

in population genetics studies, as compared to other markers, such as microsatellites.

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### PATTERNS OF SELECTION ON ANTI-MALARIAL IMMUNE GENES: ADAPTIVE EVOLUTION IN *LRIM1* IN *ANOPHELES ARABIENSIS*

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Co-evolution between *Plasmodium* and its *Anopheline* vectors may result in adaptive changes in genes that are involved in the mosquito's response to the parasite. Consequently, genes that are a crucial component of the mosquito's defense against *Plasmodium* can be recognized by identifying anti-malaria genes that show clear signs of positive selection in vectors, but not in closely related non-vector species. Therefore, we examined patterns of nucleotide variation in eight known anti-malarial immune genes: *GNBP-B1*, *LRIM1*, *CEC1*, *TEP1*, *REL2*, *NOS*, *GAMBICIN* and *FBN9*, in vector and non-vector species of the *Anopheles gambiae* complex. McDonald-Kreitman and maximum likelihood tests of selection were used to identify genes under positive selection. No signatures of positive selection were detected in six of the eight genes examined and very little differentiation in these genes was found between most species. MacDonald-Kreitman tests indicated that weak positive selection may have acted on *REL2* in *An. gambiae*, but this was not confirmed with maximum likelihood tests of selection and the data is not conclusive. However, we found strong evidence for positive selection on *LRIM1* in the major malaria vector *An. arabiensis*. In particular, two adjacent codons show clear signs of adaptation by having accumulated three out of the four replacement substitutions in this lineage. Our data also show that *LRIM1* has introgressed from *An. arabiensis* into the other major vector *An. gambiae*. Although there is no direct evidence to show that *LRIM1* evolved in response to *Plasmodium* infection, clear signs of adaptation in an anti-malarial immune gene in a major malaria vector is intriguing and suggests that this gene may play a significant role in limiting *Plasmodium* infection in *An. arabiensis*. Our data further predict that *An. gambiae* may be variable for *Plasmodium* resistance at this locus. Interestingly, *LRIM1* is located inside a recently identified QTL for *Plasmodium* resistance in field-collected *An. gambiae*.

## 873

### DIFFERENTIAL GENE EXPRESSION AMONG SUSCEPTIBLE AND REFRACTORY STRAINS OF *Aedes aegypti* MOSQUITOES FOLLOWING DENGUE 2 INFECTED BLOOD MEALS

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*Aedes aegypti* is the primary vector for dengue fever. Dengue virus must first establish infection, replicate and be able to disseminate from the midgut in order to be transmitted. Differential dissemination of dengue-2 virus was observed in our *Aedes aegypti* lab strains and we conducted an investigation to assess whether ingestion of a viral- infected blood meal induced differential gene expression in the midguts of susceptible and refractory strains. Complete midgut profiles were assessed using the Serial Analysis of Gene Expression (SAGE) method for which 5 SAGE libraries were constructed from 1) dengue-2 fed susceptible strain, MoyoS, 2) dengue-2 fed refractory strain, Moyo-In-Dry, 3) normal blood fed susceptible control group, 4) normal blood fed refractory control group, and 5) a pool of sugar fed mosquitoes. A total of 65,116 sequenced SAGE tags were obtained from these libraries and over 40% of the tags

were represented only once. Statistical analyses suggest a strain-specific (phenotypic) and dietary-specific (infected vs. normal blood meal) variation in gene expression. Comparative analysis of gene expression profiles between and among multiple SAGE libraries will be presented and will aid in understanding some of the factors that facilitate dengue-viral infection and residence within the midgut.

## 874

### COMPARATIVE TIME COURSE GENE-EXPRESSION PROFILING IN DENGUE SUSCEPTIBLE (MOYO-S) AND REFRACTORY (MOYO-IN DRY) STRAINS OF *Aedes aegypti* IN RESPONSE TO DENGUE INFECTION

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*Aedes aegypti* is the primary vector for dengue virus, an arbovirus responsible for causing large number of human morbidity and mortality particularly in tropical countries. Emergence of insecticide resistant vector populations poses great challenge to efforts aiming to control dengue virus spread. Thus it becomes imperative to characterize molecular factors that influence vector competence, which can later be targeted to develop new vector control strategies. We used cDNA microarrays to perform comparative time-course analysis of gene-expression profiles in dengue susceptible (Moyo-S) and refractory (Moyo-In Dry) strains of *Aedes aegypti* mosquitoes in response to dengue infection. We hypothesized that differentially expressed genes in the two mosquito strains might be involved in conferring vector competence to the mosquito. The differentially expressed genes observed through microarray analysis were further validated by performing qRT-PCR analysis of a small subset of genes. We used data-mining tools such as BIOMART (<http://www.biomart.org/biomart/martview/f4918791a50f44ec3b58122ef47c7194>) to annotate the EST clones, which were used to construct the microarrays. GeneCluster2, as reported previously, was used to identify clusters of differentially expressed genes in the two mosquito strains, which may have a biological role in conferring vector competence to *Ae. aegypti*.

## 875

### IMPLICATIONS OF HYBRIDIZATION, FEEDING BEHAVIOR AND PARITY RATES OF *Culex pipiens* ON WEST NILE VIRUS ACTIVITY AT STABLE ENZOOTIC STUDY SITES

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We examined various factors in *Culex pipiens* that influenced enzootic WNV activity in Delaware. Collections of mosquitoes were made at five locations that previously showed elevated epizootic activity in 2003 and 2004. We performed longitudinal comparisons of these five sites in 2005 and 2006. Several sites showed continued enzootic WNV activity based on virus-positive mosquitoes, sentinel chickens' and native birds' antibody seroconversions. The *Cx. pipiens* populations sampled from three of these sites were analyzed using 8 microsatellite DNA markers. Preliminary data indicate hybridization of *Cx. pipiens* form *pipiens* and *Cx. pipiens* form *molestus* may be seasonally related. Blood-meal preference for *Cx. pipiens* hybrids was performed using a PCR-HDA protocol for blooded females collected in the field and by carrying out mosquito choice tests in the laboratory. Parity rates over the mosquito season (June to October) were also examined to determine the age structure of the population. Parous females increase from 52% in early summer to 98.6% (n=30) in late summer indicating an older population later in the season. Other

comparisons included mosquito species composition and abundance at all five sites.

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### POLYMORPHISM IN THE GENE ENCODING GAMBICIN AND PLASMODIUM FALCIPARUM INFECTION SUSCEPTIBILITY IN ANOPHELES GAMBIAE

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A study evaluating selection patterns in immune genes of *Anopheles gambiae* revealed a signal of strong positive selection on the gene *gambicin*. Gambicin encodes an antimicrobial peptide with broad effects on bacteria, fungi, and *Plasmodium*, as reported previously. The study revealed that selection resulted in an excess of amino acid replacement mutations over synonymous mutations in a single codon. In order to determine the effect of this polymorphism, we evaluate the correlation between mosquito genotype and its infection susceptibility with *P. falciparum*. We genotype specimens from different pedigrees of *A. gambiae* that were previously phenotyped elsewhere. Specimens were selected to span a range of susceptibility based on the number of oocysts per midgut post infection with *P. falciparum*. Positive correlation will implicate gambicin as an important contributor of *A. gambiae* susceptibility to *P. falciparum* infection. Moreover, it will demonstrate the relevance of evolutionary investigations to contemporary ecological and epidemiological situations.

(ACMCIP Abstract)

## 877

### MOLECULAR IDENTIFICATION OF THE MEMBERS OF THE ANOPHELES ANNULARIS GROUP OF MOSQUITOES (DIPTERA: CULICIDAE) USING RIBOSOMAL DNA ITS2 AND DOMAIN-3

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The Annularis group of mosquitoes (Diptera: Culicidae) comprises 5 morphologically very similar *Anopheles* species - *An. nivipes* Theobald, *An. philippinensis* Ludlow, *An. pallidus* Theobald, *An. annularis* Van der Wulp and *An. schueffneri* Stanton. Two cytogenetic species in the *An. annularis* (Species A and Species B in India) and *An. nivipes* (in Thailand) complexes have also been reported. All members of this group, except *An. schueffneri*, have been incriminated as malaria vectors at one or other place in the Oriental region, based on their host preferences and the detection of sporozoite in the mosquito's salivary glands. Because of the overlapping morphological characters of these species their accurate identification is a problematic task. In particular, it has always been difficult to differentiate *An. nivipes* from *An. philippinensis*. Therefore, in a comprehensive study, we sequenced the ribosomal DNA ITS2 and domain-3 (D3) of four members of the Annularis group - *An. nivipes*, *An. philippinensis*, *An. annularis* and *An. pallidus* and developed D3-based PCR RFLP method for their molecular identification. We found that the D3 sequence of *An. nivipes* had two *Sma*I restriction sites, whereas other three species had only one site for this enzyme. An *Ap*I site was present in both *An. philippinensis* and *An. pallidus*, while an *Nco*I site was present in *An. pallidus* only. No intraspecies variations within the ITS2 or the D3 sequences were found among the specimens of *An. nivipes*, *An. philippinensis*, and *An. pallidus*. However, two types of ITS2 and D3

haplotypes were observed among the *An. annularis* specimens collected from different states of India, corresponding to Species A and B. The ITS2 sequence of Species A contained *Mva*I and *Eco*24I restrictions sites, while Species B had *Hin*fI and *Nru*I sites. Similarly, the D3 sequence of species A showed *Alw*26I restriction site, while species B showed *Kpn*I site. In conclusion, we have developed diagnostic PCR-RFLP methods to accurately identify and distinguish members of the Annularis group of mosquitoes.

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### DEMOGRAPHIC HISTORY CAN INFLUENCE PRESENT ESTIMATES OF GENE FLOW: A CASE STUDY OF THE WEST NILE VIRUS VECTOR CULEX TARSALIS

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*Culex tarsalis* is implicated as a major vector of West Nile Virus (WNV) in the western United States. Studies of field-caught individuals have indicated that there is significant spatial and temporal heterogeneity in the ability of *C. tarsalis* to transmit WNV both orally and transovarially. It is possible that this variation is in part due to genetic differences among populations. We investigated population genetic structure of *C. tarsalis* in 5 states in the western USA using microsatellites and mitochondrial sequence data. Mitochondrial sequences revealed a lack of isolation by distance, panmixia across all populations, an excess of rare haplotypes, and a star-like phylogeny. Microsatellites revealed moderate genetic structure and isolation by distance. Microsatellite clustering analysis and analysis of molecular variance indicated the presence of 3 broad population clusters. Mismatch distributions and site-frequency spectra derived from mitochondrial sequences displayed pattern's characteristic of population expansion. Fu and Li's D\* and F\*, Fu's F(S), and Tajima's D statistics performed on mitochondrial sequences all revealed significant, negative deviations from mutation-drift equilibrium. Microsatellite-based multilocus heterozygosity tests showed evidence of range expansion in the majority of populations. Our results suggest that *C. tarsalis* underwent a range expansion across the western United States within the last 375,000-560,000 years, which may have been associated with Pleistocene glaciation events that occurred in the midwestern and western United States between 350,000 and 1 MYA. Although our findings of genetic structure may not reflect the full extent of reproductive isolation across the western United States due to non-equilibrium conditions, they are indicative of significant genetic differentiation among mosquito populations and thus allow for the possibility of a relationship between genetic variation and phenotypic variation in WNV vector competence in this important vector species.

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### FREQUENCY OF MULTIPLE HUMAN BLOODMEALS TAKEN BY FEMALE ANOPHELES ARABIENSIS MOSQUITOES IN MACHA, ZAMBIA

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The mosquito *Anopheles arabiensis* is the major vector of *Plasmodium falciparum* in Macha, Zambia. Spray catches for mosquitoes were performed in the villages of Chidakwa and Lupata in Macha during the 2005-2006 malaria season. DNA was extracted from blooded mosquito abdomens and PCR were utilized to identify the animal contributor of the bloodmeal. PCRs for five human microsatellites were completed on all bloodmeals identified as human to estimate the rate at which individual mosquitoes had fed on more than one person during the last gonotrophic cycle. The frequency of multiple human bloodmeals was approximately 20%. Bloodmeals from mosquitoes positive for *P. falciparum* infection also

yielded a 20% rate for multiple bloodmeals. This finding suggests that there is no bias between selection of infective or un-infective hosts.

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### PASSERINE FILARIASIS AND THE RAPID SPREAD OF WEST NILE VIRUS - A REAL-LIFE EXAMPLE OF MICROFILARIAL ENHANCEMENT OF ARBOVIRAL TRANSMISSION BY MOSQUITOES?

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Laboratory studies have established that vertebrates concurrently infected with microfilariae and arboviruses can enhance mosquito transmission of arboviruses. When ingested, microfilariae penetrate the mosquito midgut and allow immediate dissemination of virus into the mosquito body cavity. This increases and accelerates viral infectivity of mosquitoes and can have two epidemiological consequences. First, mosquito species that are normally refractory to viral infection because of midgut barriers to viral infectivity, may now develop infections. Thus, otherwise incompetent vector species may be transformed into competent vector species, increasing the number of species involved in a virus transmission cycle. Second, microfilarial enhancement can accelerate viral development within the mosquito, significantly shortening the time required for infected mosquitoes to become infectious mosquitoes (=extrinsic incubation period [EIP]). Small reductions in EIP can lead to large increases in vectorial capacity, even with natural vector systems. In this presentation, evidence will be presented to support the hypothesis that the high prevalence of microfilarial infections in certain species of songbirds may have enhanced the enzootic transmission of West Nile virus and contributed to its rapid spread across the continental United States.

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### IDENTIFYING THE GEOGRAPHICAL CONVERGENCE OF ANOPHELES AND PLASMODIUM

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The concept of "Anophelism without malaria" has been vital to the historical development of our understanding of malaria transmission. Recently global maps of the spatial limits and endemicity of malaria parasite species have become available. Matching these maps with models of the potential distribution of malaria vectors should allow areas to be identified where particular parasite and vector combinations can coexist. Information on the concurrence of individual vector and malaria parasite species could assist the incrimination of the vector species, the identification of previously unknown vector species, and the targeting of vector control to particular species. We illustrate these concepts with ecological niche distribution models of mosquito species within the Leucosphyrus and Minimus Complexes of South East Asia.

## 882

### GENETIC POPULATION STRUCTURE IN THE MALARIA VECTOR ANOPHELES MARAJOARA IN NORTHEASTERN SOUTH AMERICA

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*Anopheles (Nyssorhynchus) marajoara* (Diptera: Culicidae) is an important regional malaria vector in eastern Amazonian Brazil. A population expansion in *A. marajoara* was estimated to have occurred in NE Amazonian Brazil during the Pleistocene using sequences from the mtDNA COI gene. Using 8 polymorphic microsatellite loci, we assessed the population structure of this species from 16 localities in Amazonian Brazil plus one in Trinidad. On the basis of microsatellite allele frequencies we detected three subdivisions: Central (along the Amazon from Manaus to near Almeirim), Northern (Boa Vista and Trinidad), and Eastern (Marajo Island and four localities around the city of Macapa in Amapa state). AMOVA detected significant differentiation among the three groups (23%), among populations within groups (about 1%) and within populations (75%). In a UPGMA tree, these three subdivisions were each supported at 100%. The range of differentiation estimates among the three subdivisions (FST = 0-0.447) combined with the AMOVA, STRUCTURE and UPGMA results and those from an earlier mtDNA sequence study, lend support to the presence of two genetically differentiated types, *A. albitarsis* E, from Boa Vista and Trinidad, and *An. albitarsis* G, from Manaus to near Almeirim, with at least partial barriers to gene flow with *A. marajoara*. Isolation by distance was inferred as one likely model to explain levels of differentiation.

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### MORTALITY DECELERATION IN LABORATORY REARED, ADULT ANOPHELES STEPHENSI MOSQUITOES

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Longevity is one of the most important factors in the overall capacity of a mosquito species to transmit malaria. The longer they live, the more chance mosquitoes have to acquire parasites, survive the extrinsic incubation period and transmit malaria. Virtually all of the current models of malaria transmission assume that the rate of mortality throughout a mosquito's lifetime is constant. However, studies using very large cohorts of adult medflies, fruit flies, seed beetles and, most recently *Aedes aegypti* mosquitoes, have demonstrated that mortality rate is not constant. Instead, the rate of mortality increases initially for younger adults, then levels off or even decreases for older adults. To determine if such "mortality deceleration" is also a feature of adult *Anopheles* mosquitoes, ca. 750 newly emerged *Anopheles stephensi* ("wild type" MR4 strain) were apportioned into five one-gallon cylindrical cardboard mosquito cages at a density of ca. 150 mosquitoes per cage and sex ratio of 1 male: 5 females. Mosquitoes were maintained at 23°C, 84% RH, provided with a sugar cube and moist sponges. Mosquitoes were not offered a blood meal. Every day, dead mosquitoes were removed and counted until all the mosquitoes had died. The on-line software program, WinModest 1.0 was used to determine which of four possible mortality models best fit the observed mortality pattern. The logistic-Makeham model (mortality deceleration) provided the best fit for the mortality pattern observed in our trial (log likelihood= - 2393), whereas the Gompertz model (no mortality deceleration) had the worst fit (log likelihood= - 2451). This is consistent with recent reports using large cohorts of *Ae. aegypti* mosquitoes. Because *Aedes* and *Anopheles* are different subfamilies within Culicidae (i.e., not closely related phylogenetically), these findings suggest that mortality deceleration may be characteristic of mosquitoes in general. Future studies will examine mortality deceleration in *Plasmodium*-infected *An. stephensi* mosquitoes to determine how (or if) *Plasmodium* infections alter the mortality trajectories of *Anopheles* mosquitoes.



## RISK FACTORS FOR THE PRESENCE OF *Aedes aegypti* IN LIMA, PERU

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In 2005, confirmed autochthonous dengue cases were reported in Lima, Peru after over 60 years of absence. We conducted household surveys from March - May 2004 assessing the presence of *Aedes aegypti* and associated risk factors in San Juan de Lurigancho, Lima, Peru. A two-stage, simple random sampling survey was conducted in 32 localities. Inspection of water containers and the collection of *Ae. aegypti* larvae and pupae were conducted. Basic socioeconomic data was recorded in addition to the source, storage, and treatment of water resources in the survey houses. Multiple logistic regression was fitted to describe factors associated with the infestation of containers by pupae, calculating odds ratios (OR) and their statistical significance. A total of 14,055 houses were inspected, evaluating 26,870 containers. Plastic containers were found to be the most frequent type of container. Only 814 containers (3.0%) were found to have pupae. The most productive containers with pupae were low cement tanks (41%), plastic containers (21%), flowerpots (15.6%) and 55-gallon drums (15%). Sealed containers (n=9,875) did not produce a single pupae and were excluded from all further analysis. In multiple logistic regression, containers without covers had an increased presence of pupae (OR=1.6, p<0.001), as well as those exposed to the sun (OR=1.6, p<0.001), when compared to partially covered containers. Low cement tanks and flowerpots had higher infestation rates (OR=2.3, p<0.001 and OR=2.2, p<0.001 respectively), followed by 55-gallon drums although with borderline significance (OR=1.4, p=0.085). Plastic containers had lower risk (OR=0.7, p=0.038) for pupae infestation. This area of Lima had a high presence of *Ae. aegypti* and concomitant risk of dengue transmission. Pesticidal treatment of the most productive containers, as well as those without covers or exposed to the sun, can reduce the total amount of insecticide applied yet provide an effective measure preventing dengue in high-risk areas of Lima.

## IMMUNE RESPONSIVE SERINE PROTEASE FROM *ANOPHELES GAMBIAE* PROMOTES *PLASMODIUM FALCIPARUM* DEVELOPMENT

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Immune responsive serine protease from *Anopheles gambiae* promotes *Plasmodium falciparum* development. Studies on acsp30 (AY995188), an immune responsive serine protease from *Anopheles culicifacies*, the main vector for transmission of malaria in India, have been reported by Rodrigues et al., (BMC Molecular Biology 2007, 8:33). Acsp30 showed high transcript abundance in naturally selected refractory (R) strain, which completely blocks transmission of the human malaria parasite, *Plasmodium vivax* compared to the susceptible (S) strain. *Plasmodium* specific upregulation of acsp30 in R but not in S strain was suggestive of its role in contributing to the refractory phenotype in *A. culicifacies* mosquito population. African vector *Anopheles gambiae* serves as a well-studied mosquito model for elucidating the role of this serine protease in regulating *Plasmodium* development. We therefore studied a homologue of acsp30 in *Anopheles gambiae* (agsp30). Agsp30 transcript levels are more abundant in laboratory-selected *Anopheles gambiae* refractory L35

compared to susceptible G3 strain. However, unlike acsp30, *Anopheles gambiae* serine protease is more abundantly transcribed, in midgut compared to bodywall and is also induced upon *Plasmodium berghei* infection in the susceptible G3 strain. Double-stranded (ds) RNA-mediated knock down of agsp30 resulted in no change in *P. berghei* oocyst numbers in G3 strain compared to dsLacZ-injected controls. Interestingly, the knock down of agsp30 in the same G3 strain impairs *Plasmodium falciparum* oocyst development, revealing its function as an agonist for human malaria parasite development in the co-evolved mosquito-parasite combination of *Anopheles gambiae-Plasmodium falciparum*.

## BLOOD MEAL PREFERENCE AND ISOLATION OF ALPHAVIRUSES AND FLAVIVIRUSES FROM MOSQUITOES IN THE CAUCA VALLEY, COLOMBIA, SOUTH AMERICA

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South America is home to several different flaviviruses and alphaviruses, including dengue, St. Louis encephalitis, West Nile virus, Ilheus, Bussuquara, Rocio, and several endemic subtypes of the equine encephalitides. Field studies in Brazil and Peru have elucidated the diversity and complexity of enzootically cycling arboviruses in mosquito populations, and also discovered unknown viral isolates. There has been little recent work on the resident mosquito species assemblage, and associated natural virus infections, and host blood meal preferences in the Cauca Valley of Colombia. Mosquitoes were collected at two sites, El Vinculo and Laguna de Sonso, during the summers of 2005-2007 using dry ice-baited CDC traps, gravid traps, resting boxes, and harborage aspiration. These were placed on dry ice and transported to the entomology laboratory at Centro Internacional de Entrenamiento e Investigaciones Médicas for identification and testing. These were identified to species where feasible; genitalia of *Culex* subgenus *Melanoconion* males were dissected to determine the prevalence of these species. Mosquitoes were initially tested with a duplex RT-PCR for alphaviruses and flaviviruses. Virus specific primers were subsequently used. Blood fed mosquitoes were separated and graded based on engorgement. Their abdomens were assayed using mammalian and avian specific primers focusing on the cytochrome b region. Mammal positive samples were tested using a multiplex RT-PCR, and negative samples were tested with horse, rat, and capybara specific primers. Samples testing positive for an avian blood meal source were assayed with order specific primers.

## A NEW ISOLATE OF *BACILLUS THURINGIENSIS* SUBSP. *ISRAESENSIS* HIGHLY EFFECTIVE AGAINST *ANOPHELES GAMBIAE*, *Aedes aegypti* AND *Culex pipiens*

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Resistance of mosquitoes to a variety of insecticides has been documented by various governmental agencies in those areas of the world where malaria is endemic. In fact, more than 125 mosquito species have been documented as resistant to one or more chemical insecticides and natural products. Alternatively, *Bacillus thuringiensis* subsp. *israelensis* (Bti) has been used successfully for a number of years to control the larval stages of various mosquitoes with no reports of resistance. There are 26 Bti products registered for use in the United States some of which include Vectobac and Teknar. Recently, we isolated a strain of Bti (M1) with greater activity against larvae of *Anopheles gambiae*, *Aedes aegypti* and *Culex pipiens*

than that of several commercially available larvicides. Importantly, the four genes (*cry4A*, *cry4B*, *cry10A* and *cry11A*) responsible for larvicidal activity are highly expressed in M1 compared to other Bti strains. We compared the efficacy of spore/crystal mixtures of M1 to the two commercial larvicides Vectobac and Teknar using the *Anopheles gambiae* strains G100, RSP, ZANU and SENN (supplied by the Centers for Disease Control and Prevention, Atlanta, GA). G100 is the wildtype strain whereas RSP, ZANU and SENN are resistant to pyrethrin, DDT and dieldrin, respectively. Toxicity (LC<sub>50</sub>) of spore/crystal mixtures against the *A. gambiae* strains was significantly greater for the M1 strain than for the two commercially available larvicides. Moreover, the LC<sub>50</sub> values for M1 against *A. aegypti* and *C. pipiens* were better than those for the two larvicides tested.

## 888

### SPATIOTEMPORAL RISK PATTERNS AND ECOEPIDEMIOLOGY OF WEST NILE VIRUS DISEASE, COLORADO, 2002-2006

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The 2003 West Nile virus (WNV) disease epidemic in Colorado, which resulted in almost 3,000 cases of reported disease in humans and more than 60 fatalities, demonstrated the vulnerability of the state to this mosquito-borne arboviral disease. Implementation of spatially and temporally targeted mosquito control campaigns to prevent future WNV disease epidemics requires a solid understanding of variability of WNV disease risk in space and time, and the relationship of risk patterns with underlying environmental factors. We analyzed spatiotemporal patterns of human risk of WNV based on 3,800 disease cases reported in Colorado from 2002-2006. We found distinct temporal and spatial risk patterns within Colorado as well as associations between environmental factors (elevation, summer temperature, commonality of ground water sources) and incidence of WNV disease. Data demonstrated greater risk of WNV disease at lower elevations, with 75% of WNV disease cases occurring at elevations <1,700 m. The epidemic curve of WNV disease at low elevations was steeper and more pronounced in comparison to higher elevations. These patterns likely reflect the spatial distribution of *Culex tarsalis* vectors, which rarely occur above 1,700 m, and at higher elevations have slower population increases leading to slower increase in the intensity of enzootic WNV transmission and subsequent transmission to humans. We also compared the spatial pattern of WNV disease incidence when calculated by county versus the smaller zip-code and census tract units and found that incidence calculated at smaller units provides enhanced ability to differentiate areas of minimal risk from elevated risk. Improved knowledge of fine-scale spatial patterns of WNV disease risk will enhance the ability of the public to make informed decisions regarding personal risk as well as the capability of vector control programs to implement spatially targeted mosquito control campaigns. This research represents the first statewide analysis in Colorado of the spatiotemporal patterns of WNV disease at sub-county scale.

## 889

### A NOVEL LETHAL TRAP FOR GRAVID AEADES AEGYPTI AND AEADES ALBOPICTUS

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A lethal trap constructed of novel materials was tested against gravid *Aedes* mosquitoes under laboratory conditions. In a walk-in cage, the lethal oviposition trap (LOT) when placed among 3 non-lethal oviposition

traps killed on average 97% of gravid *Ae. aegypti* and 90% of gravid *Ae. albopictus* within 24 h of their release. After 11 weeks of out door exposure in full sun light, the LOT achieved 87% mortality of gravid *Aedes* mosquitoes, using the same walk-in cage experimental design. The LOT is also lethal to unfed *Ae. aegypti*. After a 24-h exposure, 90% of unfed females exposed to the LOT in the walk-in cage were killed.

## 890

### DETECTING WUCHERERIA BANCROFTI IN AEADES POLYNESEIENSIS MOSQUITOES FROM AMERICAN SAMOA: A COMPARISON OF PCR WITH HAEMALUM STAINING AND DISSECTION

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Lymphatic filariasis (LF) is a deforming illness that afflicts over 120 million people worldwide. The Global Alliance to Eliminate Lymphatic Filariasis (GAELF) is a public-private partnership assisting in the implementation of a mass drug administration (MDA) program that may lead to the elimination of this disease. Critical to the success of this program is the development of metrics that can accurately monitor the reduction of filarial worms within human populations. Infection rates in mosquito populations could be used to monitor suppression of LF transmission and for estimating the proportion of the human population that is infected (xenomonitoring). Traditionally, mosquito infection rates are determined by dissection and microscopic examination. This labor intensive and time consuming method may not be appropriate for large-scale surveillance programs. Xenomonitoring (the use of infection rates in vectors to determine the presence of parasites in humans), using PCR to detect parasite DNA, should be more sensitive than dissections and is compatible with analyses of large numbers of samples. Although some studies showed discordant results between dissection and PCR, xenomonitoring with PCR may be useful to monitor transmission. A comparison study of these two techniques was undertaken to provide insight into how a PCR-based assay for large-scale surveillance of mosquito infection rates might be implemented and interpreted. We collected 4367 female *Aedes polynesiensis* mosquitoes using BG-Sentinel™ Mosquito Traps (BioGent) in three villages located on the western coast of Tutuila, American Samoa. The traps were distributed throughout the villages to minimize competition among traps and were operated during peak biting times for 4 consecutive days. Mosquitoes were collected twice daily. Collected mosquitoes were divided into two experimental groups for filarial worm detection: (1) Haemalum staining and dissection or (2) Polymerase chain reaction (PCR) analysis of the *Spl* repeat using NV1 and NV2 primers. An evaluation of these two methods along with their relative strengths and weaknesses will be discussed. In addition, we will discuss the prevalence of infection in the mosquito vector in the context of ongoing MDA in American Samoa. Finally the role that entomological monitoring could play in identifying infected individuals and intra-village spatial clustering of filarial transmission will be discussed.

## 891

### INFLUENCE OF MAIZE POLLEN ON ANOPHELES PRODUCTIVITY AND MALARIA TRANSMISSION DYNAMICS

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The force of malaria transmission is correlated spatially and temporally with the intensity of maize (*Zea mays*) cultivation in parts of sub-Saharan Africa. Maize is wind-pollinated; its tassels shed copious quantities of pollen, and these grains fall to the ground and coat surfaces of puddles nearby. Larvae of *Anopheles arabiensis* feed avidly on such pollen, develop more rapidly, and become larger and potentially longer-lived adults. It may be that adults derived from pollen-fed larvae contribute disproportionately to the force of malaria transmission. To test this hypothesis, we detasseled maize plants within 50m of *Anopheles* developmental sites in a portion of a village in western Ethiopia where maize is cultivated densely. Maize plants were permitted to shed pollen naturally near larval developmental sites elsewhere in the village. Prior to detasseling, and for two months thereafter, we recorded and compared the productivity of larval habitats in each location, measured the size of females collected as pupae, and monitored the abundance, size, parity and infection rates of adult *Anopheles* sampled within houses nearby. During a second season of observations, we shall reverse the intervention and comparison sites. Our observations will help further establish the extent of linkage between maize and malaria transmission, and guide novel agricultural intervention strategies, such as those designed to desynchronize the peaks of pollen production and larval development.

892

### USE OF FREE MAPPING TOOLS TO SUPPORT THE DEVELOPMENT OF A LOCAL DENGUE INFORMATION SYSTEM

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We are currently developing a Dengue Information System (DIS) as part of a Dengue Decision Support System project funded by the Innovative Vector Control Consortium. For our operational study areas in Mexico (Chetumal, Merida), we were able to purchase already developed Geographic Information System (GIS)-based data from INEGI with a spatial resolution fine enough to display streets and city blocks. The potential absence in some countries of this type of fine-scale GIS-based data for city structure presents a significant stumbling block to the development of a local DIS. To address this problem we are developing methodologies for use of free mapping tools, such as Google Earth or MS Virtual Earth, to generate GIS-data layers for city structure at minimal cost in the event that such data are lacking. These mapping tools provide free web-based access to imagery that for most major cities in the world is of a resolution allowing the viewer to distinguish street grids and in many cases also individual domiciles. The quality of the imagery provided is constantly improving. In addition to the imagery, Google Earth provides tools allowing the user to generate (and correct) simple data layers including lines and polygons to represent streets and city blocks and points to represent a variety of features such as location of disease cases or location of schools, hospitals, out-patient clinics, cemeteries etc. This type of "data layer" created using Google Earth or MS Virtual Earth can then be exported to GIS software and serve as the basis for spatial map outputs. We are also creating tools that will make use of the geographic files and databases developed to readily display entomological or epidemiological data in map output formats. The presentation will demonstrate how these free mapping tools were applied to our operational study areas in Mexico.

893

### PRELIMINARY ANALYSIS OF SPATIAL PATTERNS OF DENGUE ACTIVITY IN THREE STATES IN MEXICO

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Models predicting the spatial spread of dengue epidemics can facilitate national and regional vector control resource allocation and be used to detect hot-spots with high priority for emergency vector control within individual cities. As part of the Dengue Decision Support System project, which is funded by the Innovative Vector Control Consortium, we are developing a Dengue Information System that will make use of epidemiological data and of basic spatial statistics to describe the spread of dengue epidemics at different spatial scales. Initial studies have focused on dengue case data collated by municipality for 3 states in Mexico (Quintana Roo, 2006; Veracruz, 2004; Yucatan, 2001-2006). The occurrence of dengue in these states follows a distinct seasonal pattern, with peak numbers during summer and fall. We are specifically testing the hypothesis that nearby municipalities are more likely to have similar numbers of cases, relative to population size, than locations located farther from each other. Calculation of Moran's I revealed strong spatial autocorrelation in all states. Statistically significant ( $P < 0.05$ ) positive spatial autocorrelation occurred up to 25-30 km, whereas spatial autocorrelation became negative at 65-77 km. No clear spatial signature was observed at larger distances. Our results suggest that nearby locations experience similar levels of dengue activity in a given year. There were, however, differences between years in the same state and between neighboring states. For example, the 2005 pattern of dengue activity in Yucatan displayed a flat I-correlogram even with a large number of cases, possibly as a result of high levels of herd immunity. Different spatial patterns were observed for the states of Quintana Roo and Yucatan despite strong similarities in climate, vegetation and topography. These preliminary findings suggest a complex situation in Mexico and highlight the need for analyses based on long-term, multi-state data sets. A time-series analysis based on monthly, rather than annual, data is underway and will be presented.

894

### PROFILE OF MOSQUITO LARVAL HABITATS IN URBAN PUNTARENAS, COSTA RICA

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Mosquitoes are of public health importance as nuisance biters and disease vectors. Dengue, transmitted mainly by *Aedes aegypti* in urban areas, was reintroduced in Costa Rica in 1993. In Puntarenas City, two cross-sectional surveys were performed during wet and dry seasons (August 2006 and February 2007, respectively) to characterize urban mosquito larval habitats. A sample of 34 cells (100m by 100m) selected from a sampling frame developed using high-resolution satellite imagery was searched for potential (wet) habitats. Location and larval habitat characteristics were noted in each cell, as well as mosquito larvae and pupae. Location index (LI), container index (CI), Breteau index (BI), and pupae per person (PP) were calculated for *Ae. aegypti*. In total, 581 and 626 locations were evaluated in wet and dry seasons, respectively; 829 (139 larvae-positive) and 461 (27 larvae-positive) potential habitats were identified in wet and dry seasons, respectively. During the wet season, presence of one or more



potential larval habitat in a location was associated to locality ( $p < 0.001$ ), total people in house ( $p = 0.016$ ), and location type ( $p = 0.008$ ). Habitat presence was also associated to location type in the dry season ( $p < 0.001$ ). Larvae presence was associated to habitat type and disposability in wet and dry seasons ( $p < 0.001$ ); to habitat volume in the dry season; and to habitat indoor/outdoor setting, habitat type, locality, and location type in the wet season. *Aedes aegypti* was observed in 78% of larvae-positive habitats in wet and dry seasons. *Ae. aegypti* indices were higher during the wet season (LI: 17.2; CI: 13.2; BI: 22.9. PP: 0.36). Of habitats containing *Ae. aegypti*, 19% had larvae of other mosquito species, commonly *Culex quinquefasciatus* or *Limatus durhamii*. Other species identified were *Culex nigripalpus*, *Culex interrogator*, *Culex corniger*, *Ochlerotatus taeniorhynchus*, *Toxorhynchites* sp., and *Uranotaenia* sp. Results indicate that non-disposable containers are more likely to harbor mosquito larvae, perhaps due to source reduction efforts in Puntarenas households. In the dry season, large, non-disposable containers linked to human activity (like laundry tubs) possibly maintain an *Ae. aegypti* population that increases once rainfall fills outdoor habitats. There are relevant seasonal and geographical differences in mosquito habitat type and location, which should be considered in vector control programs in Puntarenas.

## 895

### ITN INTERVENTIONS ACROSS ENVIRONMENTAL AND TRANSMISSION SETTINGS: THE FUNDAMENTAL ROLE OF SPATIAL CONNECTIVITY IN DETERMINING EFFECTIVENESS

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Insecticide-treated bednets (ITNs) have become a cornerstone of efforts to address the intolerable burden from falciparum malaria in Sub-Saharan Africa. In order to help ensure that the marked benefits witnessed during ITN trials are replicated in a wide variety of settings, we present a mathematical model of malaria transmission developed to inform the design and evaluation of ITN interventions across a continuum of social, environmental, and transmission settings. The model depicts ITN action and transmission dynamics within a stochastic household-level structure to bridge prior statistical and deterministic approaches. We simulate outcomes of ITN interventions under varying patterns of human and mosquito population density, as well as malaria transmission intensity. In particular, the model analyzes how spatial connectivity between human and mosquito populations determines the extent and level of direct and indirect benefits from ITN use. Our analysis generates conceptual and pragmatic guidance for designing and evaluating ITN interventions. At one extreme, in areas of dense human settlement, productive mosquito habitats, and high transmission intensity, leveraging the 'community effect' from ITN use becomes central to generating efficient protection. Conversely, at the opposite extreme, personal protection prevails but neither users nor non-users may experience enhanced benefits from widespread use. In intermediate situations, heterogeneous distributions of human and mosquito populations within a spatially connected region determine the relative import of direct and indirect benefits. As a result, the model further demonstrates the context dependent nature of overall benefits from ITN interventions. We motivate and discuss approaches to better understand and estimate ITN effectiveness by sequentially assessing: (1) the connectivity among human settlements and mosquito habitats; (2) the interplay of malaria transmission within and between regions; and (3) the complex relationship between spatial patterns of ITN coverage and direct and indirect benefits.

