1167

CASE FATALITY OF SEVERE ACUTE RESPIRATORY SYNDROME (SARS) IN MAINLAND CHINA AND ASSOCIATED RISK FACTORS

Sake J. de Vlas[1], Na Jia[2], Dan Feng[2], Jan Hendrik Richardus[1], Wu-Chun Cao[2]

[1]Erasmus MC, Rotterdam, Netherlands, [2]Beijing Institute of Microbiology and Epidemiology, Beijing, China

This study was undertaken to analyse the case fatality ratio (CFR) and its risk factors for Severe Acute Respiratory Syndrome (SARS) in mainland China by using a comprehensive dataset of all probable cases. The data of all probable SARS cases was derived from the Infectious Disease Reporting System of the Center of Diseases Control and Hospital Information Systems, during the 2003 epidemic in mainland China. The definition of probable SARS case was consistent with the definition for clinically confirmed SARS issued by the Ministry of Health of the People's Republic of China. We performed univariate and multivariate logistic regression analysis to determine the association of CFR with age, sex, residence location, occupation, the period of the epidemic, and the duration from symptom onset to admission into hospital. he overall CFR was 6.4% among 5327 probable SARS cases in mainland China. Old age, being a patient during the early period of a local outbreak, and being from Tianjin led to a relatively higher CFR compared to young age, late stage of a local outbreak and cases from Beijing. Guangdong province resulted in an even lower CFR compared to Beijing. In conclusion, the deteriorated health status and apparent complications of SARS patients with relatively old age (> 60 years) has caused a much higher risk of dying than for younger patients. In the early stage of local outbreaks, lack of experience in patient care and perhaps treatment has also lead to a relatively higher CFR. The Tianjin SARS outbreak happened mainly within a hospital, leading to a high impact of co-morbidity. The relatively young age of the cases partly explains the low CFR in mainland China compared to other countries and areas affected by SARS.

1168

HIGH HYBRIDIZATION RATE BETWEEN *ANOPHELES GAMBIAE* MOLECULAR FORMS AT THE WESTERN EXTREME OF THEIR RANGE HIGHLIGHTS POSSIBLE GENE-FLOW IN THE X-CHROMOSOME "SPECIATION ISLAND"

Alessandra della Torre[1], Federica Santolamazza[1], Beniamino Caputo[1], Emiliano Mancini[1], Katinka Palsson[2], Davis Nwakanama[3], Musa Jawara[3], David Conway[3], Zhijian Tu[4], Vincenzo Petrarca[5], Joao Pinto[6]

[1]Dip. Scienze di Sanità Pubblica, Università Sapienza, Rome, Italy, [2]Department of Systematic Zoology, Evolutionary Biology Center, Uppsala University, Norbyvägen, Sweden, [3]Medical Research Council, Fajara, Gambia, [4]Department of Biochemistry, Virginia Polytechnic Institute and State University of Blacksburg, Blacksburg, VA, United States, [5]Dip. Genetica e Biologia Molecolare, Università Sapienza, Rome, Italy, [6]Centro de Malaria e outras Doenças Tropicais, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon, Portugal

