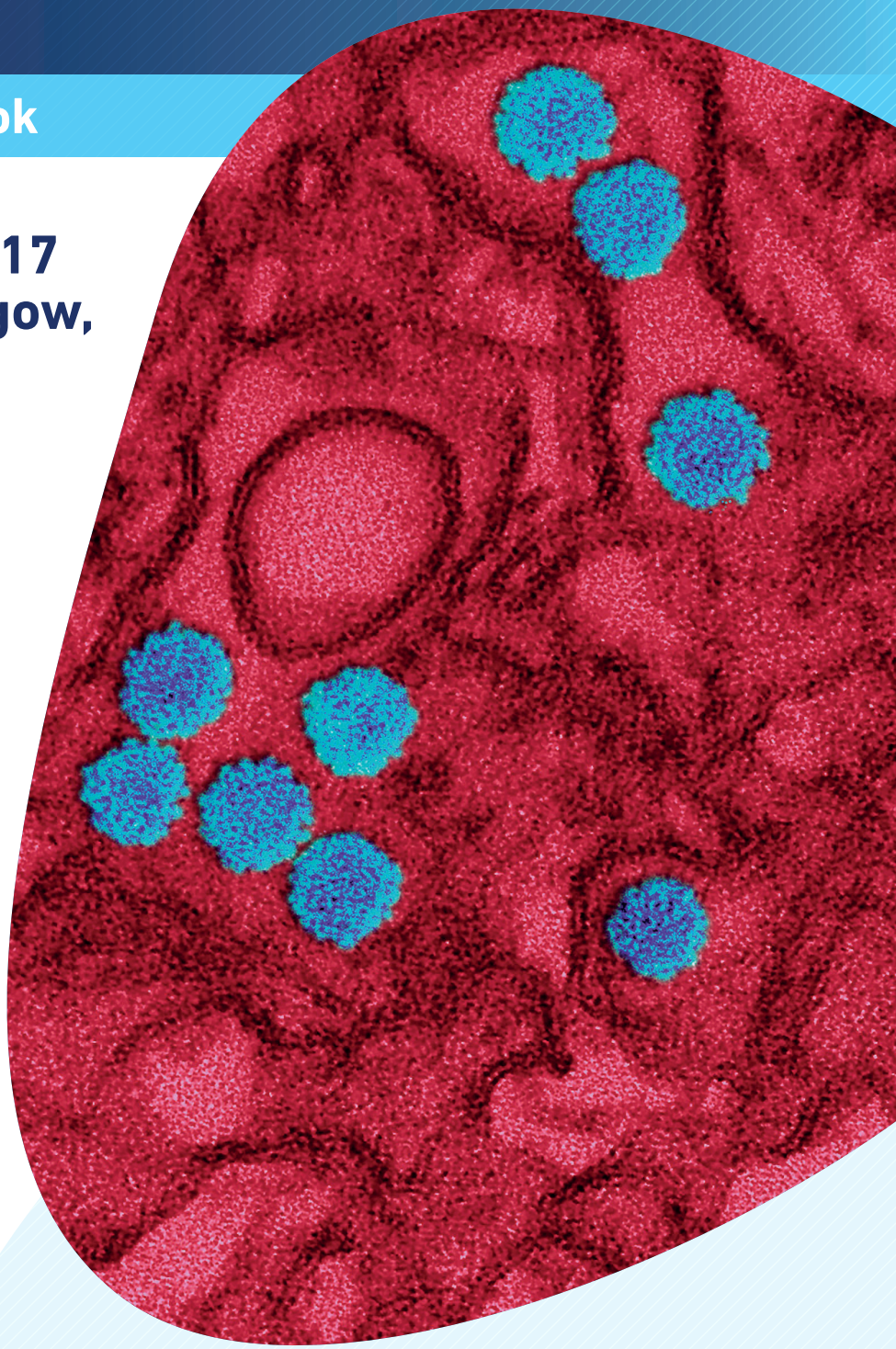
MICROBIOLOGY SOCIETY

FOCUSED MEETING 2017

2nd International Meeting on **Arboviruses and their Vectors**

Poster Abstract Book

7–8 September 2017
University of Glasgow, Glasgow, UK



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IMAV17 Poster Book Contents

Poster Sessions

All posters can be displayed for the full duration of the meeting.

There will be two poster sessions where we encourage presenting authors to stand alongside their work. Presenting times are as follows:

- Presenters of **ODD** numbers are to present on day 1, 17:30–19:00.
- Presenters of **EVEN** numbers are to present on day 2, 17:00–18:30.

Please ensure you remove your poster before you leave the meeting on Friday 8 September. The Society and the venue are unable to return any posters left behind.

Poster Number: 1

Topic: Virus-host interactions and evolution

Japanese Encephalitis Virus exploits microRNA-432 to regulate JAK-STAT signaling in human brain microglial cells