## 896

### LANDSCAPE CHARACTERIZATION OF ANOPHELINE LARVAL HABITATS IN MAPANZA, ZAMBIA

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Malaria is a significant source of morbidity and mortality in southern Zambia. In Mapanza, Zambia, where transmission is seasonal, *Anopheles arabiensis* is the dominant malaria vector. The ability to predict larval habitats can help in focusing control measures, so a survey of 17km<sup>2</sup> was conducted in March 2007 to identify and map locations of water pooling and the occurrence of anopheline larval habitats. 203 aquatic habitats were identified of which 21 percent were positive for anopheline mosquitoes. Six species of anopheline mosquitoes were identified (*An. arabiensis*, *An. quadriannulatus*, *An. squamosus*, *An. rupifex*, *An. coustani* and *An. pretoriensis*) with the majority of larval habitats belonging to *An. squamosus* and *An. arabiensis*. Global and local spatial statistics were applied to elucidate the spatial patterning of *An. arabiensis* larval sites. Global clustering of aquatic habitats was detected, although *An. arabiensis* habitats were randomly dispersed. Local clustering of *An. arabiensis* larval habitats was found to occur in a northern area, and corresponded with clustering of adult mosquitoes collected from house spray catches. The probability of predicting aquatic and *An. arabiensis* larval habitats were modeled using hydrological and environmental parameters derived from satellite imagery. Satellite Radar Topography Mission (SRTM) imagery was used to determine elevation, slope, topographic wetness and delineate drainage pathways. Indices derived from Landsat imagery were used to measure vegetation and moisture abundance. Spatially explicit modeling allows improved mosquito surveillance and more focused control of malaria at a local level.

## 897

### EFFICACY OF VECTOBAC WG, A BACILLUS THURINGIENSIS ISRAELENSIS FORMULATION, TO CONTROL DENGUE MOSQUITO VECTORS IN CAMBODIA

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In 2005 and 2006, with the approval from the National Ethics Committee Of Cambodia, an operational research trial was conducted to determine the efficacy of VectoBac WG to reduce the field dengue vector population in a commune of 9 villages with 1343 households. A direct application of VectoBac WG (0.4 g/ 50 L) was done into all containers. All treated containers were subject to routine water exchange activity. In 2005 the commune was treated in July at the peak of dengue vector season and in 2006 it was treated in May during the low dengue vector season. The efficacy of VectoBac WG treatments was measured with pupae and adult mosquito surveillance. In 2005, at the pre treatment during the peak dengue vector season (June - July) there was an average of 17 *Aedes aegypti* adults per house. The Bti treatment in July suppressed the adult density to an average of 1 - 2 adults per house for 4 months post treatment (August - November). In the untreated commune, there was a seasonal reduction from an average of 17 adults per house to 8 adults per house in August-September, with further reduction to 4 adults per house in October -November. In 2006, at the pre treatment during the dry season there was an average of 9 *Ae. aegypti* adults per house. The Bti treatment in May suppressed significantly the adult density to an average of 4 - 5 adults per house for 3 months post treatment, May - July ( $p < 0.05$ ). In the 4<sup>th</sup> month post treatment there was an average of 9 adults per house. In the untreated commune, with the seasonal rains from the month of May, the average adult density of 9 adults per house increased to an average of 12 - 18 per house during the peak dengue vector season,

May - September. A single application of VectoBac WG into all containers during the low dengue vector season significantly reduced the adult dengue vector density in the peak dengue vector season for 3 months. VectoBac WG, is an easy-to-use Bti formulation in all water receptacles and the amount used was 12.5 folds less in weight to temephos sand granules. The residents in the treated commune did not complain of any odor or change of water qualities. These attributes of VectoBac WG together with its efficacy to suppress the adult mosquito population during the peak dengue vector season makes it a potential larvicide for the control of dengue vectors in Cambodia. In May 2007, The Ministry of Health Cambodia implemented successfully a large scale use of VectoBac WG in a temephos resistance province with 58 communes.

## 898

### EFFECTS OF RESIDUAL DOMICILIARY SPRAYINGS WITH PYRETHROIDS ON POPULATIONS OF *LUTZOMYIA SPP.* IN AN ENDEMIC AREA OF CARRION'S DISEASE, IN THE NORTHERN FOREST OF PERU

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Different species of *Lutzomyia* are the potential vectors of Carrion's Disease and leishmaniasis. Where the transmission pattern is domiciliary, the residual house spraying is recommended as strategy of vector control. In Peru, there is little previous experience evaluating the effects of chemical interventions on populations of *Lutzomyia spp.* Our aim was to describe the effects of the annual residual domiciliary sprayings with pyrethroids on populations *Lutzomyia spp.* A prospective study was performed in four Villages from San Ignacio Province, Cajamarca Department, in the Northern forested area of Peru, from September 2004 to April 2007. This area is endemic for Carrion's Disease. We used CDC light traps to obtain Night Trap Collection Index (NTCI), direct collections inside the houses to obtain endophily and Domiciliary Infestation Index (DII); and Shannon Traps to evaluate wild activity. The first house spraying was made in October 2004, the second in May 2005 and the last one was made in October 2006. The main species collected was *Lu. maranonensis* (97.3%) and *Lu. robusta* (1.9%). In September of 2004 the NTCI were between 10-55 indoors and 3-12 outdoors. After the first spraying the NTCI were less than 12 y 8 indoors and outdoors respectively. After the second and third sprayings, both indexes were lower than 3. DII was 65% in the base line study, showing an important endophily. However, after the first spraying this DII is zero, observed at least after 12 months of a spraying. Wild activity always was present, yet fluctuated with environmental condition (especially rain). In conclusion, the consecutive (annual) residual houses sprayings of the inner walls with pyrethroids have important impact on diminishing *Lutzomyia spp* population in the domiciliary environment, which would reduce the human-vector contact. It appears that the repellent effect of pyrethroids also has an important role in reducing the domiciliary vector population.

## 899

### PHYSICAL, CHEMICAL AND MICROBIAL CHARACTERIZATION OF SOME LOCALLY MANUFACTURED CANNED TUNA IN OIL EXPOSED TO THE SUN FOR A LONGER PERIOD

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Fish constitute one of the great and growing food harvests of the world. Histamine, an amino acid of protein is found in fish and its high amount such as 50ppm and above can cause poisoning, which is a chemical intoxication from few minutes of several hours. Since it had

been observed that the consumption of canned fish, especially canned tuna is high, this project was aimed at accessing the changes in sensory parameters, histamine level, status of can seams and level of microbial load in mechanically damaged cans of some selected locally manufactured canned tuna exposed to the sun for long on our local markets. Sankofa tuna, starkist tuna and champion tuna were the brands used for the project. They are products from Ghana Agro Food Company, Pioneer Food Cannery Limited and Myroc Processing Company respectively. Eighteen (18) samples were obtained from the Accra Central Market Zone (Okaishie). The physical and sensory evaluation of the samples was visually and manually analyzed; the can seam was checked using a micrometer screw gauge; the histamine level of the samples was analyzed using the TD - 700 Fluorometer and the microbial analyses was done for Total Viable Count(TVA) and also for presence of *Clostridium perfringens* using plate count agar and iron sulphite agar prepared and poured into petridishes, respectively. For TVA the plates were inverted and incubated at 30°C for 72 hours and for *Clostridium perfringens* the incubation was done at 37°C for 48 hours with the petridishes inverted in an anaerobic jar with an anaerocult kit, to create anaerobic condition for the organism. Results were read using the colony counter. Obtainable results showed that all the samples analyzed were with required specifications. All the samples had their histamine level results ranging from 3.5ppm to 23.5 ppm. Proper quality control measures should be maintained and wholesalers and retailers should be advised to hygienically handle the products for the suitability of human and animal consumption.

## 900

### THE ANTIBIOTIC SUSCEPTIBILITY RATES IN THE GRAM NEGATIVE BACILLI GROWING IN URINE CULTURES AND THE PRESENCE OF EXTENDED SPECTRUM BETA LACTAMAZ (ESBL)

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In this study, the gram negative bacillies belong to 500 adult and 500 child patients which isolated from urine cultures sent from several clinics were collected. Antibiotic sensitivity tests were made with NCCLS method to be suitable Kirby Bauer disc diffusion tests. The extended spectrum  $\beta$ -lactamase (ESBL) existence was assayed by using double-disc synergy tests. There were mostly *E. coli*, *Pseudomonas spp.* and *Klebsiella spp.* were produced in urine culture. The sensitivity rates of this three microorganisms were determined. Totally, 39 GSBL existence were determined in isolated 500 gram negative bacillies from urine cultures. In conclusion, the results showed that determining of GSBL is important.

## 901

### BARTONELLA SP. INFECTION OF RED-BACKED VOLES TRAPPED FROM AN INTERIOR ALASKAN SITE WHERE TICKS ARE ABSENT

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The role of ticks as vectors of *Bartonella spp.* remains controversial. To determine whether *Bartonella spp.* may perpetuate in sites where ticks are absent, we analysed blood and spleen samples from small mammals trapped during Sept. 1999 near Cantwell, Alaska. Blood smears from 7 of 48 *Clethrionomys (Myodes) rutilus* suggested *Bartonella spp.* infection. Aliquots of Aelsevers anticoagulated whole blood from these 7 animals, held at 4°C for 2 weeks after sampling, were cultured on BHI-blood agar slants at room temperature for 2 weeks. Five of the 7 (2 became contaminated) yielded growth consistent with *Bartonella spp.* PCR and sequencing of a 1410 bp portion of the *rrs*, 915 bp of the *gltA*, and 852 bp of the *rpoB* genes confirmed *Bartonella* infection in 4 of these culture samples. Phylogenetic analysis demonstrated that the sequences clustered



with the *Bartonella vinsonii* clade, but with only 92.8%-93.8% similarity to known species for which sequence information is available in GenBank. Additional PCR analysis using *gltA* primers suggested that an additional 4 of 22 spleen samples for which blood was not available also contained the DNA of this *Bartonella* sp. for an overall prevalence of 14.3% among the 70 *C. rutilus*. *Babesia microti* was not detected by microscopy or PCR of the 48 *C. rutilus* blood samples, although this protozoan commonly infects these rodents in coastal Alaskan sites where ticks are present. We conclude that a newly recognized *Bartonella* spp. may commonly infect *C. rutilus* in a site where ticks are absent, suggesting that ticks are not required to perpetuate bartonellae within the *B. vinsonii* clade.

## 902

### A FATAL OUTCOME TREATING PLEURAL TUBERCULOSIS: IS TREATMENT WORSE THAN THE DISEASE?

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In 2000, the ATS/CDC guidelines recommended a two-month regimen of rifampin/pyrazinamide (RIF/PZA) as an alternative treatment to 9-12 month of isoniazid (INH) therapy for latent tuberculosis (TB) infection in both HIV +/- patients. The regimen was felt to be equally efficacious, ensure higher compliance with fewer side effects. However, 5 deaths were reported and guidelines were revised. We describe a case of a 55-year-old woman who developed multidrug resistant extrapulmonary TB. After six months, she developed grade 4 liver injury despite being off treatment for 1 month. Worsening LFT's and coagulopathy resulted in transfer to a liver transplant center where she eventually succumbed to the hepatic failure. A 55 year old Afrocaribbean woman diagnosed with pleural *Mycobacterium tuberculosis* by thoracentesis and pleural biopsy, complained of nausea, vomiting, changes in skin color and weakness. Initially she was started on 4 drug regimen (RIF/INH/PZA/Ethambutol). After 3 months, INH was stopped for resistance. LFT were normal. The organism showed *rpoB* gene activity which correlated with RIF resistance so rifabutin was substituted for RIF. After a few weeks she developed a maculopapular rash and neutropenia. LFT again were normal and all medications were stopped. After 3 weeks, LFT were repeated and found elevated despite cessation of medications. She developed nausea, vomiting and darker skin which sent her to the ER. Exam revealed icterus and an old rash. Labs showed abnormal LFT, coagulation profile, with episodes of hypoglycemia. Despite vitamin K, her coagulation profile worsened. In 2003, data compiled from 5 studies on RIF/PZA therapy for latent infection total 1311 patients found a rate of 5.8% with grade 3 or 4 WHO liver injury. These were only in latent infected patients. Hepatotoxicity is found more frequently in non-HIV infected patients and in those on 2 drug latent RIF/PZA therapy vs. 3 drug regimens for active TB. This likely occurs because there is impaired host immunity in HIV- infected as well as TB- infected patients so less inflammation will develop. Our patient was HIV negative, had no active viral hepatitis, denied alcohol or had collagen vascular diseases. Possible mechanisms of injury in her case include: 1- delayed hypersensitivity, 2- idiosyncratic, 3- underlying cryptogenic liver disease. Because she received 6months of therapy, the DOH rejected further TB therapy due to her condition.

## 903

### BACTERIAL CONTAMINATION IN CEBICHE PURCHASED FROM RESTAURANTS AND STREET VENDORS IN LIMA, PERU: PRELIMINARY RESULTS

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Cebiche is a popular Peruvian dish prepared with raw fish and/or shellfish marinated in lime juice. Lime's acidity supposedly kills bacteria, but some pathogens appear to remain viable. We present preliminary findings of an assessment of bacterial contamination in cebiche sold in Lima, Peru. Servings of both 'fish only' and 'mixed' cebiche (fish and shellfish) were purchased from formal restaurants and street vendors by an anonymous 'client' at the central point of each of Lima's districts between February-March 2007. Samples were obtained on Fridays between 1200-1500h and tested within 2 hours for *Vibrio* spp, *Salmonella* spp, *Staphylococcus* spp, *Shigella* spp, fecal coliforms and *E. coli*, following FDA standard methods. Pathogenic *E. coli* were tested by real time multiplex PCR methodology. We also assessed the pH of samples and the presence of *E. coli* by Colilert® in the restaurants' water supply. Data collection is still going on. To date, 20 samples from restaurants and four from street vendors were collected in 10 districts. Seventeen (71%) and 14 (58%) of the samples showed fecal coliforms and *Escherichia coli* counts over permissible limits (>10<sup>3</sup> and >10<sup>2</sup> MPN/g, most probable number per gram, respectively). Eight samples (33%) had pathogens: four *Aeromonas hydrophila*s, two *Vibrio fluvialis*, one *Vibrio cholerae* non-01, non-0139, and one Enterococcal *E. coli*. Fecal contamination was frequently present in samples from both restaurants and street vendors. Coliform counts correlated positively with pH (Spearman's Rho=0.41, p=0.05) but not with price. Water from eight restaurants had appropriate chlorine levels and no *E. coli*. Cebiche can be a source of pathogenic bacteria, but doses may be low and Peruvians are probably partially immune given their frequent intake. Our results cannot be extrapolated to restaurants where tourists eat, however, as only one restaurant listed in tourist guides was included, presenting no pathogens and *E. coli* counts within permissible limits.

## 904

### RAPID AND SPECIFIC DIAGNOSTICS OF RICKETTSIAL INFECTIONS BY A PCR-BASED HYBRIDIZATION CHIP ASSAY

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Rickettsiae, a group of fastidious intracellular gram-negative bacteria, are divided into two groups. These are the typhus group, which consists of *Rickettsia typhi* and *R. prowazekii* and the spotted fever group, which includes about 20 different species. Rickettsiae have been characterized conventionally and identified by serotyping and protein analysis. Furthermore several DNA-based techniques have been developed for the species identification of rickettsiae mainly based on sequencing of various rickettsial genes. However these methods are time-consuming, expensive and require sophisticated lab equipment. Therefore, a new identification method that counteracts these disadvantages is needed especially for rapid, sensitive and specific laboratory diagnostics of human rickettsioses. We present a method for the detection of rickettsia based on a PCR that amplifies a biotin-labelled fragment of the 23S-5S intergenic spacer region. Initial amplification can be performed either on a real-time PCR system using a broad-range rickettsial probe or on a conventional PCR cyler under basic laboratory conditions. Amplification products are hybridized to low density arrays carrying universal, group- and strain-specific oligonucleotide probes. The arrays are based on plastic photo-slide frames and are processed in a simple and purely manual procedure. Bound PCR fragments are visualized with the naked eye using a blue precipitating peroxidase substrate. A competitive internal control is included in each assay to identify sample-derived PCR inhibition. The method is highly sensitive and specific in identifying rickettsia species from both patient and arthropod samples. Additionally the generic approach used allows identifying up to now unknown pathogenic rickettsial species.

905

### CLASS-SPECIFIC ANTIBODY RESPONSES IN HUMAN BRUCellosIS

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In Egypt, brucellosis is a public health problem. Understanding the humoral immune response during infection is important for the refinement of immuno-diagnostic assays for Brucellosis. Using an enzyme-linked immunosorbent assay (ELISA), we studied the class-specific antibody (Ab) titers against whole *Brucella melitensis* (*Bm*) and *Brucella abortus* (*Ba*) in patients treated for brucellosis. Patients admitted to Abbassia Fever Hospital were screened for brucellosis using serum tube agglutination (STA). Those with STA titers  $\geq 1:320$  using whole *Ba* and/or *Bm* organisms and were diagnosed as brucellosis. Blood was collected from these cases at day 0 and 1, 2, 4 and 6 weeks after the start of antibiotics. End-point titres were assigned as the interpolated dilution giving an absorbance at 492 nm of 0.3 above background. Of 58 patients screened, 21 were diagnosed with brucellosis and 14 patients agreed to follow up. The 14 patients were aged 18-56 years (males n=6) and had fever from 8-90 days prior to admission. Four cases had positive blood culture for *Brucella* spp. Before starting antibiotic therapy 13 of 14 cases had positive IgM titers; 12 cases had high level IgM against both *Bm* and *Ba* while one case had IgM titer against *Ba* only. Also, 12 cases had IgG positive titers; 6 / 12 cases had high levels of IgG against both *Bm* and *Ba* and 5 cases had IgG against *Bm* only, while 1 case had IgG against *Ba* only. For IgA, 8/14 cases had high level IgA against *Bm* and *Ba* and 1 case had IgA against *Bm* only, while 2 case had IgA against *Ba* only. The IgM negative sample was highly reactive for IgG and IgA. During antibiotic therapy decrease in IgM and IgA titers began at week 2. In most cases IgM and IgA continued to decrease through weeks 4 and 6 (IgM 0-1000; IgA 200-5000). In 2 cases only, IgA titers remained high all through. The IgG titers decreased starting from week 2 as well, but at a slower rate, and remained positive through week 6. By end of therapy, all patients were clinically cured. In conclusion, our ELISA results suggest that IgA and IgM are effective for detecting early cases of brucellosis caused by *Ba* and *Bm*. IgA and IgM may be helpful for monitoring response to therapy after the first week. Future studies will confirm the effectiveness of anti-*Brucella* IgA as a marker for early cases of brucellosis.

906

### CASE REPORT: TRAVELER'S BRUCELLA- SPECIFIC IGA AND IGM ANTIBODIES AS EARLY SERODIAGNOSTIC MARKERS OF INFECTION

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Brucellosis is one of the most important endemic zoonotic diseases in the Mediterranean basin. We report the case of an Egyptian female, who presented with night sweats, headache, and back pain, one week post-return from a short visit to a North African country. The patient was diagnosed with acute brucellosis. We investigated the humoral immune response elicited and identified immunogenic protein bands expressed during treatment of brucellosis. The patient had a blood-borne pathogen screening prior to travel which was negative for *Brucella*-specific antibodies. Blood samples were collected from the patient at the time of presumptive diagnosis and at weekly intervals during therapy. Enzyme linked-immunosorbent assay (ELISA) was performed to detect levels of circulating immunoglobulins (Ig) IgA, IgM and IgG antibodies at different time points. Immunoblotting was used to identify specific antigenic epitopes related to corresponding stages of infection. On day one ELISA detected elevated levels of IgA specific for *Brucella* spp., and the standard tube agglutination (STA) titer was 1:160. Three days later the STA titer doubled and elevated specific IgM and IgG (Abs) along

with even higher IgA levels were observed. By then, oral doxycycline and rifampicin were started. Blood culture, yielded Gram-negative coccobacilli after one month. Using AMOS-PCR the isolate was shown to be *B. melitensis*. Immunoblot analysis of specific antibodies against electrically fractionated *Brucella* antigens revealed that the highest number of reactive bands (Ag-Ab complex) was observed during days 18 and 39. Reactivity of IgM against two protein bands of 112 and 130 kDa was observed only in the acute stage of the disease, while the reactivity of IgG against low molecular weight bands, 21 and 21.5 kDa increased with time. In conclusion, our case report demonstrates that brucellosis can be acquired during short term travel to endemic areas. Specific IgA levels can be used as an early diagnostic marker for Brucella infection. In addition, identification of high versus low MW protein bands may be useful in differentiating between acute and chronic brucellosis.

907

### CASE REPORT OF SALMONELLA TYPHI INFECTION IN A U.S. TRAVELER

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Typhoid fever, an acute febrile illness caused by *Salmonella Typhi* is still endemic in many developing countries. Since it is rare in United States, early diagnosis remains a challenge. Approximately 400 cases per year are reported to CDC every year. This easy to treat disease can be life threatening if not diagnosed and managed promptly. Here we present case of a young female who recently travelled to India and developed symptoms three weeks after returning to USA. She presented with fever, chills, malaise and abdominal pain for five days. She was seen at another hospital two days earlier and was sent home on Tylenol. On admission patient was found to be lethargic, febrile and tachycardic. She had mild abdominal tenderness with hepatomegaly. Laboratory work up was significant for leukopenia, elevated transaminases and hyperbilirubinemia. Initial working diagnosis was sub acute Tylenol toxicity versus viral hepatitis. She was started on fluoroquinolone. Abdominal imaging confirmed hepatosplenomegaly with fatty liver. Later the patient developed diarrhea. Tests for malaria, babesia, hepatitis and stool for ova and parasite were negative. Blood culture showed gram-negative rods. Patient continued to have high fever, diarrhea and worsening liver enzymes requiring transfer to the intensive care unit. There was no suspicion of typhoid fever until on fifth day of hospitalization when the patient's blood cultures grew *Salmonella Typhi*. She was given Ceftriaxone according to sensitivity results. She responded well to treatment and was discharged home. This case emphasizes the need for high index of suspicion for typhoid in patients with febrile illness returning from endemic areas. Her diagnosis was missed at another hospital. Further delay in management could have resulted in fatal complications in a young and healthy person. The need to educate our physicians about travel-related diseases should be reinforced. Travelers should also be educated about vaccinations and proper hygiene when traveling to endemic areas

908

### ACUTE INFECTION CAUSED BY A NOVEL BARTONELLA SPECIES: DESCRIPTION OF THE FIRST THREE HUMAN CASES OF B. TAMIAR -THAILAND

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*Bartonella* species are important causes of human infections worldwide, and over 10 distinct species causing human disease have been described. In Thailand, animal infections have been well-documented, but similar data on human infections are lacking. We describe the first three confirmed human cases of *B. tamiae*, a novel *Bartonella* species recently isolated from a patient in Thailand. Cases were identified through a prospective study of febrile patients and non-febrile controls at a community hospital in Khon Kaen, Thailand during February 2002-March 2003. Blood clots were screened for the presence of *Bartonella* species using either a Vero E6 cell-PCR-subculture technique or inoculation into BAPGM liquid medium. *B. tamiae* was isolated from the blood of 3 (0.9%) of 319 patients. Isolates were identified by analyses of 5 genes and 1 intergenic region. Patient 1 was a 38-year old male shopkeeper with a history of HIV and alcoholism who presented with fever (38.2°C), chills, lethargy, headache, myalgia, cough, and maculo-papular rash. Patient 2 was an otherwise healthy 12-year old boy with a 1-day history of fever (38.2°C), lethargy, headache, myalgia, vomiting, cough, and petechial rash on his arms and legs. Patient 3 was a 41-year old female rice farmer seeking care for a pterygium. She was enrolled as an afebrile control but reported headache, myalgia, diarrhea, and cough. Patient 1 had leukocytosis (23,400/mm<sup>3</sup>) and all three patients had anemia (hemoglobin 11-11.8 g/dL). Patients 1 and 2 had elevated alkaline phosphatase (648 and 373 g/dL, respectively). Patients 1 and 3 reported rat exposure in their home during the 2 weeks before enrollment. All patients had no serologic evidence of co-infection with other pathogens. Our findings support *B. tamiae* as a cause of human disease in Thailand. Although one patient was afebrile, all three patients had symptoms and laboratory findings consistent with bartonellosis. Although two patients reported recent rat exposures, further investigation is needed to determine the animal reservoir and possible vector for *B. tamiae*.

## 299

### THE DEVELOPMENT OF AN AGE-STRUCTURED MODEL FOR TRACHOMA TRANSMISSION DYNAMICS AND CONTROL

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A mathematical model of community ocular infection with *Chlamydia trachomatis* is presented that is based on the concept of multiple reinfection leading to worsening conjunctival scarring and eventually to severe disease sequelae: trichiasis, corneal opacity and blindness. The model also includes aspects of the natural history of the disease--the increasing recovery rate from infection and the decreasing chlamydial load with subsequent infections--that depend upon a (presumed) acquired immunity that more rapidly clears infection with age. Model parameters are obtained by fitting the model to infection prevalence data from hypo-, meso- and hyperendemic communities prior to control interventions, and several features of the epidemiology of trachoma are reproduced, namely: 1) the age-profile of infection prevalence, which increases to a peak at very young ages and then declines at older ages; 2) a shift in this prevalence peak, toward younger ages, in higher force of infection environments; 3) a raised overall profile of the infection prevalence with higher force of infection; 4) a rising profile, with age, of the prevalence of each of the ensuing severe disease sequelae. The suitability of the model for representing the population-level patterns of infection and disease are discussed in view of the individual processes leading to these patterns. Simplifications in e.g. the factors affecting progress towards the severe disease sequelae, may become features of future models, but here the framework is comprehensive enough to examine the outcomes of treatment on disease.

## 910

### PREVALENCE OF BACTERIAL ISOLATES FROM BLOOD CULTURES OF INFANTS ATTENDING PEDIATRIC WARDS IN UNIVERSITY OF BENIN TEACHING HOSPITAL, BENIN CITY, NIGERIA

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Infantile septicemia is a common health problem in developing Countries with inadequate health facilities especially at the pediatric level and septicemia is one of the commonest cause of infant morbidity and mortality which are often associated with pyrexia of unknown origin (PUO). The purpose of this study, therefore, was to determine the bacterial species mostly incriminated in septicemia and to determine their antibacterial susceptibility spectrum. Sixty-six children comprising thirty males and thirty-six females admitted into Pediatric wards in University of Benin Teaching hospital from March, 1999 -October, 2000 were randomly recruited into the study. Veinous blood was collected from patients and aseptically inoculated into two blood culture bottles ;dextrose broth and Thyoglycolate broth and were incubated at 37oc overnight. They were examined daily for growth. Sub culture was made onto two blood agar plates. One incubated aerobically and other anaerobic ally using gas pack anaerobic culture method and were incubated at 37oc over night overnight. The organisms were identified to species level using the protocol of Kowan and Steel. Antibiotic susceptibility spectrum of the isolates was determined using agar diffusion method of Stokes. Fifty-nine different bacterial strains were isolated as follows. *Staphylococcus aureus* 26, *Klebsiella pneumoniae*, 11, *Escherichia coli* 4, *Serratia marcescens* 2, *Alkaligenes faecalis* 2, *Enterococcus faecalis* 2, *Staphylococcus epidermidis* 2 and *Providential* 2. All the organisms were sensitive to the fluoroquinolone antibacterial agents, gentamicin and Erythromycin, but resistant to ampicillin, tetracycline and cotrimoxazole.

## 911

### HUMAN BRUCELLA ABORTUS INFECTION IN THAILAND: A REPORT OF THE FIRST TWO CASES

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Human brucellosis has rarely been reported in Thailand, and all reported cases were caused by *Brucella melitensis*. Unlike animal brucellosis, human brucellosis is not a notifiable disease and most clinical laboratories are not equipped to isolate Brucellae. We established capacity to perform real-time microbial evaluation of patients under surveillance for bacteremia in two rural Thai provinces. This enhanced surveillance led to the detection of the first 2 human cases of *Brucella abortus* reported in Thailand. Both patients presented with histories of fever, headache, night sweats, lethargy, and weight loss, and had positive hemocultures confirmed as *B. abortus* by the National Institute of Animal Health. Patient 1 was a 42 year-old female with an initial clinical diagnosis of scrub typhus and treated with doxycycline and ceftriaxone, but serologic testing for scrub and murine typhus was negative. After brucellosis was confirmed, she was treated with doxycycline and rifampin for 6 weeks and recovered. She reported eating raw beef from cattle that she owned but to which she otherwise had minimal exposure. Three (14%) of 22 cattle randomly selected from her own and neighboring farms had positive serologic testing for *B. abortus*. All 6 of her family members tested negative for *Brucellae* by serology. Patient 2 was a 58 year-old male with liver cancer, who had no



epidemiologic link to Patient 1. He reported having eaten raw beef from an aborted cow fetus, as did his family and farm staff. Serologic tests of other exposed persons and his cattle were negative for Brucellae. He died from complications of cancer. Human brucellosis is likely underdiagnosed and underreported in Thailand, and enhanced microbiologic surveillance can improve detection. These cases highlight the importance of coordinated prevention and control activities between public health and animal health sectors, and strengthening educational programs about the risks of consuming raw animal products and unpasteurized milk as well as continuous campaign and education for farmers. Thailand now plans to make human brucellosis a nationally notifiable disease.

## 912

### A MOUSE DERMAL MODEL TO STUDY EARLY INNATE IMMUNE EVENTS IN THE SKIN AFTER TRANSMISSION OF *YERSINIA PESTIS*

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A critical step in determining the outcome of bubonic plague occurs in the dermis of the host, where *Yersinia pestis* is deposited by the flea vector. Bacteria must successfully disseminate from the skin in order to establish infection in a host. Various factors produced by all three participants (flea, bacterium, and host) at this interface are known to be important to the disease process. We describe a dermal model of *Y. pestis* infection in the ear pinnae of mice. Mice were injected intradermally with live *Y. pestis*, purified *Salmonella typhimurium* lipopolysaccharide as a positive inflammatory control, or saline diluent alone. Paired ear and draining lymph node samples were taken at 3, 6, 9, 12, 24, and 48 hours post-injection. Ear skin was briefly digested with collagenase and dispersed into cell culture medium. Skin and lymph node cells were analyzed by flow cytometry to measure the nature and dynamics of the inflammatory response. Additional whole tissue samples were fixed, sectioned, and examined for histological changes. We will apply the model to study the effects of natural transmission of virulent *Y. pestis* by fleas.

## 913

### DEVELOPMENT OF REAL-TIME PCR ASSAYS FOR DETECTION AND CHARACTERIZATION OF *BARTONELLA* SPECIES IN HUMAN AND RODENT BLOOD SAMPLES FROM THAILAND

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*Bartonella* are gram-negative intracellular bacteria that infect a wide range of mammalian hosts and several arthropod vectors. Human disease caused by *Bartonella* is thought to be widely under-reported and is likely an important public health consideration in many parts of the world. A major challenge in the detection and identification of bartonellosis is the inherent difficulty in isolating and identifying many *Bartonella* species from blood samples. Commonly used approaches include culture techniques, single-step, and nested PCR to determine the presence of *Bartonella* in clinical and field samples. However, culturing techniques are often time consuming and require the handling of potentially infectious agents, single step PCR lacks the sensitivity to detect low numbers of bacteria, while nested PCR has proven to be less than 100% reproducible and contamination is a constant threat. Furthermore, recent work has shown that some *Bartonella* species are non-culturable, suggesting possible biases associated with identifying *Bartonella* strains with culture. Because of these difficulties, we developed real-time PCR assays based on conserved regions of the citrate synthase (gltA) and NADH dehydrogenase  $\gamma$  subunit (nuoG) genes to serve as general detection assays for the *Bartonella* genus. These assays have proved to be both sensitive and specific in their ability to detect all known *Bartonella* species, but no related bacteria, and have increased sensitivity and specificity of *Bartonella* detection in both human and rodent blood samples from Thailand. To further better understand the population dynamics and genetic diversity of *Bartonella*

species in humans and rodents in Thailand, we have developed a more specific real-time multiplex assay based on highly variable regions of both the gltA gene and the ITS region of the *Bartonella* genome. This assay allows simultaneous detection and quantification of 11 phylogenetic groups of *Bartonella* simultaneously, permitting the identification of multiple strains in a single blood sample. Preliminary studies using this technique have shown a higher than expected number of samples with multiple strains of *Bartonella* as well as the presence of non-culturable *Bartonella* species in both humans and rodents.

## 914

### ECONOMIC BURDEN OF COMMUNITY ACQUIRED PNEUMONIA IN CHILDREN LESS THAN FIVE YEARS OLD IN EGYPT

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Pediatric community acquired pneumonia (CAP) is a serious problem causing hospitalization in about 20% of cases. CAP results in considerable health-care utilization, burden to the health care system and society. This study estimates the direct and indirect costs of CAP in a population of children < 5 years at a pediatric hospital in Cairo, Egypt. Prospective CAP surveillance was conducted between Nov 2006 and Apr 2007. Children ( $\leq 5$  years) presenting with respiratory illness meeting the case definition of CAP were systematically identified. Economic data was collected using a questionnaire and record review, expressed in Egyptian pounds (LE) and equivalent US dollars (\$). Cost categories included laboratory, medications, transportation, lost wages, death related expenditures and other costs associated with care. Average costs are reported by type of care delivered, disposition and etiology. Of 190 cases of CAP enrolled, 143 (75%) were treated as outpatients and 47 (25%) were hospitalized. Among all cases, 74% received health care prior to presentation (81% hospitalized vs. 71% outpatient,  $p=0.2$ ). Bacterial, viral or mixed etiology was shown in 79 (42%) cases, including 62 cases due to respiratory syncytial virus (RSV). The median total costs for all cases was 63 LE (\$11) (Interquartile range [IQR]: 30-203), with 52 LE (\$9) medical and 5 LE (\$1) non-medical median costs. The median total cost per inpatient was greater (401 LE, \$70) than outpatient (46 LE, \$8) ( $p=0.0001$ ). Among those reporting treatment prior to presentation, the median prior treatment cost was 29 LE (\$5). The median total cost of RSV pneumonia cases was 99 LE (\$17), compared with 57 LE (\$10) for others ( $p=0.2$ ). Hospital services accounted for 39% of costs, treatments 36% (3/4ths of which were antibiotics), other expenditures 14% and laboratory 11%. In conclusion, based on WHO estimates of pneumonia in developing countries among children (<5 years) (0.29 episode / child-year), approximately 3 million pediatric pneumonia cases (resulting in about 300,000 hospitalizations) occur in Egypt per year. Based on the cost estimates from our study, CAP in Egypt costs about 189 million LE (\$33.2 M), of which ~10% are non-medical costs. Given the considerable economic burden to both the national health system and individual patients, further research is needed to develop and implement strategies to optimize the use of hospital treatment and antibiotics.

## 915

### COMMUNITY-ACQUIRED NON-TYPHOIDAL SALMONELLA BACTEREMIA AND PATTERNS OF ANTIMICROBIAL RESISTANCE IN THAILAND, 2005-2007

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In developed countries the most common manifestation of non-typhoidal salmonella (NTS) infection is gastroenteritis, which is usually a self-limiting and benign disease; invasive disease occurs in ~5% of patients.

In developing regions, NTS infection may be a significant source of invasive disease but has been poorly characterized, especially in Southeast Asia. We examined NTS bacteremia in rural Thailand to elucidate the pathogens involved and their antibiotic susceptibility. From 2005-2007, blood cultures from patients hospitalized in the rural provinces of Sa Kaeo and Nakhon Phanom were examined for the presence of NTS. Suspect isolates were identified by conventional methods and confirmed and serotyped at the WHO *Salmonella-Shigella* Regional Reference Center in Bangkok. Susceptibility for amikacin, amoxicillin-clavulanic acid, ampicillin, cefotaxime, ceftazidime, cephalothin, ciprofloxacin, chloramphenicol, gentamicin, imipenem, norfloxacin and co-trimoxazole was determined by disk-diffusion. Of 100 invasive NTS isolates identified, 28 (28%) were associated with pneumonia, 9 (9%) with sepsis, 2 (2%) with diarrhea, and 63 (63%) with other severe disease. Serovars identified included *S. choleraesuis*, 69 (69%); *S. enteritidis*, 22 (22%); *S. enterica*, 4 (4%); and *S. typhimurium*, 4 (4%). Of invasive NTS isolates tested to date, 4% (3/71) were resistant to amikacin, 14% (7/50) to amoxicillin-clavulanic acid, 74% (69/93) to ampicillin, 6% (5/83) to cefotaxime, 6% (3/55) to ceftazidime, 18% (9/50) to cephalothin, 19% (15/79) to gentamicin, and 12% (12/97) to co-trimoxazole. No isolates were resistant to chloramphenicol, ciprofloxacin, imipenem, or norfloxacin. For resistant isolates, MICs are being determined by Etest. The most common invasive pathogens were *S. choleraesuis* and *S. enteritidis*, suggesting that Southeast Asia may have a different NTS profile relative to other regions for which similar invasive NTS have been examined. Resistance to commonly used antibiotics was high among these isolates which will complicate the development of empiric treatment guidelines.

## 916

### TOWARDS RAPID DIAGNOSIS OF PULMONARY TUBERCULOSIS IN MALAWIAN PRISONS

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Prison inmates in Africa in general and in Malawi in particular represent a population with high prevalence and transmission rates of pulmonary tuberculosis (PTB). An active case-finding survey conducted in one of Malawi's most populous prison in 1996 showed a prevalence of 5% among those with a cough of at least one-week duration. Studies also conducted in Tanzania and Ivory Coast that employed active screening procedures found PTB point prevalence rates of at least ten times higher than national rates. Active transmission of PTB has also been documented in this population. Such high rates are mainly due to crowded living conditions that accelerate active transmission of PTB and also due to high prevalence of HIV/AIDS among the inmates. Other associated factors include poor nutrition, poor access to health services, high risk sexual behaviour including homosexuality. Previous studies on the continent have highlighted the need for a rapid and regular PTB assessment among the incarcerated populations. This research study will seek to identify such a tool in the form of a laboratory diagnostic platform. It will evaluate the current screening process and assays. Specifically, this study will compare the cost-effectiveness, efficiency, sensitivity and specificity of three assays namely: Ziehl-Nielson stain, fluorescent microscopy with auramine-rhodamine stain, and the purified protein derivative test. This study will also detect drug resistant including multi-drug resistant tuberculosis, which is an emerging global public health problem. The results of this research study will help improve tuberculosis control in Malawi. This will complement other public health interventions.