Anopheles gambiae M and S molecular forms are considered to be incipient species: in fact, only 6 M/S putative hybrids (i.e. showing a M/S rDNA-IGS-RFLP pattern) have been reported so far out of almost 7,000 *A. gambiae* s.s. from north-west Africa and none from central-west Africa (N>10,000). High genetic differentiation between M and S have been shown to be restricted to three genomic "speciation islands". One of these is the pericentromeric region of the X-chromosome, where reduced recombination and natural selection have been suggested to have contributed to the accumulation of alleles of genes involved in reproductive isolation between the two forms. We here report an unusually high degree of hybridisation between M and S forms at the western extreme of their distribution range, where no previous data were available. In fact, we recorded 35 M/S hybrid specimens out of almost 2.000 *A. gambiae* s.s. collected in The Gambia (M/S freq=0.6%-7%, in sympatric sites, with N>100) and 37/179 (20.7%) in Bissau city (Guinea Bissau, about 200 km southwards). Quite intriguingly, we also have evidence of possible gene-flow within the X-pericentromeric region between the two molecular forms in the study area. This is shown by the preliminary analysis of M/S hybrids by a novel approach based on the study of a Short Interspersed Transposable Element (SINE200) at a single locus, which we found to be consistently specific of the M-form in other geographic areas. This SINE200 locus maps about 1 Mb from the IGS-rDNA region on which the M and S-form identification is based. Unexpectedly, both IGS-RFLP and SINE200 approaches provided only partially consistent results, possibly suggesting a higher-than-expected recombination between the two markers in the X-pericentromeric area. Does selection play a role in determining the observed pattern? Are we observing a phenomenon peculiar of the study area, where the two forms hybridise more frequently? These issues will be discussed also in relation to the further analysis of laboratory offspring originated from crosses between M/S specimens.

1169

ECOLOGICAL DIVERGENCE AND REPRODUCTIVE ISOLATION ALONG AN URBANIZATION GRADIENT: HABITAT SEGREGATION OF *ANOPHELES GAMBIAE* MOLECULAR FORMS IN A FOREST AREA OF CAMEROON

Colince Kamdem[1], **Carlo Costantini**[1], Joachim Etouna[1], Diego Ayala[2], Jean-Pierre Agbor[1], Christophe Antonio-Nkondjio[1], Didier Fontenille[2], Nora J. Besansky[3], Frederic Simard[4]

[1]Institut de Recherche pour le Developpement (IRD)/Organisation de Coordination pour la lutte contre les grandes Endemies en Afrique Centrale (OCEAC), Yaounde, Cameroon, [2]Institut de Recherche pour le Developpement (IRD), Montpellier, France, [3]Eck Family Center for Global Health and Infectious Diseases, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, United States, [4]Institut de Recherche pour le Developpement (IRD)/Institut de Recherche en Sciences de la Sante (IRSS), Bobo-Dioulasso, Burkina Faso

Consequences of human activity on the biosphere include loss of biodiversity, alteration of ecological communities, and biological invasions. However, an overlooked effect of the impact of humans on the environment is the creation of new species. Anthropogenic habitat disturbance of primeval landscapes generates contrasting ecological settings where divergent selection for alternative eco-phenotypes can promote the evolution of reproductive isolation and speciation. We will present an example of how urbanization in the Central African rain forest is driving a process of ecological segregation and incipient speciation between two cryptic taxa of the major Afro-tropical malaria vector *Anopheles gambiae*. The molecular forms M and S of this mosquito, distinguishable by fixed nucleotide sequence differences in the inter-genic spacer of ribosomal DNA, sharply segregate along an urbanization cline at a geographical scale of a few kilometres. The molecular form M occurs exclusively in the most human-disturbed habitats where it can breed in polluted sites associated to waste waters, whereas the S form is found in rural settings, where it breeds in rain-dependent water puddles on bare soil. In the metropolitan area of Yaoundé, populations of the two forms come in contact along a narrow peripheral zone. Hybrids have never been found in strictly sympatric natural populations. An index of urbanization based on remotely-sensed data was used as a predictor of the probability of occurrence of the M form in 306 randomly-chosen georeferenced localities (57 positive, 249 negative) across the forest of Cameroon. The binary logistic regression model was statistically significant (P<0.001, area under the ROC curve=0.72), and correctly predicted M occurrence in about 83% of the sampled localities. With a threshold of 50%, the model was highly specific (2% false positives), but moderately sensitive (21% true positives), indicating that either the model did not capture a significant portion of the underlying explanatory variables, and/or the M form can occur at lower densities in less suitable habitat. The ongoing adaptation of the M form to a new ecological niche is of epidemiological

significance, as it may lead in the future to increased malaria transmission in urban settings, where levels of immunological premunition by the human population are currently in equilibrium with lower rates of parasite exposure.