Sunit Singh

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Abstract

Japanese encephalitis virus (JEV) is a mosquito-borne neurotropic RNA virus, which infects neuronal and microglial cells and leads to neuroinflammation and motor deficits. JEV enjoys zoonotic life cycle between pigs, water birds, and culex mosquitoes. JEV modulates the antiviral signaling pathways to dampen the anti-viral response. MicroRNAs regulate the gene expression. Viruses have been reported to modulate cellular microRNA expression to regulate anti-viral signaling. SOCS family of proteins are negative regulators of JAK-STAT signaling. JEV downregulates miR-432 levels in human microglial cells and in JEV infected mice brain. JEV infection mediated miR-432 downregulation resulted in the increase in SOCS5 levels. Cells initially produce interferon which activates Janus Kinases (JAKs), which in turn phosphorylates STAT. Activated STATs dimerize and enter the nucleus to bind to ISRE elements, to trigger the expression of ISGs. JEV initially leads to STAT1 phosphorylation and activation in cells, which dampens during the late infection. JEV utilizes various strategies to dampen the anti-viral response. The increased levels of SOCS5 during JEV infection reduces the STAT1 phosphorylation and ISRE activity, which led to increased JEV replication. The overexpression of miR-432 reduced the SOCS5 expression and hence, increased STAT1 phosphorylation and ISRE activity which suppresses JEV replication. The antimiR-432 driven silencing, led to increased expression of SOCS5 and reduced STAT1 phosphorylation and ISRE activity, which further led to the increased JEV replication. JEV exploits the expression of miR-432 in order to increase SOCS5 expression, which in turns suppress the antiviral response of the human microglial cells against JEV.

Poster Number: 2

Topic: Virus-host interactions and evolution

Pathological modelling of Tick-Borne Encephalitis Virus infection using brain cells derived from human fetal neural progenitor.

Mazigh FARES[1], Gaëlle Gonzalez[1], Marielle Cochet[1], Claire Boissart[2], Alexandra Benchoua[2], Nadia Haddad[3], Muriel Coulpier[1]

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Abstract

Tick-Borne Encephalitis Virus (TBEV), a member of the *Flaviviridae* family, genus *Flavivirus*, is the most important arbovirus of health interest in Central-Northern Europe and North-Eastern Asia. It is responsible for febrile illness and, in a subset of cases, for neurological manifestation ranging from mild meningitis to severe encephalomyelitis. So far, TBEV-induced neuropathogenesis is still poorly understood. To decipher the mechanisms by which the viruses damages the human brain, we used an *in vitro* model of primary neural cells differentiated from human fetal neural progenitors. Our results showed that neurons and glial cells are susceptible and permissive to TBEV infection. The virus replicates in these cells (peak at 48-72h) and impairs them. Neuronal clustering and death (25 % loss) are observed as early as 72 hours post-infection (*pi)*. Later, at 14 days *pi*, neuronal loss has reached 70 %, at least partly through apoptotic mechanisms, and neurites have dramatically shrunk. Astrocytes survive but a hypertrophy, suggestive of a reactive stage, was observed. Using a PCR array to analyze the antiviral response, we further report an overexpression of viral sensors (RIG-I and TLRs), cytokines and innate immune response factors (IFN-I and ISGs), indicating an activation of the antiviral response. Thus, our results show that our *in vitro* model recapitulates the main events that occur in the human brain. We are currently using this model to deepen our understanding of TBEV-induced neuropathogenesis and to screen for antiviral drug.

Poster Number: 3

Topic: Virus-host interactions and evolution

Targeting functional RNA structures in emerging positive sense arboviruses

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Abstract

The positive-sense RNA arboviruses Zika virus (ZIKV) and Chikungunya virus (CHIKV) have rapidly emerged as global pathogens. To date, there are no licensed vaccines or specific antiviral therapies available for either virus. ZIKV and CHIKV are members of the flavivirus and alphavirus genera respectively, and contain structured *cis*-acting RNA elements within their genomes that are known to be essential for virus replication. We are investigating a range of approaches for targeting such genomic RNA elements in ZIKV and CHIKV and analysing the effect on virus replication at different stages of their life cycle.

Following SHAPE mapping of the RNA structures within the 5' and 3' untranslated and adjacent protein coding regions of the ZIKV and CHIKV genomes, and validation by reverse genetics, we have designed oligonucleotides incorporating locked nucleic acids (LNAs) which target specific RNA structures within the viral genomes. Additionally, we aim to select RNA structure specific RNA aptamers using SELEX and adhiron non-antibody binding proteins (Affimers) against specific stem-loops using a novel peptide display scaffold.

Utilising these complementary approaches, we aim to target and disrupt the function of individual RNA structural elements, through inhibition of RNA-RNA and RNA-protein interactions required for genome replication. We expect such reagents to be a powerful tool for investigating mechanisms and interactions by which viral RNA elements function within the host cell environment. Furthermore, they will provide a proof of concept for the viability of therapeutic antiviral compounds targeting essential viral RNA stem-loop/*trans*-activator interactions.

Poster Number: 4

Topic: Virus-host interactions and evolution

Investigating the essential role of the nsP3 macro domain in Chikungunya virus replication

Grace Charlotte Roberts, Nicola J Stonehouse, Mark Harris

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Abstract

Chikungunya virus (CHIKV), is a positive sense, single-stranded RNA arbovirus that has recently re-emerged, causing multiple disruptive large-scale outbreaks across the globe. CHIKV encodes four non-structural proteins (nsP1-4). The functions for these proteins in the virus lifecycle have been established apart from nsP3, the precise function of which remains unclear. All alphaviruses encode an nsP3 comprised of three domains. The N-terminus being a macro domain, a highly conserved domain found in the proteins of all organisms, including many positive sense, single-stranded RNA viruses. They are known to bind RNA and ADP-ribose and to exhibit ADP-ribose hydrolase activity. We aimed to determine the function of the nsP3 macro domain in viral replication.

We generated multiple macro domain mutants at key residues in the RNA and ADP-ribose binding pocked based on the published crystal structure of the CHIKV nsP3 macro domain. In replicon, these mutants exhibited a wide range of phenotypes in RNA replication that varied between cell types. Based on our data, and published biochemical analysis of the macro domain by others, indicates a role for the CHIKV macro domain that does not involve RNA or ADP-ribose binding.