## 917

### GROUP A STREPTOCOCCUS PHARYNGITIS AMONG SCHOOLCHILDREN IN BAMAKO, MALI

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Group A Streptococcus (GAS) is one of the most common and versatile human pathogens, causing superficial and invasive infections as well as rheumatic fever and other immunologic sequelae. Recently, multivalent M type-specific vaccines have shown promise in trials. To use such vaccines in developing countries, the GAS burden and distribution of *emm*-types must be characterized. Four public elementary schools in two low-income quarters (Djicoroni-para and Sebenikoro) in Bamako, Mali were identified and a census of the student body was performed at the beginning of the study and at the beginning of the school year. Study personnel are present in each school infirmary to identify 5- to 16-year old children with pharyngitis and complete a clinical history and physical exam. A throat swab is obtained and processed to culture GAS according to standard procedures. *Emm*-typing is performed according to the Protocol. All children with GAS pharyngitis are treated with a 10-day course of penicillin or erythromycin (if allergic to penicillin). From 30 May to 12 September 2006, of the 12,508 students under surveillance, 58 presented with pharyngitis (3 cases per 10,000 person-weeks), 41 from Djicoroni-para and 17 from Sebenikoro. Of these, 15 (26%) were positive for GAS; 0.8 cases per 10,000 person-weeks. Almost half of the cases were 8-10 years of age (n=7) and most were females (n=10). Compared to those without GAS, children with GAS isolated from the throat were more likely to report pain with (p=0.31) and difficulty swallow (p=0.15) and on examination had tonsillar exudates (p=0.02) and painful lymph nodes (p=0.08). *Emm*-typing results are available for 11 isolates and represent at least 8 types, including 69.1 (n=2), 63.3 77, 8.1, 81.2, and 89.6. (Data from 30 May 2006 to 30 May 2007 will be presented.) In conclusion, these data suggest that GAS is an important cause of pharyngitis in Malian schoolchildren. *Emm*-type distribution of pharyngitis cases appears to be broad. More data is needed to determine the coverage afforded by current GAS vaccines.

## 918

### ETIOLOGY OF COMMUNITY-AQUIRED PNEUMONIA IN EGYPTIAN CHILDREN LESS THAN FIVE YEARS OLD

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Community-acquired pneumonia (CAP) remains a leading cause of morbidity and mortality worldwide. In this study we tried to identify the microbial etiology, clinical presentation and outcome of CAP. *Methods*: A prospective study of 182 children < 5 years old with clinical CAP was conducted in Cairo Children's Hospital over a 6-month period; Nov 06 to Apr 07. Nasopharyngeal aspirate (for viral and bacteriological testing), acute sera (for mycoplasmal and chlamydial serology), and blood for bacterial culture were collected. Enzyme-linked immunosorbent assay was used for diagnosis of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*. Immunofluorescent assay (IFA) was used to identify respiratory syncytial virus (RSV), influenza A&B, parainfluenza 1, 2 and 3, adenovirus, and human metapneumovirus (HMNV). *Results*: The median age of subjects was 6 months, about 85% were ≤ 2 years and 58% were males. Pathogens were identified in 55% of cases, including: *Mycoplasma pneumoniae* (3%), *Chlamydia pneumoniae* (8%), and viruses (44%). Normal bacterial flora of nasopharyngeal aspirates was found in (28%). RSV was identified in 70 patients (38%), influenza A in 4 (2%), parainfluenza 3 in 2 (1%), and adenovirus in 4 (2.2%), and mixed bacterial/viral infections in 6 (3%). Parainfluenza 2, influenza B,

and HMNV were not detected. Cephalosporin's were often prescribed empirically (67%) for suspected pneumonia. Patients received antibiotics in the week prior to presentation in 47% of cases. The mean duration of hospitalization was 5 days. The case fatality rate was 4%. Blood cultures for bacteria were negative. The most common underlying illnesses were congenital heart disease (24%), asthma (4%), malnutrition (2.4%), diarrhea (15%), vomiting (24%), unable to feed/drink (36%). **Conclusions:** Viruses play a significant role in respiratory tract infection in the population studied, followed by atypical bacteria. The results of this study suggest a remarkable role for *M. pneumoniae* and *C. pneumoniae* in childhood CAP. Knowledge of the true prevalence of these two types of infections in the community might lead to modifications in the present empirical treatment of suspected bacterial pneumonia. Bacterial etiology was difficult to determine and this may be due to the frequent antibiotic treatment prior to admission.

## 919

### CORRELATION OF HYPERHOMOCYSTEINEMIA AND CHLAMYDIA PNEUMONIAE IGG SEROPOSITIVITY WITH CORONARY ARTERY DISEASE IN A GENERAL POPULATION

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Both Chlamydia pneumonia infection and hyperhomocysteinemia have been assumed to increase the atherosclerotic risk independently of each other and independently of the classic risk factors. The correlation between hyperhomocysteinemia, *Chlamydia pneumoniae* infection and coronary artery disease (CAD) have not been investigated in the general population. In an ancillary study to the Persian Gulf Healthy Heart Study, a cohort study of men and women aged  $\geq 25$  years, a random sample of 1699 (48.9% males, 51.1% females) subjects were evaluated. Total homocysteine, high sensitivity C-reactive protein and IgG antibodies to *C. pneumoniae* were determined by ELISA. Minnesota coding criteria of a 12-lead resting electrocardiogram was used for evaluation of CAD. A total of 12.4% of the subjects had electrocardiogram-defined (Minnesota-coding criteria) coronary artery disease. Hyperhomocysteinemia ( $>14$  micromol/l) and IgG seropositivity were found in 50.8% and 37.7%, respectively. Neither of hyperhomocysteinemia and *C. pneumoniae* IgG seropositivity showed a significant association with CAD after adjusting of sex and age. Concurrent elevated CRP level ( $>8.2$  mg/l) and *C. pneumoniae* seropositivity (chronic *C. pneumoniae* infection) had a significant association with CAD [OR=1.73, C.I (1.09 -2.75);  $p=0.01$ ] after adjusting for age, sex, systolic and diastolic blood pressures, BMI, and serum levels of LDL-cholesterol, fasting blood sugar and triglyceride as covariates in a logistic regression model. This odds ratio increased to 2.11, C.I (1.18-4.12;  $p=0.02$ ) when concurrent hyperhomocysteinemia and chronic *C. pneumoniae* infection, as a single covariate entity; was adjusted for multiple risk factors in another logistic regression model. In conclusion, concurrent hyperhomocysteinemia and chronic *C. pneumoniae* infection, as a single entity, was independently associated with coronary artery disease in the general population. This synergism may have important implications for risk-stratification and intervention trials.

## 920

### DELETION OF CD36 CONFERS PROTECTION AGAINST MYCOBACTERIAL INFECTION

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Members of the CD36 scavenger receptor family have recently been implicated in the innate immune response to mycobacteria. We therefore

tested the hypothesis that deletion of the CD36 gene would result in alterations in host immunity to mycobacterial infection. Using an *in vivo* murine model we demonstrated that both wild type and CD36 deficient mice infected with *Mycobacterium bovis* BCG exhibit an initial rise in mycobacterial load in spleen, liver and lung, reaching a local maximum at 2-3 weeks post infection and declining thereafter. However, CD36 deficient mice had a lower bacterial burden overall and at peak, less splenomegaly, fewer granulomata and visible acid fast bacilli in infected organs, and lower levels of tumor necrosis factor- $\alpha$ , relative to wild type control mice. *In vitro*, initial uptake of *Mycobacterium tuberculosis* and *Mycobacterium bovis* BCG was not significantly different in macrophages derived from *Cd36*<sup>-/-</sup> mice relative to wild type macrophages. However, mycobacterial replication within *Cd36*<sup>-/-</sup> macrophages was significantly lower relative to wild type macrophages. This impaired intracellular growth occurred by an apoptosis-independent mechanism. Potential alternative mechanisms for restricted intracellular replication of mycobacteria in the absence of CD36 receptor are discussed. Our findings suggest that CD36 deficiency may be protective against tuberculosis, perhaps accounting, at least in part, for the widespread balanced polymorphisms of the CD36 gene observed in populations from tuberculosis-endemic regions.

## 921

### LEGIONELLA PREVALENCE IN SPRING RECREATION AREAS OF TAIWAN

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*Legionella* is a bacterium ubiquitous to aquatic environments. Within the genus a few species are recognized as opportunistic potential human pathogens, especially the species *L. pneumophila*, which causes pneumonia legionellosis. Outbreaks of legionellosis are frequently reported by hotel guests and hospital patients, and are spread through inhaled aerosols of contaminated institutional water systems. Contaminations in hot tubs, spas and public baths are also possible. As a result, in this study, we investigated the distribution of *Legionella* at seven hot spring recreational areas throughout Taiwan. We gathered data on factors potentially associated with the pathogen's distribution, including environment, facility operation, and physical and microbiological water quality parameters. Spring water was collected from 100 sites and *Legionella* was detected in 21 (21%). The most frequently detected was *L. pneumophila*, followed by unculture *Legionella* species, *Legionella*-like amoebal pathogen. Five species, *L. bozemanii*, *L. dumoffii*, *L. feelei*, *L. lyticum* and *L. oakridgenensis*, were all detected once. The prevalence of *Legionella* also coincided with the prevalence of indicator microorganisms. *Legionella* detection was not proportional to the frequency of cleaning. Results of this survey confirm the ubiquity of *Legionella* in Taiwan spring recreation areas. *L. pneumophila*, the organism responsible for the majority of legionellosis outbreaks, should be considered a potential public health threat in spa areas of Taiwan.

## 922

### PIRFENIDONE AS ADJUNCTIVE THERAPY PROVIDES SURVIVAL ENHANCEMENT IN A LETHAL MURINE MODEL OF SYSTEMIC STREPTOCOCCUS PNEUMONIAE

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Mortality from pneumococcal infections remains high, despite the development of potent antibiotics. The drug Pirofenidone blocks the biochemical process of inflammation and has been reported to slow or reverse pulmonary fibrosis in animal systems. It has been shown to inhibit TNF  $\alpha$  at the translational level and inhibits TGF- $\beta$  induced collagen synthesis in fibroblasts. We investigated the effect of pirofenidone as adjunctive therapy in a lethal murine model of systemic *Streptococcus pneumoniae*. Balb/c mice were inoculated intravenously with a lethal dose



( $10^8$  cfu) of *S. pneumoniae* serotype 2. Mice were separated into 4 groups. Group 1 received 25 mg/kg Ceftriaxone at 6 hours post infection. Group 2 received a combination of 25 mg/kg Ceftriaxone (i.p) and 1mg pirfenidone (i.p) 6 hours after infection and pirfenidone was then administered every 12hrs for 2 days. Group 3 received 25 mg/kg Ceftriaxone (i.p) 6hours and 1mg pirfenidone (i.p) 1hour after infection and pirfenidone was then administered every 12hrs for 2 days. Group 4 received PBS. Survival as endpoint was monitored for seven days. The survival of mice treated with the combination of ceftriaxone and pirfenidone was 80 % (n=8,  $p=0.04$ ) mice, compared to 30 %, of mice receiving ceftriaxone only (n=8) and 0% of vehicle control group (n=10). No significant survival benefit (50%, n=4  $p=0.272$ ) was observed when pirfenidone was administered 1 hour after infection (Group 3) compared to ceftriaxone treated group. In conclusion, in this mouse model, the use of an anti-inflammatory agent pirfenidone as an additional therapy to the antibiotic Ceftriaxone achieved a survival benefit in the reduction of mortality in severe systemic *S. pneumoniae* infections. We are uncertain of the mechanism of pirfenidone survival enhancement as an adjuvant in this model. Pirfenidone is a new drug currently under investigation and these results warrant further studies of pirfenidone as adjunctive therapy in this model of infection.

## 923

### IN VIVO AND IN VITRO EFFICACY OF TA-18 AGAINST HANTAVIRUS INFECTION

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At this time, there are no approved antiviral drugs for the treatment of hemorrhagic fever with renal syndrome (HFRS) or hantavirus pulmonary syndrome (HPS); two serious hantaviral illnesses. Therapeutic efforts are generally limited to supportive care, although studies performed in China on HFRS patients suggest that ribavirin provides an improved prognosis when given early in the course of disease. We hypothesize that new lead inhibitors that target hantaviral replication can be designed and synthesized based on existing compounds that are known to inhibit the production of infectious virus (ribavirin, selenazofurin and tiazofurin). Focusing on the heterocyclic- $\beta$ -riboside structure, we prepared a diverse series of 3-substituted 1,2,4-triazole- $\beta$ -ribosides. These compounds included isosteric derivatives of ribavirin and linkage isomers that exhibit altered hydrogen-bonding capacity. This series has been evaluated for antiviral activity, and we have identified a potent hantaviral antiviral activity from the novel synthetic compound TA-18. The EC<sub>50</sub> for TA-18 was 15  $\mu$ g/ml which is similar to that of ribavirin and mycophenolic acid. Mechanism of action studies suggest the compound targets the polymerase but does not cause an increase in mutation frequency as we have observed for ribavirin. TA-18 showed no toxicity in suckling mice when treated at 12.5mg/kg for 14 consecutive days *in vivo*. However, when suckling mice were treated with TA-18 at 50mg/kg and 100mg/kg for 14 consecutive days, some toxicity was observed with weight loss and 20% mortality. The clinical symptoms include loss of appetite, poor groom, hunched posture, and squinting. Clinical pathology and hematology analysis and the *in vivo* efficacy study of TA-18 against hantavirus infection is currently underway.

## 924

### PERFORMANCE EVALUATION OF SURVEILLANCE AND RAPID RESPONSE TEAMS IN THAILAND

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Thailand established Surveillance and Rapid Response Teams (SRRT) to detect and respond to public health emergencies, including pandemic influenza. SRRTs are trained to detect early human-to-human transmission warning signals such as clusters of patients with severe respiratory illnesses. Detection of such events is followed by immediate field investigation to confirm diagnoses, identify the source, and determine whether human-to-human transmission is occurring. We sought to describe SRRT response activities, evaluate performance and identify training needs. We conducted in-depth interviews and focus groups with 319 participants selected from a convenience sample of 51 of Thailand's 1,030 SRRTs in 2006; a self-administered questionnaire was also completed by all participants. Teams were chosen from each of the 12 Regional Offices of Disease Prevention and the 12 provinces where suspected human avian influenza (AI) cases had been reported. Each SRRT had at least 4 members and 1/3 of respondents were part of a team for <5 years. Team members spent an average of 4 hours per week on SRRT activities. SRRTs routinely investigated suspected human AI cases (average 2/month), reported suspected cases, and monitored events using well-established community networks. Approximately 70% of SRRT members could correctly describe standardized case finding methods, identify possible sources of AI, and communicate prevention messages. Approximately 30% of SRRTs indicated additional training was needed for specimen collection; 25% identified a need for emergency planning. All 319 participants indicated a need for standard response plans for pandemic influenza. SRRTs provide critical support for investigating suspected human AI cases in Thailand. Periodic training in specimen collection is needed to ensure accurate diagnosis during outbreaks. SRRTs will be limited in responding to pandemic influenza without well-established emergency response plans and broader knowledge of incident command structure. Continued commitment from political leaders is imperative for the program's continued success.

## 925

### ETIOLOGIES OF ACUTE FEBRILE ILLNESS IN BISHKEK, KYRGYZSTAN

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We studied etiologies of non-diarrheal, acute febrile illness (AFI) without an obvious bacterial etiology in all patients presenting with this case definition at the Infectious Diseases Hospital in Bishkek, Kyrgyzstan from fall 2004 to fall 2006. Cases were screened for Measles and Rubella with ELISA, Brucellosis using the Slide Agglutination Test (SAT) and for Typhoid using the Widal test and only samples negative with these tests were further tested for other possible causes of AFI. From 160 cases, using various ELISA methodologies, we diagnosed 29 cases of tick-borne encephalitis (TBE) and 21 cases of Q fever (as well as 14 additional cases of Brucellosis that were not detected by SAT). Results also indicated extensive possible Hantaviral infection, though definitive diagnosis of Hantaviral infection requires neutralization testing. Seropositivity to CCHF, Sindbis, Sandfly fever and West Nile virus were rarely detected. PCR confirmed TBE in 7 additional cases in which anti-TBE antibody was not detected. PCR also implicated enteroviruses as the cause of many infections. The results indicate that, in addition to commonly-diagnosed causes (Brucellosis, enteroviruses, and TBE), Q fever is also a frequent cause of AFI in and around Bishkek, Kyrgyzstan.

## 926

### MOSQUITO FEEDING PREFERENCE FOR COLD-BLOODED VERTEBRATES IN ALABAMA

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Many arbovirus infections including Eastern Equine Encephalitis virus (EEEV) are maintained primarily in an enzootic cycle involving the local avifauna. Escape from this enzootic cycle requires the involvement of mosquitoes which feed upon many different classes of organisms. Thus, vector host choice plays a central role in the dynamics of transmission of arboviral infections. Previous studies have demonstrated that targeting of avian hosts by arboviral vectors appears to be restricted. Thus, vector mosquitoes feed upon specific bird species at higher rates than would be predicted based solely on their abundance. Furthermore, a temporal analysis of the feeding upon the preferential species reveals a pattern that is generally consistent with the hypothesis that this feeding pattern is driven by preferential feeding upon nestlings and or nesting birds. We have extended an analysis of the host feeding patterns of mosquitoes found to be infected with EEEV at a study site in the Tuskegee National Forest of Alabama. In previous studies, we have demonstrated that mosquitoes at this site preferentially feed upon certain birds. We combined an analysis of host feeding patterns mosquitoes upon reptiles and amphibians with estimates of their local abundance at the site to test the hypothesis that the preferential host choice exhibited by these species when targeting birds also extends to other hosts. The data demonstrate that the proportion of blood meals taken from reptiles and amphibians was not significantly different from what would have been predicted based upon the relative abundance of these species at the site. This indicates that reptiles and amphibians are not preferentially targeted by the mosquito species, in contrast to what had been documented in studies involving host choice among birds. This serves to strengthen the hypothesis that targeting of avian nestlings and or nesting birds is an important component of mosquito host feeding behavior, as reptiles and amphibians do not exhibit a comparable behavior in rearing their young.

927

**THE MINIMAL DOMAIN OF THE EASTERN EQUINE ENCEPHALITIS VIRUS CAPSID NECESSARY FOR INHIBITION OF HOST GENE EXPRESSION IS REQUIRED FOR VIRAL PATHOGENESIS**

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Eastern equine encephalitis virus (EEEV) is a human and veterinary pathogen that causes sporadic cases of fatal neurological disease. We previously showed that the capsid protein of EEEV is a potent inhibitor of gene expression. Recently, we identified 20 amino acids within the N-terminus of the capsid gene critical for the inhibition of gene expression, suggesting that this inhibition is independent of capsid protease activity, which maps to the C-terminus of the protein. Analysis of stable EEEV replicons expressing mutant capsid corroborates these mapping data. Interestingly, deletion of 5 or 20 amino acids within this region of the capsid generated viruses with a delayed replication in Vero cells when compared to the parental virus and more importantly, with impaired replication and attenuated virulence in mice. In summary, we have identified a region within the capsid protein of EEEV that is necessary for the inhibition of gene expression and demonstrated that this function is critical for EEEV pathogenesis.

928

**EVALUATION OF THE AOTUS NANCYMAE NEW WORLD MONKEY AS AN ANIMAL MODEL FOR EASTERN EQUINE ENCEPHALITIS**

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Eastern equine encephalitis (EEE) is an arthropod-borne virus associated with life-threatening encephalitis in humans, equines and birds. To investigate the suitability of the *Aotus nancymae* New World owl monkey as a viable animal model for EEE candidate vaccine testing we used serology (IgM/IgG ELISA and PRNT), viral isolation and PCR to evaluate pathogenesis and immunity in infected animals. Monkeys were inoculated subcutaneously (SQ) and intranasally (IN) with 10<sup>4</sup> pfu of virulent EEE virus and were initially followed for 45 days. While none of the animals displayed clinical symptoms of disease, all of the SQ inoculated animals (n=6) manifested a viremia averaging 3.2 days ( $\pm$  0.8 days). Likewise, serologic responses (IgM, IgG and PRNT) were observed in all SQ infected animals. Interestingly, none of the IN inoculated animals (n=6) became viremic or mounted an antibody response and no pathological abnormalities were observed in two animals that were necropsied on Day 6 post infection (p.i.) from each group. To determine if the antibodies produced by the SQ inoculated animals were protective against homologous rechallenge, three animals from the SQ group were serologically evaluated on day 253 p.i. and were administered an inoculum identical to initial challenge on day 270 p.i. A positive control group of 4 naive animals was also infected as before. All of the naive positive control animals manifested a similar viremia as observed initially, averaging 2.75 days ( $\pm$  0.5 days) while none of the previously challenged animals became viremic. On Days 45 and 253 p.i. geometric mean PRNT titers in the SQ group were 453 and 101 respectively. This study demonstrates that the *Aotus nancymae* can be reproducibly infected with EEE and can serve as a suitable model for infection and evaluation of candidate vaccines against disease caused by this agent.

(ACMCIP Abstract)

929

**CO-CIRCULATION OF TWO DIFFERENT HANTAVIRUSES IN A HECTARE SIZED MARK-RECAPTURE SITES IN INTERIOR ATLANTIC FOREST IN PARAGUAY**

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Hantaviruses are zoonotic viruses that causes HFRS and HPS. Hantaviruses have coevolved within their unique rodent host; although they can also spill-over into nonreservoir rodents within the same geographical location. Since identification of LN virus from *Calomys laucha* in Paraguay, we identified four additional hantaviruses. They include ALPA from *Holochilus chacoensis* in western Paraguay and three in eastern Paraguay, AAI from *Akodon montensis*, IP37 from *Oligoryzomys nigripes*, and BMJ-NEBU from *oligoryzomys chacoensis*. We have established eight, one-hectare sized, mark-recapture sites in the Interior Atlantic Forest at the Mbaracayu Biosphere Reserve and in anthropogenically disturbed areas adjacent to the Reserve. Our goals are to develop and analyze; 1) several new mathematical models that describe the temporal and spatial dynamics of hantavirus in rodents, and 2) landscape tools to uncover the anthropogenic land cover change factors responsible for the apparent shifting dynamics of hantavirus prevalence in rodent species. Rodents captured in the grids were pit-tagged, identified by species, gendered and weighed. Approximately 100  $\mu$ L of blood were collected. Antibody reaction was detected by IFA, and antibody positive specimens were further analyzed by nested RT-PCR, sequencing and phylogenetic analysis. A total of 524 rodents in 10 different species were collected in eight study sites from 2005 to 2006. Among collected rodents, 51 (9.7%) rodents of five different species were antibody positive. We have amplified and sequenced 331 or 1038 nucleotides of the S segment and 1538 nucleotides of the M segment from blood specimens, of

two rodent species, *A. montensis* and *O. fornesi*. Amino acid sequence comparison between two viruses revealed 12 and 8 % difference in the S and M segments, respectively. In the S segment based phylogram, the *A. montensis* derived hantaviruses grouped with AAI, LN, RM, and ALPA, and the *O. fornesi* derived hantavirus grouped with IP37. In the M segment based phylogram, *O. fornesi* derived hantavirus grouped with IP37, but those from *A. montensis* formed a separate clade with Pergamino which was derived from *A. azarae* in Argentina. In summary, we have identified two different hantaviruses cocirculating in our hectare-sized mark-recapture sites. We show evidence for reassortment of hantaviral strains identified in *A. montensis* captured in the Mbaracayu Biosphere Reserve and previously in Itapua.

### 930

#### SEROLOGICAL EVIDENCE FOR URBAN TRANSMISSION OF VENEZUELAN EQUINE ENCEPHALITIS (VEE) VIRUS IN THE IQUITOS, CITY, PERU

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In a clinic-based (9 urban, 2 rural) passive febrile surveillance study carried out in the Amazonian city of Iquitos, Peru (population ~350,000), we observed 10-14 cases of VEE infection per year from 2000-2004. In 2005, detected cases doubled and by July 2006, 45 cases were identified, most of whom reported no history of travel. This prompted a request from local health officials for an outbreak investigation, which consisted of entomological surveys for potential vectors of VEE and an antibody prevalence study in 3 areas where most of the cases lived and in a control area consisting of a geographically stratified sample of city blocks from the central part of Iquitos. Blood samples were obtained from willing volunteers  $\geq 5$  years of age and tested by ELISA for evidence of anti-VEE specific IgG antibodies; positive samples were confirmed by plaque reduction neutralization. Seroprevalence rates ranged from 27-31% in 2 of the affected areas, whereas the remaining area was similar to that of the control area (18-19%). Overall, age-seroprevalence curves showed an increase in antibody prevalence with age. Age (OR=2.1), travel history (OR=1.3), occupation (OR=3.4), history of febrile illness in past year (OR=1.5) and presence of animals on their property (OR=1.3) were all risk factors significantly associated ( $P < 0.05$ ) with a positive VEE result as demonstrated by multivariate logistic regression. We collected 12,065 mosquitoes, 72% were *Culex quinquefasciatus* and 21% were from the sub-genus *Cx. melanoconium* over 208 trap-nights (CDC with CO<sub>2</sub>). Evidence from this study indicates that some transmission of VEE virus is occurring within the city of Iquitos, but that this transmission requires periodic introductions of the virus from nearby forest transmission cycle.

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#### MOLECULAR EPIDEMIOLOGY OF HUMAN HERPESVIRUS 8 IN HIV-POSITIVE PATIENTS WITH KAPOSI'S SARCOMA ATTENDED IN RIBEIRÃO PRETO, BRAZIL

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Kaposi's sarcoma (KS) is the most common neoplasm of AIDS patients. In 1994, a new herpesvirus, Human Herpesvirus 8 (HHV-8), was identified in HIV-related KS tissue, and has since been associated with KS-AIDS. Based on ORF-K1 variation it is possible to classify this virus in five main genotypes (A, B, C, D and E), and its variants. In this study, we aimed to assess HHV-8 genotypes infecting KS patients attended at the Hospital of Clinics of the School of Medicine of Ribeirão Preto. Either biopsy or PBMC samples of 16 patients with AIDS-associated KS were included. All patients had clinical, histopathological, and virological confirmation of HHV-8 infection. Antibodies to HHV-8 lytic-phase antigens were detected by an

indirect immunofluorescence assay. DNA was extracted using QIAamp DNA Mini Kit (QIAGEN, USA), and K1 gene (variable region 1 - VR1) was amplified by a two-step hemi-nested PCR technique. The first-step of the hemi-nested PCR amplified a 425bp amplicon and the second-step a 253bp fragment. DNA sequencing of both strands of the VR1 region amplicons was carried out. The nucleotide sequences obtained were compared to HHV-8 sequences retrieved from GenBank. Alignment was done with multiple alignment program CLUSTALW 1.8; sequence analyses were performed with MEGA vs.3.1, and unrooted trees were constructed using the distance-based neighbor-joining method and 2000 bootstrap replicates. The phylogenetic analysis showed a wide range of genotypes. Brazilian HHV-8 sequences distributed equally in A, B and C subtypes. All HHV-8 B genotypes were from B1 variant (n=5) but only three A subtype (n=6) could be genotyped and they were distributed into A1 (n=1), A2 (n=1) and A4 (n=1) variants. Subtype C samples were very diverse and could not be classified in variants. This study was the first to perform genotyping in KS-AIDS patients samples from Ribeirão Preto city and these data contribute to the understanding of the evolution of HHV-8 genetic diversity in Brazil. Also, the analysis of HHV-8 variants is important for a better understanding of the KS-AIDS pathogenesis.

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#### PHYLOGEOGRAPHIC DIVERSITY OF COLORADO SIN NOMBRE VIRUS STRAINS

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Studies of Sin Nombre virus (SNV) S segment samples obtained from Colorado were performed to examine the phylogenetic relationship between viruses currently circulating in the state. Five rodent trapping sessions were conducted to obtain SNV sequences from ecologically and geographically diverse locations, including: northern Colorado (Fort Collins, Ault) and central Colorado (Nathrop) east of the Continental Divide, and western Colorado (Molina), southwestern Colorado (Fort Lewis) west of the Continental Divide, as well as several samples from central and western New Mexico (Placitas, Navajo). Two major subdivisions exist within these S segment samples that do not cluster by simple geographic proximity. Samples from mice captured during the same trapping sessions and within meters of each other demonstrate phylogenetic differences exist within these sites. Several samples from northern and central Colorado group with the New Mexico samples while others from central and southwestern Colorado group with the northern Colorado samples. Therefore, representatives of these two phylogenetic subdivisions can be found at these locations, suggesting different SNV strains may co-circulate in the same regions at the same time. M segment analysis greatly supports that of the S segment analysis with the sequences once again forming two major subdivisions. We found a notable exception, however, in a sample from Fort Collins. The M segment of this virus groups with the New Mexico samples, while S segment analysis clearly places this sample with the other northern Colorado samples. This sample may in fact represent a reassortant virus between the two subdivisions of Colorado SNV clades. It is currently unknown if these different subpopulations of Colorado SNV have different transmission potentials to rodent or human hosts or what role such a phenomenon plays in the natural history of SNV in Colorado. Future plans for this project include collection and analyses of additional samples from seropositive deer mice from north-central (Steamboat Springs), northeastern (Wray) and southeastern (Pinyon Canyon Maneuver Site) Colorado, as well as phylogenetic analyses of their rodent hosts.



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### PREVENTING PERSON TO PERSON TRANSMISSION OF NIPAH VIRUS: CULTURAL CONTEXT

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Seven Nipah encephalitis outbreaks have been identified in Bangladesh since 2001. While the outbreaks have been associated with different exposures, epidemiological evidences suggested person-to-person transmission in five sites, increasing the potential for wider proliferation of this lethal pathogen and raising concerns over prevention and control strategies. In-depth qualitative research was carried out in six outbreak areas. Methods included focus group discussions and open-ended interviews with caregivers of the Nipah patients at household and hospital settings, care providers and other hospital-based staff, Nipah survivors, and neighbors of Nipah patients. Contagiousness was rarely mentioned as a cause of the disease. Female relatives were responsible for giving hands-on care to the Nipah patients, including feeding the patient by hand or spoon, bathing, washing after urination or defecation, and cleaning secretions such as vomit or froth. Family members continued to share utensils and glasses, eat leftovers, and share the same bed as the patients. Close physical contact took place before the deaths, involving hugging or feeding or whispering Koranic verses into the ear, and cleaning the dead body, particularly the orifices, for burial. Investigations into the understanding and acceptability of messages developed by the scientists and conveyed to the communities and caretakers in the hospital setting show very interesting contrasts. Efforts to restrict contact or isolate patients contradicted usual practices. Findings suggest that trained health workers, who are often involved in dissemination of messages, may maintain many of the same cultural beliefs and understandings as the local community. In conclusion, cultural aspects and social framework need to be taken into account to ensure that strategies and messages devised for infection control are relevant, acceptable and appropriate. Communication specialists developing preventive messages for Nipah need to make use of qualitative insights to devise approaches that take strike a balance between health ideology and social customs on one hand and sound public health strategies on the other.

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### ARTEMISININ RESISTANCE IN CAMBODIA?

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Since 2001, more than 56 countries have officially adopted ACTs for the treatment of falciparum malaria. Once it develops and spreads, resistance to artemisinin derivatives may have a major impact on malaria control worldwide. Surveillance reports of higher ACT failure rates along the Thai-Cambodia border compared to non border areas suggest the need to monitor the efficacy of the artemisinin component independently of its partner drug to determine the extent of potential artemisinin resistance. The aim of this trial was to investigate reports of developing artemisinin resistance in Cambodia using an integrated *in vivo* - *in vitro* approach. We conducted a 28 day inpatient randomized, controlled open label clinical trial designed to investigate potential clinical artemisinin resistance in Battambang Province, Cambodia along the Thai border. Sixty patients received supervised artesunate monotherapy (4mg/kg/day) over 7 days, the remaining 30 received quinine-tetracycline (30 and 25 mg/kg/day). Treatment response (PCT, FCT) and safety parameters were closely monitored throughout the study. In vitro drug sensitivity was assessed by

HRP2 assay on admission and in cases with re-emergence of parasitemia. Pharmacokinetic studies were performed to determine whether patients had adequate drug levels. Analysis of genetic markers of drug resistance is in progress. The 28-day cure rate in the artesunate arm was 93.3 % (95% CI: 83.8-98.2) vs. 100% (89.7-100%) in the control group. Mean PCT in patients who failed artesunate monotherapy was almost twice that of cures (101.2 vs. 57.5 hrs; p=0.002). Preliminary *in vitro* results indicate significantly higher IC<sub>50</sub>s for artemisinins as compared to western Thailand and Bangladesh. Patients who failed therapy had IC<sub>50</sub> values up to 5 times higher than the overall mean. Although some failures may be linked to inadequate drug levels the patient with the highest artemisinin IC<sub>50</sub> had a PCT of 133 hrs and failed therapy in spite of adequate drug levels. The proportion of artesunate-treated patients with re-emerging parasitemia appears to be low indicating that presently artemisinin resistance is not a widespread problem. However, our data suggest that there may be individual *Plasmodium falciparum* isolates resistant to artemisinins in western Cambodia. Further studies are needed to determine the rate of increase and geographical extent of this problem.

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### A HIGH THROUGHPUT IN VITRO IC<sub>50</sub> ASSAY FOR PLASMODIUM FALCIPARUM FIELD SAMPLES: ADAPTATION OF A DAPI ASSAY

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The emergence of multidrug-resistant *Plasmodium falciparum* has eroded the efficacy of almost all currently available therapeutic agents. Therefore diagnostic tests that provide regional antimalarial drug resistance data for this limited armamentarium would be crucial to guide local therapy and track the spread of drug resistance. Assays presently available for *in vitro* drug field isolate analysis are not ideal. A new assay, which is based on the DNA content by addition of the fluorescent dye 4-6-diamidino-2-phenylindole (DAPI), as reported previously, has been adapted to field isolates and provides robust data. We set out to study the IC<sub>50</sub> for five antimalarial compounds for fresh isolates obtained in Senegal. The compounds include 1) chloroquine 2) amodiaquine 3) quinine 4) pyrimethamine 5) artemisinin. Serial drug dilutions were prepared in culture medium for *in vitro* tests against blood stages of *P. falciparum*. In brief, 200  $\mu$ l of parasitized erythrocytes (starting parasitemia between 0.1% to 0.5%; hematocrit, 2%) was distributed in 96-well plates preloaded with drug, in triplicate. Parasites were cultured for 72 hours under standard gas conditions. At 72 hours, DAPI staining at a of 1:75,000 final dilution was added. To validate this assay in the presence of white cells found in field samples, we performed the DAPI assay for 3D7 in fresh blood for five antimalarial compounds and found excellent correlation to the published IC<sub>50</sub> for these drugs. A total of 20 field isolates have been analyzed. Two samples were eliminated, as they did not have adequate morphologic development at 72 hours. We determined the IC<sub>50</sub> and goodness of fit using Prism Graph Pad. For the remaining eighteen samples we found a large range of IC<sub>50</sub> for all drugs. The average IC<sub>50</sub> for: chloroquine 290nM (65% resistant), amodiaquine 147nM (47%) resistant, quinine 686nM (47% resistant), pyrimethamine 3698nM (41% resistant) and artemisinin 6.8nM. These data suggest that the DAPI assay can be adapted to field isolates and provide IC<sub>50</sub> data for a panel of antimalarial compounds in a valid and reproducible manner. Our data suggests that there is a large range of drug sensitivities to all classes of compounds and that some parasites have significant levels of multiple drug resistance.

### MODELLING ANTIMALARIAL DRUG RESISTANCE AND THE COST-EFFECTIVENESS OF DIFFERENT COVERAGE RATES WITH ARTEMISININ COMBINATION THERAPIES (ACTS)

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Most malaria-endemic countries are in the process of implementing a switch in antimalarial drug policy to an artemisinin combination therapy (ACT). The cost and impact in real-life settings with low coverage rates and using different drug choices, is largely unknown. This study was undertaken to explore the cost-effectiveness of implementing ACTs, using a comprehensive model of antimalarial drug resistance. A dynamic, population-based, deterministic model of the spread of antimalarial drug resistance and transmission is presented. This dynamic data-driven model keeps track of both the human and parasite population and incorporates the most important factors affecting the spread of drug resistance including human immunity, vectorial characteristics, coverage rates and drug characteristics. Using sub-models to incorporate human behaviour, clinical outcomes and costs, the integrated biological-economic model is used to compare the impact of different coverage rates and drug choices on the spread of drug resistance, malaria transmission and cost-effectiveness. The model is populated with data to simulate a low transmission setting (Annual Entomological Inoculation Rate of 1) where drug resistance has emerged to one of the partner drugs and initially accounts for 1% of all infections. The key predictions are: 1) That very high coverage rates (over 80%) with an ACT are required in order to substantially slow the spread of drug resistance, 2) With an ACT, rising levels of drug resistance to a partner drug may not be noticed epidemiologically until very high levels of resistance are reached 3) Compared to monotherapy, ACTs are potentially cost-effective even at low coverage rates because the epidemic rise in morbidity and mortality is averted due to the relative efficacy of the ACT 4) Combinations where there is pre-existing resistance to the partner drug such as artesunate-sulfadoxine/pyrimethamine may initially be more cost-effective than artemether-lumefantrine, but this does not take into account the potentially disastrous consequences of leaving the artemisinin component exposed as a monotherapy in terms of the increased risk of the emergence of drug resistance. In conclusion, potential low coverage rates should not be a deterrent to switching to an ACT and strong preference should be given to using combinations in which resistance has not yet emerged to the partner drug.

### THE IMPACT OF HIV-1 ON THE MALARIA PARASITE BIOMASS IN SUB-SAHARAN AFRICA AND ITS POSSIBLE CONTRIBUTION TO THE EMERGENCE AND SPREAD OF ANTIMALARIAL DRUG RESISTANCE

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We estimated the additional malaria parasite biomass in sub-Saharan Africa due to co-HIV-1 infection. Parasite biomass was defined as the incidence rate of clinical malaria multiplied by the total number of parasites in the peripheral blood during an episode. The population-level impact of HIV-1 infection on parasite biomass was estimated for each country by age group, malaria transmission intensity and urban/ rural areas. HIV-1 epidemic increases the overall malaria parasite biomass in sub-Saharan Africa by 13.5% (8.9-40.3%), with a median increase of 16.7%.

The largest relative increases (176.2-289.4%) were found in Southern African countries (Botswana, Swaziland, Namibia, Zimbabwe and South Africa). Zambia, Malawi, the Central African Republic and Mozambique, where malaria endemicity is the highest and HIV prevalence moderate, showed the largest absolute increases. HIV-mediated expansion of the malaria parasite biomass in Africa could favor the emergence of 'de novo' mutations and consequent spread of drug resistant parasites. The HIV-1 epidemic may be an important contributing factor for the emergence and spread of antimalarial drug resistance by increasing the overall malaria parasite biomass in sub-Saharan Africa by 13.5% (95% CI: 8.9-40.3%).

