antibody. The identification of each clone from not cured mice was performed by microsatellites assay. Cure in dual-clonal infection was detected in 28.4% of treated animals. Considering the cure rates of mice for all *T. cruzi* I (35.4%), *T. cruzi* II (60%) groups and their associations were not observed difference in relation to the expected benznidazole (BZ) susceptibility for combinations *T. cruzi* I + I (0%), I + II (22.1%), except II + II (60.0% susceptible). For major genotypes, combinations 19 × 32 (26.7%) and 19 × 39 (25.6%) showed their phenotypes to resistant profile and 39 × 32 (60.8%) to susceptible profile. Genotype 20 was 100% resistant to BZ in monoclonal infections, but the cure rates of their combinations ranged from 0 to 24.5%. Nine out of 24 dual infections changed their profile of BZ susceptibility: 20 + 39 ( $n = 2$ ), 20 + 32 ( $n = 1$ ), 19 + 39 ( $n = 3$ ), 19 + 32 ( $n = 2$ ) and 39 + 32 ( $n = 1$ ). Although molecular characterization had identified few mixed infections in isolates from not cured mice, very interesting results were observed. In some mixtures (sensitive + resistant), the selected clone identified after BZ treatment was that previously identified as sensitive to BZ in monoclonal infections. These results suggest that mixed infections, so current in nature, may have important impact on chemotherapy efficacy. Further studies to elucidate the mechanisms involved in this process are essential for advances in the knowledge of Chagas disease chemotherapy.

#### **(59) The epidemiology of surra (*Trypanosoma evansi* infection) in SE Asia: Can molecular tools provide the answers we need?**

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Trypanosomiasis caused by *Trypanosoma evansi* (Surra) is endemic in SE Asia where it is a significant, but often underestimated cause of mortality in livestock. Recent estimates suggest that 22% of Indonesian buffalo infected with *T. evansi* either die or are sold for salvage slaughter. In the last 10–15 years a series of severe outbreaks of surra have occurred in the Philippines. What is most puzzling is that the epidemiology

observed in these outbreaks differs from other parts of SE Asia such as Indonesia. These differences include the observation of fatal disease in small ruminants and cattle and the observation of different presenting signs. In addition, there have been reports of isolated cases of human trypanosomiasis in India. A variety of epidemiological tools are required in order to gain a better understanding of the biological basis of the epidemiological conundrum that we are faced with. In particular molecular tools can provide us with unique methods of providing us with insight but they must be used with caution to ensure that the benefit from the research reaches the key stakeholders—the farmer. This talk will describe a multidisciplinary approach to unraveling the epidemiology of surra in the Philippines.

#### **(60) Human infection by *Trypanosoma evansi* in India: Diagnosis, treatment, genetic and epidemiological investigations**

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The first case of human trypanosomiasis in Asia was evidenced in 2004 in Seoni, India. A farmer presented a fluctuating trypanosome parasitaemia associated with febrile episodes for five months. Clinical and biological examinations, including parasitological, serological and molecular biological tests confirmed the infecting species as *T. evansi* (Joshi et al., 2005). Suramin was efficient to cure the patient (Joshi et al., 2006). The *SRA* gene (Xong et al., 1998) was not detected by PCR in the *T. evansi* DNA, which had homogeneous kDNA minicircles of Type A (Truc et al., 2007). The parasite appeared to be a typical *T. evansi*, suggesting that the explanation for this unusual infection may lie with the patient. Normally humans exhibit innate immunity against *T. evansi* and *T. brucei*, and in the latter case this immunity is known to involve apolipoprotein L-I (apoL-I, Vanhamme et al., 2003). The serum of the patient



was found to be devoid of trypanolytic activity, and this was linked to the absence of apoL-I due to frameshift mutations in both *apoL-I* alleles (unpublished data). Therefore, the lack of apoL-I was sufficient to explain the human infection by *T. evansi*. Because of a mechanical transmission by insects was suspected, a serological investigation was conducted in the patient village in 2005. Out of 1806 individuals tested using the Card Agglutination Test for Trypanosomiasis/*Trypanosoma evansi* (Pathak et al., 1997), no trypanosomes were detected in the blood of 60 people who were positive at a significant serum dilution (1:4). The results indicate a frequent exposure of the human population to *T. evansi* in the study area, suggesting frequent vector transmission of parasites to humans (Shegokar et al., 2006). Further investigations are required to evaluate the importance of this phenomenon and the potential emergence of a new zoonotic disease (Brun, 2005).

**Keywords:** *Trypanosoma evansi*; Human trypanosomiasis; India; SRA gene; CATT/*Trypanosoma evansi*; Genetic markers; apoL1

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## Symposium “Medical entomology 2”

### (61) Structuration of tsetse (Diptera: Glossinidae) metapopulations according to landscape fragmentation in Burkina Faso

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The impact of landscape fragmentation due to human and climatic pressures on the structure of a metapopulation of *Glossina palpalis gambiense* (Diptera: Glossinidae) was analysed in the Mouhoun river basin, Burkina Faso. Allele frequencies at eight microsatellite loci, and morphometric features based on 11 wing landmarks, were compared among four populations. The populations originated from the Mouhoun River and one of its tributaries. The among-populations distances were 74, 61 and 81 km upstream to downstream, totalizing 216 km between the first and the fourth. Both microsatellites and wing geometry demonstrated a structuration between the populations, but no isolation. There was no clear relation between gene flow and geographic distance. Nevertheless, the type of gallery forest and particularly their disturbance level assessed using phytosociological censuses, seemed to be of tricking importance. The impact of the fragmentation of peri-riverine landscapes on tsetse metapopulations structure and its potential implications for control campaigns is discussed.

### (62) The potential use of temperate chimeric phages in arthropod vectored disease control

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Transgenic arthropod technology potentially suffers from recombination mediated loss or alteration of the transgene, especially if virus derived, and typically provides a single shot gene drive system. Paratransformation systems may theoretically overcome recombination but are both hard to stably manipulate and to get the correct transgene expression profile and secretion out of the bacteria. Likewise cytoplasmic incompatibility inducing isolates of the arthropod symbionts *Wolbachia*, *Cardinium* and *Rickettsia* as potential transgene drive candidates may provide multiple drive opportunities but are hard to transfect. However, at least some of the temperate phage isolates of *Wolbachia* reveal a chimeric nature at both the DNA sequence level and in the ability to form virions and tails. The recent development of host-specific replication or infection incompetent phage strains, cell free assembly systems, and evaluation of the control of lysogenic insertion and lytic switch mechanisms, has enabled considerable targeted manipulation of this system. Thus cytoplasmic incompatibility inducing *Wolbachia* isolates act not only as “factories” for the *in situ* production of chimeric phage within the target arthropod vector cells, but provide the gene drive system leading to rapid near fixation of the infected phenotype in the arthropod population which can be replaced by other CI types, providing the ability to replace or remove transgenic phages from the population as required. We outline (i) the development of chimeric phages that encode for defective interfering particles to reduce vector competency of arthropod vectored arboviruses or arthropod pathogens and illustrate current progress using dengue and Japanese encephalitis viruses within mosquito cell lines and mid-gut tissue culture; (ii) the adaptation of this system to control ssRNA viral pathogens of commercial shrimps and honey bees and (iii) the engineering of key genes from ichnoviruses (AgIcVak4/5) into *CI-Wolbachia* for paratransformation of insect parasitoids and their lepidopteran pests to enhance integrated pest management schemes through altered host immunity to parasitoids.

### **(63) *Ornithodoros savignyi* as a vector of AHFV—ecological, molecular and evolutionary implications**

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We recently isolated Alkhurma Hemorrhagic Fever virus (genus *Flavivirus*, AHFV), a variant of Kyasanur forest disease virus (KFDV), from *Ornithodoros savignyi* (Charrel, Fagbo, Sarah and de Lamballerie; accepted manuscript, Emerging Infectious Diseases) Manuscript) collected in Saudi Arabia; this was followed by molecular characterization and phylogenetic comparison. AHFV is the first human viral pathogen to be isolated from *O. savignyi*, a cryptic and

multiple-host seeking Argasid tick endemic is the Middle East and Africa. AHFV and KFDV are the only tick borne haemorrhagic fever viruses known to be associated with *Ornithodoros* spp vectors. AHFV was the first tick-borne hemorrhagic fever-inducing flavivirus for which the complete genome sequence was determined (Charrel, Zaki, Attoui, Fakeeh, Billoir, Yousef, de Chesse, De Micco, Gould, de Lamballerie, Biochem. Biophys. Res. Commun., 2001). Together with our tick isolate, the accumulated molecular data provides confirmation that AHFV is tick-borne and updates the nascent AHFV literature. It clearly questions, in the absence of verifiable data, recent assertions that it is a mosquito borne flavivirus. It also provides a rethinking of previously held notions on the common ancestor of both viruses. Our findings may be integrated with previous work done on the evolutionary and adaptive behaviour of *O. savignyi* in Africa vis-a-vis AHFV vectoring. Here, we discuss the ensuing and interrelated ecological, molecular and evolutionary implications in the Arabian Gulf and beyond.

### **(64) Molecular cloning and sequencing analysis of Bm91 (angiotensin converting enzymes) cDNA from salivary glands of Thai Cattle ticks, *Boophilus microplus***

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Cattle ticks, *Boophilus microplus*, are the most important ectoparasites of livestock in Thailand, and are responsible for severe economic losses through direct effects of feeding and as vectors of pathogens. The feasibility of an anti-tick vaccine against *B. microplus* has recently been successful. Excluding Bm86 and Bm95, other potential candidate proteins were searched and developed. A protein, Bm91, has regions of amino acid sequence similarity to angiotensin converting enzymes. Bm91 has also been found to be effective as the candidate antigen on its own for the anti-tick infestation. The objective of this study was to clone and sequence cDNA encoding Bm91 from *B. microplus* indigenous to Thailand. mRNA was isolated from salivary glands, and cDNA encoding Bm91 was amplified with PCR, cloned into the pPICZα vector and transformed into *Escherichia coli* DH5-α competent cells. Purified plasmid DNA was sequenced with dye terminator cycle sequencing reactions, and Bm91 nucleotide and deduced amino acid were analyzed. Nucleotide sequence analysis showed open reading frames of 1893 bps encoding proteins of 631 amino acids. By using the NCBI-Blast conserved domain search for similar domain. The result showed similarities to peptidase\_M2, the angiotensin-converting enzyme. The predicted N-glycosylation sites by NetNGlyc 1.0 server was found at amino acid

positions 51, 58, 88, 113, 207, 306, 313, 475, and 628. Comparison of both nucleotide sequence and deduced amino acids showed high identity to an angiotensin-converting enzyme-like protein precursor of *B. microplus* registered in GenBank (AC no. U62809), resulting in 96% identity. To our knowledge this work represents the first report of Bm91 sequence analysis from an Asian strain of *B. microplus*. Bm91 divergence among Thai and other *B. microplus* strains suggests that further work is warranted to determine if a geographic strain-specific vaccine would be more effective in Thailand.