We have investigated whether the macro domain of CHIKV interacts with host innate immune pathways. Our preliminary data suggests that the wt macro domain is able to disrupt the NFκB pathway, potentially through its hydrolase activity. We are also investigating possible interactions with the IFN pathway, which has recently been shown to enhance spread of the virus around the body.

Poster Number: 5

Topic: Virus-host interactions and evolution

Zika virus NS5 protein interferes with the RIG-I signaling pathway and inhibits the activation of interferon λ1 expression

Rickard Lundberg[1], Krister Mélen[2], Miao Jiang[2], Veera Westenius[2], Pamela Österlund[2], Olli Vapalahti[3], Ilkka Julkunen[1], Laura Kakkola[1]

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Abstract

Zika virus (ZIKV) is a mosquito-borne virus belonging to the *Flaviviridae* family. It is an enveloped virus with a 10.8 kb long single-stranded positive-sense RNA genome, which encodes for 4 structural proteins and 7 non-structural proteins. ZIKV infections have been associated with severe complications , such as Guillain- Barré syndrome and microcephaly. This lead to a global health concern, and ZIKV was declared by WHO a public health emergency. Recent studies have shown ZIKV to interfere with human innate immune responses, especially with interferon-induced responses. At present it is not known whether ZIKV can also interfere with the RIG-I or TLR signaling cascades and the expression of cytokine genes such as IFN genes.

The present study was initiated to analyze whether any of the ZIKV proteins can interfere with the RIG-I induced IFN-λ1 gene expression. For this purpose we cloned all 11 ZIKV genes into a mammalian expression vector. We co-transfected HEK293 cells with constitutively active form of RIG-I, IFN-λ1-luciferase reporter-plasmid and ZIKV expression plasmids. The effect of expressed ZIKV protein on RIG-I induced IFN-λ1-promoter activation was measured with luciferase assay. We found ZIKV NS2B and especially NS5 proteins to inhibit the RIG-I-mediated activation of IFN-λ1 promoter. The mechanism of action for these inhibitory effects will be determined in future studies.

Poster Number: 6

Topic: Virus-host interactions and evolution

Host range restriction of insect-specific flaviviruses occurs at several levels of the viral life cycle

Beate Kümmerer[1], Marvin Korries[1], Wolfgang Grasse[2], Janett Wieseler[1], Anne Kopp[1], Kyra Hermanns[1], Moises León-Juárez[1], Christian Drosten[1,3], Sandra Junglen[1]

[1]University of Bonn Medical Centre, Institute of Virology, Bonn, Germany, [2]Robert Koch Institute, Berlin, Germany, [3]German Centre for Infection Research (DZIF), partner site Bonn-Cologne, Bonn, Germany

Abstract

Most viruses of the genus *Flavivirus* are mosquito-borne and transmitted to vertebrates during blood-feeding of mosquitoes. Within the last decade an increasing number of insect-specific flaviviruses (ISFs) was discovered in mosquitoes. ISFs are closely related to the vertebrate pathogenic flaviviruses but their host range is exclusively restricted to insects. Elucidation of the ISF infection block in vertebrates could identify functions necessary for the host range expansion towards vertebrates.
We isolated a novel flavivirus, termed Niénokoué virus (NIEV), from mosquitoes in Côte d'Ivoire. NIEV groups with ISFs in phylogeny and grows in insect- but not vertebrate cells. To elucidate at which levels ISFs are blocked with regard to infection of vertebrates, we generated an infectious NIEV cDNA clone and a NIEV reporter replicon to study growth restrictions of NIEV in comparison to YFV. Efficient RNA replication of the NIEV reporter replicon was observed in insect- but not vertebrate cells. Initial translation of the input replicon RNA in vertebrate cells was functional, but RNA replication did not occur. Chimeric YFV carrying the envelope proteins of NIEV was recovered via electroporation in C6/36 insect cells but did not infect vertebrate cells, indicating a block at the level of entry. Since the YF/NIEV chimera readily produced infectious particles in insect- but not vertebrate cells despite efficient RNA replication, restriction is also determined at the level of assembly/release.
Together, our studies determined blocks at several levels suggesting that flavivirus host range expansion from insects to vertebrates was a complex process that involved overcoming multiple barriers.

Poster Number: 7

Topic: Virus-host interactions and evolution

Mapping protein-protein interactions between tick-borne flaviviruses and their different mammalian hosts

Sandrine A Lacour[1], Manon Lemasson[1], Yves Unterfinger[1], Sara Moutailler[2], Sarah Bonnet[2], Lesley Bell-Sakyi[3,4], Grégory Caignard[1], Damien Vitour[1], Houssam Attoui[1], Jennifer Richardson[1]

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Abstract

Tick-borne viruses represent a major threat to human and animal health, against which effective and sustainable strategies for prevention and control must be sought. Nonetheless, current understanding of the interactions between tick-borne viruses and their various host species is fragmentary, thus limiting conception of innovative approaches. Insight into virus-host relationships can be gained by large-scale mapping of virus-host protein interactions by high-throughput proteomics. As regards tick-borne viruses, such interactions are likely to be important for viral carriage in mammalian and arthropod hosts and interspecies transmission. In Europe, two tick-borne flaviviruses, tick-borne encephalitis virus (TBEV) and Louping Ill virus (LIV), are responsible for neurological disease in humans and sheep, respectively. The aim of our study is to investigate interactions between TBEV and LIV, which are of concern to human and veterinary health, respectively, and their different mammalian host species, humans and ruminants. To this end, the network of protein-protein interactions established between viral proteins and proteins encoded by cDNA librairies of *Homo sapiens* and *Bos taurus* is being resolved using the yeast two-hybrid method. Once putative interactions between viral and host proteins have been identified, these will be validated at biochemical and functional levels *in vitro* in appropriate cell lines. Moreover, interactions between selected viral proteins and innate immune pathways are being explicitly addressed. Comparison of the interactomes in different vertebrate hosts is likely to illuminate the molecular bases of viral pathogenesis for these viruses.