### RARE DRUG RESISTANCE ALLELES THRIVE IN MOSQUITO PHASE OF *PLASMODIUM FALCIPARUM*

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Preliminary studies suggest a puzzling link between vector control and reduced levels of drug-resistant *Plasmodium falciparum* malaria. The distribution of *P. falciparum* DHFR mutations was examined using PCR in contemporaneous human and vector populations of Macha. Mutated Asn-108 was found in 89.1% of human infections but in only 58.6% of mosquito sporozoite infections ( $p < 0.001$ ,  $n = 99$ ). However, vector infections also exhibited rare Thr-108 (33.3%) and Val-16 (8%) mutants, which confer cycloguanil (unused) resistance and were undetectable in humans. There was no significant difference in wild type mono-infections among humans and mosquitoes. It was proposed that the rare alleles occur in humans as minority variants, suppressed by SP pressure and biological fitness cost. Mosquitoes inferentially re-inoculate these alleles, which could escalate resistance upon introduction of cycloguanil. Vector control presumably reduces antifolate resistance by clearing Asn-108 during the survival-limiting sporogonic stage, and by slowing the replenishment of rare mutants.

### AMPLIFICATION OF THE PFMDR1 LOCUS IN *PLASMODIUM FALCIPARUM* IS LINKED TO PLEIOTROPIC TRANSCRIPTIONAL REGULATION

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The reemergence of malaria as a global threat has been driven in large part by the spread of multiple drug resistant (MDR) parasites. Identifying the alleles and mechanisms in *Plasmodium falciparum*, the protozoan pathogen responsible for the most deadly form of malaria, that confer resistance to multiple drugs will be essential for developing rational chemotherapies. Single nucleotide polymorphisms (SNPs) and their impact on coding regions have long been the focus of drug resistance studies. Increasing evidence argues that drug resistances are more likely due to complex polymorphisms (e.g. copy number variants) that influence not only coding regions but also regulatory sequences. This highlights the need for novel, unbiased approaches to discover polymorphisms contributing to MDR. The *P. falciparum* multidrug resistant gene, *pfmdr1*, was originally proposed as a chloroquine resistance (CQR) gene; however, *pfcr* was later identified as the major gene conferring CQR, and the role of point mutations or amplification of *pfmdr1* in CQR continues to be debated. Quantitative drug studies utilizing the HB3xDd2 genetic cross (a wild-type and MDR parasite, respectively) repeatedly point to the locus carrying *pfmdr1* as regulating parasite susceptibilities to various antimalarial compounds. In a growing list of organisms, variation in transcriptional regulation plays a major role in adaptation to selection

pressures; however, a role for heritable transcriptional variation is thought to be minimal in *P. falciparum*. By measuring the relative expression of more than 7000 ORFs on microarrays, we mapped gene expression quantitative trait loci (eQTL) in the progeny of the HB3xDd2 cross to test for heritable expression variation and to map the regulators of these expression differences. The progeny inherited different numbers of copies of the DNA segment carrying *pfmdr1* and 13 neighboring genes. Inheritance of the Dd2 parent allele at this locus significantly influences gene expression throughout the genome, effectively rewiring the transcriptional network. Structural polymorphisms can broadly influence phenotypes without altering gene functions traditionally associated with coding SNPs.

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### MUTANT PFCRT DOES NOT CONFER HIGH LEVELS OF CHLOROQUINE RESISTANCE TO ALL STRAINS OF PLASMODIUM FALCIPARUM

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The appearance and spread of chloroquine resistance (CQR) in the human parasite *Plasmodium falciparum* has had a devastating impact on the treatment and control of malaria, particularly in sub-Saharan Africa where CQR is a key factor responsible for documented increases in malarial mortality and morbidity. Using a genetic cross, PfcRT (*P. falciparum* chloroquine resistance transporter) was identified as a key determinant of CQR. Allelic exchange studies demonstrated that PfcRT mutations were sufficient to confer CQR to a CQ-sensitive progeny (GC03) of this cross. Transfection studies also revealed that the PfcRT K76T mutation was essential for CQR. Clinical epidemiology studies confirmed a very strong association between PfcRT and CQ treatment failure and found the K76T mutation to be a highly sensitive marker of CQR. Clinical studies have also revealed that some patients harboring mutant *pfcr*t parasites can also respond adequately to CQ treatment. This has been shown to result in part from age-dependent immunity that helps clear *in vitro* resistant infections. It is also possible that other parasite genes are necessary to augment *pfcr*t-mediated CQR and attain high-level resistance. To test this hypothesis, we have engineered mutant *pfcr*t into two CQ-sensitive lines, 3D7 and D10. Introduction of the South American-type 7G8 mutant *pfcr*t allele conferred a low level CQR phenotype as demonstrated with CQ and its metabolite monodesethyl-CQ. Neither line would accept expression of the Asian/African-type Dd2 *pfcr*t allele. PfcRT mutations downstream of K76T also were able to confer a low degree of CQR, indicating that these mutations can directly decrease parasite susceptibility to CQ and are not present solely to compensate for the K76T mutation. Studies are ongoing to determine whether the allelic exchange strategy has artificially decreased the CQ IC50 values through an adverse effect on expression of the mutant protein. Nevertheless, our data suggest that in certain genetic backgrounds, other genes are required in addition to mutant PfcRT to confer high level CQR.

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### A SECRETED ANOPHELES MIDGUT PEROXIDASE REGULATES PLASMODIUM FALCIPARUM DEVELOPMENT

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Study of *Anopheles gambiae* midgut epithelium responses during *Plasmodium* interaction showed that ookinete invasion modulates the expression of several mosquito genes. Among them the expression of five midgut peroxidases is up regulated and the expression of one peroxidase (Per1) is down regulated. We investigated the details of Per1 gene expression and its role in *Plasmodium* development. Kinetic study of Per1 expression shows that mRNA is induced at 3h and peaks around 12h after

blood feeding. Per1 protein is translated in the epithelium around 12h after blood feeding and secreted into the midgut lumen. To understand the role of Per1 on *Plasmodium* development we silenced this gene by RNA mediated interference (RNAi). Silencing of Per1 reduces the number of *P. berghei* and *P. falciparum* oocysts and also the growth of bacterial population in the midgut lumen. Interestingly we found that silencing of Per1 gene up regulates the expression of several mosquito genes including antimicrobial immune genes which explain an inhibitory effect on the growth of midgut bacterial flora. This suggests that Per1 modulates epithelial immune responses against common bacteria in the midgut lumen. The precise mechanism of this action is not known but it may be the general strategy of the gut system to avoid immune reactions against commensal bacteria under normal physiological conditions. The role of Per1 in the regulation of anti-*Plasmodium* genes is under investigation.

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### LOCALIZATION OF NOVEL $\alpha$ -CARBONIC ANYDRASES FROM THE LARVAE OF ANOPHELES GAMBIAE AND AEDES AEGYPTI

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Mosquito larvae display a unique characteristic that sets them apart from most other organisms. The anterior portion of the midgut generates a luminal pH as high as 10.5, one of the highest known in any biological system. If well understood, this characteristic could be targeted in the development of highly specific larvacides. The mechanisms for midgut alkalization are largely unknown; however strong evidence suggests a role for the enzyme carbonic anhydrase (CA). We report here the localization of a novel cytoplasmic  $\alpha$ -CA, AgCA9, from the larvae of *Anopheles gambiae*. An antibody was generated and protein expression was evaluated in both *An. gambiae* and *Aedes aegypti*. In *An. gambiae*, the AgCA9 protein localized to the ectoperitrophic fluid, the cells of the transitional region between the anterior and posterior midguts, the principal cells of the Malpighian tubules (MT), and a subset of cells on the dorsal side of the anterior extreme of the rectum. In *Ae. aegypti*, the antibody detected the CA in the cytoplasm of the gastric caeca cells, the ectoperitrophic fluid, and within the principal cells of the MT. The ectoperitrophic fluid consists of a proteinaceous matrix that flows both anteriorly and posteriorly within the ectoperitrophic space of the gut lumen. Expression of a CA within this compartment could be a key element in larval pH regulation and suggests a novel mechanism for midgut alkalization. Additionally, expression of AgCA9 within the rectum of *An. gambiae* reveals a novel subset of cells not described to date and suggests a specialized function of this region compared to the rest of the rectum. These cells may play an important role in the active excretion of bicarbonate into the surrounding media.

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### MEMBERS OF THE IMMUNOGLOBULIN SUPERFAMILY HELP CONTROL MALARIA AND BACTERIA IN ANOPHELES GAMBIAE MOSQUITOES

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Immunoglobulin superfamily (IgSF) members are known for recognizing and adhering to other molecules in a manner that is both specific and diverse. These properties make IgSF molecules adept at cell-cell adhesion, cell surface reception and recognition of invading pathogens. In mammals, IgSF proteins, such as antibodies, are well-characterized as essential immune components molecules yet implications of IgSF members in arthropod immunity is only beginning to be understood. We have described sequence and gene expression analyses of the predicted *Anopheles gambiae* transcriptome which identified 85 out of 138 IgSF proteins that have at least one immunoglobulin domain and are significantly regulated in response to bacterial or malarial challenge.



Using data from these analyses, we selected six genes as candidates for further functional analyses to confirm possible immune relevance. We refer to these candidates as Infection Responsive with Immunglobulin Domain (IRID) 1-6 and subject them to reverse genetic studies via targeted RNA interference (RNAi). We show here that IRID3, IRID5 and IRID6 contribute to mosquito viability during bacterial infection, IRID3 and IRID4 limit bacterial growth in the mosquito hemolymph and IRID4 and IRID6 help control *Plasmodium falciparum* infection. Based on these infection phenotypes, we further characterize IRID3, which contains a peroxidase domain and IRID6 which is a predicted kinase. Taken together, these data implicate IgSF gene products as important, novel contributors to anti-microbial and anti-Plasmodium action in mosquitoes.

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### PI-3 KINASE AND PTEN: DUELING INSULIN SIGNALING MOLECULES IN THE MOSQUITO *Aedes aegypti*

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The insulin signaling cascade regulates a wide range of physiological effects in invertebrates including aging, growth and reproduction. Two of these signaling molecules, phosphoinositide (PI) 3-kinase and its antagonist phosphatase and tensin homolog (PTEN), act in concert to tightly regulate insulin signaling. PI 3-kinase stimulates the signaling cascade by phosphorylating PI(4,5)P<sub>2</sub> into PI(3,4,5)P<sub>3</sub>, providing a binding site for downstream signaling molecules. In contrast, PTEN dephosphorylates PI(3,4,5)P<sub>3</sub> to PI(4,5)P<sub>2</sub>, effectively inhibiting the cascade. We have identified and characterized both of these signaling molecules in the mosquito *Ae. aegypti*. The *AaegPTEN* gene encodes six splice variants, three which arise from alternative terminal exons. These three splice variants are uniquely expressed throughout mosquito development and in female tissues. In contrast, a single transcript encodes *Aaegp110*, the catalytic subunit of PI-3 kinase. The *Aaegp110* transcript and protein are widely expressed throughout mosquito tissues, but are particularly abundant in the ovaries and fat body. In ovaries, the *Aaegp110* protein is found primarily in the follicle cells, where it translocates from the cytoplasm to the cell membrane when stimulated with insulin.

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### THE *HERVES* TRANSPOSABLE ELEMENT IN *ANOPHELES GAMBIAE*

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Transposable elements have been proposed as useful tools to spread beneficial genes to disrupt the transmission of vector borne diseases like Malaria. The study of the natural history of active transposable elements might be helpful to predict the consequences of such an approach. *Herves* is an active Class II transposable element isolated originally from *Anopheles gambiae*. It is a member of the *hAT* super-family of transposable elements which includes the gene vectors *hobo* from *Drosophila melanogaster* and *Hermes* from *Musca domestica*. *Herves* exhibits the properties of an ancient element in *An.gambiae* by its presence in all the members of *An.gambiae* species complex and its high nucleotide sequence diversity. However, it has been detected in a low copy number of 3-7 in all the *An.gambiae* s.s samples from Africa and shows a high level of structural integrity by the presence of a high frequency of complete open reading frames encoding the *Herves* transposase. 13 out of 58 complete *Herves* transposase sequences were intact with no premature termination codons indicating that they could encode a full length *Herves* transposase protein. All the 13 variants of *Herves* transposase proteins were expressed and purified in *E.coli*. Preliminary data shows that 8 out of the 13 variants show activity in a biochemical assay. Results from the

*in vitro* experiments to assay the functionality of these proteins with the possible reasons for inactivity of some of the proteins will be presented. Also, the history of *Herves* in *An.gambiae* compared to other transposable elements will be discussed.

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### EPIGENETIC REGULATION OF STACKED TRANSGENE IN YELLOW FEVER MOSQUITO, *Aedes aegypti*

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The fate of transgene expression in mosquitoes is of some interest, particularly in light of the interest in using transgenic technologies to introduce and spread transgenes through populations of disease vectors for the purposes of eliminating pathogen transmission. In gene spreading schemes that involve genetic drive agents such as transposable elements transgene copy numbers are expected to increase over time. The end-point of transgene spreading is still not well defined but many individuals will have multiple copies of the transgene. Specific aim of this work is seeking an answer for a straightforward question - what is the fate of transgene expression as they are 'stacked' or increased in copy number? For this purpose, *Drosophila* eye color *cinnabar* (*Cn*) expressing transgenic *Aedes aegypti* lines (3 independent lines) were used for the present study. Lines having upto 4 copies of *cn* (homozygous for 2 transgene) were successfully created by conventional genetic crosses. Copy numbers were confirmed in each individual for making crosses by direct PCR analysis of transgene using single leg genomic DNA without killing the mosquito. Gene expression for lines having 0, 2 and 4 copies is measured were compared both at RNA level using qRT-PCR, and eye pigment level.

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### ANALYSIS OF THE TRANSCRIPTOME OF *Aedes aegypti* MALE REPRODUCTIVE ACCESSORY GLANDS

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In *Aedes aegypti* the male accessory glands produce a secretion that, when transferred to the female during copulation, profoundly affects subsequent female behavior. In particular, mated females oviposit at a higher rate than unmated females, they are refractory to remating, and their orientation to host cues is modified. These effects are also seen in unmated females that have been injected with an aqueous extract of the male accessory glands (MAGs), suggesting that the behaviors are stimulated by components of the MAG secretion. We have begun to analyze the *A. aegypti* MAG transcriptome. Analysis of 300 cDNA clones yielded 105 distinct sequences, including at least 32 likely to encode secreted peptides or proteins, based on the presence of a signal peptide. Similarly to the transcriptome of *Drosophila melanogaster* MAGs, the inventory contains many peptides, and a similar number of proteins of unknown function. Several serine proteases and at least one metalloproteinase are present; these are matched by a similar number of protease inhibitors, primarily serpins. One protein with a TIL (trypsin-inhibitory like) domain is related to the *Drosophila* protein ACP62F; it may have an immune function. Other potential antimicrobial proteins are also present. Unlike *Drosophila*, the transcriptome includes at least two C-type lectins. The known peptide, *Aedes* head peptide 1, was represented by 15 clones. Expression of this peptide in MAGs was confirmed by immunocytochemistry, and its presence in the secretion from these glands was confirmed by HPLC and radioimmune assay. Depletion of this peptide following mating suggests that physiological amounts are transferred to the female during copulation. Transfer of this peptide reduces female attraction to host odor cues. We hypothesize this benefits the male in at least two ways. *Aedes aegypti* mates on or near the vertebrate host;

by prompting the female to leave the host, the probability of the female surviving to use the sperm is increased, as defensive behavior by the host presents a dangerous environment. Secondly, by leaving the host the female is less likely to encounter and be mated by other males during the period before mating refractoriness develops, thereby ensuring the paternity of the first male. This is the first mosquito male accessory gland peptide that can be associated with the post-mating behavior of the female.

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### TWO WEEKS OF REPEATED PARASITE EXPOSURES DO NOT INCREASE THE SUSCEPTIBILITY OF VACCINATED MICE TO HELMINTH INFECTIONS

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In the *Litomosoides sigmodontis* model of filariasis a series of three vaccinations with irradiated larvae has been demonstrated to confer partial protection against a single challenge dose of infective-stage L3 larvae. Because this vaccine induces a Th2 response and parasite-specific IgE, we hypothesized that repeated parasite exposures might reduce the effectiveness of this vaccine by inducing immunologic tolerance in a manner akin to that observed in desensitization protocols of patients with allergen-specific IgE. To test this, two groups of vaccinated mice were repeatedly injected subcutaneously with irradiated L3s or media every other day for two weeks. Afterwards, half of the animals were euthanized and splenic and lymph node cells were assessed for proliferation, regulatory T-cell frequencies, and intracellular and secretory cytokine production in response to parasite antigen. Remaining mice were challenged with infectious L3s and analyzed for worm burden 56 days later. Two weeks of repeated parasite exposures in mice resulted in decreased axillary lymph node cell proliferation compared to control mice. Although splenic cells from parasite-infected mice did not have reduced proliferation in response to parasite antigen, they did exhibit decreased IL-4 and IFN $\gamma$  release to parasite antigen and anti-CD3/anti-CD28, respectively. While parasite-exposed mice had slightly more splenic CD4<sup>+</sup>IL-10<sup>+</sup> cells than vaccinated controls there were no differences in IL-10 release. Interestingly, an increase in CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> regulatory cells was observed among spleen cells of parasite-exposed mice. Eight weeks after challenge with infectious L3 larvae spleen cells from mice repeatedly exposed to parasites continued to have increased numbers of CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> regulatory cells in response to parasite antigen and released less IL-4 and IFN $\gamma$  than the vaccinated controls. Despite these differences, however, the number of worms recovered from helminth-vaccinated mice did not differ between mice repeatedly exposed to parasites and control mice. Given the immunologic changes observed in mice that were repeatedly exposed to parasites for two weeks, we postulate that a prolonged period of repeated parasite exposures may further enhance immunological tolerance to the point that mice become more susceptible to challenge infection. Currently we are testing this hypothesis in a study with 8 weeks of repeated parasite exposures.

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### GRANZYME A AND B EXPRESSION IS ASSOCIATED WITH IMMUNOSUPPRESSION IN HUMAN AND MURINE FILARIASIS

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Granzyme (gzm) A and B, the most abundant cytotoxic serine proteases, directly induce cell death and are released by CD4<sup>+</sup> or CD8<sup>+</sup> T cells, NK cells, B cells and regulatory T cells to kill effector cells, antigen presenting cells, infected or tumor cells. Thus, granzymes help to fight infections

and suppress autoimmunity. It has not been investigated whether they play a role in helminth infection. Th1/Th2 cytokines, CD4<sup>+</sup> T cells, B cells, granulocytes and NK cells promote defence in the local response to filariae, while regulatory T cells suppress it. This immunosuppression enhances with worm burden in most onchocerciasis patients and in susceptible mice. The underlying immune mechanisms are not completely understood. Therefore, we examined the role of granzyme A and B in the murine infection with *Litomosoides sigmodontis* and in humans infected with *Onchocerca volvulus*. In the former, we show *in vivo* that gzmAxB deficient mice on the resistant C57Bl/6 background (worms are degraded before reaching maturity) had significantly lower worm loads rendering the mice for the first time in this model hyperresistant. This was associated with enhanced pleural IL-5 in the deficient mice, but with increased IgG2b (Th1) and apoptotic pleural cells in the wildtype. In the human infection, we immunohistochemically examined both granzymes in the subcutaneous nodules harbouring adult *O. volvulus*. Here, we found gzmA<sup>+</sup> cells to be abundant in nodules from patients with the immunosuppressed form of onchocerciasis, but scarce in hyperreactive ones. GzmB<sup>+</sup> cells were less frequent in both, but strongly enhanced one year after endobacterial depletion by doxycycline. This was associated with a local increase of Foxp3<sup>+</sup>/CD4<sup>+</sup> T cells, also interacting with gzmB<sup>+</sup> cells. These cell contacts occurred with both gzm<sup>+</sup> in untreated and treated nodules. In summary, we show for the first time that granzymes play a role in the immune response of humans and mice to helminths and are associated with immunosuppression and not defence in an infection.

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### MICROFILARIA POSITIVITY MODULATES THE EXPRESSION OF Fc $\epsilon$ R1- $\alpha$ ON MONOCYTES IN FILARIA-INFECTED PATIENTS

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Activation of monocytes through Fc $\epsilon$ R1- $\alpha$  has been shown to prevent apoptosis, upregulate tryptophan catabolism (which in turn down-regulates T cell responses), and prevent monocyte differentiation into myeloid dendritic cells by an IL-10 dependent mechanism. Cross-linking of this receptor on monocytes has also been shown to focus IgE-dependent antigen uptake and presentation to T cells. To examine the role of Fc R1- $\alpha$  in filarial infections, typically associated with diminished T cell responses and high IgE production, we used flow cytometry to analyze the *ex vivo* expression of Fc R1- $\alpha$  on CD14<sup>+</sup> monocytes in filaria-infected patients who also had allergen-specific IgE to a variety of environmental allergens. In 7 microfilaria (Mf)<sup>+</sup> patients, the geometric mean (GM) percent of positive monocytes was 11% vs 32% in 7 Mf<sup>-</sup> individuals (p = 0.009). Interestingly, this difference between the Mf<sup>+</sup> and Mf<sup>-</sup> patients only occurred in those that were also atopic, demonstrating a possible connection between allergen status and helminth infection. However, unlike what has been demonstrated for basophils, there was no correlation found between total IgE levels and monocyte expression of Fc R1- $\alpha$  (p = 0.582). In contrast to the flow cytometry data, quantitative RT-PCR for Fc R1- $\alpha$  did not demonstrate differences in mRNA expression in monocytes between Mf<sup>+</sup> and Mf<sup>-</sup> patients, suggesting that the regulation was not at the transcriptional level. Further phenotypic analysis of these Fc R1- $\alpha$ <sup>+</sup> monocytes in 4 patients demonstrated that some (between 15-97%) cells co-expressed CD2, a molecule previously shown to identify a monocyte subpopulation with IgE-dependent, high-level expression of Fc R1- $\alpha$ . The expression of Fc R1- $\alpha$  on monocytes, particularly in relation to antigen presentation to T cells and IgE regulation, may help elucidate the interaction between helminth infection and IgE-mediated allergic disorders.

### CYTOKINE RESPONSES TO MALARIAL ANTIGENS AND ACTIVATION OF TOLL-LIKE RECEPTOR (TLR) MEDIATED PATHWAYS IN HUMAN CO-INFECTIONS WITH FILARIAL PARASITES AND MALARIA

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Human co-infection with malaria and filarial parasites is common in regions co-endemic for these parasites. In animal models, filarial infections can influence both innate and adaptive immune responses to concurrent *Plasmodium* infections, but the effect of filarial infections on concurrent *Plasmodium falciparum* (Pf) infections in humans is largely unknown. To study potential immune interactions between filarial and malarial infections, we conducted a study among residents of a village in Mali co-endemic for Pf and two filarial parasites (*Wuchereria bancrofti* [Wb] and *Mansonella perstans* [Mp]). Blood samples were collected at the end of the malaria transmission season from a cohort of individuals with Wb and Mp infections (Fil+; n=23), as determined by a Wb Ag capture ELISA and/or circulating microfilaria (Mf), and a cohort with no evidence of active filarial infections (Fil-; n=24). All individuals had high levels of IgG to blood stage Pf antigens, indicating recent exposure to Pf. No significant differences were found between Fil+ and Fil- cohorts in percent with malaria parasitemia (9% in Fil+ vs. 4% in Fil-; p=NS) or levels of anti-malarial IgG. Whole blood samples were stimulated *in vitro* with adult worm and Mf-derived filarial Ags, recombinant blood stage malaria Ags and ligands for TLRs 1-9, and levels of pro-inflammatory (IL-1 $\beta$ , IL-6, IL-8, IP-10, TNF- $\alpha$  and IFN- $\gamma$ ) and anti-inflammatory (IL-10) cytokines in cell supernatants were determined by a fluorescent multiplex bead array assay. Spontaneous (unstimulated), TLR2, TLR4 and TLR6 ligand-stimulated IL-8, IL-10 and IP-10 levels were lower in Fil+(n=12) relative to Fil- (n=11, p=0.0005; ANOVA test). A trend towards lower IL-1 $\beta$  responses to a malarial antigen (MSP1<sub>42</sub>) was also observed in Fil+ individuals (p=0.059, Mann Whitney test). Notably, cytokine and chemokine responses to filarial adult worm Ags did not differ between the Fil+ and Fil- groups. These data are consistent with reduced activation of TLR2, TLR4 and TLR6 pathways resulting in decreased pro-inflammatory responses in filarial-infected individuals. Analysis of additional samples and examination of TLR expression on immunologically important cell populations is underway to further elucidate the influence of filarial infection on TLR- and malarial Ag-mediated responses in filaria-malaria co-infected populations.

(ACMCIIP Abstract)

### DEVELOPMENT OF A NEMATODE MICROMOTILITY ASSAY TO MEASURE KILLING OF FILARIAL PARASITES BY CULEX PIPPIENS PIPPIENS MIDGUT TISSUE HOMOGENATE

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The ability to describe mechanisms of vector competence enhances our understanding of mosquito-borne disease transmission and therefore provides more information for the development of vector control strategies. Human lymphatic filariasis is caused by the nematode parasites *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* which, depending on the parasite species, geographical location and area habitat, can be transmitted by *Culex*, *Anopheles*, *Aedes* and *Mansonia* mosquitoes. Filarial worm-mosquito host interactions have been most frequently studied in

*Brugia-Aedes* models, but little is known about the natural refractory mechanisms in *Culex* mosquitoes. Although *Culex pipiens pipiens* is the primary vector of *W. bancrofti*, it is refractory to the development of *Brugia* parasites. We have identified the *Culex* midgut as the tissue involved in the refractory mechanism. When *Culex p. pipiens* ingest *B. malayi* or *B. pahangi* microfilariae, they do not penetrate the midgut but rather rapidly die within the midgut lumen: however, if microfilariae are inoculated into the hemocoel (to bypass the midgut) the parasites develop to the infective stage. Micromotility assays have been developed to measure the influence various midgut factors have on the killing of microfilariae. These assays allow us to quantify the effects of fractionated and treated mosquito midgut homogenates.

### THE EMERGENCE OF NIPAH VIRUS IN MALAYSIA: EPIDEMIOLOGY AND HOST ECOLOGY OF PTEROPUS BATS

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Nipah virus (NiV) emerged in Malaysia in 1998 as a respiratory and neurologic disease in pigs and caused a severe febrile encephalitis in humans. Since then, Nipah virus outbreaks have occurred in India and Bangladesh, with mortality rates as high as 92%. Frugivorous bats of the genus *Pteropus* are considered a natural reservoir for Nipah virus and other related henipaviruses. *Pteropus vampyrus* and *P. hypomelanus* are the two pteropid species that occur in Malaysia. We proposed two hypotheses for NiV emergence in Malaysia: 1) Nipah virus is endemic and circulating in pteropid bats throughout Malaysia and these bats normally occurred in the area of the index farm where Nipah virus emerged; and 2) the intensification of pig farms in Malaysia enabled sustained NiV epidemics to occur in pigs, facilitating NiV emergence in humans. This report addresses the first hypothesis. We conducted cross-sectional and longitudinal serological surveys of pteropid bats from spatially disparate colonies across Peninsular Malaysia to determine the distribution and dynamics of Nipah virus in its presumed host species. We also conducted repeated population counts at several roost sites and used satellite telemetry to estimate the abundance and movement patterns of *P. vampyrus* on the mainland. Results suggested wide-spread exposure to Nipah virus in both native *Pteropus* species. Temporal variation in seroprevalence was observed between quarterly sampling periods in a single population of *P. hypomelanus*. *Pteropus vampyrus* was observed roosting within 2 km of the index farm on which NiV emerged and seropositive bats were found within 30km - within the range of nightly foraging activity (>50km). Satellite telemetry showed *P. vampyrus* travels hundreds of kilometers as part of its long-range migratory movements, including between Malaysia, Sumatra and Thailand. These data support the hypothesis that both *Pteropus* species are reservoirs of Nipah virus throughout Malaysia, and to some extent in Sumatra and Thailand. We conclude that infected bats have previously occurred in the area of the pig farm and that pig farm size (pig density), management and the presence of fruit trees near pig enclosures were key factors that precipitated NiV spillover from bats to pigs and drove Nipah virus's emergence.



## RECURRENT NIPAH VIRUS OUTBREAKS IN BANGLADESH, 2001-2007

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Nipah outbreaks have been repeatedly identified in Bangladesh since 2001. We assessed the frequency and characteristics of person to person transmission of Nipah virus in Bangladesh. We reviewed available data on Nipah virus surveillance and outbreak investigations in Bangladesh. We classified persons as Nipah case-patients if they had fever with new onset of seizures, altered mental status or severe shortness of breath, and either had specific antibody against Nipah virus, or were part of a cluster of similar cases in the same region, at least one of whom was Nipah antibody positive. We classified persons as primary cases if they developed illness without contact with any other Nipah case patients, secondary cases if they became Nipah cases at least 5 days after close contact with other Nipah case patients, and as spreaders if at least one person who had close contact with them developed Nipah after at least 5 days. Seven Nipah outbreaks were identified in northwest Bangladesh in 2001, 2003, 2004, 2005, and 2007. All occurred between January and May. A total of 122 cases were recognized. Seventy-two (59%) were male. Their mean age was 27 years (range 2-85). Eighty-eight (72%) died. Fifty-three Nipah cases (44%) developed their illness from apparent person to person transmission. The case fatality rate was higher for primary (79%) compared with secondary Nipah cases (63%;  $p=0.04$ ). Ten cases (8%) were Nipah spreaders, all of whom died, and 5 of whom were secondary cases. Nipah spreaders transmitted Nipah to a mean of 5.3 persons (range 1 to 22). Among the 7 outbreaks, 5 involved person to person transmission ranging from 1 to 5 generations of transmission. In conclusion, human Nipah outbreaks recur in a specific region and season in Bangladesh, presumably from repeated spillover of Nipah virus from its animal host. Nearly half of the identified cases result from person to person transmission. If potential Nipah spreaders could be identified, implementing selective infection control measures could reduce the size of outbreaks.

## UNDERSTANDING NIPAH VIRUS EMERGENCE IN PENINSULAR MALAYSIA: THE ROLE OF EPIDEMIC ENHANCEMENT IN DOMESTIC PIG POPULATIONS

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Nipah virus (NiV) is one of a group of recently discovered emerging zoonotic pathogens that represents a major threat to global health. NiV is a cause of human mortality across southern Asia and is a particular threat due to its broad host range, wide geographical distribution, and high case fatality, as well as the lack of available vaccine or effective therapy. The Henipavirus Ecology Research Group (HERG) is a collaborative research group that studies the ecology and emergence of Hendra virus in Australia and Nipah virus in Malaysia and Bangladesh. A primary focus of HERG research is to investigate the factors that led to a widespread epidemic of NiV encephalitis in Malaysia in 1998-1999. We analyze

livestock production data from the index farm and model within-farm infection dynamics. Results suggest that repeated introduction of the virus from the wildlife reservoir into an intensively managed commercial pig population led to changes in infection dynamics in the pigs. Initial viral introduction produced a partially immune population and led to an "enhanced" epidemic upon reintroduction of the virus. Long-term within-farm persistence permitted regional spread of the virus, ultimately producing widespread human infection. These findings have two important implications for the prevention and control of Nipah virus. First, they imply that prophylactic vaccination of commercial pig populations is unlikely to be a cost-effective option for the prevention of Nipah virus emergence, as failure to uphold expensive, rigid vaccination schedules could produce enhanced epidemics and promote widespread infection. Secondly, epidemic enhancement is most likely to occur on large farms, as these farms have sufficient population numbers and turnover to sustain long-term transmission. Targeted surveillance of these farms in areas where flying fox distributions overlap commercial pig farms is therefore extremely important to detect spillover events early-on and prevent widespread infection.

## OUTBREAK OF HUMAN RABIES IN MADRE DE DIOS AND PUNO, PERU DUE TO CONTACT WITH THE COMMON VAMPIRE BAT, *DESMODUS ROTUNDUS*

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Human rabies infections in Peru are typically associated with either vampire bat (*Desmodus rotundus*) or dog bites. Between 1975 and 2006 a total of 234 human rabies associated deaths were reported to the Peruvian Division of Epidemiology. Madre de Dios (MDD), a Department located in the jungle, reported a total of 42 rabies cases between 1987 and 2002 and a large outbreak (24 cases) occurred among gold miners and their family members in 1989. Between January and May 2007, 23 human deaths were identified from the Departments of MDD, Puno and Cusco prompting an outbreak investigation. Cases with acute febrile syndrome and neurologic manifestations (paralysis, agitation) followed by death were identified in two areas (Puno and MDD). Whole brains were recovered from decedents and sent to the Peruvian National Institutes of Health for detection of rabies antigen by direct immunofluorescent assay (DFA) and to the CDC through NMRCDC for confirmational testing by DFA, RT-PCR and sequencing. Six cases were identified in Ayapata District (Puno) and the other 17 decedents were from MDD; all cases had received a vampire bat bite at least 10 days prior to symptom onset. Six cases were migrant gold miners, which died upon returning to their homes in Cusco. Of the 23 cases, 57% were male, 35% were under 15 years of age. The incubation periods ranged from 8-87 days with a mean of 31 days. Nineteen cases (83%) were confirmed by IFA and 2 (8.7%) by DFA and sequencing. The sequence analysis identified a vampire bat (*Desmodus rotundus*) variant of rabies. Rabies postexposure prophylaxis (vaccine only) was initiated in seven cases; four at 10 days prior to symptom onset and an additional three decided to discontinue their vaccinations; all of these patients died. Common risk factors among cases were living in rural areas without protection from exposure to vampire bats (i.e., nonexistent or permeable household walls and roofs) and no history of vaccination prior to receiving a vampire bat bite. Furthermore, mosquito nets were used infrequently or not at all by cases.

### PHYLOGENETICALLY DISTINCT HANTAVIRUSES IN SOREX CINEREUS AND S. MONTICOLUS IN THE UNITED STATES

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Genetically distinct hantaviruses segregate into clades which parallel the evolution of murinae, arvicolinae, neotominae and sigmodontinae rodents. To what extent other sympatric small mammals are involved in the evolution of hantaviruses is poorly understood. In particular, the role of insectivores (or soricomorphs) in the evolutionary origins of hantaviruses has not been systematically studied. Armed with the newly acquired full genome of Thottapalayam virus (TPMV) and emboldened by the recent identification of phylogenetically distinct hantaviruses in the northern short-tailed shrew (*Blarina brevicauda*), Chinese mole shrew (*Anourosorex squamipes*) and Eurasian common shrew (*Sorex araneus*), we launched a small-scale search for soricid-borne hantaviruses by accessing the archival tissue collection of *Sorex* (family *Soricidae*, subfamily *Soricinae*), housed in the Museum of Southwestern Biology at the University of New Mexico. RNA, extracted from frozen lung and liver tissues of the masked shrew (*S. cinereus*), montane or dusky shrew (*S. monticolus*), dwarf shrew (*S. nanus*), northern water shrew (*S. palustris*), Trowbridge shrew (*S. trowbridgii*), tundra shrew (*S. tundrensis*) and vagrant shrew (*S. vagrans*), captured in the United States between 1994 and 2005, was analyzed for hantavirus sequences by RT-PCR. Of the 42 *Sorex* shrews studied, hantavirus S- and/or L-segment sequences were detected in *S. cinereus*, from St. Louis County, Minnesota, and in *S. monticolus*, from Sandoval County, New Mexico, and Jackson County, Colorado. The newly identified hantaviruses were designated Ash River virus (ASRV) and Jemez Springs virus (JMSV), respectively, based on their capture sites. Pair-wise alignment and comparison of the S and L segments showed low sequence similarities between JMSV and ASRV with TPMV, as well as with rodent-associated hantaviruses. JMSV strains from New Mexico and Colorado differed by 11.2-12.4%, whereas ASRV and JMSV differed by 20.5-20.7% at the nucleotide level. Phylogenetic analyses of these newest members of the *Hantavirus* genus suggest the possibility of host switching of ancestral hantaviruses among soricids or between soricids and rodents in the long co-evolutionary history of hantaviruses and their hosts. This gateway project heralds the discovery of additional soricid-borne hantaviruses, one or more of which may have significant medical importance.

### THE ROLE OF PREDATORS IN REDUCING PARASITES IN PREY POPULATIONS: AN EXAMPLE IN URBAN AREA

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The role of predator-prey interactions in reducing parasite levels in prey populations has been proposed as a primary utility for maintaining biodiversity and indirectly improving human health. Initial theoretical analyses indicated that predators nearly always have this effect. Recent work, however, shows this conclusion is based on a previously unrecognized assumption. This general model result is reviewed. Wild Norway rat (*Rattus norvegicus*) is prevalent in many urban areas,

globally, and is a reservoir of many zoonotic pathogens. We examined the occurrence of selected zoonotic pathogens of wild *R. norvegicus* populations where domestic cats (*Felis catus*) served as predators. Twenty city blocks were surveyed for cats, rats, and viral parasites of rats. In this system, cats preyed on weaned but juvenile rats that were unlikely to be infected by the viruses studied. Perturbation studies suggested that cat populations were not limited by rat population size, nor was the rat population limited by either the cat or parasite populations. Given the estimated population parameters and the forms of population interactions, the models predict that under some conditions predation by cats may support higher levels of parasitism in the rat population.

### OUTBREAK OF FATAL CARDIOPULMONARY FAILURE AMONG CHILDREN CAUSED BY AN EMERGING STRAIN OF ENTEROVIRUS 71 - NAKHORN RATCHASIMA PROVINCE, THAILAND, 2006

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Fatal outcomes following enteroviral infection are rare in Thailand. In late June 2006, four deaths among children with fever and cardiopulmonary failure were reported from Nakhorn Ratchasima Province. We conducted an investigation to identify the etiology and to implement control measures. Medical records of the four fatal cases were reviewed. Active case finding was conducted in the affected district. A case was defined as a child aged <15 years who developed fever (>38°C) and/or Hand Foot and Mouth Disease (HFMD) July 5<sup>th</sup>-August 5<sup>th</sup>, 2006. Laboratory investigation included viral isolation from stool, throat and nasopharyngeal swabs. Paired sera were tested for Enterovirus 71 antibody by microneutralization technique. Nucleotide sequencing was done in confirmed fatal case and electron microscopy was evaluated in autopsy case. The four fatal cases ranged in age from 4 to 39 months; three were male. Illness onset occurred June 22-25. Two cases resided in the same district and had a history of close contact. All cases exhibited abrupt onset of high (39-41°C) fever, tachycardia, acute dyspnea, respiratory failure and coma. Bilateral pulmonary edema without cardiomegaly was noted on all chest roentograms. Brain tissues in autopsy case revealed diffuse brain edema, small numbers of lymphocytes and histiocytes focally presented in the subarachnoid spaces, scattered foci of necrosis presented in thalamus, pons and medulla. Lungs tissue appeared diffuse pulmonary edema and hemorrhage and cardiac tissues was diffuse congestion, no definite pericarditis, myocarditis or endocarditis and no area of infarction in pathological finding. An enterovirus 71 isolate from the stool of 1 case was subsequently identified as serogroup C4, Shenzhen strain. Of 39 children surveyed, 21% (3 HFMD and 5 non-HFMD) exhibited positive antibodies for enterovirus 71. Electron microscope of formalin-fixed tissues in fatal case revealed viral-like particle, average 20 nm in diameter with spherical structure in brain tissue without cardiac and lungs tissues. An emerging strain of enterovirus 71 (C4, Shenzhen) was likely the cause of this outbreak. Control measures included improved hygiene and isolation of sick children at home. Pediatricians were informed about atypical clinical characteristics of enterovirus 71. Surveillance was implemented nationwide for fever and pulmonary edema cases among children younger than 15 years of age.