**Keywords:** Bm91; Angiotensin converting enzymes; Salivary glands; *Boophilus microplus*

**Abstracts poster sessions (by alphabetical order of first author)**

#### Proteome analysis by mass spectrometry

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Proteomics is an experimental approach to understand the message present in genomic sequences by analysis of proteins expressed in cell or tissue.

**Objective:** The first requirement for proteome analysis is the separation and analysis of proteins from organism or cell by electrophoresis. In two-dimensional gel electrophoresis (2D-E). Isoelectric focussing (IEF), separates proteins according to their isoelectric point (pI), the second step, SDS-PAGE separates proteins according to their molecular weights. Mass spectrometry has become an important and powerful tool for large-scale protein and polypeptide analysis and as the niche methodology in the emerging field of proteomics.

**Methods:** In this investigation, lysates were prepared from a B lymphoma cell line (A20) and the proteins were resolved across a pI range of 3–10 using immobiline DryStrip gel. This was followed by the second dimension, on a 12% polyacrylamide gel. Gel protein digestion was carried out by trypsin and sample became ready for electrospray (ESI) mass spectrometric analysis. ESI coupled on-line with high-pressure liquid chromatography (HPLC), shortened the time to solve the primary structure of proteins and peptides (LC–MS). ESI produces multiply charged species extending its capability to analyze a mass of over 100,000 Da. The ionization process took place in atmosphere and the charged species were transferred into mass spectrometer with high efficiency for analysis.

**Results:** Mass spectrum was formed by charged species for B cell proteins. Charges and masses were indicated on the top of the respective peaks. They were Max.75.9 counts. The highest molecular weight was 198.5977 kDa with pI 4.98 and the lowest was 16.4186 kDa with pI 9.59.

**Conclusion:** Mass spectrometry is an indispensable technique for proteomics. This facilitates the study of all protein complexes and organelles that can be purified.

#### Monitoring of influenza A viruses in synanthropic birds in South of Western Siberia in H5N1 epizootic and postepizootic period

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Wild birds are a natural reservoir of influenza viruses from which influenza can penetrate in population of domestic birds. Migrations of wild birds promote geographical spreading of various strains of influenza A viruses. It is necessary to monitor the circulation of influenza viruses among wild birds in regions located on birds' migration ways. The special importance should be given to monitoring of influenza viruses among synanthropic birds because of their close contacts with man and domestic animals on the one hand, and with wild biocenoses on the other. The susceptibility of synanthropic birds to high pathogenic avian influenza (HPAI) virus was shown in many works. Since September 2005 till March 2006 we have collected samples from synanthropic birds in Omsk and Novosibirsk region (South of Western Siberia) to investigate the influenza virus infection carrier state. We have investigated 458 samples from 10 bird species. As a result of screening research in chicken embryos we have isolated five viruses that showed hemagglutination activity. Serological typing of isolates was carried out with the help of serums kindly given by Dr B. Webster (Memphis, USA). Isolate of H4 serotype was isolated only from one bird (from *Pica pica*) during the epizootic period, that is equal to 0.2%. All the other four isolates belonged to a New Castle virus. Thus, we have not registered high pathogenic avian influenza viruses in synanthropic birds during epizootic and postepizootic periods. Probably, it accounts for weak susceptibility of synanthropic birds or small amount of samples.

#### The problems of property preservation of museum and collection strains of mycobacteria of the tuberculosis complex at long-term cultivation

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Cultivating microorganisms on an artificial medium, we recover it from the process of interaction with macroorganism and start the process of changes in genotype stability. The recent data are indicates on property changes of many bacterial pathogens after two to three passages while cultivation on artificial media.

We have developed a new method of cultivation of pathogenic mycobacteria on a basis of continuous cell culture. The investigated strains of pathogenic mycobacteria are passaged with the help of two methods (on Levenstein–Yensen medium and on DM Eagle's MEM growth medium with *Vero* cell culture). Sensitive laboratory animals (guinea pigs) were used for pathogenicity studies. The guinea pigs

were euthanased on day 80 post-infection. Estimation of pathological changes in internal organs of guinea pigs showed that pathologic changes started eliminating with third passage while cultivating on Levenstein–Yensen medium. Evaluation of internal organs of guinea pigs infected with *M. tuberculosis* grown on DM Eagle's MEM Vero medium showed that pathological changes are observed at least up to passage 13. The examination of internal organs of guinea pigs infected with *M. tuberculosis* grown on DM Eagle's MEM-Vero medium showed that pathological changes were high and constant.

The following conclusions can be drawn from the obtained results: the virulence of mycobacterium of the tuberculosis complex decreases while cultivation on Levenstein–Yensen medium. Pathological pictures differ in animals infected with mycobacteria cultivated using a continuous cell culture and in those infected with mycobacteria grown on Levenstein–Yensen medium. We have developed a new method of cultivation that allows to remain the most important biological properties unchanged at long-term cultivation on a continuous cell culture.

#### Identification of genetic diversity within *Brugia* species in feline based on internal transcribed spacer regions

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It has been reported that *Brugia malayi* have infected not only human but also animals such as cats, monkeys, and dogs, whereas *B. pahangi* causes morbidity only in cat reservoirs. Due to their similarity in morphologies and others, the identification of both brugian species in such carriers based on traditional detection tools can be difficult and mostly lead to misdiagnosis. Hence, the data based on genes is the alternative useful information for not only parasite identification but also their genetic diversities. The internal transcribed spacer (ITS) regions were used to determine the genetic diversity of *Brugia* spp. within domestic cat reservoirs from different geographical areas in Thailand. Microfilaria was separated and their DNA was extracted prior to PCR amplification. The specific primers of ITS1 and ITS2 regions were used to yield the PCR products of 580 bp and 660 bp in size, respectively. The fragments were cloned, sequenced, and aligned in comparison to the reported data of *B. malayi* and *W. bancrofti*. It was found that ITS1 and ITS2 phylogenetic trees demonstrated the genetic variation among *Brugia* spp. Phylogenetic trees based on Neighbor Joining (NJ) and DNA Parsimony (DNA PARS) revealed both single infection of either *B. malayi* (cats 1, 3 and 4) or *B. pahangi* (cats 6 and 7) and mix infection of both *Brugia* spp. (cats 2 and 5). It can be proposed that ITS regions could be used for studying genetic diversity of *Brugia* spp., especially, in cat reservoirs which will be beneficial for epidemiological survey.

#### Spatial approach of the production of *Aedes aegypti* pupae using GIS and remote sensing

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DHF is a permanent challenge for Public Health authorities in Thailand, as epidemics in 1997–1998 and 2001, spread over most of the country. Wide variations of level of incidence over areas mean that to be efficient the control strategy needs the delineation of risk areas. Classical entomological indices are used by public health authorities to launch local vector control activities but their reliability to identify areas with higher incidence and to reduce it, is limited. In the frame of a WHO-TDR program to develop new entomological indices based on pupae counts, an exhaustive survey of potential breeding sites has been done in areas with different types of urbanization in Thailand. A GIS has been developed, using the precise localization (GPS) of houses as a basic layer. The characterization of the most productive breeding sites in terms of pupae, the density of human population and socio economical indicators, such as the field description of the type of dwellings (unmanaged urban environment, town houses, residential and administrative areas, villages) were additional layers of information. Most productive BS were similar in the different areas. The containers for water storage produced up to 90% of the pupae which density could reach 0.1–2.6 pupae per person. The correlation between the number of potential BS and the number of pupae is higher (0.9) if we consider groups of neighboring houses (density of attributes). A minimal threshold was defined under which stochastic process in BS colonization may lead to an interruption in pupae production. Spatial patterns in the distribution of pupae allows to identify areas where targeted vector control should be easier and more efficient. This method, combining field survey for the characterization of productive breeding sites and GIS technology to delineate areas with a specific type of urbanization, will help to identify similar environments likely to evolve simultaneously in response to the emergence of epidemic phenomena. Control strategies can therefore target the most productive containers but also key areas in the transmission network, for a better efficiency.

#### The RNA virus database

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As concern mounts over the threat posed by RNA viruses, and the amount of genomic information we have for them increases, a single web application providing key genomic resources for all species is timely. We have created the RNA Virus Database to perform six main services for all 700 RNA viral species:

Identify submitted viral nucleotide or amino acid sequences, provide curated whole genome nucleotide alignments – with corresponding phylogenetic trees – for each virus, Align submitted nucleotide sequences to the above, provide amino acid sequences for all viral genes, and allow the user to extract the corresponding region from the above whole genome alignments, provide whole translated genomes for each viral species, show links to the more specialised web sites for the viruses of greatest medical importance (including genotyping tools). We also link to other sites providing further taxonomic or biological information for each virus. We are currently using the database to analyse the deep phylogeny of RNA viruses and the relationship between genome size and genome architecture. We expect the website version of the database to facilitate and encourage research into many other aspects of RNA viral evolution. It is freely accessible at <http://virus.zoo.ox.ac.uk/>.

#### Genetic diversity in clinical and environmental samples of *Legionella pneumophila*

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*Legionella pneumophila* are waterborne bacteria responsible for legionellosis, an emerging disease causing respiratory illness when a susceptible human host inhales contaminated aerosolized water. Molecular epidemiology and diversity studies of *L. pneumophila* usually compare PFGE and/or AFLP patterns from bacterial cultures derived from respiratory and environmental samples. Recently, a sequence based typing scheme based on six loci has been developed for this species, which is also applied on cultured isolates. But culture of this species on selective media, despite its high specificity, presents low sensitivity (only 5–15% of the samples produce cultured isolates). In order to improve the efficiency of sequence based analysis of *L. pneumophila*, we have developed a protocol to amplify and sequence DNA extracted from uncultured respiratory samples and we have used it to sequence and compare three intergenic regions and internal fragments of six genes of the *L. pneumophila* clinical and environmental origin. Sequences of these nine markers were derived from 40 environmental samples and 40 clinical samples taken from different years and were used to study the genetic diversity and population structure of *Legionella* strains in a Spanish region that includes an area where legionellosis has become almost endemic, with continuous bouts of sporadic cases and several outbreaks affecting tens even hundreds of people. We have also studied the phylogenetic relationships among all these 80 isolates. Phylogenetic analyses have revealed that isolates recovered from patients in years 1999, 2004 and 2005 from this area were almost identical. Moreover, genetic differentiation between clinical isolates from this area and the

remaining clinical strains was detected. Statistics measuring genetic differentiation between clinical and environmental data sets also showed a significant differentiation for all 9 markers, both when analyzed independently and when combined. These results are suggestive of an unexpected differentiation between clinical and environmental isolates of *L. pneumophila*.