Poster Number: 8

Topic: Virus-host interactions and evolution

Investigating *Wolbachia*-mediated arbovirus inhibition

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Abstract

Wolbachia are maternally transmitted bacterial endosymbionts found in a broad range of arthropods, including some mosquito species. Some Wolbachia strains can manipulate host reproduction to ensure their propagation throughout a population. Wolbachia are naturally found in Aedes albopictus, but not the closely related Aedes aegypti, the primary vector of Dengue virus. When artificially transinfected into Ae. aegypti Wolbachia can provide a pathogen-blocking phenotype, limiting the transmission of some viruses, including Dengue, Chikungunya and Zika. Wolbachia-infected Ae. aegypti are currently being implemented as a vector control strategy, yet the precise mechanism of pathogen-blocking is unknown. Pan et al (2012) showed higher levels of Reactive Oxygen Species (ROS) in Wolbachia-infected mosquitoes which, they hypothesise, activates the Toll immune pathway, resulting in Dengue inhibition. However, others have shown that immune upregulation is not required for pathogen blocking, therefore the role of ROS in Wolbachia-mediated inhibition is somewhat ambiguous.

We investigated the role of Reactive Oxygen Species (ROS) in Wolbachia-mediated pathogen blocking in the Aag2 - Aedes aegypti cell line. Results suggest higher levels of ROS in Wolbachia-infected cells, but similar levels of lipid peroxidation. We found reduced Xbp1 splicing and Calnexin levels associated with Wolbachia infection – both of which are involved in endoplasmic reticulum (ER) homeostasis. These data suggest Wolbachia can interfer with the ER which may prevent some viruses from using this crucial organelle for replication.

Poster Number: 9

Topic: Virus-host interactions and evolution

Should I stay or should I go? Opal stop codon read-through during Chikungunya virus non-structural polyprotein expression

Raymond Li[1], Andres Merits[2], Andrew Tuplin[1], Mark Harris[1]

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Abstract

Read-through of stop codon signals during translation can result in the production of different virus polyprotein precursors. In this regard, some strains of Chikungunya virus (CHIKV) possess a naturally occurring opal stop codon near the end of the non-structural protein 3 (nsP3) coding region. Read-through at this site results in the expression of all four nsPs, while termination would only allow the production of nsP1, nsP2 and a C-terminally truncated nsP3.

The effect of this naturally occurring opal stop codon on CHIKV genome replication and translation was investigated using both a subgenomic replicon and full length infectious virus. Opal, ochre or amber stop codons were substituted into a dual luciferase reporter CHIKV subgenomic replicon derived from the East Central South African (ECSA) strain of the virus that naturally lacks the opal stop codon. In human cells and C2C12 murine muscle cell lines, replication of the opal and amber mutants was comparable to that of the wildtype CHIKV replicon, while the ochre mutants were unable to replicate; suggestive of specific read-through of the opal and amber stop codons.

Intriguingly, our results suggest that the ochre and amber mutants were able to replicate in C6/36 *Aedes albopictus* but exhibited a delay in replication. We are currently investigating whether this delay is a genuine replication phenotype or due to reversion of these mutants to wildtype. Further work into how the presence or absence of an opal stop codon in different isolates of CHIKV influences early replication events is also underway.

Poster Number: 10

Topic: Virus-host interactions and evolution

Interactomic high-throughput mapping for Bluetongue virus in its natural hosts: towards the identification of new factors of pathogenicity and interspecies transmission.

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Abstract

Bluetongue virus (BTV) is an arbovirus responsible for bluetongue (BT), a non-contagious disease that affects a wide range of wild and domestic ruminants, inducing. It is transmitted by blood-feeding midges belonging to the *Culicoides* genus. BTV induces a large panel of clinical manifestations depending primarily on the infected host and the viral strains. Despite many studies, we still have little understanding of the molecular determinants of BTV virulence. We took advantage of the yeast two-hybrid (Y2H) method to map interactions between BTV and cellular proteins. As a starting point, this approach has been applied on serotype 8 of BTV (BTV-8), which has caused considerable economic losses between 2006 and 2010 and has recently re-emerged in France. Viral proteins were used as baits to screen two cDNA libraries originating from cattle and *Culicoides*. Therefore, 43 screens were performed and 1,478 positive yeast colonies were analyzed, allowing us to identify a hundred of new virus-host interactions. A preliminary global analysis of these interactions has uncovered many signal transduction factors involved in autophagy, apoptosis and the ubiquitin-proteasome system. All the cellular interactors are currently re-tested by several protein-protein interaction methods including GST-pulldown and GPCA (*Gaussia princeps* protein complementation assay) with the viral proteins encoded by BTV-8 and also other serotypes. Conserved protein interactions will be instrumental to design generic drugs against multiple serotypes. On the other hand, interactions that are highly specific of a particular virus would shed light on the molecular mechanisms responsible for its virulence/pathogenicity and transgression of cross-species barriers.