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### EFFICACY OF PYRVINIUM PAMOATE AGAINST *CRYPTOSPORIDIUM PARVUM* INFECTION *IN VITRO* AND IN A NEONATAL MOUSE MODEL

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The antihelminthic drug, pyrvinium pamoate, was tested for growth-inhibitory activities against the intestinal protozoan parasite, *Cryptosporidium parvum*. A quantitative alkaline phosphatase immunoassay was used to measure growth inhibition in human enterocytic HCT-8 cells. An IC<sub>50</sub> for pyrvinium was observed at ~150 nM using this assay. For comparative purposes, *in vitro* activity against *C. parvum* was also measured for paromomycin for which the IC<sub>50</sub> was observed at ~10 μM. *In vivo* anticryptosporidial activity of pyrvinium pamoate was measured using a neonatal mouse model. Beginning three days after infection, pyrvinium at 5 mg/kg or 12.5 mg/kg was administered to the treatment group mice for four consecutive days. Three days after the final dose the mice were sacrificed and drug efficacy was determined by comparing numbers of oocysts present in fecal smears of treated versus untreated mice. Infection intensity of the developmental stages was also compared using H&E stained histological slides of the ileocaecal intestinal region. We observed ~90% reduction in infection intensity in the pyrvinium treated mice compared to the untreated controls, along with a substantial reduction in tissue pathology. Based on these results, pyrvinium pamoate is a potential drug candidate for treatment of cryptosporidiosis in immune compromised individuals.

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### IMPACT OF BATHERS ON LEVELS OF *CRYPTOSPORIDIUM PARVUM* OOCYSTS AND *GIARDIA LAMBLIA* CYSTS IN RECREATIONAL BEACH WATERS

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Recreational beach water samples collected on weekends and weekdays during 11 consecutive summer weeks were tested for potentially viable *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts using the multiplexed fluorescence *in situ* hybridization (FISH) method. The levels of oocysts and cysts on the weekends were significantly higher than on the weekdays ( $P < 0.01$ ). Concentrations of oocysts in weekend samples ( $n = 27$ ) ranged from 2 to 42 oocysts/L (mean; 13.7 oocysts/L), and cyst concentration ranged from 0 to 33 cysts/L (mean; 9.1 cysts/L). For the samples collected on weekdays ( $n = 33$ ), the highest oocyst concentration was 7 oocysts/L (mean; 1.5 oocysts/L), and the highest cyst concentration was 4 cysts/L (mean; 0.6 cysts/L). The values of water turbidity were significantly higher on weekends compared to weekdays, and were correlated with the number of bathers and concentration of *C. parvum* oocysts and *G. lamblia* cysts ( $P < 0.04$ ). The study demonstrated positive relationships between the number of bathers and levels of the waterborne *C. parvum* oocysts and *G. lamblia* cysts in recreational beach water. It is recommended to test recreational waters for *Cryptosporidium* and *Giardia* when numbers of bathers are greatest, or limit the number of bathers in a recreational beach area.

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### A MOLECULAR *IN VITRO* ASSAY TO ASSESS THE PARASITOCIDAL ACTIVITY OF TOLTRAZURIL AGAINST *NEOSPORA CANINUM*

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*Neospora caninum* is an intracellular parasite affecting mainly cattle and dogs. Attempts to treat neosporosis have been restricted to dogs, and no efficient treatment strategy has been elaborated for cattle so far. In this work we present a molecular assay that allows to distinguish between live and dead parasites. This method is anticipated to be used for *in vitro* drug treatment experiments, i.e. to determine the efficiency of a drug and also for the detection of live parasites *in vivo*. Live parasites can be detected by measuring the mRNA level of specific genes, making use of the specific mRNA available in live cells. Recently, the dense granule NcGra2 gene was identified with a 56% sequence similarity to the related parasite *Toxoplasma gondii* TgGra2 gene. The subcellular location and function of this dense granule protein in *N. caninum* is currently not known; however, it is likely to fulfil a similar role as TgGra2. TgGra2 is constitutively expressed in tachyzoites and bradyzoites. The NcGRA2 gene was used to establish a PCR, based on mRNA isolation and cDNA synthesis. We could show that this PCR is highly sensitive. With the NcGRA2-PCR, different *Neospora* strains can be detected, but it is negative for *Toxoplasma*. The PCR can also be used for real-time PCR to quantify parasites in a sample. To test the system, an already established *in vitro* drug treatment experiment with Nitazoxanide (NTZ) was repeated. *Neospora* infected cells were treated for three or five days with 10 g/ml NTZ and then grown in absence of the drug. It is known, based on DNA detection, that a three-day treatment did not kill the parasite and *Neospora* starts growing again in the absence of NTZ, whereas a five-day-treatment exerts a true parasitocidal activity. These results could now be backed up with the new PCR based on mRNA. The same approach is presently being carried out with toltrazuril. We are using 30 g toltrazuril per ml medium, as this concentration was shown (by others) to cause severe destruction that could be lethal for the parasite. So far, effects of Toltrazuril on *N. caninum* were only studied by light and electron microscopy by others. With the *in vitro* assay, we found out that a 10-day-treatment exerts a parasitostatic activity, whereas a 14-day-treatment was able to kill all of parasites. Thus, no proliferation could be detected anymore in infected and 14-day-treated monolayers when maintained in the absence of the drug for up to 4 subsequent weeks.

(ACMCIP Abstract)

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### THE STUDY OF ASSOCIATIONS BETWEEN *ENTAMOEBIA HISTOLYTICA* INFECTION AND DISEASE WITH SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN IMMUNE RESPONSE GENES

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A cohort of children has been prospectively studied at a field site in Dhaka, Bangladesh, for the last 6 years. There have been 529 children enrolled in the cohort since 1999, of whom 419 remain active participants. The



field site is endemic for *Entamoeba histolytica* with a 40% overall annual incidence of infection. Despite this intense exposure, 10% of the children have not been infected with *E. histolytica*, and only 20% of infections result in diarrhea or colitis. Our goals are to identify host genetic variants that influence the host's immune response to infection with *E. histolytica*. Using SNPlex technology, 2800 single nucleotide polymorphisms (SNPs) in 92 immune response genes were genotyped in 458 Bangladeshi children. In order to incorporate any possible promoter regions, 10kb upstream and downstream of the genes of interest were included. Tagging SNPs with an allele frequency >5% were genotyped. We have screened 10 of the 92 genes to date. There were 77 tag SNPs genotyped for the *leptin receptor* gene (LEPPR) in 186 children in our original Mirpur, Bangladesh cohort. Upon analysis, the *LEPR* was divided into 9 LD haplotype blocks. The greatest SNP allelic associations with *E. histolytica* asymptomatic infection ( $p=0.0004$ ) were found for haplotypes within LD blocks 7 and 8. This strong association persisted after 100,000 permutation tests ( $p = 0.02$ ). In addition, multiple SNP genotypes were associated with individuals having 0 or 1 asymptomatic *E. histolytica* infections compared to individuals with multiple *E. histolytica* asymptomatic infections (e.g. rs4655539 OR = 3.8,  $p$  value = 0.008). In conclusion, the leptin pathway may influence the susceptibility of children to *E. histolytica* infection and the severity of *E. histolytica* disease. We are currently evaluating SNPs in other genes involved in the leptin pathway for associations with *E. histolytica*, such as Stat 3 and Stat 1.

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### THE IMPORTANCE OF IRON IN ANAEROBIC METABOLISM OF *ENTAMOEBAS HISTOLYTICA* AND *E. INVADENS*

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*Entamoeba histolytica* has cytosolic fermentation enzymes possibly acquired through horizontal gene transfer from bacteria to survive within the human intestine. We study *E. histolytica* alcohol dehydrogenase 2 (EhADH2) to understand the origin and evolution of anaerobic enzymes. EhADH2 is a fusion-protein with N-terminal ALDH and C-terminal ADH domains and is essential in glycolytic fermentation of *E. histolytica*. We have identified a member of the ADHE family from *E. invadens*, a reptilian and amphibian pathogen. Expression of EhADH2 and EiADHE restored the ability of a mutant *E. coli* strain to grow anaerobically. EiADHE showed ADH and ALDH activities with higher resistance to oxygen inactivation. Molecular and biochemical studies demonstrate these bifunctional enzymes require iron for both ADH and ALDH activities. Zinc and EDTA dramatically reduced enzymatic activities and anaerobic growth of the recombinant *E. coli* strains transformed with *pEhADH2* and *pEiADHE*. Addition of iron to *E. coli*<sub>*pEhADH2*</sub> and *E. histolytica* trophozoites significantly enhanced bacterial and amebic growth while zinc or EDTA inhibited or halted it. We are currently testing metal effects on *E. invadens* trophozoites. These results are comparable to the enzymatic behavior of Adh11 from *Zimomonas mobilis*, a "unifunctional" alcohol dehydrogenase. Comparisons between ADHE enzymes (human, reptilian, amphibian pathogens) and their response to inhibitors will provide insights to their evolutionary history and host immune responses.

(ACMCIP Abstract)

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### REAL-TIME PCR ASSAY FOR DETECTION OF *ENTAMOEBAS HISTOLYTICA* DNA IN SALIVA AND URINE SPECIMENS OF AMEBIC LIVER ABSCESS (ALA) PATIENTS

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*Entamoeba histolytica* is the cause of amebic colitis and liver abscess, resulting in an estimated 100,000 deaths annually. Diagnosis of amebic liver abscess (ALA) is difficult since it has to be differentiated from pyogenic liver abscess (PLA) as well as from other space occupying lesions of the liver. Serologic tests for anti-amebic antibodies are positive in only two-thirds of patients at presentation, and the parasite is detected in stool in less than half. A recent advance has been the development of techniques to detect *E. histolytica* DNA and antigen in liver abscess pus specimens. Disadvantages of this approach include that collection of liver abscess pus is an invasive procedure and also requires technical expertise. Here we report the detection of *E. histolytica* DNA in saliva and urine specimens of patients with ALA use of a real-time PCR (qPCR) assay. Saliva, urine, and liver abscess pus specimens were collected from 7 ALA patients and tested by qPCR. All the liver abscess pus specimens were positive for *E. histolytica* DNA by qPCR. 5 out of 7 saliva specimens were positive by the real-time PCR assay. Of 6 urine samples 3 were positive by the real-time PCR assay. All 6 patients from whom both saliva and urine were available to analyze had *E. histolytica* DNA identified in one or both specimens. These preliminary data suggest that detection of *E. histolytica* DNA in saliva and urine may prove useful for the non-invasive laboratory diagnosis of ALA. This study is ongoing and more data will be presented at the meeting.

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### DRUG DISCOVERY: TARGETING ATTACHMENT IN *GIARDIA LAMBLIA* PATHOGENESIS

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*Giardia lamblia* is a prevalent intestinal parasite and major cause of diarrheal disease throughout the world. *Giardia* trophozoites avoid clearance by peristalsis, and consequently establish an infection, by attaching to the lining of the small intestine. Current drug treatments for giardiasis have efficiency rates that are less than ideal and examples of drug resistance have been reported, giving rise to the need for novel anti-giardial drug alternatives. While several drugs currently in use, including the benzimidazole derivatives albendazole and mebendazole, target microtubule polymerization, preliminary data from our laboratory indicates that disruption of microfilaments perturbs attachment, and therefore also merits attention in new drug development. *In silico* homology modeling in combination with phalloidin binding studies has predicted structural differences in the phalloidin binding site of human and *Giardia* actin, and therefore provide a proof-of-concept for the validity of actin as a drug target. This has been further supported by our failure to detect *Giardia* actin with fluorescently labeled phalloidin. To screen potential drug candidates against *Giardia* infections, we have developed a medium-throughput microscopy assay to rapidly measure *Giardia* attachment in response to the large number of drugs available to us in the NCI diversity set library (1,990 drugs). Drugs that reduce attachment by greater than 50% when compared to untreated cells are being further evaluated at a broader spectrum of concentrations. Additionally, we are developing a second medium-throughput assay using Biacore technology to rapidly measure and compare the binding affinities for each of the small molecule drugs against both recombinant *Giardia* and human actins. While drug candidates identified in our study will need further evaluation to determine their mode of action and potential toxicity, this study is a significant first step in the discovery and development of novel drug therapies.

(ACMCIP Abstract)

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## RELATIONSHIP BETWEEN TREATMENT OUTCOME AND MOLECULAR MARKERS OF RESISTANCE IN *PLASMODIUM FALCIPARUM*: A SYSTEMATIC REVIEW AND META-ANALYSIS OF PUBLISHED DATA

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*Plasmodium falciparum* has developed resistance to nearly all antimalarial drugs currently used, although the geographical distribution of resistance to any single drug varies greatly. The development of resistance to CQ probably requires successive gene mutations and evolves slowly. Evidence indicates that some of these mutations occur in a transporter-like gene on the surface of the parasite food vacuole. A better understanding of the genes involved in resistance, and the mutations of these genes, will enable predictions to be made on the likelihood of resistance developing in a parasite population when a given drug regimen is administered. The controversy regarding the predictive value of these point mutations for drug efficacy *in vivo* is ongoing. To build a broader knowledge of the value of molecular markers of drug resistance against quinoline and antifolate drugs, large prospectives and well-defined studies are urgently needed. However, the discrepancies between first-line treatment policies, malaria transmission levels, and local medical conditions, are major concerns for the feasibility of multi-center studies. It has yet to be determined how closely molecular changes are correlated with true phenotypic resistance *in vivo*. We report the results of a meta-analysis done to address the question of whether the presence of single nucleotide polymorphisms in *Plasmodium falciparum* genes involved in drug resistance is predictive of therapeutic failure in patients treated with various antimalarial drugs. Our meta-analysis indicates that most of the drug resistance molecular markers investigated, including single nucleotide polymorphisms on *Pfmdr1*, *pfcr*, *pfdhfr*, *Pfdhps* genes are significantly related to minimal risk of therapeutic failure. When applying the complete quality criteria set, few studies fulfill all criteria for this meta-analysis compared to the relative huge amount of papers on this subject. This meta-analysis used data from 1180 patients for MDR, 1648 patients for CRT, 576 patients for MDR+CRT and 919 patients for DHFR+DHPS quintuple mutant.

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## MOLECULAR EVOLUTION OF THE RETURN OF CHLOROQUINE-SUSCEPTIBLE *FALCIPARUM* MALARIA IN MALAWI

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We recently documented the return of chloroquine-susceptible malaria in Malawi, twelve years after chloroquine use was discontinued. Chloroquine was highly effective in treating children with uncomplicated malaria. We conducted molecular analyses to determine the genetic basis of the re-emergence of chloroquine susceptibility. Parasite DNA was extracted from pre-treatment blood spots collected in a clinical trial comparing chloroquine and sulfadoxine-pyrimethamine efficacy in 2005. Polymorphic regions of the *Plasmodium falciparum* chloroquine resistance transporter gene (*pfcr*) that are associated with chloroquine resistance were subjected to direct DNA sequencing, and microsatellites on chromosome 7 that flank *pfcr* from -4 kb to +10 kb were analyzed to examine the ancestral origin of this region of the chromosome. One hundred clinical samples underwent sequencing of the codons of interest. In all samples the *pfcr* haplotype at amino acid positions 72-76 was CVMNK. In 99/100 samples, codons 97, 220, 271, 326, 356 and 371 had the expected wild type haplotype HAQNIR. One sample had the resistant allele at codon

220 but the remainder of alleles were wild-type. The infection with the A220S mutation was cleared by chloroquine treatment. Microsatellite analysis revealed genetic heterogeneity in the microsatellites flanking *pfcr*. In conclusion, whereas the spread of chloroquine-resistant malaria in Africa resulted from a selective sweep of resistant parasites with a single evolutionary origin, the return of chloroquine-susceptible malaria is characterized by expansion of parasites with genetically diverse backgrounds in the chromosomal region surrounding *pfcr*, consistent with the neutral variation generally observed in parasites with wild type *pfcr*. This finding suggests that antimalarial drug susceptibility may return after withdrawal of antimalarial drugs in high transmission settings in Africa because of the persistence of a diverse population of susceptible parasites even in the face strong drug pressure favoring resistant parasites.

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## NO GENETIC BOTTLE-NECK IN *PLASMODIUM FALCIPARUM* WILD TYPE *PF CRT* ALLELES RE-EMERGING IN HAINAN ISLAND, CHINA FOLLOWING HIGH-LEVEL CHLOROQUINE RESISTANCE

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Chloroquine resistant (CQR) *Plasmodium falciparum* became highly prevalent in Hainan Island, China in the late 1970s with 84% of clinical cases and 98% of *in vitro* susceptibility test samples classified as CQR. In 1979, CQ was replaced by piperazine as first-line therapy for falciparum malaria. Since then the prevalence of CQR *P. falciparum* and the *pfcr* 76T mutation has been reported to be declining. However, the rate of CQR decline in Hainan has been much slower than in Malawi; a decrease in prevalence of mutant *pfcr* alleles from 90% to 53% in 20 years for Hainan Island compared to a decrease from 85% to 13% in 8 years in Malawi. While several possible causes for the slow reversal of CQR in Hainan have been proposed, the genetic diversity of re-emerging chloroquine sensitive (CQS) parasites has not been examined. The population structure of the re-emerging sensitive parasites may impact on the sustainability of the parasite population and future control efforts. Furthermore the allelic type of CQR *pfcr* in Hainan has not been elucidated. In this study we performed detailed molecular epidemiological investigations to define the allelic type of re-emerging CQS parasites and CQR parasites on Hainan Island. The results suggest that CQS parasites on the island did not arise from clonal expansion of one or a few CQS parasites, instead, from parasites having diverse origins. Therefore, despite the prevalence of mutant *pfcr* nearly reaching fixation under CQ selection, the pressure was not sufficient to create a genetic bottle-neck in CQS parasites.

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## INDEPENDENT EVOLUTION OF MUTANT *DHFR* AND *DHPS* ALLELES IN AN AREA OF HIGH TRANSMISSION IN WESTERN KENYA

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The origin and spread of sulfadoxine-pyrimethamine (SP) resistant *Plasmodium falciparum* is a major public health concern. Mutations in the genes *dhfr* and *dhps* have been shown to yield parasite resistance to SP. Previous studies have demonstrated multiple origins of resistance in South

America, Melanesia, Southeast Asia, and Kenya, with the predominant mutant *dhfr* lineage arising in Southeast Asia and subsequently spreading to Africa. Our goals in the present research were to assess the extent of lowered variation surrounding both *dhfr* and *dhps* as a result of selection and the relationships between the mutant alleles. We used 236 blood samples collected between 1992 and 1999 from children enrolled in the Asembo Bay Cohort Project in western Kenya. The *dhfr* and *dhps* genotypes for each sample were determined by pyrosequencing. We have characterized microsatellite markers spanning 138 kb around *dhfr* on chromosome 4 and *dhps* on chromosome 8 as well as neutral markers spanning approximately 100 kb on chromosomes 2 and 3. We found that *dhfr* has a surrounding region of about 11kb with reduced variation and *dhps* has a larger region of 35kb with reduced variation; the implications of this difference will be discussed. We find multiple lineages for the mutant *dhfr* and *dhps* alleles, with one predominant lineage for the *dhfr* 511V/108N, 511V/59R/108N, and *dhps* 437G/540E alleles. Using the eBURST algorithm, we show that the mutant *dhfr* alleles (511V/108N, 59R/108N, and 511V/59R/108N) are all distinct lineages from one another. In addition, minor frequency triple mutant alleles are not related to the predominant triple mutant allele lineage that was originally described in Southeast Asia. These results support the use of microsatellite markers as molecular surveillance tools in a parasite population with high amounts of variation.

## 972

### DECLINE IN SULPHADOXINE-PYRIMETHAMINE RESISTANT *DHFR* AND *DHPS* ALLELES AFTER CHANGES IN DRUG POLICY IN THE AMAZON REGION OF PERU

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Recent studies have shown that chloroquine (CQ)-resistant *Plasmodium falciparum* parasites have disappeared in Malawi after CQ was removed from use for more than a decade. Further, a clinical trial in Malawi has confirmed that CQ has become efficacious again for malaria treatment. It is not known if in an area with a high prevalence of sulphadoxine-pyrimethamine (SP)-resistant parasites, sensitive parasites could also increase over time in the absence of drug pressure. In this study, we investigated the fate of SP-resistant genotypes in the Peruvian Amazon basin where SP was replaced in 2001 for primary treatment of uncomplicated *Plasmodium falciparum* malaria. In a previously published study in 1998, the prevalence of pyrimethamine-resistant *dhfr* and sulphadoxine-resistant *dhps* triple mutant alleles was reported to be about 47% in the Iquitos region of Peruvian Amazon Basin. To investigate if there has been any change in the frequency of SP resistant alleles after this drug was replaced in 2001, we genotyped *dhfr* and *dhps* mutations using a pyrosequencing method in 208 *P. falciparum* isolates obtained between 2005 and 2006 in the Iquitos region of Peru. The highly resistant *dhfr* triple mutant allele N511V/S108N/I164L was found in 16.2% of the samples. The triple mutant *dhps* allele A437G/K540E/A581G was completely absent and only 11.4 % of the samples had the double mutant *dhps* allele A437G/A581G. We also found that the microsatellite markers surrounding the triple mutant *dhfr* and the double mutant *dhps* alleles have a similar profile, which suggests that each of these two alleles may have derived from a common ancestor in this population. Thus, our findings show evidence for a decline in the SP-resistant parasite genotypes after a change of drug policy. If this trend continues, it may be possible to use SP as one component of combination malaria treatment in the future in the Amazon basin of Peru.

## 973

### ASSOCIATION OF MUTATIONS IN *PLASMODIUM VIVAX* *DHFR* AND *MDR1* AND *IN VIVO* RESISTANCE TO AMODIAQUINE PLUS SULPHADOXINE-PYRIMETHAMINE IN PAPUA NEW GUINEA

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Molecular mechanisms and markers for sulphadoxine-pyrimethamine (SP) resistance in *P. vivax* have been reported. However, molecular correlates involved in resistance to 4-amodiaquine and data on their relationship with *in vivo* treatment response are still scarce. We assessed *P. vivax* *dhfr* (F57L/I, S58R, T61M, S117T/N, and I173F/L) and *mdr1* (Y976F and F1076L) mutations in pre-treatment samples from 98 patients with a *P. vivax* monoinfection who received amodiaquine (AQ) in combination with SP in Papua New Guinea and investigated the association between infecting genotype and treatment response. Treatment failure rate reached 11% with the new combination regimen. Polymorphisms in *pvdhfr* codons T61M, F57L/I, S58R, S117T/N, and *pvmr1* codon Y976F were detected in 20-60% of the samples. The single mutant *pvdhfr* 57 showed the strongest association with treatment failure. Presence of the quadruple mutant *pvdhfr* 57L+58R+61M+117T with *pvmr1* mutation 976F best predicted treatment failure. The difference in failure rates between sites was reflected in the genetic drug resistance profile of the respective parasite populations. In conclusion, our study identified a novel molecular marker in *pvmr1* to be associated with *in vivo* response to AQ plus SP. Our results suggest *pvdhfr* F57L/I, T61M, and S117T/N plus *pvmr1* Y976F as a suitable marker set for the molecular monitoring of *P. vivax* resistance to amodiaquine + SP in PNG.

## 974

### THE CONTRIBUTION OF MÉDECINS SANS FRONTIÈRES TO THE ASSESSMENT OF EFFICACY OF ANTIMALARIAL TREATMENT, 1996-2004

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At the turn of the millennium, most countries in which Médecins Sans Frontières (MSF) was operating were affected by the emergence of resistance to antimalarial treatment, but evidence of this phenomenon was often lacking. A review was conducted of 43 *in vivo* antimalarial efficacy studies performed by MSF in Africa and Asia from 1996 to 2004. We identified all *Plasmodium falciparum* (*Pf*) treatment efficacy studies conducted during this period. We calculated the proportion of MSF studies among the total *Pf* efficacy studies conducted in a given country during the same period. We used this proportion (combined with two other criteria) to classify MSF studies as having made a definitive, probable, low or negligible contribution to the changes in antimalarial treatment policies in a given country. We also calculated the proportion of publications resulting from these studies among all published articles, both overall and for individual countries. Overall, we included 12,145 patients in 43 efficacy studies or clinical trials conducted in 18 countries. Eight (17%) took place in Asia and 35 (83%) in Africa. Most studies (88%) were conducted between 2001 and 2004 and had a post-treatment follow-up of 28 days or more (n=34, 79%). These studies represented 24% of



the total studies conducted in these countries. MSF's contribution to the drug policy change was considered definite or probable in ten (55%) countries and low or negligible in eight. Publications resulting from these studies accounted for 58% (11/19) of the total articles published in the six countries where the role of MSF in policy change was considered definite. This proportion was lower in the four (26%, 12/45) and in the eight (11%, 8/73) countries where our role was considered probable, low or negligible, respectively. In conclusion, the evidence provided by MSF was important to stimulate and inform policy change, especially in conflict-affected countries, although implementation of this change occurred usually with some delay. Had some national and international institutions stimulated a systematic process of monitoring antimalarial efficacy from the very onset of reports of drug resistance, changes might have occurred much earlier.

## 975

### TRANSCRIPTIONAL REGULATION OF PROTEASE GENE EXPRESSION IN THE MIDGUT OF *Aedes aegypti* MOSQUITOES

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One of the key metabolic events in blood meal digestion by anautogenous mosquitoes is the transcriptional induction of protease genes in the midgut which is essential for digestion of blood meal proteins. We have been investigating molecular mechanisms that control the coordinate expression of numerous serine protease genes in *Aedes aegypti* using both *in vivo* and *in vitro* approaches. For the studies reported here, we have focused on the expression of the late trypsin, 5G1-14, serine collagenase, and CxLT protease genes in the midgut using quantitative real-time PCR. In order to identify physiological processes that contribute to the onset of protease gene expression in response to feeding, we performed abdominal ligations 30 mins. post feeding, as well as, decapitations prior to administration of a protein meal enema. Surprisingly, both of these conditions led to normal levels of protease gene induction (~1000-fold), suggesting that protein in the midgut is sufficient to activate the transcriptional program in the absence of neuronal or hormonal signaling from the head or thorax. Since the fat body and ovaries could play a role in controlling protease gene expression in the midgut through hormonal signaling, dissected midguts from unfed mosquitoes were cultured with increasing amounts of 20-hydroxyecdysone (20E). We found that 20E treatment led to as much as a ~10-fold increase in protease gene expression under these *in vitro* conditions. Similar effects on midgut protease gene expression were obtained by directly injecting unfed female mosquitoes with 20E. Although it is unlikely that ecdysone signaling alone cannot account for the observed transcriptional induction of midgut protease genes, these data together provide new insights into signaling components that control blood meal digestion.

## 976

### FUNCTIONAL CHARACTERIZATION OF LIPID SYNTHESIS, TRANSPORT, AND STORAGE IN *Aedes aegypti* MOSQUITOES

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In order to identify key components of lipid metabolism in blood fed female mosquitoes, we have recently cloned and characterized numerous *Aedes aegypti* genes required for fatty acid synthesis, transport, and triacylglycerol metabolism in the midgut, fat body, and ovaries. These genes include orthologs of mammalian acetyl-coA carboxylase (ACC), two fatty acid synthases (FAS<sup>1</sup>, FAS<sup>2</sup>), fatty acid binding proteins (FABP, FABPm), the fatty acid membrane translocases (CD36A, CD36B), the lipase/transacylase isoforms (iPLA2-epsilon, zeta, and eta), and three enzymes involved in the final fatty acid acylation step in triacylglycerol

synthesis (DGAT1, DGAT2A and DGAT2B). Initial studies have focused on gene expression in the midgut, fat body, and ovaries at 0, 3, 6, 12, 24, 36, 48, 72, 96, and 120 hours after blood feeding using quantitative real-time RT-PCR. Transcript levels of most genes were found to be altered by blood-feeding, indicating that lipid metabolizing enzymes are transcriptionally-regulated by nutrient signaling. Interestingly, we found that expression of all three fatty acyl transferase genes was coordinately-induced, and moreover, that the peak levels of expression were 24 hours post-feeding in the midgut during food bolus digestion, 48 hours in the fat body, and 24 and 72 hours in the ovaries. The gene expression pattern of the DGAT genes suggests that the movement of fatty acids from the midgut epithelial cells to the fat body and ovaries may be facilitated by temporal and spatial gene regulation. Current efforts are aimed at investigating the functional role of selected genes in lipid metabolism using RNAi-mediated knock-down strategies and metabolic labeling studies. Our long term goal is to exploit the knowledge gained by these analyses to develop novel metabolic inhibitors for use as vector control agents.

## 977

### HEMOZOIN-ACTIVATED INNATE IMMUNE RESPONSES IN *Anopheles* MOSQUITOES

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Nitric oxide (NO)-mediated inactivation of malaria parasites is a defense mechanism that is common to mammals and mosquitoes. We have previously shown that *Plasmodium falciparum* glycosylphosphatidylinositols (PfGPIs) can induce *Anopheles stephensi* NO synthase (AsNOS) expression in the midgut epithelium *in vivo* in a manner similar to the induction of cytokines and NO by PfGPIs in mammalian cells. In mosquito cells, signaling by PfGPIs and *P. falciparum* merozoites is mediated through Akt/protein kinase B (PKB), the mitogen-activated protein kinase kinase (MAPKK) DSOR1, and extracellular signal-regulated kinase (ERK). Malaria pigment or hemozoin is a second important parasite-specific signal which can induce production of proinflammatory molecules and NO in mammalian cells through ERK- and nuclear factor kappa B (NF- $\kappa$ B)-dependent pathways. Hemozoin has also recently been identified as a novel ligand for Toll-like receptor (TLR) 9. In this study, we demonstrate that hemozoin can also induce AsNOS gene expression in immortalized *A. stephensi* and *A. gambiae* cell lines *in vitro* and in the *A. stephensi* midgut epithelium *in vivo*. In mosquito cells, hemozoin signaling is mediated through transforming growth factor- $\beta$  (TGF- $\beta$ )-associated kinase 1 (TAK1), Akt/ PKB, ERK and atypical protein kinase C zeta/lambda (aPKC $\zeta/\lambda$ ). Our results indicate that hemozoin is a prominent parasite-derived signal for *Anopheles* and that signaling pathways activated by PfGPIs and hemozoin have both unique and shared components. Taken together with our previous findings, our data indicate that parasite signaling of innate immunity is conserved in mosquito and mammalian cells. Dissection of cell signaling pathways involved in mosquito immune response to parasite-associated molecular patterns such as PfGPIs and hemozoin will ultimately lead to the identification of novel anti-parasite effector genes that can be targeted directly to enhance mosquito resistance to *Plasmodium*.

## 978

### THE REGULATION OF *ANOPHELES GAMBIAE* FEEDING BEHAVIOR BY ITS CIRCADIAN CLOCK

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Circadian clocks regulate rhythmic phenomena in all organisms and control almost all biochemical, behavioral and physiological processes and have wide implications in health and disease. Genetic and molecular analyses in *Drosophila melanogaster* have revealed important insights into the molecules and mechanisms underlying circadian oscillator functions,

and they mainly comprise different transcriptional feedback loops. The most important clock entrainment cues are light, heat and food. The olfactory response to food, odorants, mating signals, and predators, etc., is also controlled by the circadian rhythm. Despite the extensive progress in our understanding of the circadian rhythm, little is known on how it control the hosts seeking and blood feeding behavior of hematophagous insect vectors like *Anopheles gambiae*, which spreads *Plasmodium falciparum*, the causative agent of malaria. We have initiated a systematic dissection of the mosquito *A. gambiae* circadian clock and how it is linked with processes such as host seeking and blood feeding. Towards this, we have studied the influence of light cues in modulating feeding behavior and analyzed this response at the molecular level with high throughput gene expression assays and reverse genetics. This effort is aimed at a deeper understanding of how different cues can modulate the vectorial capacity of malaria transmitting mosquitoes.

## 979

### INVESTIGATIONS OF QUANTITATIVE GENE EXPRESSION ANALYSIS AS A METHOD FOR PREDICTING THE AGES OF MOSQUITOES

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Pioneering studies in malaria epidemiology and vector biology showed that mosquito survival was a major variable defining the spread of mosquito-borne disease. A new disease control proposal involves reducing mosquito survival by infecting mosquitoes with a strain of *Wolbachia* bacteria (maternally-inherited endosymbionts of insects) that causes life-shortening in *Drosophila melanogaster*. Suitable tools are required to measure the age structure of mosquito populations (number of mosquitoes in different age classes) to determine the parameters for a field release of mosquitoes trans-infected with *Wolbachia* bacteria, and to monitor the potential life-shortening effect following release. Until recently, the most accurate method of determining mosquito age involved measuring the relative abundance of cuticular hydrocarbons from mosquito legs using gas chromatography. However, we have recently demonstrated that more accurate predictions of the age of *Aedes aegypti* mosquitoes can be made by measuring the expression of age responsive genes using quantitative RT-PCR. The gene expression (GE) method predicts mosquito age using calibration analysis with multiple gene expression profiles as predictor variables. Age predictions of *Aedes aegypti* are currently within  $\pm 5$  days of actual age. We will present investigations of the influences of environmental variables such as ambient temperature on this assay. Additionally we will discuss whether GE analysis will be broadly applicable as an age grading tool for a range of Australian mosquito vector species. Given the absence of published sequence data for most mosquito species, orthologs of age responsive transcripts were sought from various species using degenerate RT-PCR and confirmed by sequence analysis. Orthologs of age responsive genes identified from five mosquito species ranged in nucleotide similarity from 45 to 71% to age responsive genes originally identified in *D. melanogaster*. Expression of a subset of these has been confirmed as being age-related and there is, therefore, a large potential that GE age-grading will be broadly applicable to mosquitoes and other insects. Prediction of the age of mosquitoes based on gene expression is an exciting new tool in vector biology.

## 980

### A DENSONUCLEOSIS VIRUS FROM ANOPHELES GAMBIAE

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Densonucleosis viruses (DNVs) are attractive agents for mosquito control by transducing expression of anti-pathogen or toxin genes. *Anopheles*

*gambiae*, the major vector of human malaria, has been previously shown to be refractory to DNV dissemination. We have identified and characterized the first DNV (AgDNV) capable of infection and dissemination in *An. gambiae*. The AgDNV genome is 4139 nt in length and has 3 overlapping reading frames (capsid protein and 2 non-structural (NS) proteins). The 5-prime and 3-prime ends of the genome consist of inverted hairpin repeats and are predicted to fold into Y-shaped secondary structure formations. AgDNV is infectious, but non-lethal to *An. gambiae* *in vivo*. We have cloned the entire AgDNV genome into an infectious plasmid. Recombinant viral genomes with EGFP fused to the capsid or NS1 protein can be used to transduce EGFP expression in cultured *Anopheles* cells and mosquitoes. AgDNV will form the basis for simple viral-based systems for malaria control and manipulation of *An. gambiae* in the laboratory.

(ACMCIP Abstract)

## 981

### TOWARDS A VACCINE AGAINST CANINE VISCERAL LEISHMANIASIS BASED ON VECTOR SALIVARY ANTIGENS

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Visceral leishmaniasis is a major parasitic zoonosis which affects worldwide humans and dogs. *Leishmania infantum* is transmitted during the blood meal of sand flies in their mammalian host. There is now an urgent need for an efficient vaccine to protect dogs in enzootic and outbreak-susceptible areas, and to strongly reduce the incidence of human disease by breaking the epidemiological cycle in dogs, main reservoir of the disease. We have focused on the role of *Lutzomyia longipalpis* salivary proteins as potential vaccine candidates in dogs against *L. infantum* infection. In order to explore and characterize the immune responses of dogs against sand fly salivary antigens, we first developed a model reproducing natural exposure of dogs to sand fly saliva. From these experiments, we observed that a strong delayed-type hypersensitivity (DTH) is developed overtime to salivary antigens in dogs pre-exposed to sand fly bites. Because a DTH response could be protective by inducing indirect killing of *Leishmania* parasite at the skin site, as it was demonstrated in rodent models, we needed to identify first the DTH-inducing sand fly salivary molecules in dogs. For this, we developed a new type of antigen screening, "reverse antigen screening", for the selection of valuable candidates for vaccination. From the 35 most abundant transcripts coding for *L. longipalpis* salivary proteins we identified three promising candidates which induce a cellular response in the skin of pre-exposed dogs to sand fly bites. This approach was validated by using recombinant proteins encoding these proteins. This novel reverse antigen screening approach is accelerating the discovery of salivary proteins producing a cellular immune response in the skin of dogs and identified three potential vaccine candidates to be tested for the control of *L. infantum* on these animals.

(ACMCIP Abstract)

## 982

### NEW MECHANISMS IN THE EXPULSION OF GUT NEMATODES: A IMMUNOCYTOCHEMICAL AND MICRO-ARRAY ANALYSIS

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The mechanisms responsible for the expulsion of nematodes from the intestine remain unclear. Using the standard *Nippostrongylus brasiliensis*

model in rat tissues from the Peyer's patch containing segments and non-patch containing segments were compared for cellular content at the time of adult worm expulsion (12 days). The same tissue samples were also analyzed using a micro-array system to determine the RNA profiles; increases in specific candidates were confirmed by PCR analysis. Immunocytochemical analysis of the Peyer's patches showed a specific increase in eosinophils and dendritic cells in a cap formation in the dome of the patch. Increases in TNF were detected by PCR in the patches at this time. Micro-array analysis comparison of the 12 day tissues with controls revealed differences in RNA profiles that suggest that There were 98 genes that showed >2-fold increase in their expression. About 31 genes showed >2-fold decrease in their expression. Significantly in the up-regulated genes were transcripts for a number of mast cell proteases. At least six different mast cell proteases (mast cell protease 1, 2, 3, 4, 9, and 10) showed up-regulation to varying levels. In addition to these mast cell proteases, other proteolytic enzymes such as carboxypeptidase A3, chymase, matrix metalloproteinase 7 and matrix metalloproteinase 10 also showed up-regulation to varying extents in the infected animals. Another mast cell associated transcript that was seen to be up-regulated in the infected animal was the high affinity  $\alpha$  peptide of Fc receptor for IgE. Specific enzymes that were greatly increased at the time of rejection will be described and are believed to be central to expulsion. Discussion of the mechanisms that might contribute to expulsion will be discussed.