#### *Borrelia afzelii* gene expression in *Ixodes ricinus* ticks

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Spirochetes belonging to the *Borrelia burgdorferi* sensu lato (s.l.) complex have evolved remarkable ability to survive in diverse ecological niches during transmission cycles between ticks and vertebrate hosts by variable gene expression. To understand the events during spirochaete transmission from feeding ticks to hosts, mRNA levels of selected *B. afzelii* genes (*bbk32*, *dbpA*, *ospA*, *ospC* and *vlsE*) were measured by quantitative real-time Sybr Green PCR. *B. afzelii* infected *Ixodes ricinus* nymphs fed on laboratory Balb/c mice for 0, 24, 48, and 72 h. The mRNA levels of the constantly expressed *flagellin* gene were used for the relative quantification of selected genes. Differences in gene expression profiles were observed in unfed ticks and during tick feeding. mRNA levels of *bbk32* and *dbpA* showed distinctive decreasing patterns during the first 24 h post-attachment, while *OspC* and *vlsE* mRNA levels increased significantly during the feeding process. In contrast, *ospA* levels decreased for the 48 h of tick feeding and slightly increased by 72 h. More detailed and comprehensive studies on regulation of gene expression in different borrelia genospecies on the vector–host interface would aid to develop effective strategies in preventing pathogen transmission.

**Keywords:** *Borrelia burgdorferi* sensu lato; *Ixodes ricinus*; Pathogen transmission; Gene expression; *bbk32*; *dbpA*; *ospA*; *ospC*; *vlsE*

#### First report on lectin-related gene in Iranian main malaria vector *Anopheles stephensi*

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Malaria is the major vector-borne infectious disease. Nearly 40% of the world's population is at risk, and close to 2 million persons (mostly children under the age of five) die every year. Many insects possess lectins with distinct sugar specificities

which play important recognition and protective roles in immune defense against microbial pathogens and parasitic protozoans. Interactions between parasites and vector gut walls may be mediated by the carbohydrates on the surface of parasites and lectin in the vector gut. Fibrinogen-related proteins are a kind of lectin, so in this study we used primers that flanking a sequence of fibrinogen gene based on *Anopheles gambiae* genome. *Anopheles stephensi* specimens were collected from Sistan and Baluchistan Province, followed by DNA extraction, amplification of a 380 bp fragment and sequencing by using FBN9 primers. Two samples originated from Sarbaz and Nikshahr districts had 100% similarity with each other and a GC count of 56.84%. However, its nucleotide similarity with this fragment in *Anopheles gambiae* is about 90%, while amino acid sequences had 92% similarity in these two main vector species of malaria in Africa and Asia. In order to study the role of these genes in interaction between *Plasmodium* and *Anopheles* and to find candidate molecules in designing transmission blocking vaccine, it is necessary to do further research to obtain more information on the structure of lectin related genes in this important but neglected *Anopheles* vectors.

**Keywords:** *Anopheles stephensi*; Lectin; Fibrinogen; Iran

#### Analysis of genetic heterogeneity of POL variants in the HIV-1 circulating in Nivisibirsk (RUSSIA)

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**Objective:** The goal of the work was to assess the heterogeneity of the regions encoding reverse transcriptase and protease of the HIV variants circulating in Novosibirsk and to analyze the emergence of the virus variants carrying drug-resistance mutations.

**Patients and methods:** The peripheral blood samples of HIV-infected patients from the Novosibirsk AIDS Center were used in the work. The patients were divided into two groups—those received antiretrovirus therapy (ART) and not received it. HIV-1 RNA was isolated from blood sera. DNA nucleotide sequences were determined in a Beckman CEQ2000XL (Beckman Coulter, USA) automated sequencer. Mutations were assayed for their connection with drug-resistance using the Stanford University HIV Database Drug Resistance.

**Results:** Overall, the fragments for the regions of HIV-1 reverse transcriptase were obtained and analyzed for 84 HIV-1 variants; of protease, for 54 variants. Phylogenetic analysis of the obtained reverse transcriptase sequences demonstrated that the HIV variants cluster into two main clades, one belonging to HIV-1 subtype B and the other, to subtype A. The mutations belonging to the secondary resistance mutations described for HIV-1 subtype B were detectable in all the samples. The mutations connected with the drug resistance to protease inhibitors were recorded in the following codons: I13V (40% of the samples studied), H69K (70%), M36I (100%), V77I

(50%), and I93L (100%). The detected occurrence rate of mutation A62V in reverse transcriptase gene amounted to 81%. Analysis of the samples for the presence of primary mutations rendering HIV-1 resistant demonstrated that the HIV-1 variants isolated from the not received ART patients had no primary resistance mutations. The mutations determining resistance to a wide range of drugs were found in 78% HIV variants from the ART patients. In the studied HIV variants isolated from the patients receiving reverse transcriptase inhibitors, the most frequently met mutations occur at codons 215, 184, 41, and 210 in the case of administration of nucleoside inhibitors and at codons 103, 181, 184, 188, and 190 in the case of non-nucleoside inhibitors. In the case of treatment with protease inhibitors, the resistance mutations emerge at a considerably lower rate.

**Conclusion:** As no resistant variants were isolated from the patients that did not receive ART, we may assume that the resistant variants had not distributed in Novosibirsk. The HIV variants isolated from ART patient treated for a period over 1 year developed multiple drug resistance. This work demonstrates that it is of the utmost importance to monitor the HIV-1 drug resistance in the clinical practice. Administration of the anti-retrovirus therapy in our country is only at its very beginning; therefore, emergence of HIV-1 drug resistant variants is possible in the nearest future.

**Keywords:** HIV-1; Reverse transcriptase; Protease; Genetic heterogeneity; Drug-resistance mutations

#### The association of host HLA type and the sequence variation of Gag gene of HIV-1 CRF\_01AE subtype

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Human immunodeficiency virus or HIV is one of the most well-known viruses. As other viruses, the survivability of HIV is hanged in the balance with two arms. First side is the selection pressures, such as an immune response and anti-retroviral drug, and another is the capability of the virus for escaping from these selection pressures. Because HIV has an extraordinary of genetic diversity and the ability that can infect and replicate in the cellular component of the immune system, these seem to make HIV undefeatable. However, the immune pressure also leaves the scare, as a traceable history, on the viral genome. To determine the effect of host immune response, we analyzed the viral genetic diversity of 370 HIV-1 CRF\_01AE Gag sequences obtained from 116 HIV-1 infected couples, and examined the association between host HLA and

the sequence polymorphism. We found that the rate of sequence variability was depended on the region of *Gag* gene. The part of *Gag* gene that had highest mean sequence variability was p2 spacer region followed by p6, matrix, p1, nucleocapsid, and the most conserved capsid region, respectively. We had identified 52 variable sites that were associated with 12 host HLA alleles. Most associations were observed in the positions that had high polymorphism. The variable sites that associated with the same HLA were clustered in the specific region of *Gag*, especially HLA-A. HLA-A\*02, A\*11, and A\*24 associated variable sites were clustered in the matrix protein, whereas the variable sites that associated with HLA-B were distributed in all parts of *Gag* protein. Fifteen variable sites located within HLA-specific CTL epitopes. Although most of variable sites did not locate within HLA-specific CTL epitopes, we found that some of them were correlated with the variable sites that located within HLA-specific CTL epitopes. This correlation might be a compensatory mechanism of the escape mutation. Reversion of the mutated to wild type amino acid was commonly found in many variable sites, when the virus was transmitted from the HLA-presented patient to their wives or husbands that did not have that HLA. The rate of reversion depended on the strength of the association. Our result showed that the HLA-restricted immune response and the reversible escape mutation were the basic mechanism that caused the sequence variation of HIV-1.

#### **Molecular identification of *Anopheles superpictus* (Diptera: Culicidae) complex from Iran and Pakistan**

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Malaria is one of the most important parasitic and vector-borne diseases in southeast provinces of Iran. Eight out of 19 species of *Anopheles* in Iran incriminated as malaria vectors. *Anopheles superpictus* usually in its distributed regions acts as secondary vector. This species is more prevalent in northern Pakistan and Afghanistan, through the Caucasus into southern Tadzhikistan and middle Asia, and through the Middle East and Asia Minor to Southeastern Europe. In this study, *An. superpictus* specimens collected from Sistan & Baluchistan, Ilam and Khorassan provinces in Iran and Karachi in Pakistan, followed by morphological identification, DNA extraction and PCR amplification of ITS2 region. Total size of amplified fragment in sequenced specimens was 525 bp with ITS2 region of 352–373 bp and GC count of 53–56%. This is the first world report on identification of ITS2 region in *An. superpictus* as a complex species including two suspected species A and B from Iran and Pakistan. Detailed analysis and differences in these two suspected species will be discussed.

**Keywords:** *Anopheles superpictus*; Suspected species A and B; ITS2; Iran; Pakistan

#### **Polymorphism in the P-glycoprotein (Pgp) gene from *O. volvulus* Mexican isolates**

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Onchocerciasis is caused by the filarial parasite *Onchocerca volvulus*, it is transmitted by *Simulium* spp. It is the second infectious disease that leads to blindness in the world. Ivermectin is the drug administered to the population at risk. Nematodes of veterinary importance under pressure with ivermectin develop resistance to this drug. Simple strand and conformational polymorphism analysis (SSCP) and PCR-RFLP of the associated multidrug-resistance P-glycoprotein (Pgp) gene, from *O. volvulus* Mexican isolates from Oaxaca and Chiapas foci were done. By SSCP was found genetic variation in three out of six samples from Chiapas, but there was no variation in samples from Oaxaca. The pattern of the PCR-RFLP of Pgp were analyzed by using the NTSYS-PC program and the corresponding phenogram was built with the UPGMA method. The corresponding phenogram showed three groups, one formed with samples from Oaxaca, other with the samples from Chiapas and the last is formed with samples from both, Oaxaca and Chiapas. The role of the geography and idiosyncrasy of people living in Chiapas and Oaxaca foci is highly important in variation. Oaxaca has no migration and excellent control of ivermectin administration, Chiapas state has high illegal migration without a control of dosages of ivermectin and there are no geographical barriers.

#### **Random amplified polymorphic DNA technique for the identification of *Leishmania* species recovered from *P. papatasi* in north-east of I.R. Iran**

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Zoonotic cutaneous leishmaniasis (ZCL) is an important endemic disease in Sabzevar district, north-east of Iran. It has been shown that *L. major* is the species encountered and *Rhombomys opimus* is the main animal reservoir host. In this study, promastigotes were isolated from infected *P. papatasi*, inoculated subcutaneously into the base tail of BALB/c mice separately. The isolates were successfully reisolated from the BALB/c and then cultured in Schneider's media supplemented by 10% FBS, subsequently *Leishmania* species were determined by RAPD-PCR with four oligonucleotide primers including AB1-O7, A4, 327 and 329. The identification was also confirmed by RFLP-PCR method with nagt gene by Dr. K.P. Chang from Chicago University, USA. Out of five



*Leishmania* spp. isolated from *P. papatasi*, all of them were identified as *L. major* by above techniques. The results showed that *L. major* is the principal agent of ZCL and *P. papatasi* is the main vector of the disease in studied area in Iran.

#### What evolutionary ecology tells us about dengue control

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Many current vector-borne disease control efforts narrowly focus on mosquito control. Often, these projects lack a broader, evolutionary ecology viewpoint of the disease dynamics. We propose a transdisciplinary approach to analyze disease transmission by evaluating interactions between the vector, pathogen, human and environment within a defined ecosystem. Included in this paper is summary of classic ecological concepts vital to understanding population, community, and ecosystem biology, as well as key ideas regarding the evolutionary ecology of pathogens and disease, with a focus on dengue and its vectors. Mosquito control and avoidance methods are summarized, noting the environments and situations in which they could effectively reduce or eliminate the risk of dengue infection. A protocol was designed to assist in creating an efficient dengue control program including guidelines on how to select a study site and identify the study population and ecosystem, as well as monitoring techniques to evaluate program success.