1170

A TEP1 MEDIATED RESPONSE IS REQUIRED BUT NOT SUFFICIENT FOR MELANIZATION OF *PLASMODIUM FALCIPARUM* IN THE *ANOPHELES GAMBIAE* MIDGUT

Alvaro Molina-Cruz[1], Corrie Ortega[2], Randall DeJong[2], Janneth Rodrigues[2], Giovanna Jaramillo-Gutierrez[2], Ekua Abban[2], Carolina Barillas-Mury[2]

[1]National Institutes of Health, Bethesda, MD, United States, [2]National Institutes of Health, Rockville, MD, United States

It is known that the survival of the *Plasmodium* parasite in its mosquito vector is in part determined by the innate immune response of the mosquito. A powerful tool to identify mosquito genes that mediate killing of Plasmodium are mosquito strains refractory to the parasite. An *Anopheles gambiae* laboratory strain (L3-5), selected from an African mosquito line to be refractory to *P. cynomolgi*, was used to study the nature of the innate immune response of the mosquito against *P. falciparum*. The L3-5 strain was found to be susceptible to African *P. falciparum* but it was highly refractory to a *P. falciparum* strain from Brazil, melanizing 98% of the parasites in the mosquito midgut. Using dsRNA mediated gene knockdown was found that *P. falciparum* killing and melanization in *An. gambiae* L3-5 requires TEP1. In order to test whether activation of the TEP1 mediated pathway is sufficient to kill different *P. falciparum* strains, a coinfection of *P. falciparum* strains was done in the L3-5 mosquito. Coinfection of *P. falciparum* from Africa and from Brazil gave rise to a mixed phenotype with live oocysts and melanized parasites in each of the mosquito midguts analyzed. There was no indication of any of the two parasite phenotypes predominating over the other, indicating that even when the mosquito immune system is activated and is able to melanize parasites through TEP1, this is not sufficient to kill different invading Plasmodium lines. This suggests that besides activation of the immune response of the mosquito, there are Plasmodium factors that determine whether the parasite is susceptible or not to the mosquito defenses.

1171

LARVAL ANOPHELINE MOSQUITO RECTA EXHIBIT A DRAMATIC CHANGE IN ION TRANSPORT PROTEINS IN RESPONSE TO SHIFTING SALINITY

Kristin E. Smith[1], Leslie A. VanEkeris[1], William R. Harvey[1], Peter J. Smith[2], Paul J. Linser[1]

[1]University of Florida, Saint Augustine, FL, United States, [2]BioCurrents Research Center, Program in Molecular Physiology, Marine Biological Center, Woods Hole, MA, United States

Mosquito larvae live in dynamic aqueous environments which can fluctuate drastically in salinity due to ecological events such as rainfall and evaporation. Larval survival depends on the ability of tissues such as the rectum to regulate hemolymph osmolarity by absorbing and excreting ions. The recta of several culicine genera (including Aedes and Culex) have been studied in detail, but very little is known about the recta of anopheline larvae. Here we report several lines of evidence which suggest anopheline larvae differ from culicine larvae both in rectal structure and regulation of protein expression. Whereas obligate fresh-water and saline-tolerant culicines have structurally distinct recta, immunolocalization patterns of carbonic anhydrase and Na+K+-ATPase reveal that all anophelines examined (regardless of saline tolerance) have structurally similar recta composed of distinct DAR (dorsal anterior rectal) cells and non-DAR cells. In larvae reared in fresh water, carbonic anhydrase localizes to the cytoplasm of DAR cells and Na+K+-ATPase localizes to the basal membrane of non-DAR cells. Additionally, saline-tolerant anopheline larvae undergo a dramatic shift in rectal Na+K+-ATPase protein localization from the non-DAR cells to DAR cells when reared in saline water compared to those reared in fresh water. A similar shift in protein localization is not seen in any culicine larvae examined. We also report preliminary physiological data obtained using a self referencing potassium selective electrode which suggests a change in potassium flux in the non-DAR cells of Anopheles albimanus larvae reared in fresh versus saline water. From these data we suggest that saline-tolerant anopheline larvae adapt to saline water in a distinctive way by shifting ion regulatory proteins such as Na+K+-ATPase to alter the primary function of specific rectal cells. This likely changes the overall regulatory functionality of the tissue from adsorption to secretion of specific solutes.