## 983

### SELENIUM (SE) DEFICIENCY DIMINISHES DIAPHORASE ACTIVITY ASSOCIATED WITH ALTERNATIVELY ACTIVATED MACROPHAGES AND BLOCKS RESISTANCE TO *HELIGMOSOMOIDES POLYGYRUS*

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A diet deficient in Selenium (Se) blocks resistance to a secondary infection with *Heligmosomoides polygyrus*. IL-4 and IL-13 dependent increases in intestinal smooth muscle hyper-contraction and decreased glucose absorption correlate with resistance expressed as intestinal expulsion of adult worms following a challenge infection. Se deficiency, however, does not affect local intestinal gene expression for IL-4 and IL-13 or associated STAT6-dependent changes in smooth muscle and epithelial cell function. This suggests another protective mechanism compromised by Se deficiency. Parasitic *H. polygyrus* larvae encyst in the submucosa of the duodenum prior to development and emergence of luminal dwelling adult worms. Neutrophils and alternatively activated macrophages (AAM) surround the larval stage, and an intense NADPH/NBT-dependent diaphorase activity indicative of localized production of reactive oxygen species (ROS) envelops the larvae. Inactivation of AAM reduces diaphorase activity and contributes to enhanced emergence of worms into the intestinal lumen. Se deficiency does not affect AAM infiltration around the larvae, but does reduce diaphorase activity and also enhances emergence of worms from the tissue. This suggests a role for Se in expression of localized oxidants linked to alternatively associated macrophage function that is important for host defense.

(ACMCIP Abstract)

## 984

### EXPRESSION AND INTRA-CELLULAR LOCALIZATION OF FKTF-1 IN TRANSGENIC *STRONGYLOIDES STERCORALIS*

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The dauer hypothesis uses the developmental arrest of *Caenorhabditis elegans*' dauer larvae as a model for the development of infective parasitic larvae. One well-characterized signal controlling entry into dauer arrest is the insulin-like signaling cascade which negatively regulates the key transcription factor DAF-16. Unphosphorylated DAF-16 remains in the nucleus and initiates the dauer developmental program. Our lab has identified components of the insulin-like cascade in the human parasitic nematode *Strongyloides stercoralis*. *Fkttf-1* (forkhead transcription factor-1) is the *S. stercoralis* ortholog of *daf-16*. *Fkttf-1b* complements *C. elegans daf-16* mutants in heterologous rescue assays. Using our transgenesis system, we undertook investigation of the expression and potential function of *fkttf-1* in directing infective larval development. Free-living female *S. stercoralis* were microinjected with a GFP reporter construct containing an *fkttf-1B* promoter comprised of 2.8 kb of the 5' flanking sequence, *fkttf-1B::gfp*. Transgenic larvae show GFP expression in hypodermal cells and a region of the pharynx homologous to the *C. elegans* pharyngeal metacarpus. Furthermore, when transgenic larvae develop to the infective stage, GFP fluorescence continues in the hypodermal cells and the pharynx. The cuticle, secreted from hypodermal cells, and the pharynx undergo significant alteration during infective stage larval development supporting a function for *fkttf-1b* in tissue remodeling. Female *S. stercoralis* were then transformed with a construct composed of *gfp* fused 5' to *fkttf-1b* under control of the *fkttf-1B* promoter, *fkttf-1B::gfp::fkttf-1b*. The transgenic first-stage larvae exhibited expression of the GFP::FKTF-1b fusion protein in the same tissues expressing the *fkttf-1B::gfp* construct. Significantly, the GFP::FKTF-1b signal showed nuclear localization indicating translocation into the nucleus. The tissue specificity and sub-cellular localization of *fkttf-1b* in transgenic larvae are consistent with a role for insulin-like signaling in the development of infective larvae.

(ACMCIP Abstract)

## 985

### IDENTIFICATION OF DIFFERENCES IN PROTEIN SECRETION FROM INFECTIVE LARVAE AND FREE-LIVING STAGES OF *STRONGYLOIDES RATTI* WITH PUTATIVE RELEVANCE FOR THE FORM OF LIFE

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The intestinal nematode *Strongyloides stercoralis* affects about 100 million individuals mostly asymptotically. In immunosuppressed hosts, however, either due to corticosteroid treatment or HTLV-1 co-infection, hyperinfection often occurs with a fatal outcome. *Strongyloides* exhibits an unusual developmental plasticity characterized by obligate parasitic and facultative free-living generations. The closely related *S. ratti* has been used as a rodent model for strongyloidiasis. The objective of the present study is to identify genes and gene products with potential relevance for establishing and containing of a parasitic stage. Excretory/secretory (ES) products were generated from infective larvae, parasitic females and free-living stages of *S. ratti* under unstimulated or stimulated conditions. One dimensional gel electrophoresis shows differences in the protein composition of ES products from different developmental stages. These results are also subsidized by preliminary mass spectrometric data. The obtained sequenced peptides were identified by searching against contigs generated from *S. ratti* and *S. stercoralis* expressed sequence tags. Sequence comparisons detected conservation to mostly low molecular weight proteins from related nematode species. Among candidates that warrant more detailed investigation are a metalloproteinase, various anti-oxidative enzymes, allergens such as the polyprotein allergen, abundant larval transcript and galectins. An secretory astacin metalloproteinase has already been identified in *S. stercoralis*, *Ancylostoma* as well as



*Onchocerca volvulus*. These results advance our understanding of the biology of *Strongyloides* living at the gateway between free-living and parasitic form of life. Identified genes may prove to be relevant for the establishment of the parasitic life style and may possibly serve as target for intervention against *Strongyloides* and/or other nematodes with related biology.

## 986

### CD4+CD25+FOXP3+ T-REGULATORY CELLS ARE EXPANDED IN HTLV-1 PATIENTS WITH STRONGYLOIDIASIS

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Clinical presentation of human strongyloidiasis varies among host populations from a chronic, but limited infection in normal hosts to hyperinfection in patients treated with corticosteroid or with HTLV-1 co-infection. How human strongyloidiasis is controlled and how HTLV-1 infection dampens the protective responses are not clear. We hypothesized that T regulatory cells (Treg) are involved in suppressing specific immune responses to *Strongyloides* larval antigen. This study was undertaken to evaluate peripheral blood T regulatory cells and *Strongyloides* larval antigen specific cytokine responses in strongyloidiasis patients with HTLV-1 co-infection. Peripheral blood mononuclear cells (PBMCs) were isolated from 13 newly diagnosed strongyloidiasis patients in Lima, Peru, 5 of them with HTLV-1 co-infection, 8 of them without HTLV-1 co-infection. We characterized T-regulatory cells by flow cytometry with intracellular staining for Foxp3, defining T-regulatory cells as CD4+CD25+Foxp3+ T cells. In addition, PBMCs were cultured in complete media in the presence or absence of crude *Strongyloides* larval antigen. Supernatants were collected and stored at -80°C until cytokine analysis. The median proportion of CD4+ cells that were also positive for CD25 and Foxp3 was increased in HTLV-1 positive subjects with strongyloidiasis compared to strongyloidiasis-only patients (median= 32.8 % vs 4.8 %, p=0.002, Mann-Whitney test). *Strongyloides* antigen-specific IL-5 responses were blunted in strongyloidiasis/HTLV-1 co-infected patients (5.0 vs 187.5 pg/ml, p=0.03, Mann-Whitney test). Also, the median peripheral blood eosinophil count was decreased in the HTLV-1 and *Strongyloides* co-infected subjects (70.0 vs 502.5 cells/mm<sup>3</sup>, p=0.09, Mann-Whitney test). In conclusion, T-regulatory cell population is increased in patients with HTLV-1 and *Strongyloides stercoralis* co-infection and correlates with low circulating eosinophil counts and blunted IL-5 production. These findings suggest a role for T-regulatory cells in the susceptibility to *Strongyloides* hyperinfection observed in these patients. Larger sample sizes are needed to confirm these findings and study the effect of HTLV-1 co-infection on Treg cells and strongyloidiasis.

(ACMCIP Abstract)

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### EXPRESSION, REFOLDING AND NEUTRALISATION OF CONFORMATIONALLY ACTIVE FORMS OF THE HOOKWORM VACCINE ANTIGEN, NA-APR-1

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The hookworms *Necator americanus* and *Ancylostoma duodenale* infect 730 million people in developing countries where they are a leading cause of intestinal blood loss and iron-deficiency anaemia. Adult parasites ingest and lyse erythrocytes and subsequently digest the released haemoglobin in their intestines via a proteolytic cascade that begins with the aspartic protease, APR-1. Vaccination of canines using recombinant APR-1 from the dog hookworm *Ancylostoma caninum* (Ac-APR-1) significantly reduces

both parasite load and blood loss and supports the development of APR-1 as a vaccine against human hookworm. We are currently producing high yield, insoluble forms of Na-APR-1 in *E. coli* and are investigating strategies to refold the enzyme into a conformationally active state, at yields that are sufficient for cost-effective production of a vaccine for developing countries. Polyclonal and monoclonal antibodies have been raised against catalytically active and inactive forms of Na-APR-1 and their ability to neutralise enzyme activity will be discussed.

(ACMCIP Abstract)

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### IMPACT OF MASS DRUG ADMINISTRATION ON THE DEVELOPMENT OF POSSIBLE BENZIMIDAZOLE RESISTANCE OF HUMAN HOOKWORMS IN HAITI

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Hookworms are intestinal parasites infecting about one billion people in much of the tropical and subtropical world. These parasites feed on blood, producing an iron-deficiency anemia that leads to malnutrition, stunting of growth, intellectual and cognitive retardation in children, and adversely affects intrauterine growth resulting in premature births and low birth weight. The prevalence and intensity of hookworm infections have been significantly reduced in treatment campaigns in the context of the WHO-sponsored global program to eliminate lymphatic filariasis using mass drug administration (MDA). However, mass treatments hold the risk of selecting alleles related to drug resistance. We are studying the potential impact of the MDA on the development of resistance against benzimidazole (BZ) drugs in hookworms of the species *Necator americanus* in rural areas in Haiti, where re-emergence of hookworms after five periods of MDA calls for investigation. In several nematode parasites of livestock, resistance to BZ is associated with point mutations (TTT to TAT or TTC to TAC) in positions 167 and 200 of  $\beta$ -tubulin gene, which replaces a phenylalanine (Phe) with a tyrosine (Tyr). However, resistant phenotypes also may be caused by mutations in other positions of the protein. In this study we used a broad range of techniques including real time PCR specific for above mentioned alleles and sequencing of the whole gene to track sequence polymorphisms associated with drug resistance and microsatellites as genetic markers for population genetics of drug resistance development and spread.

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### FORECASTING THE TEMPORAL AND SPATIAL DISTRIBUTION OF A RIFT VALLEY FEVER OUTBREAK IN EAST AFRICA: 2006-2007

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El Niño/Southern Oscillation (ENSO) related climate anomalies have been shown to have a direct impact on Rift Valley fever (RVF) disease outbreaks. Knowledge of the links between ENSO driven climate anomalies and RVF can allow us to provide improved long range forecasts of an epidemic or epizootic. We used satellite generated data to detect that sea surface

temperatures (SSTs) in the equatorial east Pacific ocean anomalously increased significantly during July - October 2006 indicating the typical development of El Niño conditions. The persistence of these conditions and the concurrent elevation of the SSTs in the Indian Ocean was similar to extremes in global-scale climate anomalies that have been observed during similar conditions in the past that produced excess rainfall in East Africa. Subsequent normalized difference vegetation index (NDVI) anomalies for Africa showed positive NDVI patterns with the largest departures concentrated over East Africa especially eastern Kenya, Somalia southern Ethiopia and northern Tanzania following above normal rainfall from September through December. A RVF risk map derived from thresholding NDVI anomaly data indicated for the period October to December 2006 that there was elevated risk of RVF activity in northern Kenya, central Somalia, and subsequently Tanzania. An outbreak of RVF occurred in northeastern Kenya in early December and has continued in east Africa through April 2007. We describe the spatial and temporal accuracy of our RVF risk map forecasts. Forecasting disease is critical for timely and efficient planning of operational control programs. In this paper we describe how we can refine our RVF risk model to give decision makers additional tools to make rational judgments concerning implementation of disease prevention and mitigation strategies.

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### EXAMINATION OF RIFT VALLEY FEVER VIRUS ENTRY DETERMINANTS USING SIRNA

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Rift Valley Fever Virus (RVFV), a member of the *Phlebovirus* genus in the *Bunyaviridae* family, is transmitted by mosquitoes and infects both humans and domestic animals, particularly cattle and sheep. RVFV is typically non-pathogenic in its insect host, but causes severe disease in mammals. The host and viral determinants of this difference are unknown. Furthermore, relatively little is known about the host determinants necessary for RVFV entry, assembly or budding. We have developed an unbiased screening strategy to identify host determinants essential for the viral lifecycle taking advantage of genome-wide RNAi technology in *Drosophila* cells. *Drosophila* cells are an ideal system for high-throughput screening because siRNAs against the entire *Drosophila* genome have been validated, and siRNAs are readily taken into cells without transfection. Using the RVFV strain MP12, we have productively infected *Drosophila* S2 cells and optimized infection conditions for image-based high-throughput screening in a 384 well format. We have validated our screening technique with several siRNA panels, and identified genes that affect RVFV infection in insect cells. Additionally, we have used a library of 1,200 pharmacologically active small molecules to screen for RVFV inhibitors both in *Drosophila* and human cells. This complementary approach will allow us to compare the requirements for infection between the insect and mammalian hosts. Using this strategy, we have discovered similarities and differences in the viral response to drug treatment. Whereas inhibitors of endosome acidification and microtubule polymerization block infection in both *Drosophila* and human cells, actin polymerization appears to be important for entry into *Drosophila* cells, but not mammalian cells. Infection differences between cell types may provide insight into the mechanism of infection in mosquito cells, where infection is nonpathogenic, and mammalian cells, where infection is cytopathic. Using the information from both cell types, we have investigated the roles of several important cellular pathways in the lifecycle of RVFV.

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### CHARACTERIZATION OF A NOVEL BRAZILIAN VACCINIA VIRUS ISOLATED FROM HUMAN AND COMPARATIVE ANALYSIS WITH ORTHOPOXVIRUSES

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In the last ten years the Brazilian rural community has experienced outbreaks of exanthematous disease affecting animals and humans. *Vaccinia virus* (VACV) has been consistently isolated in association with the outbreaks which have had substantial impact on local economies and public health. Retrospective epidemiological data revealed that 100% of the patients develop classical poxvirus infection symptoms after direct contact with symptomatic cows. During a 2005 outbreak, VACV sample was collected from a 31 year old male milker. This virus, named Serro 2 (S2V), is further analyzed here. S2V was isolated in chorioallantoic membrane of chicken embryos (CAMs) where typical VACV pocks were observed. Biological properties of the virus, such as plaque morphology and comet-shape plaque production in BSC40 cells were analyzed and compared with other VACV strains. S2V produced small round plaques and did not induce the production of comet-shape plaques. A molecular analysis of gene deletions associated with the small plaque phenotype (A33R, A34R, A36R, F12L) was carried out to investigate possible factors associated with this morphology in S2V. These genes are all present in the S2V genome; however, alterations in amino acid sequence or expression of these genes may be responsible for the small plaque phenotype. The complete genome of S2V was sequenced by the sequencing-by-synthesis approach implemented by the GS 20 Sequencer (454 Life Sciences, Roche Diagnostics). Phylogenetic analysis showed that S2V clustered together with four Brazilian Vaccinia viruses (BVV) isolated previously. The results suggest that this group of BVV could represent an autochthonous source of VACV. In recent years, concerns over the emergence of OPXV infections have increased and poxviruses have been brought to the center of discussions on bioterrorism and emerging infectious diseases. The origin and natural host of VACV remains unknown but the virus clearly persists today in Brazil. In this study we present the characterization of a novel VACV isolated from a human case. Preliminary results show that this virus could be an autochthonous attenuated VACV, and raise questions regarding virulence, host interactions, drug susceptibility and the origins of the virus.

(ACMCIP Abstract)

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### PATHOGENESIS OF MONKEYPOX IN CYNOMOLGUS MACAQUES: DEVELOPMENT OF A NON-HUMAN PRIMATE MODEL FOR THERAPEUTIC AND VACCINE TESTING

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Monkeypox (MPX) infection causes severe disease in both humans and nonhuman primates. Due to the discontinuation of vaccinia vaccination upon global smallpox eradication in 1980, monkeypox has re-emerged as naturally-occurring human pathogen. However, little is known about the development of MPX, particularly the early stages of infection, viral dissemination, and pathogenesis throughout major organ systems leading to systemic disease. In order to better understand the temporal progression of MPX infection, 18 cynomolgus monkeys

were experimentally infected with MPX and examined sequentially over a 10-day period to investigate the dissemination and pathogenesis of MPX infection. We observed high virus titers in major organ systems and in circulating blood. We determined initial cellular targets of infection and potential mechanisms for viral dissemination. A significant increase in CD14+ monocytes and neutrophils was accompanied by massive production of pro-inflammatory cytokines. Using high-density DNA microarrays we analyzed genome-wide host expression patterns in a variety of different cell types (CD4+ T cells, CD8+ T cells, CD14+ monocytes, CD19+ B cells, and neutrophils) from sequential blood samples. We are currently collecting samples from human monkeypox infections in the Democratic Republic of Congo, in order to compare the non-human primate model with human disease. These results represent the first sequential study of the pathogenesis of monkeypox in a non-human primate model, and the first picture of global gene expression responses during live monkeypox infection. We developed a detailed molecular picture of the viral pathogenesis and spread as well as the putative host defenses during a controlled, synchronous infection. Further analysis and additional experiments will elucidate mechanisms of host defense subversion and potential viral targets for therapeutic and prophylactic intervention.

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#### EVALUATION OF CATIONIC LIPID DNA COMPLEX (CLDC) IN SMALL ANIMAL MODELS AS A PLATFORM FOR BOTH THERAPEUTIC TREATMENT AND VACCINE DEVELOPMENT FOR ALPHAVIRUS INFECTIONS

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CLDC's have been shown to be protective against other arboviruses but the utility of these complexes is unknown against alphaviruses. We have undertaken experiments to examine the possible protective effects of CLDC's against encephalitic arboviral infection. In our initial studies mice were pre-inoculated intraperitoneally with 200 ul of CLDC's or diluent. Twenty four hours later, mice were subcutaneously challenged with a dose of VEEV or WEEV that is lethal in 4-6 days for all mice. Initial results demonstrated that CLDC's provided a protective effect for the pre-treated mice. Mice receiving the CLDC prior to infection showed no (or only mild signs of illness) while those that were mock-treated demonstrated significant and typical signs of illness. Additionally, serum from all of these mice has been tested for the presence of the cytokines interferon (IFN)- $\alpha$ , IFN- $\beta$ , and IFN- $\gamma$  and examined by immunohistopathology analysis of the brains. Mice that received CLDC treatment induced a high IFN- $\alpha$  response at 48h indicating that the CLDC's were indeed priming the immune response. Further, the IFN- $\gamma$  levels were slightly increased at 48h but persisted to 120h. We have demonstrated the utility of CLDC's as a therapeutic treatment for alphaviral infection. Expansion of these studies to include additional (earlier) timepoints, pre-, post-, and co-infection cohorts, use of other encephalitic alphaviruses to determine if this protection is universal or species specific, and to test the ability of these complexes to protect if viral infection occurs via the aerosol route will be discussed.

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#### GENETIC CHARACTERIZATION OF HIGHLY PATHOGENIC H5N1 AVIAN INFLUENZA A VIRUSES ISOLATED FROM SOUTHWESTERN NIGERIA

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From February to April 2006, several outbreaks of highly pathogenic avian influenza (HPAI) in poultry were reported in different parts of Nigeria. However, prior to these reported outbreaks, efforts were made to isolate viruses from naso-pharyngeal/cloacal swab samples collected from sick and dead birds at the poultry clinic of the University of Ibadan Veterinary Teaching Hospital and some farms in Ibadan, Nigeria. During these efforts, one H5N1 influenza A virus isolate was recovered several months before the outbreak in February while two others were isolated from samples from a farm that was affected in Ogun State of Nigeria. A/chicken/Nigeria/228-5/2005 was isolated in August 2005 and A/chicken/Nigeria/228-6/2006 and A/chicken/Nigeria/228-10/2006 were isolated in February 2006. The complete nucleotide sequence of these viruses has been deduced and confirms that these viruses are highly similar. We confirmed in all three isolates the presence of the multiple basic amino acid at the HA cleavage site. This sequence was identical to those of HPAI from Europe and Asia and from recent isolates from Lagos state of Nigeria. No known amantadine or oseltamivir resistance-linked mutations were identified in these isolates. As with other recent H5N1 HPAI isolates, the NS1 open reading frame encodes a five-amino-acid deletion at positions 80 to 84, as had been observed since 2001 in isolates from poultry in HongKong. Isolate A/chicken/Nigeria/228-10/2006 also has a C-terminal amino-acid extension characteristic of a recent Vietnamese NS1 sequence. Finally, it is of interest that the PB2 protein of these viruses possesses a lysine residue at position 627. This amino acid was previously reported to be important for mammalian adaptation of H5N1 viruses. Phylogenetic analysis of the HA and other viral genes shows that these viruses belong to the same lineage as the viruses isolated from the SO farm in Lagos State of Nigeria and to those from Egypt, Iraq and Turkey. Pathogenesis studies confirmed that following intranasal exposure, the current Nigerian H5N1 HPAI virus produced in chickens a systemic infection with virus replication, and associated necrotic and inflammatory lesions in critical internal organs such as the heart, brain and lungs. These features are similar to those of other H5N1 HPAI virus infections in chickens.

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#### GLOBAL TRENDS IN EMERGING INFECTIOUS DISEASES

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Emerging infectious diseases (EIDs) are diseases that have recently increased in incidence, impact or geographic range, are caused by pathogens which have recently evolved or have entered the human population for the first time. These diseases are a significant burden to global economies and public health. Socio-economic, environmental and ecological factors are considered to be the drivers of disease emergence, however no comparative study has explicitly analyzed these linkages to understand the global temporal and spatial patterns of EIDs. Here, we employ a new database of 391 disease emergence 'events' (origins of EIDs) between 1940 and 2004 to demonstrate non-random global patterns. EID events show a significant rise since 1940, with the highest number in the 1980s concomitant with the rise in HIV incidence in Europe and North America. EID events are dominated by zoonotic pathogens, and the incidence of those originating in wildlife (e.g. SARS, Ebola) has increased significantly, with the highest proportion occurring in the last decade. In contrast to previous work, we find that most EIDs originate



in higher latitudes, from relatively few regions in developed countries, and that their origins are significantly correlated with human population density and growth, latitude, rainfall and wildlife biodiversity. Our results provide a basis for developing predictive models for the regions where new EIDs are most likely to originate (emerging disease 'hotspots'). Importantly, they suggest that there is a substantial risk of zoonotic EIDs originating from wildlife at lower latitudes. We conclude that global resources for EID surveillance and investigation are poorly allocated, with the majority of the scientific and surveillance effort currently focused on countries from where the next important emerging pathogen is least likely to originate.

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### COMPARISON OF BLOOD DONOR CHAGAS RISK ACROSS THREE CALIFORNIA SITES

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The US is not endemic for *Trypanosoma cruzi*, but up to 100,000 US immigrants may be unknowingly Chagas infected. Following the recent FDA approval of an ELISA test for detection of *T. cruzi* antibodies, blood banks must assess likely donor risk without clear data. This study was undertaken to compare donor Chagas risk by travel/residence history and characterize at risk donors across 3 distinct CA locations: San Diego (SD) a border area, Stockton/Modesto (SM) an agricultural area, and San Francisco (SF). We screened 2029 donors with a broad risk based question: If they or their birth mother was born or ever lived/traveled in Latin America for > 2 weeks. The 481 that screened positive took a survey about residence/travel history, Chagas knowledge and risk factors. Each donor was assigned a risk based on known risk of places lived/traveled. Pearson's Chi-square 2-sided statistical test was used to compare data across sites. We found significant differences in % screening positive for any risk (24%) (PChi=20.6, Pr<0.001) with more in SD (28%) and less in SM (15%) than expected. Calculated Chagas risk of all donors (0.004) was highest in SF (0.007) and lowest in SM (0.002). We found a significant difference in live (PChi=15.9 Pr=0.014) and travel (PChi=29.0 Pr<0.001) history across sites. In SD more donors and in SM fewer donors lived/traveled in Mexico than expected. More SF donors lived/traveled in Central/South America than expected. There was no sig. difference in % of donors surveyed who consider themselves Hispanic despite sig. differences in risk history and census identified Hispanics across sites. In conclusion, verbal screening may lessen the US testing burden by as much as 68%. The calculated risk of CA blood donors varies across sites but is higher than the recently reported 0.0003 serology risk. We caution against strategies testing only in areas with high % Hispanics because surveyed risks did not follow surveyed Hispanic self-identity.

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### ACUTE CHAGAS DISEASE (ACD) OUTBREAK RELATED TO SUGAR CANE JUICE DRUNK IN SANTA CATARINA STATE, SOUTH BRASIL

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During March 2005, the deaths of three previously healthy people following acute icterohemorrhagic fever of unknown cause was reported. We present results of a field investigation which showed illness was acute Chagas' disease (ACD) in area without previous autochthonous human infection. This outbreak resulted from the consumption of contaminated sugar cane juice. Medical records were reviewed and patients interviewed using standardized questionnaires. Confirmation of ACD was based on: 1) *Trypanosoma cruzi* identified by microscopy of blood smears stained with Giemsa; 2) serum reactive for Chagas-specific IgM or IgG by indirect immunofluorescent [IF] testing and IgG using ELISA; 3) in fatal cases, PCR. An unmatched case-control study was conducted comparing confirmed ACD case-patients with asymptomatic controls. Among 24 persons with confirmed ACD identified, median age was 26 years; three died (mortality rate=12,5%). All case-patients had consumed unpasteurized sugar cane juice produced on February 13<sup>th</sup>, 2005 at a single retail store; no dose-response effect was observed; no other risk factor was identified. Time interval between consumption and illness onset (incubation period) was a median of 12 days (range: 3-17 days). Of 21 persons hospitalized and treated with Benzonidazole, all (100%) survived. Eight (33%) had gastrointestinal hemorrhage, 88% had elevated transaminases, 42% hyperbilirubinemia. Three case-patients had gastric ulcer; one had amastigote forms of *T. cruzi* identified upon biopsy. Fourteen (87%) of 16 tested had abnormal electrocardiogram; of 16 with chest x-ray 92% had cardiomegaly or pleural effusion, and 100% with echocardiography had pericardial effusion or thickening. Thirteen cases (54%) had false-positive ELISA results for leptospirosis and 5 (21%) for hantavirus. Triatomine insects and marsupials captured were infected with *T. cruzi*. In conclusion, autochthonous human illness due to *T. cruzi* infection has not been observed in this area of southern Brazil for many years. This outbreak was unusual because transmission occurred due to food contamination. We observed the unusual icterohemorrhagic presentation in this ACD outbreak. It demonstrates the importance of ACD due to ingestion, and suggests this mode of transmission may be more widespread than currently understood.

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### EPIDEMIOLOGY OF *TRYPANOSOMA CRUZI* FROM THE SOUTHEASTERN UNITED STATES

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*Trypanosoma cruzi*, the causative agent of Chagas disease in humans, has become a parasite of increasing interest in the United States since the diagnosis of two new autochthonous human cases within the last year and an increase in canine cases. While considerable research has been conducted on *T. cruzi* from South America, emphasis on North American isolates is imperative to understand its transmission and pathogenicity within the US. In particular, it is necessary to understand the association between the genetic type of the parasite, virulence, and primary reservoir hosts. While molecular characterization of South American isolates of *T. cruzi* has demonstrated recombination and hybridization, as well as the presence of two major phylogenetic lineages (Type I and II), few studies have investigated such exchange events and genetic diversity in North American isolates. In the current study, we genetically characterized US isolates from wildlife (raccoons, opossums, armadillos), dogs, humans, nonhuman primates, and reduviid vectors. To determine genotype, the mismatch repair (MSH2) and glutathione-s-transferase (TC52) genes were amplified and sequenced from parasite cultures. To determine genotype and investigate genetic exchange, nuclear genes (mismatch repair (MSH2), glutathione-s-transferase (TC52), and DHFR-TS) and mitochondrial gene targets (NADH dehydrogenase-COXII) were amplified and sequenced. Initial sequence typing of MSH2 and TC52 genes from selected isolates showed single nucleotide polymorphisms and support for the existence of the two primary genotypes. Some host species were highly associated with certain genotypes of parasites; Type 1 parasites were associated with opossums and human cases, Type IIa parasites were associated with raccoons, nonhuman primates, and dogs. Both genotypes were

detected in armadillos and vectors. Genetic exchange was observed in several US isolates as demonstrated by incongruent mitochondrial and nuclear phylogenies. Future studies will be conducted to characterize the biological and virulence properties of these genetically classified isolates.

(ACMCIP Abstract)

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### THE ROLE OF SOCIAL EXCLUSION AND DEFORESTATION IN THE SPATIO-TEMPORAL PATTERNS OF CUTANEOUS LEISHMANIASIS IN COSTA RICA

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Risk of American Cutaneous Leishmaniasis (ACL) has been associated with changes in the relationship between humans and forests, but other factors may be important. We analyzed county-level incidence of ACL in 81 counties of Costa Rica during 1995 through 2000 as a function of several variables hypothesized as relevant to transmission ecology, including proximity to forest, climate variability and social status. Analyses used various methods, including spatio-temporal cluster statistics, principal component analysis, multidimensional scaling, generalized additive models, and linear mixed effects models. In addition, negative binomial generalized linear models with break-points were used to capture major qualitative changes in the relationship between disease and hypothesized determinants. Once social marginalization was considered, the effect of living close to the forest was small or even protective depending on the value with respect to the breakpoint (below or above, respectively). Forest cover modulated the temporal effects of ENSO at small spatial scales, revealing a multifaceted interplay of environmental and social factors on disease risk. Our results suggest that understanding the complex etiologies of infectious diseases with strong environmental and social influences will be enhanced by expanding analyses to multivariate, non-linear, and multi-level methods.

## 1000

### CLIMATE VARIABILITY AND LEISHMANIASIS IN COLOMBIA

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Leishmaniasis are transmitted in Americas by *Lutzomyia spp.*, mostly in endemic zones, being involved many animal reservoirs. Previous asian, european and southamerican studies indicated potential changes in vectors climate-related-distribution, but impact outcomes are still need to be furtherly studied. For this reason we report possible climatic impacts and El Niño events during 1985-2002 on leishmaniasis (ATL/AVL) in all the 31 departments of Colombia, South America. Climatic data was satellitally obtained; and epidemiological from Health Ministry. NOAA climatic classification and SOI/ONI indexes were used as global climate variability indicators. Yearly variations comparisons and medians trends deviations for disease incidence and climatic variability were made. Statistical analysis used SPSS (conf. 95%). During this period a considerable climatic variability was present, strong El Niño during 6 years and strong La Niña for 8. In this period, 86,678 leishmaniasis cases were registered in the country (21.4% from Antioquia, 13.8% Norte de Santander and 7.9% Santander), mean 6808.94 cases/year (ranging in the departments from 1.93 to 408.41 cases/year). During El Niño years disease increase in a mean of 2.62% (for the whole country) was observed, in comparison to La Niña years when a mean reduction of 7.22% was observed, but this was spatially heterogeneous in the country with departments evidencing

increases during El Niño up to 15.07% (in Norte de Santander) and decreases during La Niña up to 42.58% (in Guajira). These differences were significant in 15 departments ( $p < 0.05$ ), and for the whole country ( $p = 0.002$ ). In conclusion, climate is changing at an unprecedented registered-rate. Shifts in insect and animals distribution indicate their importance. Climate is a relevant temporospatial vectors and reservoirs distribution determinant. This data and other previous published by our group reflected climate importance on leishmaniasis transmission in different areas of Colombia, and opens further investigations in the area related to forecasting and monitoring systems in public health systems of these emerging and reemerging diseases.

## 1001

### EFFICACY OF MILTEFOSINE FOR BOLIVIAN CUTANEOUS LEISHMANIASIS

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Oral Miltefosine (2.5 mg/kg/day for 28 days) was compared to intramuscular antimony (20 mg/kg/day for 20 days) in the treatment of cutaneous leishmaniasis due to *Leishmania braziliensis* in Palos Blancos, Bolivia. The cure rates with 6 months of follow up were statistically similar: 36 of 41 evaluable miltefosine patients [88%] vs 15 of 16 [94%] evaluable antimony patients [94%]. However, antimony cured more rapidly, since by one month post therapy, 31 of 44 miltefosine patients [70%] compared to 16 of 16 antimony patients [100%] had achieved cure. The two conclusions from this work are: 1) oral miltefosine can be used with confidence for cutaneous disease in this part of Bolivia. 2) The relative efficacy of miltefosine to antimony for *L. braziliensis* in Palos Blancos, Bolivia was far superior to the relative efficacy in Guatemala. This suggests that chemotherapy needs to be evaluated in each endemic region, even if the "same" species of *Leishmania* causes disease in the several locales.

## 1002

### PARASITE TUBULIN AS A DRUG TARGET

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Due to their selective effects on parasite tubulin, dinitroaniline herbicides such as trifluralin and oryzalin have been viewed as potential lead compounds against protozoan pathogens. The antimetabolic and antiparasitic activity of these compounds against kinetoplastids increases dramatically when the N1 position of the dinitroaniline sulfanilamide ring is substituted with a phenyl ring. For example, GB-II-150 (N1-phenyl-3,5-dinitro-N4,N4-di-n-butylsulfanilamide) displays an IC<sub>50</sub> value of 120-180 nM against *T. brucei* subspecies *in vitro* and causes the accumulation of trypanosomes in mitosis, as reported previously. Recently, a consensus dinitroaniline binding site on *Leishmania*, *Toxoplasma*, and *Plasmodium* tubulin was identified through homology modeling and molecular docking simulations, revealing several key interactions between this protein and its dinitroaniline ligand. Analog synthesis has provided support for this binding site model with *Leishmania* tubulin: substitution of the N1-phenyl ring with substituents larger than hydrogen or fluorine decrease activity, steric bulk is permitted in one but not both of the substituents at the N4 atom, and activity is retained when the potentially mutagenic nitro moieties are substituted with cyano groups, as reported previously. Docking studies using AutoDock and Glide showed that GB-II-150 and its nitrile-containing analog displayed similar binding clustering, while less active analogs possessing different substitutions for the nitro group showed much worse clustering. Parallel quantum mechanical studies also demonstrated that substitutions at various parts of the compound could

give significant improvements in the electrostatic interactions with tubulin. This tubulin-dinitroaniline binding site model is currently being used to facilitate structure-based drug design efforts and in silico screening approaches aimed at exploiting this inviting target in protozoan parasites.

## 1003

### THE EFFECT OF COMPLIANCE UPON CLINICAL EFFECTIVENESS OF CHLORPROGUANIL-DAPSONE (CD) AND ARTEMETHER-LUMEFANTRINE (AL) WHEN COMPARED TO SULFADOXINE-PYRIMETHAMINE (SP) FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN MALAWI - A RANDOMISED CONTROLLED TRIAL

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Malawi is in the process of switching its first line therapy for uncomplicated malaria from sulfadoxine-pyrimethamine (SP) to artemether-lumefantrine (AL). Resistance to SP is high, associated with a high prevalence of resistance mutations in the parasite folate synthesis pathway. Chlorproguanil-dapson (CD), another anti-folate drug, is thought to have activity against SP resistant parasites in Africa. The aims of this study were to compare the effectiveness of SP, CD and AL for the treatment of uncomplicated malaria and to investigate the effect of poor compliance upon the clinical response to CD and AL. Children  $\geq$  12 months and adults with uncomplicated malaria were recruited from a health centre in Blantyre Malawi, and randomised to receive SP, CD or AL in the ratio 1:2:1. Routine follow up was on days 7, 14, 28 and 42. Effectiveness was measured using modified WHO criteria. Compliance was measured in three ways; a drug history questionnaire, use of electronic monitoring devices, MEMS<sup>®</sup>, pill bottles which recorded the date and time of each opening and by measurement of dapson or lumefantrine blood concentrations on day 7 and use of pharmacokinetic modelling to predict the number of doses taken. The study took place between May 2004 and May 2006. 7536 patients were screened and 841 recruited. The median age of those recruited was 10 years. The day 28 ACPR rates (not PCR adjusted) in 705 patients with available data were; SP 62.5% (95% CI 54.7 - 69.8), CD 75.6% (70.8 - 79.9), and AL 98.3% (95.2 - 99.7). AL was significantly more effective than both SP and CD,  $p < 0.001$ . CD was more effective than SP,  $p = 0.003$ . In the intention to treat analyses,  $p$  remained  $< 0.017$  for each difference (the corrected significance level for pairwise comparisons). Compared to the SP group, there was no significant change in haemoglobin (HB) level between day 0 and 28 in the CD group. For the AL group, there was a significant rise in HB by day 28 compared to the SP group,  $p < 0.001$ . Compliance data for AL and CD will be presented and discussed. CD in combination with artesunate is currently undergoing phase 3 trials.