Poster Number: 11

Topic: Virus-host interactions and evolution

The Chikungunya virus nsP3 alphavirus unique domain (AUD) has multiple functions during the virus lifecycle.

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Abstract

Chikungunya virus (CHIKV) is a re-emerging arbovirus characterized by fever and joint pain. Antiviral therapies and effective vaccines are urgently required. This project seeks to investigate the functions of CHIKV nsP3, especially the alphavirus unique domain (AUD) in virus replication to identify targets for antiviral intervention and vaccine development.

Firstly, informed by Sindbis virus AUD structure and alignment of the amino acid sequences of various alphaviruses, a series of AUD mutations were generated in the context of a CHIKV sub-genomic replicon. These mutants exhibited distinct species- and cell-type specific phenotypes, revealing that the AUD plays an essential role in CHIKV replication. One mutant (R243A/K245A) replicated in mosquito cells but not in mammalian cells. A second mutant (M219A) only exhibited a phenotype in mosquito cells that had a defect in RNAi pathway, suggestive of a role for the AUD in counteracting host defences. The phenotypes of the mutant panel were also analysed in infectious CHIKV, the results largely correlated with the replicon data. Interestingly, mutant E225A was replication competent but defective in the production of infectious virus.

In parallel we investigated the RNA-binding activity of AUD. RNA filter binding assay demonstrated that purified AUD could bind to CHIKV 3'UTR. Interestingly, the R243A/K245A mutant was found to be defective in RNA-binding activity.

Our data reveal for the first time that the AUD may be a pleiotropic protein domain, functioning in both genome replication and virus assembly, as well as playing a role in counteracting host defences in both mammalian and mosquito cells.

Poster Number: 12

Topic: Virus-host interactions and evolution

Zika Virus Infection of Human Umbilical Vein Endothelial Cells Induces Cell Death and Activation of Secondary Hemostasis

Fatih Anfasa[1,2], Marco Goeijenbier[1], Widagdo Widagdo[1], Jurre Siegers[1], Noreen Mumtaz[1], Nisreen Okba[1], Debby van Riel[1], Marion Koopmans[1], Joost Meijers[1], Byron Martina[1,3]

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Abstract

Introduction: Zika virus (ZIKV) is an emerging arbovirus that belongs to the *Flaviviridae* family. Symptomatic patients generally develop a mild febrile illness which lasts for 3-7 days. However, during the recent outbreaks, several hemorrhagic manifestations were reported. In contrast, a recent study found segmental thrombosis in the umbilical cord of pregnant rhesus macaques that were infected with ZIKV. Other study also showed that ZIKV-infected *Ifnar*$^{-/-}$ mice show signs of vascular damage in the placenta and fewer fetal blood vessels.

Aim: We intend to characterize the effect of ZIKV infection in human umbilical vein endothelial cells (HUVECs) and determine whether infection induces activation of secondary hemostasis.

Methods: We infected HUVECs with two ZIKV strains and performed virus titration, immunostaining, and flow cytometry to determine replication kinetics and induction of cell death on HUVECs. We also performed ELISA to determine interleukin (IL)-6, IL-8 and von willebrand factor (vWF) production. A thrombin generation test (TGT) was performed as a functional test to asses secondary hemostasis.

Results: Both ZIKV strains infected and replicated to high titers in HUVECs. There was a significant increase of IL-6 and IL-8 production after infection. Flow cytometry data revealed that ZIKV infection induces cell death mainly through direct infection. Furthermore, we found evidence that infection induces shortened TGT time.

Conclusion: Here we demonstrate that ZIKV replicates efficiently on HUVECs and induces production of pro-inflammatory cytokines and cell death mainly through direct infection. Additionally, we also showed for the first time that infection induces activation of secondary hemostasis.

Poster Number: 13

Topic: Virus-host interactions and evolution

Structural and biochemical analysis of protein/RNA interactions during initiation of dengue virus genome replication

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Abstract

The dengue virus (DENV) genome contains *cis*-acting RNA structures forming alternative linear and cyclised conformations - essential for viral replication. Genome cyclisation, stabilized by direct RNA-RNA interactions, is essential for initiation of viral replication requiring remodelling of local RNA *cis*-acting structures, to transfer the RNA-dependent RNA polymerase (RdRp) to the 3' end of the genome. However, the mechanisms and RNA/protein interactions involved in stabilising alternative RNA structures and initiating conformational switching remain unclear. This study aims to determine RdRp and genome conformational changes associated with initiation of DENV replication.

DENV-2 RdRp was purified and activity confirmed by *de novo* α-^{32}P nucleotide incorporation and Fluorescent Polarisation Anisotropy. We used X-Ray crystallography to determine the structure of the RdRp, solved by molecular replacement, to a resolution of 2.2 Å. RdRp/RNA co-crystallisation trials are ongoing to determine structural changes to the polymerase when bound to promoter RNA.

We are investigating initiation/stabilisation of RNA conformational changes within the DENV genome, associated with RdRp binding (in the context of varying viral/host cell factors) using SHAPE - in which RNA is folded and probed in the presence of *trans*-activating factors. Studies suggest that full-length DENV genomes, in the absence of *trans*-activating factors, favour the linear conformation. Furthermore, we observe that polymerase binding destabilises local RNA structures and increases the flexibility of adjacent unpaired regions. We hypothesise that these structural rearrangements are involved in preparing the genome for cyclisation and RdRp transfer to the 3' promoter – essential for initiation of DENV genome replication.