#### Development of immunological test kit for detection of porcine reproductive and respiratory syndrome in swine

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Two types of virus, European type (EU PRRSV) and North American type (US PRRSV), are known to cause porcine reproductive and respiratory syndrome (PRRS). An enzyme-linked immunosorbent assay (ELISA) for the simultaneous detection of serum antibodies against these two PRRS types was developed. Fusion nucleocapsid proteins of EU PRRSV and US PRRSV (USEU-N protein) expressed in *Escherichia coli* were partial purified and used as antigens. Determined by checkerboard titration optimal condition was obtained using USEU-N protein at 1:1600 and serum at 1:40. The optimal cut-off value for developed USEU PRRS ELISA was found to be 0.4, having sensitivity and specificity at 97.5% and 100%, respectively. Comparison was made with IDEXX<sup>®</sup> HerdCheck PRRS ELISA using two graph-ROC program testing with 200 positive sera and with 200 negative sera. The degree of agreement ( $\kappa$  value) was highly obtained at 0.7652. The kit is

considered reliable for routine diagnostic, epidemiological surveys and outbreak investigations.

**Keywords:** PRRS; Recombinant protein; ELISA

#### Characterization of avian influenza virus (H5N1) from Asian open-billed storks in Thailand

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Emergence of a highly pathogenic avian influenza virus (H5N1) was started in Thailand since late 2003. Asian open-billed stork is one of the waterfowl species infected by H5N1 virus. To investigate whether it could be a reservoir or carrier of this virus, during February 2004–September 2006, 1959 cloacal swabs of this waterfowl from central part of Thailand were collected for H5N1 surveillance. All together, 30 H5N1 viruses were isolated. To characterize the molecular epidemiology, 244 full-length genes were sequenced. All viruses had multiple basic amino acids at the hemagglutinin (HA) cleavage site. Uniquely, 16 neuraminidase (NA) sequences had no glycosylation at position 235 which was associated with the presence of IERRRKKR in the cleavage site of HA. Additionally, all isolates possessed at least 1 human specific residue at position 79 of PB1-F2. Phylogenetic analysis was demonstrated that these viruses originated from the Gs/Gd/1/96-like lineage and formed distinct sub-lineages corresponded to Thailand isolates. Our study presents molecular basis and provides insight to understand the evolution of H5N1 viruses as well as assists in planning for pandemic influenza surveillance.

#### Identification of *Trypanosoma* sp. based on hypervariable region of 18S rDNA

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The complete nucleotide sequences of 18S rDNA (2100 bp) from 42 isolates of trypanosomes were aligned against those of *Euglena viridis* and *Khawkinea quartana* by using ClustalX program. The DNA parsimony (DNA PAR) phylogenetic tree showed five hypervariable regions (HV) within 18S rDNA sequences. Upon analysis of each region, it was found that DNA PAR tree of the HV region 2 (272 bp) was corresponded to DNA PAR tree of 18S rDNA in classification of *Trypanosoma* sp. The HV regions 1 and 3 (242 bp and 314 bp) the distinction of section *Salivaria* from other *Trypanosoma* sp. whereas HV regions 4 and 5 (292 bp and 402 bp) could discriminate only section *Stercoraria* from the others. Based on the analysis, it can be concluded that DNA PAR tree inferring from HV regions of 18S rDNA could represent the tree of 18S rDNA. Thus, this could be applicable for identification and discrimination of *Trypanosoma* sp. which will be useful for epidemiological study and the control of the disease.

### Genetic diversity of *Trypanosoma evansi* in buffalo based on internal transcribed spacer (ITS) regions

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The nucleotide sequences of 18S rDNA and internal transcribed spacer (ITS) regions were used for studying the relationships of *Trypanosoma evansi* isolate from a buffalo. The sequences were analyzed and compared to 18S rDNA and the ITS regions of the other *Trypanosoma* spp. Maximum likelihood phylogenetic trees were constructed using *Leishmania major* as the outgroup. The tree of 18S rDNA indicated that *T. evansi* (buffalo B18) isolate was closely related to those of Taiwan and *T. brucei* stock. The ITS tree showed the genetic diversity among 32 clones of *T. evansi* (B18) within a single host. This data will be useful for epidemiological and dynamic studies for designing the rational control programs of the disease.

**Keywords:** *Trypanosoma evansi*; Phylogenetic; Nucleotide sequence; Small subunit rDNA; 8SrDNA; Internal transcribed spacers (ITS); Buffalo

### Genetic diversity as an indicator to the activity of *Echinococcus multilocularis* on the French region of the Ardennes

Jenny Knapp, Jean-Mathieu Bart, Marie-Hélène Guislain, Francis Raoul and Renaud Piarroux

*Echinococcus multilocularis* is a cestode responsible for one of the most important zoonosis in the Northern hemisphere, the Alveolar Echinococcosis disease. The parasite involves in its live cycle mainly foxes as definitive hosts (DH) and rodents as intermediate host (IH). Human can accidentally act as IH and develop the disease after a long incubation period (10–15 years). In France, about 15 new cases are recorded each year. The parasite is chiefly present in the East and the Centre of the country. Since 1984, 7 human cases were described in the department of the Ardennes, and then could be considered as an emergent endemic focus. Moreover the prevalence in foxes reached 53%. Due to the long incubation period, the number of human cases could not reflect the current activity of the parasite in this area. The genetic diversity of the parasite could reflect its activity in the Ardennes. In this aim, we have studied two microsatellite DNA targets, the tandem repeat multilocus microsatellite EmsB and the single locus microsatellite NAK1 on a panel of 25 red foxes, presenting different worm burdens. These two targets allowed us to defined 6 main genotypes among 145 adult worms. A fox, presenting the more important worm burden ( $n = 73380$  worms), harboured individuals with a heterozygote genotype for the NAK1 locus, suggesting cross-fertilization event. From the microsatellite analysis, we proved to be able to detect genetic variability in

restricted areas, and thus to track dynamic transmission of *E. multilocularis* at local scale.

### The origin of European and North American infectious salmon anemia virus (ISAV) revisited by relaxed molecular clock analysis

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An emerging infectious disease, called infectious salmon anemia (ISA), among farmed salmon has raised the concern of its impact on salmon-farming industry. The causative agent was identified to be a segmented RNA virus and named as infectious salmon anemia virus (ISAV). Earlier phylogenetic analyses classified the ISAV, predominating in European and North American aquacultural farms separately, into two genetically distinct strains: European (EU) and North American (NA) type. It was suggested that human traffic and trading have transported their common ancestor between these continents, and led to their local emergences. A recent evolutionary study on ISAV hemagglutinin-esterase (HE) and fusion protein (FP) gene suggested, under global molecular clock assumption, atypically low substitution rates ( $10^{-6}$  and  $10^{-5}$  substitutions/site/year, respectively) comparing to other RNA viruses. This result has new implications on the viral epidemiology and the hypothesis of origin: EU and NA isolates may have separated long before any human traffic could transport the virus. We examined the genetic sequences of HE gene from both EU and NA strains of ISAV. Our result demonstrated a large disparity of the substitution rates with in the phylogeny, which disrupted the molecular clock assumption, was caused by the presence of a small group of virus isolates with extremely low rate. The presence of these isolates in the phylogeny can mislead the substitution rate estimation in global clock analysis. By using relaxed molecular clock models implemented in the Bayesian framework which accommodating the rate variation, the time of the most recent common ancestor of EU and NA strains was extrapolated to around 300 years ago. This finding reasserts the original hypothesis suggesting the translocation of ancient ISAV between North America and Europe could be a consequence of human cross-ocean traffic and trading, which had been refuted by the recent global clock analysis.

**Keywords:** Infectious salmon anemia virus; Relaxed clock; Molecular dating; Penalized likelihood

### Foot-and-mouth disease virus isolated from pig in Thailand in 2005 related to Cathay Topotype in Vietnam

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Cathy Topotype of Food and Mouth Disease Virus Moved From Vietnam to Thailand.

Two samples of pigs infected Food and Mouth Disease Virus (FMDV) in the central part of Thailand in 2005 were identified by RT-PCR and nucleotide sequencing. The nucleotide sequence was analyzed of multiple alignment and constructed dendrogram by MEGA3.1 program. The dendrogram data of partial VP1 sequence showed that two viruses isolated in Thailand closely related with the Cathay topotype isolated pig from Vietnam in 2004. Moreover, 3A non-structural protein of both isolations showed ten amino acid deletions (position 93–102). In addition, the virus was not found in cattle in the endemic area. This is the warning system for control the outbreak of FMD in Thailand because this is the first reported of this topotype in this country.

**Keywords:** FMDV; Cathay topotype; Phylogenetic tree

#### Identification of *Babesia canis vogeli* from domestic dogs in Nakhon Phathom, Thailand in 2005

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The reverse line blot hybridization (RLB) and a restriction fragment length polymorphism (RFLP) analysis were analyzed *babesia* species in dogs. Eighteen blood samples of dogs were found to be *babesia* infections by microscopical analysis of blood smear at Kasetsart University Veterinary Teaching Hospital of Kasetsart University in Nakhon Phathom province in 2005. The blood samples were performed partial 18s rRNA gene amplification by polymerase chain reaction (PCR) technique, and identified *babesia* species by species-specific probe of TBD-RLB KIT (Isogen<sup>®</sup>). All PCR products were also analyzed with RFLP with TaqI restriction enzyme to confirmed *Babesia* species. The PCR combined with species specific probe (TBD-RLB KIT) and RFLP with TaqI restriction enzyme were showed *Babesia canis vogeli* infection in these eighteen domestic dogs. The partial 18S rDNA spanning the V4 region of three samples were chosen for analysis and revealed the identical sequences with *Babesia canis vogeli* (accession no. AY072925).

**Keywords:** *Babesia canis vogeli*; 18s rRNA; Reverse line blot hybridization; RFLP

#### Production of monoclonal antibodies against 3AB non-structural protein of foot and mouth disease virus

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Recombinant 3AB protein of a foot and mouth disease virus (FMDV) was expressed from *Escherichia coli* and immunized in mouse after gene amplification and gene cloning into pQE30 vector (Qiagen<sup>®</sup>). The immunized-mouse was collected spleen to fusion with myeloma cells strain P3-X63-Ag8.653. Hybridoma propagated in HAT medium was eval-

uated by recombinant 3A and 3B enzymed linked immunosorbent assay (ELISA). The results showed that positive clones produced antibodies against 3A and 3B proteins and also detected FMDV infected BHK-21 cell by immunoperoxidase monolayer assay (IPMA). The monoclonal antibodies against 3AB protein will be used for serological development to discriminate between infected and vaccinated animal.