## 1004

### RESULTS OF A RANDOMISED, MULTICENTRE, PHASE II, DOSE-RANGING CLINICAL STUDY TO ASSESS THE SAFETY AND EFFICACY OF FIXED DOSE, ORALLY ADMINISTERED PYRONARIDINE AND ARTESUNATE IN ADULT PATIENTS WITH ACUTE UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA

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ACT therapies are effective antimalarials and are considered to afford a lower propensity to resistance. The results from a randomised, blinded phase II clinical trial of the fixed dose combination of pyronaridine: artesunate in uncomplicated malaria are presented. This double-blind, multicentre, randomised, parallel group, dose-finding trial studied orally administered pyronaridine+artesunate (PA) combination tablets, for the treatment of patients with acute, symptomatic, uncomplicated *Plasmodium falciparum* malaria. Patients were enrolled for treatment on Day 0, hospitalised for at least 4 days and followed up to Day 42. Patients from 7 sites South East Asia and Africa were randomised into one of 3 PA treatment groups (6:2; 9:3; 12:4 mg/kg). Safety assessments evaluated vital signs, ECG, laboratory tests, adverse events. The primary efficacy endpoint was the PCR-corrected adequate clinical and parasitological response (ACPR) on Day 28. Secondary efficacy endpoints included: cure rate on Day 14, parasite and fever clearance time. 477 patients were randomised and 476 patients (75% males vs. 25% females) were treated with PA: 160 with 6+2 mg/kg, 157 with 9+3 mg/kg and 159 with 12+4 mg/kg. The mean age was 27.8 years (range 15 and 60 years). The 3 treatment groups were closely matched with respect to demographic characteristics. Treatment with PA for 3 days resulted in cure at Day 28 for 99% of patients treated with 9+3 mg/kg or with 12+4 mg/kg and in 97% of patients treated with 6+2 mg/kg. Early treatment failures did not occur. The treatment failures observed were either late clinical failures (2 patients) or late parasitological failures (5 patients). Up to 99% of patients were clear of parasites by Day 3 and median parasite clearance time was 24 hours in all dose groups. 94% to 99% of patients cleared fever by Day 3. The most common treatment-related AEs were bradycardia, headache, vomiting and transient increased transaminases. There were 6 serious adverse events of which two were possibly treatment related occurring in the same patient. Overall, PA was very well tolerated and there was no dose-response relationship between PA and the AEs reported in this study. This Phase II trial demonstrated that both 9+3 mg/kg and 12+4 mg/kg doses of pyronaridine+artesunate, given as a 3 day course, were well tolerated and very effective in the treatment of *P. falciparum* malaria.

## 1005

### EFFICACY OF ARTESUNATE-AMODIAQUINE (ASAQ) FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN SUB-SAHARAN AFRICA: AN INDIVIDUAL PATIENT DATA META ANALYSIS (IPDM) IN 3,455 PATIENTS

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Artemisinin-based combination therapies (ACTs) are now recommended by the WHO for the treatment for falciparum malaria. The combination of artesunate (AS) with amodiaquine (AQ) is being used increasingly in Africa and has been studied in clinical trials. An individual patient data meta-analysis (IPDM) of comparative randomized and non-comparative trials based on the WHO *in vivo* protocol. Analysis was by intention-to-treat. Efficacy was assessed by survival analysis (Kaplan-Meier) for both crude (unadjusted) and Polymerase Chain Reaction (PCR) adjusted results. 3,455 patients (66% children under 5 years of age) received ASAQ at 23 sites in 11 African countries (Angola, Burkina Faso, Congo, Guinea, DRC, Rwanda, Senegal, Sierra Leone, Sudan, Uganda, and Zanzibar) and followed for 28 days were included in the analysis. Unadjusted efficacy was 72.7% (95%CI 70.2%-75.2%) in 3450 patients with a median time to failure of 21 days (range 7-28.) Using multivariate analysis and controlling by sites, a higher risk of failure was associated with young age ( $P=0.001$ ), gametocytaemia on admission ( $P=0.001$ ), or anaemia ( $P=0.002$ ). Genotyping was available in 2139 patients (62%). PCR-



adjusted efficacy was 93.7% (95%CI 89.4%-98.0%). Children aged of 4 years old and above were at lower risk of failure ( $P < 0.05$ ) than 2 year olds. ASAQ lead to a rapid clearance of fever (Day 1) and parasites (Day 2); the proportion of patients remaining parasitaemic on Day 3 was 1.3%. The incidence density of gametocyte appearance was 19.8% or 43 per 1000 person-week. Overall, the median time to gametocyte clearance was 14 days. Mean haematocrit decreased by 4.7% between Day 0-7 followed by full recovery by Day 28. Using multivariate analysis, younger patients were more likely to be anaemic on admission ( $P = 0.001$ ), and gametocyte carriers ( $P = 0.001$ ) were at higher risk for anaemia. In conclusion, ASAQ efficacy for the treatment of uncomplicated falciparum malaria varies in different regions of Africa. In the majority of cases it meets the WHO criteria of >90% efficacy after genotyping but reinfections are common.

## 1006

### QTC INTERVAL CHANGES FOLLOWING MEFLOQUINE AND ARTESUNATE COMBINATIONS IN MALARIA PATIENTS AND NORMAL VOLUNTEERS

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Heightened awareness of Torsades de Point arrhythmia due to antimalarial drugs necessitates QTc interval evaluation during drug development. We evaluated QTc interval changes (Fridericia correction formula) of artesunate (AS) and mefloquine (MQ) given as a new fixed (F) dose combination or standard loose (L) tablets in 25 *Plasmodium falciparum* infected adults (Days 0, 3, 7) and 12 volunteers [Day (D) 0, 1h, 2h, 24h, D 90 cross over design, washout 90 days]. Total doses were: (i) patients: L: 12 mg/kg AS, 15 mg/kg MQ, F: 600 AS, 1200 mg MQ over 3 days, and (ii) volunteers: 200 mg AS + 400 (F) or 500 (L) mg MQ once. All QTcF values between arms were very similar and are combined. In patients, the mean Day 0 QTcF (389.2 ms) increased on Day 3 (407.3 ms) and fell on Day 7 (399.2 ms). Parallel changes in the: (i) mean temperatures were 38.4, 37.1, 37.1°C, (ii) mean pulse rates were 82, 67, 73 b/min, and (iii) mean MQ concentrations were 0, 3095 (range 1346-5796) and 1721 (range 600-2917) ng/ml. Regression analyses showed no correlations between: (i) the QTcF and [MQ] on D3 ( $r = -0.14$ ,  $p = 0.35$ ) and D7 ( $r = -0.16$ ,  $p = 0.29$ ), (ii) the D7 D0 change in the QTcF and D7 [MQ] ( $r = 0.32$ ,  $p = 0.83$ ), (iii) the D3-D7 QTcF change and D3-D7 change in [MQ], and (iv) changes in mean temperature and mean QTcF. The D0 QTcF and pulse rate ( $r = -0.34$ ,  $p = 0.03$ ), and the change in D3-D0 QTcF and D3-D0 pulse rate ( $r = -0.32$ ,  $p = 0.029$ ) were inversely related. The change in the D3-D0 QTcF and the D3 [MQ] ( $r = 0.32$ ,  $p = 0.034$ ) was positively correlated.

Changes in the volunteers were consistent. There were no significant increases, over baseline (395 ms), in the mean QTcF at all post drug time points: 397.5 (1h), 391 (6h), 398.5 (24h), 395.5 (D90) ms. Mixed effects regression models will provide further information but these data suggest that mefloquine at therapeutic doses had very little effect on the QTcF interval.

## 1007

### PHARMACOKINETICS AND EFFICACY OF PIPERAQUINE AND CHLOROQUINE IN MELANESIAN CHILDREN WITH UNCOMPLICATED MALARIA

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The disposition of chloroquine (CQ) and the related 4-aminoquinoline, piperazine (PQ), were compared in Papua New Guinean children with uncomplicated malaria. Twenty-two children were randomized to three-days of PQ phosphate 20 mg/kg/day (12 mg PQ base/kg/day) co-formulated with dihydroartemisinin (DHA-PQ: Duocotexin TM) and 20 to three days of CQ 10 mg base/kg/day with a single dose of sulfadoxine-pyrimethamine (CQ-SP). Following a 42-day intensive sampling protocol, QC, CQ and its active metabolite monodesethyl-chloroquine (DECQ) were assayed in plasma using high performance liquid chromatography. A two-compartment model with first-order absorption was fitted to the PQ and CQ data. There were no significant differences in age, gender, body weight or admission parasitemia between the two groups. The PCR-corrected 42-day adequate clinical and parasitological response was 100% for DHA-PQ and 94% for CQ-SP but *Plasmodium falciparum* re-infections during follow-up were common (33% and 18%, respectively). For QC, the median [interquartile range] volume of distribution at steady state allowing for bioavailability ( $V_{ss}/F$ ) was 431 [283-588] l/kg, clearance (CL/F) was 0.85 [0.67-1.06] ml/min/kg, distribution half-life ( $t_{1/2\alpha}$ ) 0.12 [0.05-0.66] h and elimination half-life ( $t_{1/2\beta}$ ) 413 [318-516] h. For CQ,  $V_{ss}/F$  was 311 [261-451] l/kg, CL/F 1.53 [1.02-1.87] ml/min/kg,  $t_{1/2\alpha}$  0.43 [0.05-1.82] h and  $t_{1/2\beta}$  233 [206-298] h. The non-compartmentally derived DECQ  $t_{1/2\beta}$  was 290 [236-368] h. Combined molar concentrations of DECQ and CQ were higher than those of PQ during the elimination phase. Although PQ has a longer  $t_{1/2\beta}$  than CQ, its prompt distribution and lack of active metabolite may limit its post-treatment malaria suppressive properties.

## 1008

### DOXYCYCLINE HYCLATE TOLERABILITY AND COMPLIANCE AS DAILY ORAL MALARIA PROPHYLAXIS IN FIELD CONDITIONS: EXPERIENCE OF THE 10<sup>TH</sup> MOUNTAIN DIVISION (LI), OEF VII

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Doxycycline is one of the three available drugs approved by the FDA for the chemoprophylaxis of chloroquine resistant *Plasmodium falciparum*. From clinical trial and field experience in small populations, doxycycline has a well known adverse event profile to include gastrointestinal upset, especially on an empty stomach. Phototoxicity is also of concern to troops in the field. Doxycycline hyclate was used as the drug of choice for malaria chemoprophylaxis by the US military in Afghanistan in 2006. A survey of compliance, tolerability and safety of anti-malarial medications was carried out for all soldiers (approximately 5000) returning from Afghanistan to Fort Drum, NY between January and June of 2007. Final data from this survey will be presented, including rates of compliance, rates and severity of side effects, and the rates of intolerance among alternative anti-malarial medications including mefloquine. This will be one of the largest surveys of its kind ever conducted. Interim data analysis shows correct use of doxycycline in only 55% of respondents, with nearly a third reporting limiting side effects, and 11% needing to switch or discontinue therapy. Doxycycline used as malaria prophylaxis in deployed soldiers may have higher rates of side effects and non-compliance than those previously reported in the literature.

## 1009

**LICENSED CGMP INTRAVENOUS ARTESUNATE AVAILABILITY IN THE DEVELOPED WORLD: LIGHT FINALLY AT THE END OF THE TUNNEL**

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Walter Reed Army Institute of Research (WRAIR) started in 2000 to develop a replacement for quinidine gluconate, the only current FDA approved intravenous therapy for severe malaria, by looking at two similar artemisinin derivatives. In the period of four years, WRAIR was able to complete extensive preclinical work, down-select to Artesunate, overcome some difficult parenteral formulation issues to produce a clinical lot of the drug, and file an Investigational New Drug Application with the U.S. Food and Drug Administration (FDA). Over the last three years WRAIR began and completed some pivotal clinical trials with this product, obtained Orphan Drug and Fast Track Status, and partnered with Sigma-Tau Pharmaceuticals Inc. for manufacture and distribution of the drug after licensure. WRAIR completed two intensive Phase 1 trials with this product at up to almost 4 times the anticipated therapeutic dose thereby formally proving the remarkable safety of this product in healthy volunteers. WRAIR's laboratory in Kenya, the United States Army Medical Research Unit - Kenya, executed a Phase 2 trial last year with outstanding efficacy and continued evidence of the safety of this product. This year, a dose ranging trial in both Thailand and Kenya are adding to the body of evidence to be brought to the FDA for the final package. In an innovative regulatory approach to accelerate application to the FDA for licensure, WRAIR and the Wellcome Trust are collaborating to in an analysis of the entire clinical database for the landmark SEAQUAMAT trial. This analysis will provide a major piece in the FDA submission in support of the efficacy and safety of the drug. Sigma-Tau's contribution on the current Good Manufacturing Practices scale-up final packaging solutions improved the already exceptional clinical lot and will make this product available not only in the United States, but hopefully throughout both the developed and developing world.

## 1010

**PIGGYBAC TRANSPOSON MEDIATED TRANSGENESIS OF THE HUMAN BLOOD FLUKE, *SCHISTOSOMA MANSONI***

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The transposon *piggyBac* from the genome of the cabbage looper moth *Trichoplusia ni* has been observed in the laboratory to jump into the genomes of key model and pathogenic eukaryote organisms including mosquitoes, planarians, human and other mammalian cells, and the malaria parasite *Plasmodium falciparum*. Introduction of exogenous transposons into schistosomes has not been reported but transposon-mediated transgenesis of schistosomes might supersede current methods for functional genomics of this important human pathogen. In the present study, we examined whether the *piggyBac* transposon could

deliver reporter transgenes into the genome of *Schistosoma mansoni* parasites. A *piggyBac* donor plasmid modified to encode firefly luciferase under control of schistosome gene promoters was introduced along with 7-methylguanosine capped RNAs encoding *piggyBac* transposase into cultured schistosomula by square wave electroporation. The activity of the helper transposase mRNA was confirmed by Southern hybridization analysis of genomic DNA from the transformed schistosomes and the hybridization signals indicated the *piggyBac* transposon had integrated into numerous sites within the parasite chromosomes. *piggyBac* integrations were recovered by retrotransposon-anchored PCR, revealing characteristic *piggyBac* TTAA footprints in the vicinity of the endogenous schistosome retrotransposons *Boudicca*, *SR1* and *SR2*. This is the first report of chromosomal integration of a transgene and somatic transgenesis of this important human pathogen, in this instance accomplished by mobilization of the *piggyBac* transposon.

(ACMCIP Abstract)

## 1011

**EVIDENCE OF GENE-SPECIFIC TRANSCRIPTIONAL SILENCING BY RNAI IN *STRONGYLOIDES STERCORALIS***

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Our laboratory seeks a method for disrupting the expression of specific genes as a means of studying their function in the parasitic nematode *Strongyloides stercoralis*. RNA interference (RNAi) forms the basis for functional genomic study based on targeted transcriptional silencing in many taxa, including the free living nematode *Caenorhabditis elegans*, where the effect was first applied for this purpose. Consequently, we and others have sought to develop this methodology for parasitic nematodes. We selected *Ss unc-54* as a target gene for a proof-of-principle experiment in *S. stercoralis*. This gene's ortholog in *C. elegans*, *unc-54*, encodes a body wall muscle myosin heavy chain and is associated with a strong paralysed RNAi phenotype, which we have duplicated consistently in our laboratory. A 569 bp dsRNA, complementary to the coding sequence of *Ss unc-54*, was microinjected at 1 µg/µl into the ovaries free-living *St. stercoralis* females. Control worms were similarly microinjected with 1 µg/µl of a 615 bp stretch of dsRNA complementary to *gfp*, a gene that does not occur in *St. stercoralis*. Larval progeny of dsRNA-injected worms as well as progeny of buffer-injected and non-injected controls, were cultured for 5-7 days at 20° C to the third stage (L3) and examined daily for phenotypes. Cohorts of 20 L3 were sampled at random from each group for assay of *Ss unc-54* message via Real Time RT-PCR. mRNA from the constitutively expressed small subunit ribosomal protein gene, *Ss rps-21*, was the internal control. In three experiments, *unc-54* message levels in worms treated with gene specific dsRNA were significantly (P=0.01) decreased, averaging 39.3% of that in worms treated with *gfp*-specific dsRNA. None of the worms showed a clear motility phenotype. Thus, partial sequence-specific transcriptional silencing does occur in response to dsRNA treatment in *St. stercoralis*. The lack of a phenotype in *Ss unc-54* dsRNA treated worms may derive from the magnitude of mRNA knockdown or possibly redundancy of function between this and other gene products in *St. stercoralis*.

(ACMCIP Abstract)

## 1012

**DEVELOPMENT OF TRANSGENIC *PLASMODIUM BERGHEI* EXPRESSING *P. FALCIPARUM* SEXUAL ANTIGEN Pfs25 FOR *IN VIVO* ASSESSMENT OF TRANSMISSION BLOCKING IMMUNITY**

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Currently, there is no animal model for *Plasmodium falciparum* challenge to test the activity of malaria transmission blocking antibodies. The membrane feeding assay (MFA) is considered to be the "gold standard" for testing transmission blocking potential of anti-sera. MFA is an *in vitro* method that involves mixing antibodies with cultured *P. falciparum* gametocytes and feeding them to mosquitoes through an artificial membrane followed by assessment in the mosquitoes. We describe studies that would obviate the need for performing MFA. We developed a *P. berghei* transgenic parasite expressing a well established *P. falciparum* transmission blocking candidate antigen Pfs25. The asexual as well as sexual growth kinetics of the transgenic parasites were comparable to those of wild-type parasites and transgenic *P. berghei* displayed Pfs25 on their mosquito stage ookinetes. Immune sera from primates immunized with Pfs25 (Coban et al. 2004, Infect Imm. 72, 253-259) passively transferred to mice blocked transmission of transgenic parasites to *An. stephensi*. More importantly, antibodies elicited by immunization by pfs25 DNA vaccine were also highly functionally effective in blocking transmission of transgenic *P. berghei* used for challenge. These studies describe development of an animal model that can be used to test and assay *P. falciparum* transmission blocking activity of sera without performing standard MFA. We argue that using an animal model to test transmission blocking would be superior to MFA since there could be additional immune factors that may synergize transmission blocking activity of antibodies *in vivo*.

(ACMCIP Abstract)

## 1013

**STAGE-SPECIFIC REGULATION OF TRANSCRIPTIONAL ACTIVITY IN *PLASMODIUM FALCIPARUM* DURING THE INTRAERYTHROCYTIC DEVELOPMENTAL CYCLE**

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*Plasmodium falciparum* develops through several distinct morphological stages while replicating in human red blood cells (RBCs). Microarray studies of these stages report dramatic changes in the steady-state mRNA levels of many genes, suggesting that differential gene expression is important for development. However, the interplay between mechanisms that control RNA levels in the parasite remains poorly understood. We assayed bulk transcriptional activity by nuclear run-on in synchronized *P. falciparum* cultures and observed a sharp increase in the total incorporation of radiolabeled UTP by nuclei from populations containing late developmental stages (late trophozoites and schizonts). Following the hypothesis that each of the morphological stages has a characteristic level of transcriptional activity, we used the stage composition and the transcriptional activity of the population at each timepoint to mathematically infer these characteristic levels. According to this model, which consistently fits the data well, early schizonts possess a ~16-fold higher level of transcriptional activity than the other stages (averaged). We observed a similar peak in the transcriptional activity of several individual genes when radiolabeled RNA was hybridized to filters carrying gene-specific probes, despite their variation in steady-state mRNA profile across the life cycle. These findings suggest that transcription in the

*P. falciparum* RBC life cycle is globally regulated, with the bulk of new transcripts being produced during a distinct period in the cycle. We have further characterized this critical period with respect to S-phase and mechanisms of transcriptional activation. Our data suggest that gene- or operon-specific, sense-only transcription -- the mode of regulation in so many other organisms -- may be the exception, not the rule, in the intraerythrocytic developmental cycle of *P. falciparum*.

(ACMCIP Abstract)

## 1014

**STAGE-SPECIFIC DETECTION OF *BRUGIA MALAYI* INFECTIVE LARVAE IN MOSQUITOES**

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Existing molecular assays for filarial parasite DNA in mosquitoes cannot distinguish between mosquitoes that contain microfilariae or early larval stages (alive or dead) and infective mosquitoes that harbor third stage infective larvae (L3) that can establish new infections in humans. We now report development of a molecular L3-detection assay for *Brugia malayi* in vectors based on RT-PCR detection of a L3-activated gene transcript. Candidate genes were identified by bioinformatics analysis of EST datasets across the *B. malayi* life cycle and initially screened by PCR using cDNA libraries to select candidates for further testing. Insectary-reared mosquitoes were fed on blood from microfilaremic cats. Mosquitoes were sampled daily for 15 days after feeding for RNA isolation and RT-PCR analysis with primer sets that are specific for individual candidate genes. A large number of promising candidates with strong expression in L3 were excluded because of low-level transcription in less mature larvae. The expression of one transcript (encoding a particular form of collagen) was initially detected by RT-PCR and qRT-PCR (using a TaqMan probe) when L3 first appear in mosquitoes (9 days after mosquito feeding). This L3-detection molecular assay can be used to test mosquito pools to efficiently estimate infectivity rates in wild caught mosquitoes. We have also identified a transcript that is constitutively expressed by all filarial larvae in mosquitoes that can be used in a multiplex qRT-PCR assay to detect infected and infective mosquitoes in the same sample. These assays are promising new tools for assessing the infection and infectivity status of mosquitoes and for measuring the impact of interventions such as mass drug administration on filariasis transmission potential. This general approach (detection of stage-specific gene transcripts from eukaryotic pathogens) may also be useful for detecting infective stages of other vector-borne parasites.

(ACMCIP Abstract)

## 1015

**SELECTION OF MUTATED *PLASMODIUM FALCIPARUM* MALARIA PARASITES FOR LONG-LIVED INVASIVE MEROZOITES BY LIMITING THEIR CONTACT WITH ERYTHROCYTES (RBC) USING LOW RBC CONCENTRATIONS IN CONTINUOUS MOTION SUSPENSION CULTURES**

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Malaria merozoites are small pear-shaped parasites released from malaria schizont-infected RBC. Merozoites remain extracellular in the blood stream (or culture) very briefly before they either contact and invade another RBC or die and degrade. Merozoite antigens are prime targets for malaria blood-stage vaccines, but little is known about intact antigens on merozoites in their undegraded extracellular viable (invasive) state.



The relatively long-lived merozoites of the monkey malaria *Plasmodium knowlesi* die (become non-invasive) within about 10 minutes at 37 C, as reported previously; but the merozoites of the human malaria *P. falciparum* die before their life-span can be measured. To produce *P. falciparum* merozoites that stay alive long enough to purify and to study their intact antigens, their signal transduction, and other invasion-related processes after mixing with RBC, a mutated parasite line was selected for long-lived merozoites by increasing the average distance (and time-to-contact) between merozoites and RBC. Parasites mutated by  $\gamma$ -irradiation (5 different doses) were put into long-term continuous motion suspension cultures with RBC concentrations low enough to decrease the invasion efficiency and growth rate of the parent line of parasites. Parasite growth rates eventually increased (first in parasites receiving the 150 rad dose). The suspension-selected line was compared to two control lines (the cryopreserved parent line and a static-selected line that had been cultured in parallel without motion). Compared to the control lines, the suspension-selected line showed (a) better invasion efficiency at low RBC concentrations in suspension cultures; (b) other evidence for less rapid degradation of merozoites (a smaller percentage of small sized merozoites out of those that had not invaded); (c) but no differences in invasion efficiencies or growth rates in static cultures, or in suspension cultures at higher RBC concentrations. The life-span of invasive merozoites in the suspension-selected line appeared to be four (4) times that of the control lines.

(ACMCIP Abstract)

## 1016

### ..... APOPTOSIS STALKS AN EXPONENTIALLY GROWING PLASMODIUM FALCIPARUM CULTURE

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*Plasmodium* cell-death is mediated by a number of proteases, including metacaspase and the recently described apoptosis related protein (PfARP). However, the components of apoptotic machinery in the blood stages of *P. falciparum* have not been explored largely because the asexual forms are obligatory intra-erythrocytic unlike the vector stages that can exist freely. In this paper we traced the process of apoptosis in an exponentially growing *P. falciparum* culture. Synchronized cultures were initiated at a parasitemia of 0.8 % and maintained under conditions that were not limiting for red blood cells and nutrients. Parasite growth doubled over and over again every 48 hours and at about 8 % parasitemia, the culture consistently crashed. The following apoptotic processes were evaluated at every stage of the growth curve: Calcium re-distribution using calcium indicator dye Fluo-4/AM, mitochondrial membrane potential collapse by TMRE (Tetramethylrhodamine, ethyl ester) and DNA fragmentation by TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling). Expression of PfARP and metacaspase genes was evaluated by RT-PCR and blotted membranes were probed with polyclonal antibodies to PfARP and human caspase. Although the expression of these apoptotic features was highest just before the cultures crashed, they were also expressed to varying levels at low parasite density. These findings suggest existence of quorum sensing mechanisms that allow malaria parasites to auto regulate their density even when the growth requirements are not limiting.

(ACMCIP Abstract)

## 1017

### ..... MOLECULAR MARKERS OF THE PATHOGENESIS OF CEREBRAL MALARIA IN THE MURINE MALARIA PLASMODIUM BERGHEI

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Cerebral malaria (CM) is a rare but severe consequence of infection with *Plasmodium falciparum* malaria and is responsible for a significant portion of the 1-2 million deaths due to malaria every year, mostly in young children in sub-saharan Africa. Nonetheless, not all *P. falciparum* infections in African children progress into CM suggesting that host genetics contributes towards the pathogenesis of CM. The host factors and their associated biological pathways that culminate in the pathogenesis of CM are poorly defined. The *P. berghei* ANKA murine model of experimental cerebral malaria (ECM) closely mimics the pathogenesis of human CM. Using a combination of approaches such as comparative genome-wide transcriptional expression profiling (comparison between C57BL/6 mice with ECM to C57Bl/6 mice displaying no symptoms of ECM), immunohistopathology studies, and differential detection of biomarkers of ECM (identified in microarray studies) by western blot, we have identified novel host molecules and biological pathways that are clearly associated with expression of pathogenesis of ECM in mice. Specifically, by high density oligonucleotide microarray analysis, we have identified 210 host molecules in the brain that are strongly associated with the manifestation of ECM. Through in-depth functional analysis, we were able to classify these proteins into eleven categories primarily based on biological function and subcellular localization. Several molecules of the immune system, including OX40 and CD14, as well as a wide variety of host genes encoding transcription factors, receptors, transporters and proteins involved in signal transduction and cell adhesion are transcriptionally altered during ECM. These findings have significant relevance as anti-disease drug and vaccine targets. Research is in progress to determine if any of the identified biomarkers could be used to determine whether a *P. berghei* infection in susceptible mice will progress into ECM. An early detection test that could predict whether a *P. falciparum* infection in a non-immune child will progress into cerebral malaria or result in a less benign form of infection could significantly reduce mortality caused by *P. falciparum* malaria.

(ACMCIP Abstract)

## 1018

### ..... APOPTOSIS-RELATED AND INTERFERON-RESPONSIVE TRANSCRIPTS CHARACTERIZE DIFFERENTIAL WHOLE BRAIN RESPONSES IN RESISTANCE AND SUSCEPTIBILITY TO EXPERIMENTAL CEREBRAL MALARIA

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The pathogenesis of cerebral malaria is currently poorly understood. Specific local brain responses, influenced by parasite sequestration and general host immune system activation have been implicated to play key roles in the development of cerebral malaria. This study assessed brain transcriptional responses over the course of experimental cerebral malaria, by comparing genetically resistant and susceptible inbred mouse strains infected with *Plasmodium berghei* ANKA and using computational biology to identify differential patterns of biological regulation. Overall, genes that showed the most transcriptional activity changed in susceptible mice 1-2 days prior to the point when mice display symptoms of cerebral malaria. Most of the differentially expressed genes identified were associated with immune-related gene ontology categories. Further analysis to create interaction networks and to examine patterns of transcriptional regulation within the set of identified genes suggested a role for interferon-regulated processes and apoptosis in the pathogenesis of cerebral malaria. The biological relevance of these genes and pathways was confirmed using quantitative RT-PCR and histopathological examination of brain apoptosis. Additionally, several genes or pathways identified have been previously associated with malaria in independent studies, reinforcing their potential importance in disease progression. The application of computational tools to rigorously examine disease progression in cerebral malaria not

only identified important transcriptional patterns in pathogenesis, but may also be a promising approach to identifying targets for therapeutic intervention.

(ACMCIP Abstract)

## 1019

### ISOLATION OF HOST RESISTANCE FACTORS TO LIVER STAGE *PLASMODIUM BERGHEI* INFECTION BY GENETIC MAPPING

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Genetic mapping of liver resistance to *Plasmodium berghei* was performed in a cross between two mouse strains that differ in liver parasite burden, after sporozoite infection. A QTL controlling this phenotype was mapped on mouse chromosome 17 within a region of 37 Mb that encompasses the MHC region. Fine mapping with congenic strains confirmed this locus and narrowed down the linked region to 28 Mb, excluding the MHC region; this locus was named Ber1. A panel of 10 subcongenic mouse strains was generated to fine map the Ber1 locus. Preliminary analysis of this subcongenic strains will be presented. Experiments on infection time course, intra-hepatic infection and primary hepatocyte cultures strongly suggest that this phenotype is controlled by genetic factors operating within the hepatocytes. This project aims to test whether candidate genes within the Ber1 region are associated with the control of molecular pathways involved in liver response to infection by the malaria parasite.

(ACMCIP Abstract)

## 1020

### REGULATION OF *PLASMODIUM FALCIPARUM* GLYCOSYLPHOSPHATIDYLINOSITOL-INDUCED CYTOKINE RESPONSES BY MAPK-ACTIVATED PROTEIN KINASE 2 AND P38 MAPK

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Over production of proinflammatory mediators play important role in *Plasmodium falciparum* malaria pathogenesis. The parasite glycosylphosphatidylinositols (GPIs) are thought to be the major factors involved in proinflammatory responses during malaria infection, contributing to malaria pathogenesis. Recent studies have shown that malarial GPIs activate macrophages through TLR2/MyD88-dependent signaling, leading to the activation of ERK, p38 and JNK mitogen-activated protein kinase (MAPK) and NF- $\kappa$ B pathways, and cytokine responses. The MAPK pathways and NF- $\kappa$ Bs differentially contribute to the expression of various proinflammatory mediators. However, very little is known about the regulation of GPI-induced inflammatory responses by various signaling pathways. Here, we present the results of our recent studies on the roles of p38 MPK and MAPK-activated protein kinase 2 (MK2) in GPI-stimulated TNF- $\alpha$  and IL-12 production by macrophages. The level of TNF- $\alpha$  produced by MK2<sup>-/-</sup> macrophages was ~50% of that produced by WT cells. Inhibition of either ERK or p38 pathway each caused ~25% lower TNF- $\alpha$  production and significantly lower MK2 activity, whereas inhibition of both ERK and p38 caused ~50% lower TNF- $\alpha$  and complete abrogation of MK2 activity, suggesting that both ERK and p38 pathways regulate the GPI-induced TNF- $\alpha$  production through MK2. The level of IL-12 however was ~2-fold higher in GPI-stimulated MK2<sup>-/-</sup> macrophages compared to WT cells. The induction of I $\kappa$ B $\zeta$ , the nuclear factor critical for IL-12 expression, and NF- $\kappa$ B binding to IL-12 promoter were markedly decreased in p38 inhibited WT macrophages but not in MK<sup>-/-</sup> cells. The levels of inhibitory nuclear factors, c-Maf and GAP-12, were drastically decreased in MK2<sup>-/-</sup> cells. Together our data show that whereas p38-dependent induction of I $\kappa$ B $\zeta$  and enhanced NF- $\kappa$ B binding to gene promoter are

essential for GPI-induced IL-12 production, MK2 regulates IL-12 level by upregulating c-Maf and GAP-12 expression.

(ACMCIP Abstract)

## 1021

### MOLECULAR AND IMMUNOLOGICAL ANALYSES OF A MAJOR SEQUENCE POLYMORPHISM IN THE *PLASMODIUM FALCIPARUM* INVASION LIGAND PFRH2B

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Invasion of human erythrocytes by *Plasmodium falciparum* involves multiple receptor-ligand interactions. The Reticulocyte binding protein homologue (Pfrh) family has been implicated in the invasion process through binding to alternative receptors on the red cell surface, known as invasion pathways. Laboratory and field studies have shown that variations in the levels of expression of Pfrh family members is associated with invasion pathway usage. Here we present molecular and immunological analyses of the Pfrh family in uncultured isolates. We have identified considerable heterogeneity in the invasion pathways used by uncultured and clonal isolates from a hypoendemic region in Senegal. Minimal variation in the levels of expression of Pfrh1, Pfrh2a and Pfrh2b was observed; however, we identified a large deletion (~0.58 kb) in the C-terminal region of the Pfrh2b protein. The deletion occurred at a strikingly high prevalence (64%) within two sites in Senegal. We have used PCR-based typing methods to assess the global distribution of the Pfrh2b deletion by analyzing isolates from other sites within Senegal, Thailand, Malawi, and Tanzania. We find that the deletion is prevalent throughout the world, and are currently using Fst statistical analysis to measure differences between these populations to infer selection. We hypothesize that the high prevalence of the deletion may imply that it is under balancing immune selection. To determine the immunogenicity of Pfrh2b, we have measured total IgG reactivity from malaria-exposed Senegalese serum by ELISA and find that the Pfrh2b domain encompassing the deletion is recognized by 16% of patient sera. Future studies will determine the IgG subclass of the positive responses in addition to expanding our analysis to consider additional domains of the Pfrh proteins. Understanding the selection pressures exerted upon invasion ligands and the nature of the immune response have important implications for the development of erythrocytic stage vaccines.

(ACMCIP Abstract)

## 1022

### SWARM SEGREGATION IS THE MAIN MECHANISM THAT PREVENTS MATING BETWEEN SYMPATRIC MOLECULAR FORMS OF ANOPHELES GAMBIAE

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Speciation involves the "construction" of barriers to genes exchange between diverging populations. For pathogens and disease vectors it implies new epidemiological complexities. Understanding speciation requires identifying the reproductive barriers involved in the reduction of gene flow between populations. The molecular forms of Anopheles

gambiae, the major African malaria vector, display a pattern of habitat segregation, but are sympatric in many places, and a strong assortative mating in nature. The forces driving their separation and the mechanisms facilitating restricted gene flow have to yet be identified. To understand the role of swarm segregation as mechanisms of premating barriers, we collected 810 males from 51 different swarms in Donegubougou, Mali - an area where the forms are sympatric. Identification of the males revealed a remarkable spatial segregation among the mating swarms, i.e., swarms were composed either of M or of S forms but no mixed swarm was observed, so the molecular forms clustered in distinct form-specific mating units within the village. Moreover, distinctive markers were associated with their swarming sites. Additionally, 480 males and females were collected in different houses near each swarming site to see if the specific composition of the swarms was a by-product of the spatial clustering of the molecular forms across the village. The molecular M and S forms were equally represented in the indoors collections across the village with 45.3% and 43.9% respectively and *An. arabiensis* represented 10.8%. Both M and S forms and *An. arabiensis* were more or less uniformly distributed across the village suggesting that the males and females use specific cues to locate swarms of their own. Our results therefore provide strong support that swarms segregation is the main mechanism facilitating the genetic isolation of the forms.

## 1023

### AUDITORY INTERACTIONS BETWEEN MALES AND FEMALES OF MEDICALLY IMPORTANT *CULEX* SPECIES

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Following our discovery that males and females of the non-bloodfeeding mosquito *Toxorhynchites brevipalpis* enter into complex auditory-motor exchanges that may be used to identify and locate suitable mates, we have investigated the mechanical and biophysical properties of the hearing organ of *Culex quinquefasciatus*, and analysed the behavioral responses to flight tones in free-flying mosquitoes of opposite-sex and same-sex pairs. Pairs of *Tx. brevipalpis* in tethered flight respond to each other through feedback-like interactions, such that each mosquito alters its own flight tone in response to the flight tone of the other. This interaction continues until, in the case of male-female pairs, the tones converge, or, in the case of same-sex pairs, dramatically diverge. This form of sound communication effectively serves as a mechanism for bringing together mosquitoes only of opposite sexes during pre-mating encounters, and may hold the key to understanding how closely related species recognise con-specifics. In the case of *Cx. quinquefasciatus*, the typical fundamental wing-beat frequency of a male is about twice that of a female. Rather than converging on fundamental wing-beat frequencies, therefore, the male's fundamental converges with the first harmonic of the female. In principle, this should not be possible, since the antennae are not tuned to these frequencies. These results indicate that the mosquitoes cannot simply be listening for the absolute fundamental of other individuals, and that the auditory interactions are far more complex than we first imagined from the *Tx. brevipalpis* data. There is some evidence to suggest that mosquitoes may 'compare' their flight tones, and respond to changes in the difference between them. The auditory interactions may involve difference tones created by non-linearities in the neural coding of the antennal response. Such an interaction may account for discrepancies between our mechanical and behavioural data. The auditory system is clearly capable of far more complex interactions than we have explained thus far.

## 1024

### A NEW ROBUST DIAGNOSTIC POLYMERASE CHAIN REACTION (PCR) FOR DETERMINING THE MATING STATUS OF FEMALE *ANOPHELES GAMBIAE* MOSQUITOES

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The principal malaria vector in Africa, *Anopheles gambiae* contains two pairs of autosomes and one pair of sex chromosomes. The Y-chromosome contains a male determining factor and other Y-chromosome specific DNA sequence, which are transferred to females during mating. A reliable tool to determine the mating status of dry wild *An. gambiae* females is currently lacking. In this study, DNA was extracted from dried virgin and mated females and used to test whether Y-chromosome specific PCR markers can be successfully amplified and used as a predictor of mating. Three male-specific primers were used in this study. Here we report a new PCR based method to determine the mating status among successfully inseminated and virgin wild *An. gambiae sensu stricto* and *An. arabiensis* females. This dissection-free method has the potential not only to screen large numbers of recently mated and dried specimens, but also to facilitate both population demographics and gene flow studies from dried mosquito samples routinely collected in epidemiological monitoring, and aid existing and new malaria-vector control approaches.

## 1025

### COMPARISON OF KAIROMONES UTILIZED BY SEVERAL MEDICALLY IMPORTANT INSECT AND TICK TAXA

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Mosquitoes, biting flies and ticks utilize kairomones throughout their life cycle. Kairomones are utilized to find a mate, a suitable host for a bloodmeal, to locate sources of nectar/sugar and oviposition sites. This paper will review the projects conducted so far with various taxa of mosquitoes, other biting flies and ticks conducted by the author. It will show how similar kairomones are utilized across taxa for similar behavioral activities. These results will be compared with those found by other scientists conducting similar studies throughout the world. Among the kairomones studied were carbon dioxide, 1-octen-3-ol, various fatty acids, phenols, alcohols, aldehydes and ketones. Carbon dioxide is a universal attractant used by all blood-feeding insects and ticks. Its relative importance, however, varies by species. 1-Octen-3-ol is a widely used kairomone. Studies conducted with structural analogues of this compound will be heavily emphasized in this presentation. The importance of chain length and stereoisomers will be presented. Studies conducted with commercial lures, such as the BG-lure, will also be presented. The BG-lure consists of lactic acid, ammonia and hexanoic acid. The practical use of these kairomones in epidemiology and pest management will be included in this presentation.