Poster Number: 14

Topic: Virus-host interactions and evolution

Phosphorylation sites in the C-terminal domain of Chikungunya virus nsP3 are uniquely distributed

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Abstract

Chikungunya virus (CHIKV) is a medically relevant alphavirus of the *Togaviridae* family. The alphaviral replicase consists of four non-structural proteins nsP1–4, which act as mediators of a wide range of host-pathogen interactions. The specific functions of nsP3 are not yet completely understood, but as it interacts with multiple cellular proteins, its C-terminal domain (CTD) is phosphorylated by cellular kinases in model alphaviruses Semliki Forest virus (SFV) and Sindbis virus (SINV). We aimed to investigate if CHIKV nsP3 is phosphorylated altogether, and whether the probable phosphorylation sites map to similar regions as known in said examples. We radiolabelled cells infected with various CHIKV constructs containing suspected phosphorylation site mutations, and determined the incorporation of phosphate groups by immunoprecipitation of nsP3 and autoradiography. Supporting complementary data was collected by mass-spectrometry. We confirmed that CHIKV nsP3 of both the East/Central/South African and Asian genotypes, as well as nsP3 of a close relative, O'nyong'nyong virus, is indeed a phosphoprotein. Deleting the beginning of nsP3 CTD, which in SFV has been shown to include all phosphorylated amino acid residues, did not abolish phosphorylation of CHIKV nsP3. Thus, differing from those of SFV, CHIKV phosphorylation sites do not distribute as a cluster in the beginning of nsP3 CTD, and are possibly more disperse along the protein. Further research is needed to study the importance of this unique distribution regarding the effect on host-pathogen interactions, including the location of replicase complexes in cells and *in vivo* properties of phosphorylation-altered constructs.

Poster Number: 15

Topic: Virus-host interactions and evolution

Variable inhibition of dengue virus replication by different *Wolbachia* strains in mosquito cell cultures

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Abstract

Globally, dengue is the pre-eminent arboviral disease in terms of morbidity and mortality. A biocontrol strategy pioneered in Australia and currently being trialled in several countries, utilizes the insect endosymbiotic bacteria *Wolbachia* to stop dengue transmission. Several strains of *Wolbachia* are now available for biocontrol but their effects on different strains of dengue virus (DENV) are unknown. Using insect cell lines, we tested the effect of two strains of *Wolbachia*, *w*MelPop and *w*AlbB, on nine strains of DENV belonging to all four serotypes. Time-course experiments showed that the strongest DENV blocking occurs at later time-points during infection. Both *w*AlbB and *w*MelPop showed significant blocking of all DENV strains versus a *Wolbachia*-uninfected control. At various multiplicities of infection (MOIs), by days 6 to-8 post infection, both *w*AlbB and *w*MelPop showed similar blocking strength across all 9 strains (69.4% and 77.8% respectively, $P>0.05$; Chi-squared test of association). However, earlier in the time course, *w*MelPop provided stronger blocking effect than did *w*AlbB (41.7% v 19.4%; $P<0.05$). These results suggest that DENV blocking by *w*AlbB in cell lines may be comparable to *w*MelPop, probably due to similar *Wolbachia* density levels. Our results also showed that the extent of blocking was more dependent on virus strain than on serotype and that blocking effects may vary in strength as *Wolbachia* densities are dynamic through time. Our findings may have implications for the use of *w*AlbB as a biocontrol strain.

Poster Number: 16

Topic: Virus-host interactions and evolution

Interaction of the natural RNA virome with Anopheles vectors of malaria

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Abstract

Mosquitoes are colonized by a largely undescribed natural virome which probably influences mosquito biology and immunity, but tractable experimental models are lacking. We collected human-seeking wild Anopheles in disease-emergence zones in Senegal and Cambodia, and screened for actively replicating RNA viruses by de novo assembly of small RNA sequences. Two novel members of the virome, a dicistrovirus (Anopheles C virus, AnCV), and Anopheles cypovirus (AnCPV), were developed into an experimental model for bloodmeal infection of Anopheles to study mosquito-virus interactions. Abundance of the two viruses is negatively correlated in individual mosquitoes, and functional genomic analysis reveals differential impact of mosquito immune pathways on replication of the two viruses. Sequences of AnCPV are highly polymorphic in individual mosquitoes, while AnCV is virtually devoid of variation, but both viruses display worldwide distribution in Anopheles, suggesting radically different mechanisms for their successful host adaptation. In addition to transmitting vertically, AnCPV appears capable of transmitting like an arbovirus through a mammalian host to uninfected mosquitoes, suggesting that the evolutionary pathway from vertical “insect-specific” to infectious blood transmission may be remarkably simple.

Poster Number: 17

Topic: Virus-host interactions and evolution

Structure-function studies of dengue virus NS1 reveals new roles of the protein in the virus life cycle

Richard Kuhn, Michael White, Thu Cao, Andrew Miller

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Abstract

Dengue virus (DENV) is an arthropod borne human pathogen within the *Flaviviridae* family. Of the ten DENV viral proteins, NS1 remains the most elusive in terms of function. Currently NS1 has been linked to vascular permeability and disease progression, and was recently suggested to have a role in virion assembly in addition to its role in viral RNA synthesis. Although NS1 has been implicated in these various functions, the mechanistic bases for these are unknown. To interrogate these functions, we have carried out a large scale molecular genetic analysis of DENV, West Nile and Zika NS1 proteins and identified several residues that influence NS1 and structural protein secretion, virus particle assembly and infectivity. We have now further characterized several of these variants and have come across a novel role of NS1 in DENV particle infectivity. An amino acid substitution of E343K located in a solvent exposed region on the β-ladder domain of NS1 showed an equivalent number of capsid, envelope and RNA containing particles to be secreted as wild-type DENV, but this E343K virus was significantly reduced in viral titer. After further characterization, it appears that this mutation in NS1 produces virus particles with an altered density while apparently being less efficient in virus attachment to host cells. Additional mutants that display wild-type RNA synthesis but varied phenotypes with respect to assembly and secretion will be presented. This is the first report of the flavivirus NS1 protein affecting virus infectivity and potentially influencing entry into cells.