**Keywords:** FMDV; 3AB protein; Monoclonal antibody

#### Spoligotypes of *Mycobacterium tuberculosis* strains from Brazilian tuberculosis patients

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Brazil is in 14th place among the 23 countries that concentrate 80% of Tb cases in the world, with 50 millions of infected and 6000 deaths annually. The goal of this study is to evaluate *Mtb* strains diversity on a national level. Brazil is a Republic composed by 26 states, a Federal District and has a population of around 170 million. Seventy percent live in the eleven states of which *Mtb* isolates were genotyped (Amazonas, Ceara, Goias, Minas Gerais, Para, Parana, Pernambuco, Rio Grande do Sul, Rio de Janeiro, Sao Paulo, and Sergipe). A total of 2000 *Mtb* isolates was submitted to spoligotyping and 42% of the isolates were from the Southeastern region, the richest and most industrialized Brazilian region. A considerable variability of spoligotypes was observed and upon comparison with already described genotype families, 515 were classified as the Latin American and Mediterranean (LAM) family, 20% as the T

family and 13% as the Haarlem family. Within the LAM family, 27% was LAM 9 and within the Haarlem family, 55% belonged to the Haarlem 3 class; 69% of the T family was T1. Another 16% were recognized as other families, including the X family, S family, East African and Indian Family and the Beijing family (3 strains in Rio de Janeiro city). Unrecognized profiles (U) and profiles that were classified only by the type number in the SpoIDB4 database were also observed. Besides genotypes already described in literature, 15% were classified as new types and include profiles belonging to LAM, Haarlem, T, and other families. Interestingly was the absence of LAM class 7, and 10. Furthermore, no genotypes characteristic for other species of the MTBC were observed. Our data demonstrate the high prevalence of some strain families that are probably deeply rooted in the phylogeny of Brazilian Mtb, probable consequence of the colonization process started in the beginning of XV century. Representative of many other families were found in a small frequency (Manu, Beijing, Class LAM 11 and 8); this could represent cases resulting from modern migratory flux between countries and continents.

#### Transcriptional levels of *Helicobacter pylori* *cagA* and *vacA* genes in Lebanese patients with gastritis and peptic ulcer disease

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**Background:** The prevalence and clinical relevance of *Helicobacter pylori* *cagA* and *vacA* virulence genes in the pathogenesis of disease phenotype was assessed by a novel approach for this organism consisting of gastric mucosal *H. pylori* gene transcription levels determination and comparisons made according to disease phenotype.

**Materials and methods:** Gastric mucosal biopsies were collected from patients with peptic ulcer disease (PUD), gastritis, and normal mucosa in an academic medical center in Lebanon. *H. pylori* was detected in these biopsies by rapid urease (CLO<sup>TM</sup>) test and PCR amplification of the *ureaseA* gene. *H. pylori* virulence genes, their transcription and transcription levels were determined respectively by PCR, RT-PCR and real time RT-PCR.

**Results:** Forty-five percent of patients were *H. pylori* positive by PCR of the *ureaseA* gene, 37.5% of whom had *cagA* and 59.4% had *vacA*. The *vacA* s1a allele was more prevalent in our study population and appeared to be associated with increased virulence. The *cagA* and *vacA* genes were detected

and transcribed more frequently in PUD patients and the transcription levels of both *cagA* and *vacA* genes were observed to be higher in endoscopically apparent disease phenotypes (i.e., PUD, gastropathy) than in controls suggesting that they likely contribute to disease pathogenesis.

**Conclusions:** This study provided insight into the virulence potential of *H. pylori* encountered in Lebanese patients with gastroduodenal pathology. The results of our investigation in this regard need to be corroborated through larger studies.

#### The study of TH<sub>1</sub> and TH<sub>2</sub> cytokines profile (FN $\gamma$ , IL-12, IL-4, IL-10) in PBMCs of patients with MDR-TB and newly diagnosed treated cases tuberculosis

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Multi-drug-resistant strains of *Mycobacterium tuberculosis* seriously threaten TB control and prevention efforts. Studies of the immune response mechanisms are essential. In this article, the status of cytokines release is examined after stimulating the mononuclear cells of patients. 16 MDR patients, 14 newly diagnosed TB cases were selected according to clinical/radiological data. 10 apparently healthy PPD negative individuals selected as control. Blood was obtained and PBMCs were isolated by differential centrifugation over ficoll-Hi Paque and plated at  $2 \times 10^5$  cells per well. PPD and PHA were then added to proper wells and were cultured at 37 °C. Supernatants were harvested and frozen at -70 °C. Cytokine concentrations were measured by ELISA. Concentrations of IL-10 AND IL-4 in supernatants from tuberculosis patients (responsive to treatment and MDR) does not differ significantly, while IL-12 and IFN $\gamma$  was much higher in patients compared to control group. The present finding is not compatible with the data reported by John F. McDyer and J.S. Lee and it seems in our patients groups no marked imbalance in Th<sub>1</sub>/Th<sub>2</sub> activity is noticeable.

#### Independent evolution of pyrimethamine resistance in *Plasmodium falciparum* in Melanesia

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A single focus origin of pyrimethamine-resistant *Plasmodium falciparum* is suggested to have spread from Southeast Asia to

Africa. We have compared the genetic profile of pyrimethamine-resistance in Melanesia, where unique chloroquine resistance developed independently, including the polymorphism of the dihydrofolate reductase gene (*dhfr*) and microsatellite haplotypes flanking *dhfr* in a total of 285 isolates from different regions of Melanesia (Papua New Guinea, Vanuatu and Solomon) and Southeast Asia (Thailand and Cambodia). Nearly all isolates (92%) in Melanesia harbored a *dhfr* double mutant (CNRNI at positions 50, 51, 59, 108 and 164), whereas 98% of isolates were either triple (CIRNI) or quartet (CIRNL) mutants in Southeast Asia. Microsatellite analysis revealed two distinct lineages of the *dhfr* double mutants in Melanesia. One lineage had the same microsatellite haplotype as reported in Southeast Asia and Africa, suggesting the spread to Melanesia from Southeast Asia. The other lineage had microsatellite haplotype not found elsewhere. This study therefore provides evidence for independent at least partly unique evolution of *P. falciparum* pyrimethamine-resistance in Melanesia, in contrast to the apparent common evolution in Southeast Asia and Africa.

#### Variability among *Taenia solium cysticerci* from Mexico state of Mexico

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In Mexico, neurocysticercosis has an incidence of 0.2–3.4%. High human neurocysticercosis mortality rates are found in the State of Mexico, situated around Mexico City, comprising both urban areas with a high human population and rural areas with traditional breeding of pigs that often lacks appropriate hygienic conditions. Mitochondrial COI, ribosomal ITS1, and 28S rDNA from 23 *T. solium cysticerci* isolates from pigs from several districts in Mexico State and *Cysticercus racemosus* and *C. cellulosae* from patients with neurocysticercosis were PCR-RFLP analyzed with several restriction enzymes. The PCR-RFLP data were analyzed with the Li Nei ( $S_{ij} = 2n_i - j/n_i + n_j$ ) index, the similarity matrix was constructed among the different isolates of cysticerci using Ntsys-PC Version 2.0 software and the corresponding dendrogram with the UPGMA program. The statistical analysis was done with the Mantel test. These analyses showed three groups of cysticerci isolated from pigs and three groups from human brain. The dendrograms demonstrated that there is intraspecific variability in *T. solium* isolates from Mexico State. Sequencing and polymorphism analysis showed that the phylogenetic tree of ITS1 has two related groups, the first Mexican and the second Philipino. The consensus tree of the COI gene shows four groups and the highest nucleotide diversity of the three analyzed sites.

#### Acknowledgements

A. Monroy-Ostria and G. Zúñiga-Bermúdez are supported by COFAA and EDI, IPN, Mexico. A.C. Hinojosa-Juárez was sponsored by CONACYT, Mexico.

#### Variation in the O-150 *Onchocerca* specific repeat sequence family of *Onchocerca volvulus* Mexican isolates

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Onchocerciasis has been the world's second leading infectious cause of blindness. It is endemic to Africa, the Arabian Peninsula, and the Americas. Around 120 million people worldwide are at risk of onchocerciasis. In Mexico there are two endemic foci one in Oaxaca and the other in Chiapas States. The disease is caused by the nematode *Onchocerca volvulus*. DNA from adult worms of *Onchocerca volvulus* from Oaxaca (24 onchocercosmata) and Chiapas (28 onchocercosmata), Mexico were used as templates to amplify and sequencing members of the O-150 *Onchocerca* specific repeat sequence family. The O-150 sequences of Mexican *O. volvulus* isolates were aligned and compared with the O-150 sequences of savanna and rain forest strains of Africa, one strain from Guatemala and one from Brazil. The statistical analysis was done and the corresponding trees were built. It was found higher variation in the Mexican isolates sequences than the Brazilian and African. Mexican and Guatemala isolates are more related to the African rain forest and the Brazilian with the African Savanna strains.

#### Acknowledgements

A. Monroy-Ostria was supported by COFAA and EDI, IPN, Mexico. A. Ramírez-Ramírez was sponsored by CONACYT, Mexico.

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#### Study on iron uptake regulation in *Agrobacterium tumefaciens*

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Iron is critical for bacterial growth, but problems arise from the toxicity of excess iron; thus, iron uptake is subject to tight control. The most widely found and best studied iron-responsive regu-

lator in Gram-negative bacteria is the ferric uptake regulator Fur. In recent years, it has become apparent that iron regulation in rhizobia differs from that in many other bacteria. New regulators, RirA were identified which appear to mediate functions that in other bacteria are accomplished by Fur. *Agrobacterium tumefaciens* causes crown gall disease of a wide range of dicotyledonous plants. The *A. tumefaciens* Fur protein does not exhibit significant role on iron regulation. Mutation of *rirA* shows the sensitive phenotype to iron, hydrogen peroxide, tert-butyl hydroperoxide, menadione and streptonigrin which resulted from iron overload. The result form reverse transcriptase PCR (RT-PCR) indicated that Fur regulates *sitABCD* (an ABCs-type transporter). RirA plays an important role in regulation of iron responsive genes which are siderophore production and *suf* operon; an [Fe-S] cluster formation. Blast searched indicate putative binding box of RirA; IRO-like motif lie on the siderophore and *suf* gene. The ability to infect plant is affected by mutation of *rirA* shows in decreasing of both *virB* and *virE* promoter activity and slightly reduced tumor formation.

**Keywords:** *Agrobacterium tumefaciens*; Iron regulation; Fur; RirA

#### Occurrence of two heterophyid metacercariae from freshwater fish in reservoirs

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According to current information, the metacercaria of heterophyid trematode found in freshwater fishes, especially the cyprinoid group. A parasitological investigation was made using 241 fishes from 12 species: *Barbodes schwanenfeldi*, *Labiobarbus siamensis*, *Barbodes gonionotus*, *Osteochilus hasselti*, *Henicorhynchus siamensis*, *Cyclocheilichthys armatus*, *Cyclocheilichthys apogon*, *Mystacoleucus marginatus*, *Cirrhinus cirrhosus*, *Notopterus notopterus*, *Oxycleotris marmoratus* and *Pristolepis fasciatus*. The fishes were caught over the summer period (February 2006–May 2006) in the Chiang Mai water reservoirs (Mae Ngud and Mae Kwong) and the Chiang Rai water reservoirs (Nong Luang and Mae-Kataa). The prevalence of heterophyid metacercariae infection at Mae Ngud, Mae Kwong, Nong Luang and Mae Kataa were 15.04%, 5.39%, 12.37% and 0.07% respectively. The highest prevalence of heterophyid metacercariae infection in *H. siamensis* in Mae Ngud was 17.46%. The freshwater fish, *C. cirrhosus*, *N. notopterus*, *O. marmoratus*, and *P. fasciatus* were not found to be infected with heterophyid metacercariae.