## 1026

### DISCRIMINATIVE FEEDING BEHAVIOR OF *ANOPHELES GAMBIAE* S.S ON DIFFERENT PLANT SPECIES AND EFFECTS ON ITS SURVIVAL, FECUNDITY, AND VECTOR COMPETENCE IN A MALARIA ENDEMIC AREA OF WESTERN KENYA

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*Anopheles gambiae* s.s. (Diptera: Culicidae) is known to feed on plant sugars, but this is the first experimental study to determine whether it discriminates between plant species, and the fitness consequences of this behavior. Feeding responses of *An. gambiae* on 13 perennial plant species growing in Mbita, a malaria endemic area of western Kenya, were determined in a choice and no-choice bioassays. *An. gambiae* exhibited a preferential feeding pattern on different plant species, with five plant species: *Hamelia patens* J., *Parthenium hysterophorus* L., *Ricinus communis* L., *Senna didymobotrya* F., and *Tecoma stans* L. being more preferred. Survival, fecundity, and *Plasmodium falciparum* development in the midgut of *An. gambiae* were assessed on groups of mosquitoes exposed *ad libitum* to each plant species. Except for *P. hysterophorus*, mosquitoes that fed on the more preferred plant species lived significantly longer ( $P < 0.001$ ) and laid more eggs ( $P = 0.01$ ) than those fed on the least preferred plant species. The infection rates were significantly ( $P < 0.01$ ) reduced for mosquitoes fed on *R. communis* ( $7.1 \pm 2.00\%$ ) and *P. hysterophorus* (0%), than for those fed on the control glucose 6% ( $15.21 \pm 2.85\%$ ). Inhibition of infection by *P. hysterophorus* was only obtained when mosquitoes were infected with less than 200 gametocytes/ $\mu$ l of blood. However, when infected with higher gametocytemia (mean 566.57 gametocytes/ $\mu$ l of blood), significant reductions ( $P < 0.001$ ) of infection prevalence ( $12.4 \pm 1.59\%$ ) and oocyst intensity ( $1.6 \pm 0.15$  oocyst/infected midgut) were still obtained in mosquitoes fed on *P. hysterophorus* than those fed on the glucose ( $32.31 \pm 2.43\%$ ) and ( $14.3 \pm 0.64$  oocyst/infected midgut). These results indicate that *An. gambiae* feed preferentially on plants species that provide them with either direct fitness benefits (increased longevity and fecundity), or inhibit *P. falciparum* development in their midgut. This suggests that the availability of some plant species in malaria endemic area can increase or reduce malaria transmission by affecting the fitness and the competence of the vectors.

## 1027

### A MEANS TO AN END: COMPARATIVE ANALYSIS OF CHROMOSOMAL INVERSIONS FREQUENCY AND DISTRIBUTION IN THE MAJOR MALARIA VECTORS *ANOPHELES GAMBIAE* AND *AN. FUNESTUS* ACROSS ECOLOGICALLY DIVERSE ENVIRONMENTS IN CAMEROON

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Recent data obtained on various organisms strongly suggest that chromosomal polymorphism is a mechanism species use to rapidly adapt to their changing environment. Population studies have shown that differential adaptations of mosquitoes to various environments are often associated with dramatic changes in composition and frequency of polymorphic chromosomal inversions. We hereby present biological evidence that strengthens this view, through a detailed comparative analysis of chromosomal polymorphism and distribution of paracentric inversions in populations of the two main African malaria vectors, *Anopheles gambiae sensu stricto* (s.s.) and *An. funestus* s.s. collected across the wide range of geographical, ecological and climatic heterogeneities that exist in Cameroon. We karyotyped more than 2,000 *An. gambiae* and over 800 *An. funestus* collected in 305 villages located along a latitudinal transect ( $12^{\circ}\text{N}$ - $2^{\circ}\text{N}$ ) ranging from dry sahelian savanna in the North to equatorial forest in the South. The frequency of chromosomal

inversions in each locale was entered as a separate layer in a Geographical Information System containing 17 eco-geographical variables related to climate, land use and topography. Maps of habitat suitability for each major chromosomal inversion in each anopheline species were constructed by a specialized multivariate factor analysis. The spatial distribution of homologous chromosomal inversions was highly correlated between *An. gambiae* and *An. funestus* (Spearman's  $\text{Rho} > 0.67$ ;  $P < 0.001$ ). Furthermore, Principal Component analysis pointed toward the very same explicative environmental variables for each pair of homologous arrangements. These results are consistent with former cytogenetic, molecular, and comparative genomics studies that revealed colinearity and extensive synteny between *An. gambiae* and *An. funestus* chromosomes: they provide further evidence that both malaria vectors use homologous chromosomal inversions to adapt to the same environments, by producing highly specialized ecotypes. Chromosomal inversions are the means used by both species to release their full adaptive potential allowing them to occupy such a wide range of eco-climatic conditions across Africa.

## 1028

### CHROMOSOMAL EVOLUTION IN MALARIA MOSQUITOES OF SUBGENUS CELLIA

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Chromosomal rearrangements are often associated with differential adaptations of malaria mosquitoes to various environments. Polymorphic inversions tend to cluster in certain genomic regions suggesting existence of hot spots for generating and maintaining rearrangements. Fixed inversions are non-randomly distributed among chromosomes in the *Anopheles gambiae* complex; the chromosomes X and 2R are most rearranged. However, it is not known whether such pattern is a characteristic of the subgenus Cellia. Localization of hot and cold spots for rearrangements could be useful for identification of genes involved in ecological adaptations and biologically important gene clusters. The information on functional gene clusters should be taking into consideration for developing transgenic mosquitoes because expression of a transgene depends of the genomic environment. Physical maps are useful tools for studying the genome evolution and for relating sequence information to the chromatin structure. We compared physical maps of *A. stephensi*, *A. funestus*, and *A. gambiae* which belong to subgenus Cellia. The gene order comparison at the 2.5 Mb resolution has been performed using the Multiple Genome Rearrangements (MRG) and Sorting Permutation by Reversals and block-Interchange (SPRING) programs. We have found that inversions fixation rates vary significantly among the chromosomal arms. The small and large blocks of the conserved gene order have been identified among *A. stephensi*, *A. funestus*, and *A. gambiae*. The largest conserved blocks (up to 6 Mb long) have been found on the chromosomal arms 3R and 2L of *A. gambiae*. Interestingly, these genomic regions are free from polymorphic inversions in the three species. The analysis of the physical maps and polytene chromosomes has revealed extensive variations in morphology of chromatin within conserved regions among the three species. Euchromatin-heterochromatin evolutionary transitions represent the extreme cases of such variations. On the other hand, clear examples of preservation of the banding pattern can be seen within the subgenus.

## 1029

### TRANSCRIPTOME ANALYSIS OF *BIOMPHALARIA GLABRATA*, SNAIL HOST OF *SCHISTOSOMA MANSONI*

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The large extent to which transmission and subsequent epidemiology of schistosomiasis are determined by the distribution of gastropod intermediate hosts of schistosomes merits detailed study of the biology of the snail *Biomphalaria glabrata*. Along with ongoing efforts that include

traditional and molecular methods, the current sequencing of the genome of *B. glabrata* is anticipated to yield extensive novel data. Cataloguing of the mRNA transcripts that are expressed by the snail will inform on the genomic sequence by assisting gene finding and prediction. Analysis of the response to various immune challenges (including parasites) has the potential to reveal previously uncharacterized aspects of the immune function (regulation and effectors) of *B. glabrata*. Moreover, the study of immune transcriptome of *B. glabrata* may be informative to benefit our understanding of the biological processes that determine snail-schistosome immune interactions and compatibility. This study presents comparative analysis of circa 10,000 cDNA sequences (traditional ESTs and open reading frame ESTs) collected from M line strain *B. glabrata* (susceptible to *Schistosoma mansoni*) at 12 hours post exposure to various pathogens, including *S. mansoni*, *Schistosoma haematobium*, *Escherichia coli* (Gram -), and *Micrococcus luteus* (Gram +) and from untreated control snails. In addition to yielding several (groups) of immune-relevant genes, this approach revealed multiple novel (unknown) candidate immune sequences that were recovered only in association with particular immune challenges. Results will be presented in light of general biology of *B. glabrata* and of parasite-host interactions.

(ACMCIP Abstract)

### 1030

#### GENE CLONING AND FUNCTIONAL CHARACTERIZATION OF A TANDEM-REPEAT GALECTIN FROM CELLS OF THE *BIOMPHALARIA GLABRATA* EMBRYONIC (BGE) CELL LINE

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Galectins represent a large family of highly conserved  $\beta$ -galactoside-binding lectins found in both vertebrate and invertebrate organisms. Vertebrate galectins have been shown to be multi-functional, including playing important roles in the regulation of innate and adaptive immune responses. By contrast, although galectins also are widespread in diverse invertebrates species, little is known regarding their specific role in regulating immune responses to invading pathogens. In the present study, using an expressed sequence tag (EST) to a galactoside-binding protein present in the *Biomphalaria glabrata* EST-database, we obtain from Bge cells a full-length cDNA of 855 bp whose predicted amino acid (aa) sequence had significant homology to a tandem-repeat galectin. An identical sequence was subsequently cloned from *B. glabrata* hemocytes. The predicted Bg-galectin (Bg-gal) protein of 32 kDa contained 2 carbohydrate recognition domains (CRDs) and a unique peptide link of 12 aa residues. In order to confirm the lectin-like function of the Bg-gal, a recombinant protein was expressed in *E. coli*, purified and subjected to hemagglutination tests using fixed rabbit RBCs. The minimum concentration of recombinant Bg-gal (rBg-gal) producing a positive hemagglutination reaction was 2.5  $\mu\text{g/ml}$ . Moreover, in hemagglutination sugar inhibition assays, characteristic of galectins, lactose and galactose inhibited RBC agglutination at minimum inhibitory concentrations of 10 and 50 mM, respectively, whereas none of the other sugars tested (fucose, trehalose, glucose, mannose, gluNAc, galNAc) were inhibitory at the highest sugar concentration of 200 mM. Polyclonal antibodies raised against the rBg-gal reacted with Bge cell and *B. glabrata* hemocyte protein(s) in Western blot analyses, and localized to the cytoplasm and surface of hemocytes by immunofluorescence imaging. Finally, rBg-gal bound to *in vitro* cultured schistosome sporocysts in a lactose-inhibitable fashion, suggesting a potential immune interaction between this lectin and early developing larval schistosomes.

(ACMCIP Abstract)

### 1031

#### NOVEL MODULATORY ACTIONS OF SCHISTOSOME CALCIUM CHANNEL $\beta$ SUBUNITS ON VOLTAGE-GATED CALCIUM CURRENTS

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Voltage-gated  $\text{Ca}^{2+}$  ( $\text{Ca}_v$ ) channels regulate intracellular levels of  $\text{Ca}^{2+}$ , and may play a role in the mode of action of the antischistosomal drug praziquantel. The function of  $\text{Ca}_v$  channels greatly depends on coupling to cytoplasmic accessory  $\beta$  subunits, which promote surface expression of the pore-forming  $\alpha_1$  subunit, and also modify biophysical properties of  $\text{Ca}^{2+}$  currents. We have previously shown that schistosomes and other platyhelminths express two  $\beta$  subunit subtypes: a conventional  $\beta$  subunit (Sm $\beta$ B) and a variant  $\beta$  subunit (Sm $\beta$ A) that has unusual functional properties and confers susceptibility to praziquantel. Here, we have used whole-cell patch clamp to characterize the functional modulation of the schistosome  $\beta$  subunits Sm $\beta$ A and Sm $\beta$ B on the human  $\text{Ca}_v2.3$   $\alpha_1$  subunit stably expressed in HEK-293 cells. As described previously, the variant Sm $\beta$ A subunit significantly decreased the amplitude of the currents produced by  $\text{Ca}_v2.3$  channels. In contrast, the conventional Sm $\beta$ B subunit dramatically increased  $\text{Ca}_v2.3$  currents (~5-fold), slowed macroscopic inactivation and shifted the steady state inactivation in the hyperpolarizing direction, effects similar to those found for mammalian  $\text{Ca}_v\beta$  subunits. Interestingly, currents produced by the  $\text{Ca}_v2.3/\text{Sm}\beta\text{B}$  complex run down to about 75% of their initial amplitudes within two minutes of establishing the whole-cell configuration. This rundown was  $\text{Ca}^{2+}$ -independent; recordings using  $\text{Ba}^{2+}$  as the charge carrier rundown at the same rate. In contrast, currents produced by  $\text{Ca}_v2.3$  subunits alone or coexpressed with a mammalian  $\beta$  subunit were stable over the same time frame. Since the kinetics of inactivation and steady-state properties were the same before and after rundown, we hypothesized that the interaction between  $\text{Ca}_v2.3$  and Sm $\beta$ B remains intact, and that rundown is caused by dialysis of one or more intracellular factors that are necessary for maintenance of the Sm $\beta$ B-mediated current increase. Our preliminary data show that protein kinase A (PKA) is necessary for this Sm $\beta$ B-mediated current increase; including the catalytic subunit of PKA in the intracellular whole-cell patch-clamp solution suppressed rundown. These data provide new insights into the properties of schistosome  $\text{Ca}^{2+}$  currents, which show rapid rundown in recordings from schistosome cells, and also into the unusual mechanisms employed by schistosome  $\text{Ca}_v\beta$  subunits to modulate voltage-gated  $\text{Ca}^{2+}$  currents.

### 1032

#### IDENTIFICATION AND CHARACTERIZATION OF A R-SMAD ORTHOLOGUE (SMSMAD1B) FROM *SCHISTOSOMA MANSONI*

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Smad proteins are the cellular mediators of the TGF $\beta$  superfamily signals. We describe the isolation of a fourth Smad gene from the helminth *Schistosoma mansoni*, a Receptor-regulated Smad (R-Smad) gene termed SmSmad1B. The SmSmad1B protein is composed of a 380 amino acids and contains conserved MH1 and MH2 domains separated by a short 42 amino acid linker region. The SmSmad1B gene (>10.7kb) is composed of 5 exons separated by 4 introns. Based on phylogenetic analysis, SmSmad1B demonstrates homology to Smad proteins involved in the BMP pathway. SmSmad1B transcript is expressed in all the stages of schistosome development and exhibits the highest expression level in the cercaria stage. By immunolocalization experiments, the SmSmad1B protein was detected in novel cells of the parenchyma of adult schistosomes as well

as in female reproductive tissues. Yeast two-hybrid experiments revealed an interaction between SmSmad1B and the Common Smad, SmSmad4. As determined by yeast three-hybrid assays and MBP pull-down assays, the presence of the wild type or mutated SmTβRI receptor resulted in a decreased interaction between SmSmad1B and SmSmad4. These results suggest the presence of a non-functional interaction between SmSmad1B and SmTβRI that does not give rise to the phosphorylation and the release of SmSmad1B to form a heterodimer with SmSmad4. SmSmad1B, as well as the schistosome BMP-related Smad SmSmad1 and the TGFβ-related SmSmad2, interacted with the schistosome co-activator proteins SmGCN5 and SmCBP1 in pull-down assays. In all, these data suggest the involvement of SmSmad1B in critical biological processes such as schistosome reproductive development.

### 1033

#### IDENTIFICATION BY SUPPRESSION SUBTRACTIVE HYBRIDIZATION OF IMMEDIATE RESPONSE-GENES DOMINANTLY EXPRESSED IN *BIOMPHALARIA GLABRATA* SNAILS UPON EXPOSURE TO *SCHISTOSOMA MANSONI* INFECTION

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Intermediate snail hosts of parasitic infections are an integral part of the transmission of intractable chronic diseases such as schistosomiasis. An understanding of the snail host-parasite relationship at the molecular level is probably the best chance for the identification of novel tools that will help to block parasite development. Non-self responses in *Biomphalaria glabrata* towards parasite infection depend on an innate defense system, characterized by an immediate response against miracidia that completely eliminate traces of parasite in snail. In this study, we focused on the identification of immediate response-transcripts that may be involved in miracidia destruction before they can develop into sporocysts. Suppression subtractive hybridization (SSH) was used to reveal the up-regulation of dominantly expressed transcripts in either resistant (LAC) or susceptible (NMRI) snails 5 hrs post exposure to *S. mansoni*. SSH cDNA libraries were also constructed from parasite-exposed juvenile resistant (BS-90) and susceptible (NMRI) snails. From ESTs generated from these libraries transcripts shown by blastx to be significant and predominantly expressed between snails that are either resistant or susceptible were assembled into contigs. From these, we identified 41 dominantly expressed genes from parasite exposed susceptible snails. These included antioxidant, cell structure, signaling, immune related, metabolic, transduction/translation and enzyme encoding transcripts. From the resistant snail specific SSH libraries, ESTs generated included 25 genes that were up-regulated in these parasite-exposed snails. These comprised of a similar repertoire of gene transcripts as those found in the susceptible parasite exposed snails. Real time PCR was used to verify the specificity of transcription in both snails at different times post-exposure. From the resistant snail, immediate defense response genes were identified including cytidine deaminase and 2 defense related transcripts that were dramatically up-regulated shortly after parasite exposure. Similarly, by RT-PCR, results showed that several receptors for a lectin-like, low density lipoprotein and protein kinase C, were significantly up-regulated in parasite exposed susceptible but not resistant snails. Differences in the relative expressions of the SSH transcripts identified in resistant and susceptible snails pre-and post exposure will be discussed.

### 1034

#### EFFECT OF HURRICANE KATRINA ON CONTAINER BREEDING *Aedes* MOSQUITO SPECIES IN UPTOWN NEW ORLEANS, LOUISIANA: DISPLACEMENT IS REVERSED

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Prior to August 29, 2005, container inhabiting mosquitoes in the genus *Aedes* were widespread in the Greater New Orleans Area of Louisiana. Although *Ae. aegypti* was the species historically associated with yellow fever and dengue virus transmission in Louisiana and the rest of the United States, introduction of *Ae. albopictus* in the mid 1980's was associated with a drastic reduction in *Ae. aegypti* populations in all but the most southern areas of the country. Populations of *Ae. aegypti* remained in south Florida, south Texas, and south Louisiana. In Louisiana through 2005, only New Orleans continued to support *Ae. aegypti* and the population was limited more or less to the Uptown area of New Orleans. During the summer of 2005, collections in the area known to support both *Ae. albopictus* and *Ae. aegypti* showed ratios of 3:1 for human landing collections, 2:1 for CO2 trap collections, and 6:1 for aspirator collections of resting mosquitoes, respectively. The overall ratio of total *Ae. albopictus* to *Ae. aegypti* collected was 3.7:1 (total collected = 6060:1637). In 2006, 10 months after Hurricane Katrina and associated flooding, the ratio of the two species had changed markedly. Ovitrap collections ranged from a low ratio of 2.4:1 in July to 8.7:1 in late October for *Ae. aegypti* compared to *Ae. albopictus*. Collections in 2006 made at sites similar to 2005, showed ratios of 7.3:1 in CO<sub>2</sub> trap collection and 1.6:1 in aspirator collections for *Ae. aegypti* to *Ae. albopictus*, respectively. The overall ratio of total adult *Ae. aegypti* to *Ae. albopictus* collected was 2.6:1 (total collected 4027:1573). Possible reasons for this reversal in relative density of the two species and public health implications will be discussed.

### 1035

#### SPATIAL MODELLING OF HABITAT SUITABILITY, DISTRIBUTION, AND RELATIVE ABUNDANCE OF SPECIES AND MOLECULAR FORMS OF THE *ANOPHELES GAMBIAE* COMPLEX IN CAMEROON

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Spatial modelling based on Remote Sensing (RS) and Geographical Information Systems (GIS) has received growing attention in landscape ecology to predict species distributions and to build maps of habitat quality for many animal and vegetal species, as well as in the epidemiology of vector-borne diseases to predict and monitor the risk of transmission. Spatial approaches can be used also to explore the genetic basis of ecological adaptations in species with known genome sequences, by a detailed analysis of gene-environment interactions. As a first step towards this goal, a country-wide analysis to predict the habitat suitability, distribution, and relative abundance of the taxa of the major African malaria vector, *Anopheles gambiae s.l.*, was performed



across Cameroon. *A. gambiae* s.l. mosquitoes were collected in 305 georeferenced villages, chosen according to a stratified random sampling protocol along a North-South transect (LAT 12°N to 2°N). Over 5,000 mosquitoes were characterized to species and molecular form. The habitat profile was characterized by extracting environmental data from spatial databases and remote-sensed data. Environmental and entomological data were entered as separate layers in a GIS where environmental data were further processed to construct ecogeographical variables related to climate, land use, and topography for spatially explicit modelling. A specialized multivariate factor analysis was used to build maps of habitat suitability and to rank the ecogeographical variables on the basis of their association with each of the M and S molecular forms of *A. gambiae* and *A. arabiensis*. The relative abundance of the taxa of the complex was modelled by kriging their relative frequencies across the study area. We conclude that habitat suitability maps and geostatistical predictors are useful tools providing a spatially explicit basis to explore the mechanisms underlying ecotypic adaptations in this group of highly specialized mosquitoes.

### 1036

#### INTEGRATED VECTOR MANAGEMENT FOR THE PREVENTION OF MALARIA IN WESTERN KENYA: INTERACTIONS OF LARVAL CONTROL AND INTENSIVE ITN IMPLEMENTATION ON *ANOPHELES GAMBIAE* DENSITY

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Integrated control of vectors of human malaria in subSaharan Africa involves strategic and tactical attacks on the identifiable resources of the different stages of these mosquitoes. We took advantage of an intensively managed program of implementation of insecticide treated bed nets (ITNs) in an area of western Kenya to impose an anti-larval control program, with *Anopheles gambiae* s.l. and *An. gambiae* s.s. in particular as the target vector species. The goal was to determine if anti-larval methods will synergize with ITNs in reducing malaria transmission. The study site is extremely well characterized with a large GIS data base and a landscape model under development which aims to predict location and distribution of larval habitats. We divided the study area into twenty 2x2 km zones covering 44% of the total study area. Half of these were used as intervention clusters and half as non-intervention clusters. Each of the intervention zones had one or two larvicide applicators depending on whether the zone was a high larval habitat density area or not. All habitats in the intervention zones were treated weekly using a formulation of the microbial *Bacillus thuringiensis* var. *israelensis* (Bti) from Valent BioSciences Corp, Illinois, USA. The control program was evaluated by sampling in both intervention and non-intervention zones of outdoor adult *Anopheles gambiae* densities using the recently developed AgREPOT system, of indoor surveys by pyrethrum spray collections, and larval densities at larval habitats using area samplers. Larvicide application methodologies employed, logistics involved in the implementation, and sampling results will be presented.

### 1037

#### *ANOPHELES GAMBIAE* GUT FLORA DYNAMICS AND MICROARRAY ANALYSIS OF GUT GENE EXPRESSION IN RESPONSE TO BACTERIA

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The mosquito *Anopheles gambiae*, a primary malaria vector, undergoes a major proliferation of its gut flora during blood digestion. It has been shown that gut bacteria can affect negatively the infection of the midgut by *Plasmodium*, which could be due to activation of mosquito gut immunity by bacteria. In the present study the midgut flora dynamics of *An. gambiae* (G3) laboratory colonies was characterized after control or *Plasmodium* infected blood meals, and changes in mosquito gut gene expression in response to lumen bacteria were analyzed with microarrays. Direct cloning and sequencing of 16s RNA genes in midgut homogenates detected the same bacterial species in control blood fed or *P. berghei* blood fed mosquitoes: Flavobacteria, Chryseobacteria, Acinetobacter, Pseudomonas and Asia. Most bacteria detected were gram-negative aerobic non-fermenters. Bacteria determination by real-time quantitative PCR indicated that gut bacteria grows (20-30 times) by 24 h after a control blood meal and levels up to 48h. Interestingly, a *P. berghei* infected blood meal led to higher levels of lumen bacteria compared to the control meal by 40h post feeding. This indicates that lumen bacterial growth is at its highest by the time *P. berghei* invades the midgut and the presence of *Plasmodium* in a blood meal allows further bacterial growth. Microarray analysis of mosquito gut gene expression 3h after a feeding with a meal containing BSA and a gram-negative (*E. coli*) presented significant changes in expression of 700 genes compared to a BSA fed control. The up regulated genes (408) included several categories including immune defense genes like: peptidoglycan recognition proteins, Toll receptor, cecropin, dual oxidase, peroxidases and lysozyme. This indicates that the mosquito gut can mount an immune response against the presence of bacteria in its lumen, which potentially could affect *Plasmodium*.

### 1038

#### INTROGRESSION OF THE CARB77 TRANSGENE INTO A GENETICALLY DIVERSE LABORATORY STRAIN OF *Aedes Aegypti* FROM TAPACHULA, CHIAPAS STATE, MEXICO

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Introgression of the dengue refractory CARB77 transgene into a genetically diverse laboratory strain (GDLS) of *Aedes aegypti* was conducted to determine the effects on CARB77 on net fitness. For each experiment GDLS was established by combining 10 *Ae. aegypti* collections from the Tapachula region, along the coast of Chiapas. We intercrossed CARB77xGDLS in 50 isofemale lines and performed the reciprocal intercross in another 50 isofemale lines. We obtained 33 GDLSxCarb77 and 31 Carb77xGDLS isofemale lines. There were no readily apparent pre-zygotic barriers to mating between the GDLS and CARB77 strains. Sixty four isofemale lines were hatched and in each we checked the F<sub>1</sub> larvae for expression of green fluorescent protein. Heterozygous larvae exhibited clear, bright green anal papillae and bright green slits posterior to the eye. No problems were encountered in separating CARB77/+ (transgene heterozygotes) from +/+ (wild types). In each isofemale line we counted and recorded numbers of green and wild type larvae. Wild type larvae were discarded. The backcross generation 1 (BC<sub>1</sub>) was started from 521 heterozygous CARB77/+ offspring. In BC<sub>1</sub> and BC<sub>2</sub> we performed reciprocal crosses of CARB77/+ hybrid females with GDLS males. We recovered the expected ~50% of CARB77/+ offspring and the hybrid males were better carriers of CARB77 than the females. We have also developed a melting curve PCR assay to genotype CARB77 homozygotes, CARB77/+, and +/+ for final production of a CARB77 homozygous strains for the next stage of testing.

**TRANSCRIPTOMIC ANALYSIS AND TEMPORAL EXPRESSION PROFILING OF THE MIDGUT OF THE SAND FLY *LUTZOMYIA LONGIPALPIS* IN BLOOD FEEDING AND INFECTION WITH *LEISHMANIA CHAGASI***

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The midgut of a sand fly is a key organ for Leishmania parasites successful proliferation and differentiation into an infective and transmissible form, but little is known about the molecular interactions that occur between *Leishmania chagasi* and the principle vector, *Lutzomyia longipalpis*. To understand the repertoire of molecules present within the midgut of *L. longipalpis* we used a transcriptomic approach, constructing and randomly sequencing transcripts from five cDNA libraries including sugar-fed, blood-fed, gravid, *Leishmania chagasi*-infected blood-fed, and *L. chagasi*-infected gravid sandflies. Bioinformatic analysis of 11,520 cDNA clones resulted in the identification of the most abundant *L. longipalpis* midgut-specific transcripts. A comparative analysis of the five cDNA libraries was used to identify transcripts which may be differentially modulated at certain time points after blood meal ingestion in the presence and absence of *L. chagasi*. Several transcripts which were found in higher numbers in midgut cDNA libraries from sugar fed, blood fed, or *L. chagasi*-infected sand flies were chosen for multiplexed quantitative RT-PCR analysis. Temporal expression profiles of transcripts such as those encoding putative trypsin, chymotrypsin, carboxypeptidase, peritrophin-like and microvilli associated-like proteins showed strong modulation by the ingestion of a blood meal. Furthermore, transcript profile analysis allowed the comparison between blood fed and *L. chagasi* infected sand flies as the blood meal is digested and the parasite proliferates within the midgut. This tissue-specific transcriptome provides a comprehensive look at the repertoire of transcripts present in the midgut of the sandfly *L. longipalpis*. Additionally, temporal profiling of specific transcripts provides insights into the processes of blood meal digestion as well as demonstrating the effects of the presence of *L. chagasi* may have on the transcripts of *L. longipalpis* midgut tissue.

## 1040

**IDENTIFICATION OF MALE SPECIMENS OF *CULEX PIPPIENS* COMPLEX (DIPTERA: CULICIDAE) MOSQUITOES BY MORPHOMETRIC INVESTIGATION OF THE PHALLOSOMES AND BY MOLECULAR TECHNIQUES**

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Knowledge of the population structure of arthropod vectors is crucial to the understanding of the eco-epidemiology of the diseases that they transmit. Despite the introduction of molecular methods, the identification of the *Culex pipiens* L. complex in arbovirus surveillance programs still relies heavily on the use of morphological identification keys. In this study, we investigated males of *Cx. pipiens* mosquitoes collected from 9 different locations, encompassing northern, southern, and the hybrid zone sites in North America, as well as specimens from Cairo (Egypt) and Nairobi (Kenya). The species or hybrid identifications based on D/V/D ratios were compared to the amplification of the acetylcholinesterase (ACE.2) gene by both conventional and real-time PCR, and examination of the CQ11 locus. The morphological and molecular identifications did not always agree, particularly in regions of extensive introgression. Whereas

*Culex p. quinquefasciatus* Say populations were consistently identified by D/V/D ratio and ACE.2 amplification from all sites, *Culex p. pipiens* Linnaeus and hybrid forms were highly polymorphic, especially in areas of known hybridization. An increased frequency of hybrid forms in late summer and early fall was detected in Champaign Co., (an area north of the hypothetical hybrid zone). Although the overall distribution of the complex agreed with previously reported works, our study suggests a spatial expansion of the hybrid zone and the presence of self-sustained hybrid population in Champaign Co. The impact of a late season increase in hybrids on flavivirus transmission is discussed.

## 1041

**LONG-TERM OUTCOMES OF JAPANESE ENCEPHALITIS IN BANGLADESH**

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Japanese encephalitis (JE) virus is an important cause of viral encephalitis throughout Asia. Data on long-term functional and neurologic outcomes in survivors of JE is limited. In May 2006, we assessed neurologic and functional sequelae in 15 Bangladeshi patients with laboratory-confirmed JE that occurred from 2003-2005. We performed neurologic evaluations, electroencephalograms (EEG), and brain magnetic resonance imaging (MRI), and administered a questionnaire on persistent symptoms and functional impairment. Eight (53%) of the 15 subjects were female; median age was 12 years (range 5-37). Median interval between illness onset and follow-up was 30 months (range: 10 - 35). Four subjects (27%) had severe persistent neurologic deficits at followup, including a 5 year old with developmental regression and seizures, a 10 year old with severe focal arm dystonia, a 22 year old with parkinsonism and severe cognitive deficits, and a 37 year old with persistent hemiplegia. Three (75%) of these four patients had MRI abnormalities (including bilateral thalamic lesions [1], and confluent subcortical hyperintensities [2]) and abnormal EEG's. Three additional patients had MRI or EEG abnormalities but no objective neurologic deficits at followup. Nine subjects, including all with ongoing deficits, reported persistent functional difficulties including fatigue (9), memory difficulties (7), and mood problems (7). In seven patients, these neurologic or functional problems prevented return to normal daily activities. In conclusion, neurologic sequelae, including cognitive impairment, parkinsonism, and dystonia, were persistent in some patients over 2 years following acute JE. Confluent subcortical hyperintensities on MRI have been infrequently reported with JE. Persistent functional impairment limiting normal daily activities was reported by over half of patients. In countries in which JE is endemic, persistent neurologic dysfunction and functional impairment may cause significant long-term morbidity among survivors.

## 1042

**A COHORT STUDY TO ASSESS THE NEW WHO JAPANESE ENCEPHALITIS SURVEILLANCE STANDARDS**

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Approximately half the world's population lives in areas affected by Japanese encephalitis (JE). JE can be controlled through vaccination, but disease surveillance is needed to support countries in their decisions on vaccine implementation. New surveillance standards for JE have been produced by the WHO, but it is unclear how good they are. In this

study we assessed the field test version of the new WHO JE surveillance standards. We applied the clinical case definition of acute encephalitis syndrome (AES), laboratory diagnostic criteria and case classifications to patients with suspected central nervous system (CNS) infections in southern Vietnam. 380 patients (149 children) with suspected CNS infections were recruited and evaluable, of whom 296 (96 children) met the AES case definition. 54 children were infected with JE virus (JEV), of whom 35 (65%) had AES, giving a sensitivity of 65% (95%CI 56-73%), and specificity 39% (30-48%). 9 adults with JEV all presented with AES. The 19 JEV-infected children missed by the surveillance included 10 with acute flaccid paralysis, 2 with a flaccid hemiparesis, and 6 with meningism only. Altering the case definition to include limb paralysis and meningism improved the sensitivity to 89% (83-95), whilst reducing the specificity to 23% (15-30). An acute serum sample diagnosed 41(68%) of 60 JEV positive patients; an admission CSF diagnosed 33(72%) of 46 patients with this sample, including 7 that were serum negative. Examining a 2<sup>nd</sup> sample at day 10 diagnosed 61 of 62 patients. 5 patients with neurological manifestations of dengue infection had JEV antibodies in serum, and would have been misdiagnosed had we not tested for dengue antibodies in parallel. In conclusion, the case definitions detected about two thirds of the children infected with JEV, missing those presenting with acute flaccid paralysis. A modified case definition which included acute paralysis and meningism detected nearly 90% of children. An acute CSF sample is more sensitive and specific than an acute serum sample. This formal evaluation of surveillance standards during their development provides an evidence base to support their recommendation, and should be encouraged for future WHO standards.

### 1043

#### EPIDEMIC CHIKUNGUNYA FEVER, INDIA AND INDIAN OCEAN, 2006: LABORATORY-BASED SURVEILLANCE FOR IMPORTED CASES, UNITED STATES

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Chikungunya virus (CHIKV) is a mosquito-borne alphavirus endemic to Africa and Asia. Chikungunya fever (CHIKF) is characterized by fever, rash, arthralgia, and sometimes arthritis; joint symptoms can be severe and prolonged. In 2005-2006, an unprecedented outbreak of CHIKF occurred on islands in the Indian Ocean and in India. Viremic travelers from epidemic areas could introduce CHIKV to the United States (U.S.) through infection of competent local mosquito species, including *Aedes aegypti* and *Aedes albopictus*, which are distributed throughout the southeastern U.S. and Hawaii. We investigated all cases of CHIKF among U.S.-bound travelers in 2006 that were confirmed at CDC. We searched the CDC Arboviral Diagnostic and Reference Laboratory's database for all patients with laboratory-confirmed CHIKF with onset in 2006, and abstracted demographic and travel information. Cases were confirmed using serology (IgM enzyme-linked immunosorbent assay and plaque reduction neutralization test), viral culture, and reverse transcriptase-PCR (RT-PCR). Thirty-eight people from 16 states and the District of Columbia had laboratory evidence of recent CHIKV infection. Their median age was 49 years (range, 22-78 years); 55% were female. India was the travel destination most frequently reported (87%), followed by Sri Lanka (11%), Réunion (3%) and Zimbabwe (3%). One person reported travel to both India and Sri Lanka. Evidence of recent infection was found by serology in 31 (82%), by viral culture and RT-PCR in 5 (13%), and by RT-PCR alone in 2 (5%). In contrast, only 3 cases of CHIKV infection among U.S.-bound travelers were diagnosed at CDC during the preceding 15-year period from 1991-2005. An unprecedented number of CHIKF cases were confirmed at CDC among travelers to the U.S. in 2006. The 5 culture-positive travelers, and others who might have had undetected viremia, posed a risk of introducing CHIKV into local mosquito populations. There was no evidence of local CHIKV transmission in the U.S. in 2006, but the potential for introducing CHIKV to the U.S. from areas with ongoing transmission still exists. Travelers to tropical areas of Asia and Africa should

take precautions against mosquito bites. Travelers returning from epidemic or endemic areas with fever and joint symptoms should be tested for CHIKV infection, and positive cases reported promptly to local public health authorities.

### 1044

#### PERSISTENT SEROPREVALENCE OF ANAPLASMA PHAGOCYTOPHILUM IN NEW ENGLAND BLOOD DONORS

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The incidence of human granulocytic anaplasmosis (HGA) has doubled since 1999. The causative agent, *Anaplasma phagocytophilum*, is transmitted to humans primarily by the ixodid tick, *Ixodes scapularis*, endemic in New England. *A. phagocytophilum* causes an illness that ranges from asymptomatic to severe. There has been one reported case of transfusion-transmitted *A. phagocytophilum*, but blood donors are not currently screened for HGA. To determine the potential blood safety risk posed by this agent, we determined its seroprevalence in Connecticut (CT) and Massachusetts (MA) blood donors. Consenting CT and MA blood donors were enrolled in a comprehensive tick-borne disease study. Blood samples were collected during the late spring to early winter (2001-2005) and year round beginning in 2006. Serum collected from participating donors was tested for human IgG antibodies to *A. phagocytophilum* using an indirect immunofluorescent assay (IFA). A donor was considered positive if their IFA titer result was  $\geq 1:64$ . Of 15, 828 donor sera tested by IFA, 432 (2.7%) were positive by IFA for *A. phagocytophilum* antibodies. The distribution of titers was as follows: 256 (59%) donors at 1:64, 115 (27%) at 1:128, 42 (9.7%) at 1:256, 14 (3.2%) at 1:512 and 5 (1.2%) at  $\geq 1:1024$ . MA donors had a seroprevalence rate of 2.2% (30/1346), while the rate of CT donors was slightly higher, 2.8% (402/14,482). Seroprevalence peaks occurred in the following months: February (4.7%), December (3.7%) and September (3.4%). Overall, the seroprevalence data demonstrated variable yearly rates with a low of 1.7% in 2004 and a high of 4.1% in 2001. Observed fluctuations in yearly seroprevalence rates are likely the result of climactic and environmental factors that influence the complex lifecycle of *A. phagocytophilum*. The observed persistence of relatively high seroprevalence rates reinforces the need to examine the possible impact that *A. phagocytophilum* may have on blood safety. Limited transmission evidence to date may be attributable to the agent's short bacteremic phase, the effect of leukoreduction on this intra-granulocytic organism, or to transmission of primarily sub-clinical infection and resultant under-recognition.

(ACMCIP Abstract)

### 1045

#### FAILURE OF STANDARD BABESIOSIS THERAPY IN IMMUNOCOMPROMISED HOSTS

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