Poster Number: 18

Topic: Virus-host interactions and evolution

Discovery, characterization and functional analysis of novel WNV-derived small noncoding RNAs

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Abstract

Innate immune response to West Nile virus (WNV) in vertebrates primarily relies on type I IFN pathway and WNV has evolved to counterpart this response by employing viral non-structural proteins and 3'UTR-derived noncoding RNA (sfRNA). However, we have now found that sfRNA is not the only noncoding RNA generated from the 3'UTR of WNV. Using RNA-Seq analysis we have identified novel noncoding RNAs derived from the 3'-terminal stem loop (3'SL) of the WNV 3'UTR. The production of these RNAs by different strains of WNV in all tested cell lines from mammalian and mosquito hosts and also in the brain of WNV-infected mice was confirmed by Northern blot. The biogenesis of these RNAs was found to be independent of host miRNA/siRNA processing machinery, RNaseL and XRN-1, and required viral RNA replication and/or viral non-structural proteins. It involves two cleavage steps undertaken by different nucleases. First, the larger 100nt RNA that includes the 3'SL and the preceding small hairpin is excised from viral RNA by an RNase that produces 5'OH-end. Subsequently, this RNA is cleaved into two smaller RNAs (32 and 68nt), by another RNase that produces 5'-P-end. Unlike siRNAs and miRNAs, these 3'SL-derived noncoding RNAs did not incorporate into RISC and did not regulate expression of reporter mRNAs containing WNV noncoding RNA-complementary sites in their 3'UTR. Our results suggest that the identified here novel WNV 3'-UTR-derived noncoding RNAs abundantly produced during infection may employ potentially novel regulatory mechanism unrelated to RNAi to exert their putative biological function(s).

Poster Number: 19

Topic: Virus-host interactions and evolution

Molecular Determinants of Epidemiological Fitness of Dengue Virus

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Abstract

Mutations in the dengue virus (DENV) genome has been associated with major outbreaks without any change in the relative sero-prevalence. These observations indicate that the DENV genome encodes factors that define its epidemiological fitness. We recently identified that mutations in the non-coding 3' untranslated region (UTR) of the genome resulted in increased production of sub-genomic RNA that inhibited type-I interferon expression for increased viral fitness. These mutations could have contributed to the 1994 DENV2 epidemic in Puerto Rico, which coincided with a clade replacement of the endemic clade PR1 to the epidemic PR2B, despite the lack of a serotype switch. However, our study also identified several other amino acid substitutions in the coding region of the DENV2 genome in the epidemic compared to pre-epidemic isolates. To define which of these mutations could have also contributed to the increased epidemiological fitness of the Puerto Rico DENV2, we performed an ancestral state reconstruction analysis of the DENV2 codon phylogenies to study its molecular evolution. This analysis identified mutations in the NS5 gene as critical for the divergence of the epidemic strain PR2B. With a reverse genetic approach, using Gibson assembly multi-site directed mutagenesis, we synthesized infectious clones of these viruses to examine how these mutations could affect fitness. Our results show that mutations in the NS5 gene altered viral replication rate and induction of interferon (IFNb) response in host cells. We therefore speculate that NS5 regulates DENV2 replication and with the 3'UTR synergistically modulate the innate immune response for viral fitness.

Poster Number: 20

Topic: Virus-host interactions and evolution

Rift Valley fever virus modulates immune responses in *Aedes aegypti* Aag2 cells.

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Abstract

Rift Valley fever virus (RVFV) is a mosquito-borne *Phlebovirus* of the *Bunyaviridae* family. The virus infects both ruminants and humans and infection can be fatal. Recent outbreaks of Rift Valley fever outside Africa raised concerns about the virus spreading to previously non-endemic areas including Europe and Asia. In the absence of licenced vaccines and effective disease treatment measures, scientific efforts are more and more focussing on interrupting the viral transmission by mosquitoes. However, little is known about the interactions of RVFV with its mosquito hosts. In this study we aimed to better characterize mosquito innate immune responses to RVFV infection that may determine viral replication and transmission.
Aedes aegypti Toll, immune deficiency (IMD) and JAK-STAT immune pathways were stimulated in Aag2 cells with Gram-positive and Gram-negative bacteria and the effect of immune activation on RVFV replication was assessed. Our findings indicate that bacterial stimulation led to a marked cross-activation of all immune pathways and, interestingly, a significant increase of RVFV replication. RVFV infection of Aag2 cells itself resulted in a minor upregulation of the expression of the antimicrobial peptides attacin and diptericin and in an enhancement of immune responses to bacterial stimulation. These findings imply that, while mosquito innate immunity is not able to restrict RVFV *in vitro*, infection with RVFV causes changes to the immune system of vector mosquitoes that should be investigated further to aid the development of disease prevention strategies.