#### Recovery of *Haplorchis taichui* and *Stellantchasmus falcatus* from small intestines of *Gallus gallus domesticus*

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Parasitic zoonoses are widespread in Southeast Asia, particularly Thailand. Among fish-borne trematode infection caused by many species. It is a universally observed characteristic of parasites that they infect a restricted group or a few restricted groups of hosts. This phenomenon has been known as “host specificity” *Stellantchasmus falcatus*, one of the minute intestinal flukes of fish-eating birds and mammals, was first described by Onji and Nishio (1915). *Haplorchis taichui* is a minute intestinal fluke (MIF) that parasitizes the small intestine of birds and mammals including human (Faust and Nishigori, 1926). An experimental study was performed to observe the recovery of minute intestinal flukes, *H. taichui* and *S. falcatus* from small intestines of chicks (*Gallus gallus domesticus*). Metacercariae of *Haplorchis taichui* were isolated from *Labiobarbus siamensis* and *Henicorhynchus siamensis* and metacercariae of *S. falcatus* were isolated from *Dermogenys pusillus* which were collected in the Chiang Mai Province, Thailand, by using 1% acid pepsin solution in a blender. The digested material was incubated in shaking water bath for one and a half hour at 37 °C incubation and subsequently passed through two layers of wet gauze. The digested material was rinsed with 0.85% sodium chloride solution and examined for metacercariae under the stereomicroscope. The identification of metacercariae was carried out by morphological examination based on Sholz et al. (1991) and Wongsawad et al. (2000) under a compound microscope. The 25 of one day-old chicks were orally force fed with a dose of fifty metacercariae of *H. taichui* and one hundred metacercariae of *S. falcatus*. The worms were recovered from small intestines of chicks by Dearman’s apparatus technique. The intestine of the chicks were examined in seven day post-infection (PI). The infection rate of *H. taichui* and *S. falcatus* were 12.32 and 1.44% respectively.

**Keywords:** *Haplorchis taichui*; *Stellantchasmus falcatus*; *Gallus gallus domesticus*

#### Phenotypic resistance prediction from genotypes for human immunodeficiency virus type 1 (HIV-1) protease inhibitors using neural networks

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The 598 HIV-1 protease sequences and their corresponding phenotypic fold change values for six drugs were retrieved from the Stanford HIV RT and Protease Database for neural network modeling using software Clementine Version 7.0. The results were compared with those from the rule-based method from the Stanford HIV RT and Protease Database and the support vector machine method from the Geno2Pheno. The amino acid input pattern encodings gave higher total correlation coefficient values than the binary input pattern encodings, which ranged from 0.83 to 0.93 and the best total correlation coefficient value was 0.93 from “AA Rb” input pattern encoding of the ritonavir resistance dataset. The neural network system provided a high correlation coefficient of 0.96 and high accuracy of 95%, both

of which were higher than the other two systems, when compared with experimental phenotypic testing values. Regarding consensus based prediction; neural network system predicted values also showed better results (97%) than the other two systems.

**Keywords:** Virtual phenotype; Neural networks; HIV-1 protease inhibitors; Resistant mutations; Fold change; Inhibition concentration

### Detection of vancomycin resistance genes and virulence factors in vancomycin-resistant enterococci isolated from patients hospitalized in large university clinical hospital during 2-year period

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We analyzed group of 98 VRE strains isolated from patients hospitalized in large university clinical hospital in Warsaw, Poland during 2-years period (2000–2002). Strains were isolated from blood, wound, peritoneal cavity, bile and feces. We tested VRE strains for their ampicillin and glycopeptides susceptibility, type of van genes and virulence factors genes carried. Strains were identified with phenotype-based methods and checked for species-specific *Enterococcus faecium* and *Enterococcus faecalis* *ddl* genes by PCR. MICs for ampicillin and glycopeptides were determined. *vanA*, *vanB*, *vanC* and *vanD* genes were detected by PCR. Virulence factors genes were detected using PCR method with primers specific for five genes from cytolysin complex, gelatinase, aggregation substance, enterococcal proteins (EfaA) and surface protein genes. Among 98 strains of VRE, the most prevalent was *E. faecium* (82.7%), according to biochemical identification results. Other VRE species embraced *E. faecium*, *E. durans*, *E. gallinarum* and two strains of *Enterococcus* spp. Discrepancy between phenotypic and genetic identification results was noted in case of 11.2% of strains. Resistance to teicoplanin was detected in 96.9% of strains. Three VRE strains were ampicillin susceptible. The most prevalent glycopeptide resistance gene was *vanA* (92.9%). Other types of glycopeptide resistance genes were *vanB* (three strains) and *vanD* (one strain). In two VRE strains, we were unable to detect any van gene. The most prevalent virulence factor gene was *efaA* (88.8% of strains). Other virulence factors genes were also present: *esp* in 72.4% of strains, *gelE* (12.2%) and *agg* (10.2%). Various genes of cytolysin complex were found in 11.2% of strains, but there was no strain carrying complete set of *cyl* genes. We characterized two groups of epidemic strains, and seven groups of VRE not responsible for epidemic infections. Only in case of *esp* gene we found significant correlation between site of infection and carried virulence factor.

**Keywords:** VRE; Hospital acquired information; Virulence factor

### Understanding the interaction and the structure–activity correlation of efavirenz derivatives and WT and K103N HIV-1 RT by molecular docking and 3D-QSAR approaches

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Molecular docking and 3D-QSAR analyses were performed to understand the interaction between a series of efavirenz derivatives with WT and K103N HIV-1 RT. To model the potential binding modes of efavirenz derivatives in the binding pocket of WT and K103N HIV-1 RT, molecular docking approaches by using GOLD and Autodock 3.0 programs were performed. The results show that the docking results obtained from both methods reveal a good ability to reproduce the X-ray bound conformation with rmsd less than 1.0 Å for both WT and mutant enzymes. The docking calculations of all efavirenz derivatives in the data set were, consecutively, performed to elucidate their orientations in the binding pockets. The results derived from docking analysis give additional information and further probes the inhibitor–enzyme interactions. The correlation of the results obtained from docking models and the inhibitory activities validate each other and lead to better understanding of the structural requirements for the activity. Therefore, these results are informative to improve the development of more efficient HIV-1 RT inhibitors, especially, active against mutant enzyme. Based on the molecular alignment of conformations obtained from molecular docking procedures, the high predictive 3D-QSAR models were produced by using CoMFA and CoMSIA approaches. The CoMFA models reveal the importance of steric and electrostatic interactions through contour maps. The resulting CoMSIA models enhance the understanding of steric, electrostatic, hydrophobic, electron donor and acceptor requirements for ligands binding to the K103N HIV-1 RT. Consequently, the results obtained from structure-based and ligand-based design approaches can be integrated to identify the structural requirements of HIV-1 RT inhibitors in the class of efavirenz compounds. The principle derived from the present study provides a beneficial guideline to design and predict new and more potent compounds active against K103N HIV-1 RT.

### Characterization of *sod* genes involved in oxidative stress response and tumor formation in *Agrobacterium tumefaciens*

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Phytopathogenic bacteria are always exposed to reactive oxygen species (ROS) generated from aerobic metabolisms as well as from the host plant first line defense. To avoid oxidative



damages, bacteria produce an array of ROS-scavenging enzymes to detoxify them before reaching harmful level. Superoxide dismutase (SOD) capable of catalyzing the dismutation of superoxide anions to  $H_2O_2$  and molecular oxygen. The removal of superoxide blocks the secondary reactions which lead to formation of the highly reactive hydroxyl radicals. Here, we cloned and characterized multiple SODs from *Agrobacterium tumefaciens*, the causative agent of crown gall disease in plants. Analysis of its genome revealed three genes encoded iron superoxide dismutases (FeSODs). Sod1 and Sod3 are cytoplasmic while Sod2 is a periplasmic isoenzyme. *sod1* was expressed at relatively high level and appears to be increased during stationary phase. The expression of *sod2* is a phase-dependent and produced at detectable level at stationary phase. *sod3* is a member of SoxR regulon whose expression could be strongly induced upon exposure to superoxide anion. Deprivation of *sod1* markedly alleviated resistance to superoxide generator. Moreover, the strain increases tolerance to  $H_2O_2$  due to a compensatory expression of catalase-peroxidase. Inactivation of *sod2* or *sod3* alone slightly affected ability of bacteria to cope superoxide toxicity. The SOD null mutant is extremely sensitive to killing treatment with superoxide generator and attenuated the ability to cause tumor on plant leaves. All the evidences indicate that superoxide dismutases are not only critical enzymes responsible for protection of superoxide anion but also required for a virulence of *A. tumefaciens*.

**Keywords:** *Agrobacterium tumefaciens*; Oxidative stress; *sod*  
**RNA interference in the malaria parasite and its possible use in treatment**

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**Introduction:** Malaria is still a major parasitic disease despite efforts spanning more than a century to eradicate or control it because of the poor understanding of the functions of the malaria parasite. One of the main reasons for the slow progress in the development of new anti-malarial or for an effective malaria vaccine has been the poor understanding of the functions of most of the malaria parasite proteins. Recently RNA interference (RNAi) has emerged as a powerful tool to understand the gene function in variety of organisms and to inhibit the gene expression on the level of single gene, gene families and the entire genome. The major goals of this study is to develop RNAi technologies for functional genomic studies in malaria and follow it, for next studies in future, is to understand molecular mechanisms of malaria drugs and host-parasite interactions.

**Method:** *Plasmodium falciparum*, and to a much lesser extent *Plasmodium vivax*, are the main causes of disease and death from malaria.

The genome sequencing project of the human malaria parasite, *P. falciparum* has identified 5300 proteins, of which 60% (3208) have not been assigned any function. Even though gene targeting by homologous recombination has been successfully used to understand the functions of a number of

parasite proteins, it still has limitations. Following methodology has been used for carrying out RNAi for the two cysteine protease genes of *Plasmodium falciparum*:

1. Selection of dsRNA/siRNA sequences: the length requirement of dsRNA has been recommended greater than 500 bp.
2. dsRNA preparation: using PCR with appropriate RNA polymerase.
3. Preparation of siRNA (Donze and Picard, 2002).
4. Treatment of parasite with dsRNA/siRNA.
5. FACS analysis of dsRNA/siRNA treated GFP parasite lines.
6. Detection of reduction in protein by Western blotting.