1172

FUNCTIONAL CHARACTERIZATION OF A PLATELET AGGREGATION INHIBITOR FROM THE SALIVARY GLANDS OF *AEDES AEGYPTI*

Saravanan Thangamani[1], Venkata D. Boppana[1], Francisco Alarcon-Chaidez[1], Jianxin Sun[2], José M.C Ribeiro[3], Stephen K. Wikel[1]

[1]University of Connecticut Health Center, Farmington, CT; Current address: Department of Pathology, University of Texas Medical Branch, Galveston, United States, [2]University of Connecticut Health Center, Farmington, CT, United States, [3]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

A complex repertoire of pharmacologically active molecules in blood feeding arthropod saliva is responsible for modulating host hemostasis, immune defenses, pain/itch, and wound healing, which facilitates blood feeding and pathogen transmission. Genomic strategies yielded previously unobtainable insights into the nature and diversity of salivary gland molecules. Understanding the function(s) of these molecules is vital to development of novel vector and disease transmission control strategies. Salivary glands of *Aedes aegypti* contain a Kazal serine protease inhibitor (AeKSPI) that inhibits fibrinogen mediated platelet aggregation. AeKSPI is a 7kDa protein containing a single Kazal domain with 6 cysteine residues forming three disulfide bonds, and shares high sequence homology to a potent thrombin inhibitor from the triatomine bug, *Dipetalogaster maximus*. Recombinant AeKSPI (rAeKSPI) specifically inhibits trypsin; is less inhibitory of α-thrombin; and, lacks activity against γ-thrombin. Mass spectrometric analysis of the protein bands obtained from a quartz crystal microbalance capture strategy and His-tag pull down experiments revealed that recombinant AeKSPI binds to the γ domain of human fibrinogen. Fibrinogen γ domain recognizes sequences for the platelet receptor GPIIa-IIIb, which mediates aggregation. We observed dose dependant inhibition of fibrinogen mediated platelet aggregation and adhesion by rAeKSPI. Far-Western blots revealed the mechanism of inhibition as the binding of rAeKSPI to fibrinogen regions that recognize GPIIa-IIIb. Results suggest that AeKSPI is a platelet aggregation inhibitory protein contributing to successful blood feeding by *Ae. aegypti*.

1173

SURVIVAL AND REPLICATION OF *WOLBACHIA PIPIENTIS* IN *ANOPHELES GAMBIAE*

Chaoyang Jin, Jason L. Rasgon

Johns Hopkins Malaria Research Institute, Baltimore, MD, United States

Cytoplasmic incompatibility (CI), induced by Wolbachia endosymbionts is of extreme interest to control vector insects for disease control. The mosquito Anopheles gambiae is the primary vector of Plasmodium parasites in Africa. No naturally occurring Wolbachia infections have ever been identified in Anopheles mosquitoes. Artificial horizontal transfer of Wolbachia not yet succeeded in Anopheles mosquitoes, although Wolbachia can infect cultured Anopheles cells in vitro. To assess the ability of Wolbachia to colonize Anopheles cells in vivo, we purified

Kamdem C., Costantini Carlo, Etouna J., Ayala Diego, Agbor J. P., Antonio-Nkondjio C., Fontenille Didier, Besansky N.J., Simard Frédéric (2008)

Ecological divergence and reproductive isolation along an urbanization gradient : habitat segregation of anopheles gambiae molecular forms in a forest area of Cameroon

In : 57th American Society of Tropical Medicine and Hygiene annual meeting. The American Journal of Tropical Medicine and Hygiene, 79 (6), 343-art. 1169

ASTMH Annual Meeting, 57., La Nouvelle-Orléans (USA), 2008/12/07-11

ISSN 0002-9637