**Results:** This study earlier carried out an in-depth study to establish RNAi in *P. falciparum* *in vitro* for the two cysteine protease genes (*falcipain 1* and *2*) of the parasite. Using dsRNAs corresponding to cysteine protease genes of *P. falciparum*, we demonstrated that *falcipains* play an important role in hemoglobin degradation. In this process, specific dsRNA elicits the degradation of cognate mRNA.

#### **Trypanosoma cruzi infection reactivation after immunosuppression is correlated with the parasite genetic diversity**

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The factors involved in the reactivation of chagasic infection are not clear enough and may be related to selective host immune depletion or parasite genetic diversity. To evaluate the role of the parasites genetic in *T. cruzi* infection reactivation induced by cyclophosphamide immunosuppression, groups of 32 Swiss mice were inoculated with *T. cruzi* clonal stocks classified as *T. cruzi* I (Cuicacl1, P209cl1, Gambacl1, SP104cl1) and *T. cruzi* II (Bug2148cl1, MNcl2, IVVcl4, MVBcl8) were used. Infected animals were treated with cyclophosphamide when was with subpatent parasitemia still during the acute phase (AP), and chronic phase (CP). Animals infected with *T. cruzi* I stocks showed 82,6% and 47,5% of parasitemia reactivation during the AP and CP, respectively, being observed 0%, 100%, 100% and 100% of parasitemia reactivation in animals inoculated with clones SP104cl1, Gambacl1, Cuicacl1 and P209cl1, respectively in the AP, and 80%, 40%, 50% and 20% in CP. On the other hand, animals infected with *T. cruzi* II showed only 4.1% of parasitemia reactivation when immunosuppressed during the AP and 0% in CP. However the heart and skeletal muscle lesions of animals infected by *T. cruzi* I were similar to those observed in controls infected and not immunosuppressed group (CI). By the way an increase of encephalic lesions in animals immunosuppressed during the CP in relation to CI was observed. Although parasitemia reactivation was not observed

in animals infected with *T. cruzi* II clones, an increase of inflammatory process in the heart and skeletal muscle, but not in the brain, was observed among animals infected with Bug2148c11. These results showed that the genetic diversity of *T. cruzi* has an important role on the reactivation of the infection after immunosuppression and corroborates the working hypothesis subjacent to the model clonal theory in *T. cruzi*.

#### Identification of *Theileria* in endangered serow and endangered eld's deer in Thailand

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The blood samples of eld's deer and in the zoo and poaching serow were collected for DNA extraction. Polymerase Chain Reaction (PCR) was performed with the 18s rRNA specific primer for babesia and theileria. The PCR products were determined species specific by species-specific probe of TBD-RLB KIT (Isogen<sup>®</sup>). Two PCR products of Eld's deer and also of the serow hybridized to *Theileria/Babesia* common probe but not found hybridization to species specific probe position. Moreover, nucleotide sequences of PCR products were determined by using BigDye Terminal Cycle Sequence Kit and analyzed with blast program which closely related with *Theileria* sp. The sequence of the parasite from the serow, *Theileria* sp. (Khao Yai), showed most similarities with *Theileria* sp. (OT1) of the ovine reported in Spain and from elder's deer, *Theileria* sp. (Khao Khaew) was highest similarity with *Theileria* sp. of deer in Japan. The completely sequence of 18srRNA will be done soon. This is the first report of theileriosis in the wildlife serow and elder's deer in the zoo in Thailand.

**Keywords:** Theileria; 18s rRNA; Reverse line blot hybridization

#### Using a climate dependent matrix model to predict mosquito abundance: Application to *Aedes (Stegomyia) africanus* and *Aedes (Diceromyia) furcifer* (Diptera: Culicidae), two main vectors of the yellow fever virus in West Africa

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Mosquitoes, acting as vectors of diseases, are particularly involved in the transmission of viruses. Thus the abundance of some tree-hole breeding species strongly depends on weather, specially rainfall. The aim of this paper is to provide a tool to predict vector abundance. In order to describe the dynamics of these mosquito populations, we developed a matrix model integrating climate fluctuations. The population is structured in five stages: two egg stages (immature and mature), one larval stage and two flying stages (nulliparous and parous adult females). We considered the water availability in breeding-sites as the main environmental factor affecting the

mosquito life cycle. The model represents the evolution of the mosquito abundance in each stage over time, in connexion with water availability. This model was used to simulate the abundance trends over three years of two mosquito species, *Aedes africanus* (Theobald) and *Aedes furcifer* (Edwards), vectors of the yellow fever in Côte d'Ivoire, West Africa. Water dynamics in the tree-hole was reproduced from daily rainfall data. The results we obtained show a good match between the simulated population and the field data over the time period considered.

**Keywords:** Vector population; Mathematical model; Climate dependency

#### Human immunodeficiency viruses type 1 circulating in the Comunitat Valenciana (Spain)

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Human immunodeficiency virus type 1 (HIV-1) mutates rapidly and nucleotide substitutions, deletions, insertions, and rearrangements resulting from recombination events are the main factors contributing to its high degree of genetic heterogeneity. Nucleotide sequence analyses allow the recognition of phylogenetic relationships and the classification of HIV-1 into different subtypes and recombinant forms. The prevalence of multiple HIV-1 subtypes in a single geographic region might result in an increased frequency of mixed infections. Eventually, recombinants between more than one variant can be found. HIV-1 subtype B is the most prevalent variant in Spain, although non-B subtypes have been reported, mainly among African immigrants. Classification of HIV-1 into subtypes is based primarily on the analysis of sequences coding for the *env* gene. However, the *pol*-coding region has also been validated for this purpose and is currently used much more since drug-resistance testing is undertaken routinely at a large scale. Here we describe the main features of HIV-1 subtypes circulating in the Comunitat Valenciana (Valencia and Alacant provinces). We have amplified and sequenced the HIV-1 protease and partial reverse transcriptase (PR-RT) genes from isolates of 75 patients. Samples were obtained from two Centers of AIDS Information and Prevention and 40% of them correspond to immigrants. Subtype identification was performed by phylogenetic analyses, taking as reference a panel of 120 HIV-1 sequences representing all subtypes and recombinant forms described. We have found 60 (80%) subtype B sequences. Non-B subtypes were mainly represented by recombinant forms (14/15, 18.7%) from patients coming from Africa and South America and only one for subtype A. At least one drug-resistance mutation in the *pol* gene was detected in 86.7% of sequences. This study demonstrates that most viruses circulating in Spain (Valencia-Alacant) are in fact inter-subtype recombinants, with CRF02\_AG being the most prevalent recombinant form.

### Evidence of recombination in hepatitis C virus intrapatient populations

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Hepatitis C virus (HCV) is a major cause of liver disease worldwide and a potential cause of substantial morbidity and mortality in the future. The prevalence of HCV infection is estimated to be 2%, representing about 170 million people in the world. Hepatitis C virus is characterized by a high degree of genetic heterogeneity. Although homologous recombination has been demonstrated in many members of the family Flaviviridae, to which HCV belongs, there are few studies reporting recombination on natural populations of HCV, suggesting that these events are rare *in vivo*. Furthermore, these few studies have focused on HCV recombination between different genotypes/subtypes but there are no reports about the extent of intra-genotype or intra-subtype recombination between viral strains infecting the same patient. Given the important implications of recombination for RNA virus evolution, our aim in this study has been to assess the existence and eventually the frequency of intragenic recombination on HCV. For this, we have analyzed two regions of the HCV genome (NS5a and E1–E2) in viruses obtained from patients belonging to two different groups: (i) infected only with HCV (either treated with interferon plus ribavirin or treatment naïve), and (ii) HCV–HIV co-infected patients (with and without treatment against HIV). The complete data set included more than 16000 clonal sequences from 215 samples of 119 patients. Recombination analyses were performed using 6 different methods implemented in RDP3 program. We have detected recombination events (by at least 3 of the 6 methods used) in 12% of the samples, which belonged to all the groups described and to the two genomic regions studied. Consequently, intragenic recombination cannot be disregarded as a potentially important mechanism generating genetic variation in HCV.

### Identifying deeper taxonomic relationships among RNA viruses

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Although approximately 200,000 gene sequences (and ~1000 complete genomes) from RNA viruses are available on GenBank, the origin, phylogenetic relationships and mechanisms of genome evolution of these important infectious agents are largely unknown. This is due in part to the rapid rate of mutation of RNA viruses, which results in very little sequence similarity remaining even between closely related viruses and after relatively little evolutionary time. As a consequence, traditional phylogenetic methods are inappropriate for reconstructing evolutionary relationships between RNA viruses. Here, we use an alignment-free method to assess the relatedness between RNA viruses. We perform pairwise comparisons

between all available RNA virus genomes, and between each viral genome and 1000 simulated genomes to assess the extent to which short fragments of amino acid sequences are conserved across various taxonomic levels. We find significantly more conservation of amino acid fragments than is expected by chance when comparing within genera, within subfamilies, and within families. These results suggest that, despite the high rate of mutation, it may be possible to use phylogenetic methods based on identifying sequence homology to reconstruct deeper taxonomic relationships among RNA viruses.

### Towards high-throughput molecular diagnosis of *Plasmodium*: New approaches and molecular markers

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Malaria epidemiologic studies require diagnosis tools that have to deal with a large scale. The molecular approach offers this flexibility. Thus, two PCR assays based on the SSU rRNA and cytochrome *b* genes were developed in our laboratory. These new ways of diagnosing malaria are designed for being compatible with high-throughput methods, namely Dot Blot and SNP analysis. In Cambodia, a cross-sectional malaria survey was conducted in the Rattanakiri province which is characterized by a high malaria transmission rate and low levels of drug-resistance compared with the rest of the country. In three selected villages, 337 blood spots were collected. Based on these samples, our new diagnosis techniques were compared with two reference methods: microscopy and a nested PCR method published by Singh et al. (1999). Our results confirmed the previously reported high sensitivity and specificity of molecular methods. Indeed, the prevalence of *Plasmodium* infections in the three studied villages increased from 41.5%, using Giemsa-stained thick blood smears, to 76%, using the reference PCR method. The new high-throughput methods resulted in a prevalence of 80% (Dot Blot) and 85% (SNP analysis), respectively. For the majority of samples, species typing was also confirmed by these three methods. Contradictory results were mostly related to detection of minor species (*P. malariae* and *P. ovale*) in mixed infections. However, similar results were obtained in 83% of tested isolates. Molecular large-scale methods provide more accurate informations of the malaria epidemiology in a country or region. In particular, they reveal a much larger distribution of *Plasmodium* infections than previously supposed and thus are useful for a better follow-

up of malaria control measures. The implication of these new tools will be discussed, in particular the possibility to adapt cytochrome b SNP detection on DNA microarrays.

### **CD40L is a type II membrane protein comprised of 261 amino acids**

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CD40L is a type II membrane protein comprised of 261 amino acids. CD40L plays a crucial role in the immune system where it is primarily expressed on activated T cells and triggers immunoglobulin class switching. The genetic disease X-linked hypergammaglobulinemia (HIGM1, XHIGM, or XHIM) is caused by mutations in the CD40L gene. Individuals with HIGM1 have are susceptible to recurrent infections to pathogens and a relationship has been shown to exist with malaria (Sabeti et al., 2002). The CD40L gene is under strong and recent natural selection in humans (Sabeti et al., 2002). In this paper, we phylogenetically examine the promoter region of CD40L in primates and other mammals via phylogenetic shadowing. This analysis revealed several regions of the promoter of CD40L that were highly constrained and thereby inferred to be functional. These constrained regions confirmed known regulatory sites that had been studied *in vitro*. In additional, a highly constrained region with an NF-AT recognition site was also identified. This region would be an excellent target for studies of CD40L regulation *in vitro*. These analyses also showed that the primate and rodent CD40L do not share a similar set of promoter binding sites, and instead that a ‘mouse specific’ and a ‘primate specific’ promoter has evolved, suggesting that this gene is differently regulated in these species.

### **The SARS-coronavirus nucleocapsid protein: A protein with multifarious activities**

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The SARS-CoV nucleocapsid (N) protein is a major structural component of the virus capsid and is postulated to play important roles in viral pathogenesis, replication, and RNA packaging. Besides these, the N protein has also been predicted to be involved in a variety of other important functions in the viral life cycle. Here we present and discuss recent data obtained from our laboratory showing the diversity of host pathways that this protein may be involved in. We have tested the capability of N protein to self-associate using its ~140 amino acid interaction domain implying that N may be involved in various regulatory activities in the infected cell. Mammalian cell expression studies further proved our *in-silico* predictions that the 46 kDa N protein is a phosphoprotein. Immunofluorescence, *in-vitro* phosphorylation and c-DNA subtraction tech-

niques subsequently were used to prove that the serine-phosphorylated N was stable and localized in the cytoplasm and co-precipitated with the membrane fraction. N was a substrate of cyclin dependent kinase (CDK), glycogen synthase kinase (GSK3) and casein kinase II (CKII). Phosphorylated N translocated to the cytoplasm by binding 14-3-3 (tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein) thus revealing a phosphorylation dependent nucleoplasmic shuttling mechanism. The N protein directly inhibited the activity of the cyclin-CDK complex resulting in hypophosphorylation of retinoblastoma protein with a concomitant down-regulation in E2F1 mediated transactivation. Our data clearly points towards N having a major and multifarious role to play in SARS-CoV life cycle and pathogenesis.

### **Designing and searching of highly effective anti-virus compounds as preparations for prophylaxis of AIDS and Flu pandemic**

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Annually in the world there are some millions new cases HIV infection, and FLU infections emerging constantly. At present, the whole humanity can be considered as a risk group for AIDS and FLU. Radically efficient medicines, which could allow curing HIV infection, have not been created yet; available preparations only somewhat slow down the development of AIDS. It is necessary creation of new highly effective preparations for prophylaxis and treatment HIV-1/2 infection. To preparations against a FLU the drug-resistance quickly develops, therefore creations of new effective compounds also are actually. On the basis of co-polymers of divinyl ether with maleic anhydride, polymeric matrixes modified with Norbornane have been synthesized and adamantane, and/or peptide imitators chemokine receptors for complex original anti-virus compounds have been designed and synthesized, also. By evaluating the newly synthesized complex compounds *in vitro*, their low toxicity was revealed ( $CC_{50} > 2.0$  mg/ml), as well as high anti-FLU and anti-HIV activity (by means of viral procedure: suppression of reproduction of virus ( $EC_{50}$  from 0.5  $\mu$ g/ml), and by means of ELISA measuring inhibition of the production of p24 HIV-1 protein ( $IC_{50}$  from 0.1  $\mu$ g/ml). Maximal level of antiviral efficiency was revealed when preparations were introduced at the stage of virus adsorption or/and was during the whole of cultivation. From our point of view, these compounds can be promising for the development of anti-FLU and anti-HIV microbicide preparations. Questions concerning the development of optimal means of transporting the complex compounds of this class are investigated and the effective medicinal forms also are prepared. In order to eliminate irritating action we included efficient anti-virus compounds into the pH-dependent interpolymeric complex (IPC) which is stable into weakly acidic media and decompose with the release of active anti-virus preparation in neutral and/or alkaline media. The anti-virus efficiency of some IPC proposed has been demonstrated experimentally *in vitro* (up to 99% HIV-1 suppressed), along with a decrease in local toxic action of

preparation included in the IPC in vivo when applied on vaginal mucous membrane of white mice (morphological studies and histology). Thus, now we have high efficient candidates of anti-FLU compounds and anti-HIV microbicide gel for local intra vaginal using for anti-HIV prophylaxis and therapy.

### Quantitative real-time PCR applications proposed for investigations of environmental leptospirosis

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Leptospirosis is a globally emergent infectious disease involving complex ecological processes that as such requires a systems analytic approach in order to elucidate patterns of pathogen transmission within the host reservoir community. An ecosystems-level model of leptospirosis acknowledges the cross-scale interactions that are relevant to the ecology of this disease and recognizes the necessity of a trans-discipline solution that includes consideration of the molecular, cellular, and organismal factors likely to be involved in disease emergence in human populations. We highlight three specific contributions to an understanding of leptospirosis in the environment that can be made by application of a recent advance in molecular techniques, specifically quantitative polymerase chain real-time reaction or Q RT-PCR methods. The use of Q RT-PCR in ecological investigations of pathogenic leptospires allows for: (1) The ability to quantify an environmental disease risk to humans; (2) an estimate of pathogen type abundance which thereby allows for an understanding of pathogen community assembly; and (3) the ability to investigate thresholds of pathogen persistence in the environment. While Q RT-PCR will help little in elucidating the evolutionary relationships amongst leptospiral types of interest, this promising modern molecular technique should be useful for identifying the scale and magnitude of transmission patterns of pathogenic leptospires in the environment.

### Cat! Has any role in zoonotic transmission of giardiasis in Tehran/Iran

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In order to investigate the genotype of *Giardia duodenalis* from domestic and stray cats in Iran, feces were collected from 181 cats, screened by microscopy and examined by molecular method which included DNA extraction, triosephosphate isomerase gene amplification (PCR) and restriction fragment length polymorphism (RFLP). 21 cats were found to be infected with *Giardia* and harboring cysts or trophozoites belonging to assemblage A and subtypes of this assemblage. Also, the human isolate of *Giardia* in Iran has been clearly specified to be assemblage A and its subtypes. These findings suggest that infection of humans by zoonotic genotypes from domestic

and stray cats and vice versa, is possible and could be of high significance. This is the first report on the genotype of *Giardia* isolate from cats in Iran.

### Workshops/debates

#### Modern morphometrics, a cheap and advanced tool for medical entomology

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Morphological characters of medically important insects were usually described on a qualitative mode (color, texture, aspect, etc.) with limited quantification, and on a few specimens only. By reconsidering morphological characters quantitatively, at the population level, modern morphometrics brought new and important perspectives. Among them: (i) an identification tool challenging the accuracy of molecular tools, and (ii) a sensible detection tool of current population structure. In this latter application, modern morphometrics seems best suited to detect recent events affecting the population structure, including isolation or environmental changes. In addition, (iii) it is able to evaluate the level of adaptation of an insect to its local environment. Since it is cheap and does not require any special entomological skill, modern morphometrics should be the first line technique associated with entomological surveillance, an important epidemiological question not well negotiated as far.

#### Phylogeography

J.P. Hugot, J.F. Cosson

#### How old is HIV?

J.P. Hugot, Fran Van Heuverswyn

#### Infections, Genetics and Evolution: Past and future of the journal. The new webportal Infections, Genetics and Evolution

M. Tibayrenc, B. Straub

#### Ecoepidemiology of *Aedes aegypti*

Philippe Barbazan

#### Characterization of a novel *IL7RA* mutation (444\_450insA) caused marked reduction in CD127 expression highlighting an important role of interleukin-7 receptor $\alpha$ on T-cell development

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Susceptibility to infectious diseases involved pathogen, environment and importantly, host defense mechanism combating exogenous factors. Molecular evidence addressing the important of human (host) genetics in predisposition to infectious pathogen was mainly derived from studies in primary immunodeficiency syndrome. Severe combined immunodeficiency (SCID) is one of the most common primary immunological defects in man characterized by blocking in T-cell development and/or functions with variable degree of simultaneous association of B-cell or natural killer (NK) cell dysfunction. Previous studies indicated that there was a genetic heterogeneity underlying SCID including mutation in *IL2RG*, *IL7RA*, *JAK3* and *CD3D* gene. Recently, we have molecularly characterized a 2 year-old girl presenting with BCGosis and recurrent serious bacterial infections including sepsis. This patient had distinctive immunological profiles of T<sup>-</sup>, B<sup>+</sup>, NK<sup>+</sup> SCID. Therefore, we firstly analyzed *IL7RA* and *CD3D* genes, which have been previously shown to cause such phenotype. We identified a novel adenine insertion at an adenine tract located between nucleotide 444–450 of *IL7RA* encoded CD127, resulting in a frameshift and premature stop codon. A quantitative real-time PCR analysis revealed that the relative mRNA expression were markedly reduce (0.037) in the patient and considerably decrease and her parents (0.119, 0.167) compared to normal (1,  $n = 8$ ). This suggested that this mutation hampered mRNA expression, possibly, due to the non-sense mediated decay mechanism (NMD). Using flow cytometric analysis, we demonstrated that there was a significant reduction of CD127 positive-T cells in the patient confirming *in vivo* reduction at the protein level. Finally, this truncated CD127 protein was identified as expected by 2-Dimension electrophoresis-western blotting assay. Our study provided, for the first time, the molecular basis of SCID in Southeast Asian population and

characterization of further cases will provide more insights on immunological mechanism controlling interaction between host and pathogen at last.

### Specific mechanisms of genomic plasticity in the tick-borne Rickettsiale *Ehrlichia ruminantium*

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*Ehrlichia ruminantium* is the causative agent of a major tick-borne disease of livestock in Africa known as heartwater or cowdriosis. Three genomes corresponding to two different groups of differing phenotype, Gardel and Welgevonden, have been completely sequenced. The three strains display genomes of differing sizes with 1,499,920 bp, 1,512,977 bp and 1,516,355 bp. The genome organization is highly conserved with *A. marginale* whereas no synteny is conserved with the other *Rickettsiales*. 56 unique sequences and 19 truncated genes differentiate the two phenotypic groups but only 10 CDs are associated to major genomes rearrangements (i.e. deletions or extensive mutations). *E. ruminantium* displays a strong strand-specific compositional bias as well as a specific group of membrane proteins. *E. ruminantium* displays a strong GC bias resulting in the presence of different codon usages in leading and lagging strands. Moreover, *E. ruminantium* displays an active specific process of genome expansion/contraction targeted at tandem repeats in non-coding regions and based on the addition or removal of ca. 150 bp tandem units. Two populations of tandems repeats are present: short tandems displaying a period of 12–15 bp, associated to coding sequences, and long tandems displaying a period of 150 bp and associated to non-coding regions. The long tandems are affected by a GC bias whereas the short tandems are not. This specific mechanism of genome plasticity might be related to the low efficiency of vaccines in the field.