Poster Number: 21

Topic: Virus-host interactions and evolution

Silent infection by African and Asian strains of Zika virus in human dendritic cells

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Abstract

While Zika virus (ZIKV) circulated for decades (strains of the African lineage) without report of outbreaks and severe complications, the emergence of ZIKV in French Polynesia and subsequently in the Americas (strains of the Asian lineage) was associated with description of severe neurological defects in newborns/neonates and adults. With the aim to identify viral lineage-dependent factors, we compared cell susceptibility, virus replication, cell death and innate immune responses following infection with three Asian and two African lineage strains of ZIKV. To this end, we employed two cell lines frequently used for virus propagation including, green monkey kidney Vero and *Aedes albopictus* C6/36, as well as human monocyte-derived dendritic cells (MoDCs). The latter are involved in the pathogenesis of several mosquito-borne *Flavivirus* infection. In Vero and C6/36 cells, we observed strain- but not lineage-dependent differences in infection profiles. Nevertheless, in human MoDC no significant differences in susceptibility and virus replication were found between ZIKV lineages/strains. ZIKV did not or only in a limited fashion induce antiviral interferon type I and III, with the exception of one strain of the African lineage. None of the ZIKV strains induced cell death or DC maturation in terms of MHC class II, CD40, CD80/86 or CCR7 expression.

Poster Number: 22

Topic: Virus-host interactions and evolution

domino is antiviral and regulates the siRNA pathway in *Aedes aegypti* mosquitoes

Melanie Macdonald[1], Floriane Almire[1], Joy Kean[1], Mathilde Laureti[1], Claire Donald[1], Margus Varjak[1], Marie Vazeille[2], Anna-Bella Failloux[2], Alain Kohl[1], Emilie Pondeville[1]

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Abstract

Aedes aegypti female mosquitoes are vectors of arboviruses such as Zika virus (ZIKV) and Chikungunya virus (CHIKV). *Ae. aegypti* mosquitoes possess different immune pathways of which the major small interfering (si)RNA pathway that limits arbovirus replication. To identify new antiviral genes in *Aedes*, we performed a screen in mosquito cells on candidates previously shown to be involved in the siRNA pathway in the fly *Drosophila melanogaster*. Among candidate genes, we identified the gene *domino* (also called *p400*) as antiviral in mosquito cells and also *in vivo* in *Ae. aegypti* infected females. Moreover, dom is regulating *in vitro* and *in vivo* the expression of *argonaute-2*, a key player of the siRNA pathway, strongly suggesting that dom is antiviral by mediating the antiviral siRNA pathway. Tissue-specific analysis of *dom* gene expression and immunofluorescence assays show that dom is expressed in different female tissues. Interestingly, tissue-specific analysis of *dom* function revealed that this gene regulates the siRNA pathway in a tissue-specific manner.

Poster Number: 23

Topic: Virus-host interactions and evolution

Inhibition of Semliki Forest Virus Replication Through Disruption of Lipid Homeostasis

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Abstract

Cellular metabolic processes are fundamental to replication strategies of viruses. Aspects of lipid metabolism may be important from the perspective of energy production, virus envelope structure, and compartmentalization of virus nucleic acid synthesis and replication. In this study we have examined the effects of statin and non-statin drugs on replication of Semliki Forest virus in arthropod and mammalian cell lines. The data indicate that dynamic replenishment of plasmamembrane cholesterol is sufficient to facilitate the entry of virus particles in both mammalian and insect cells. Although insects lack the cholesterol biosynthetic pathway, drugs that down-regulate cholesterol synthesis in mammals are shown to inhibit virus replication in both mammalian and insect cell lines. A cholesterol-free source of fatty acids is shown to rescue translation of virus encoded genes from the effects of the drugs, but interestingly does not rescue virus replication.

Cholesterol is known to be important for the entry/fusion step in both insect and mammalian cell culture, and insects are able to obtain cholesterol from blood meals or by conversion of phytosterols ingested from plants. However the inhibitory effect of statin drugs in insect cell cultures is difficult to explain, as these are inhibitors of cholesterol biosynthesis. This, and the rescue of virus translation by non-sterol lipids in cholesterol-depleted cells, suggests a complex interaction of sterol and non-sterol lipids, potentially at multiple stages of the replicative cycle.

Poster Number: 24

Topic: Virus-host interactions and evolution

Mosquito biting modulates Zika virus infection in vivo

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Abstract

Arboviruses are a large, diverse group of viruses that cause a wide spectrum of diseases in humans. This heterogeneity, combined with our inability to accurately predict the nature and timing of future epidemics, makes developing and stockpiling *virus-specific* drugs and vaccines very challenging. Studying common aspects of all these infections provides an opportunity to target mechanisms common to a large number of arboviruses. All mosquito-borne viruses share a common attribute; their site of inoculation at mosquito bite sites. Infected mosquitoes transmit virus to the mammalian host as they probe the skin and deposit saliva. Establishment of viral replication in the skin represents a key stage of infection, in which virus replicates rapidly and then disseminates to remote tissues. We have recently shown that inflammatory responses to mosquito bites inadvertently enhances severity infection by virus. As such, our seminal finding identifies an important and novel aspect of the host immune response to mosquito bites that paradoxically promotes arbovirus infection. Here, we show how host responses to mosquito bites modulate infection with Zika virus (ZIKV). ZIKV could not replicate in immunocompetent mice, irrespective of the presence of a mosquito bite. However, when type I interferon responses are repressed, ZIKV was able to replicate and disseminate to multiple tissues. We report the effect of mosquito bites in this model, and identify cell types infected. Ultimately these findings have the potential to inform the development of novel, broadly applicable therapeutic strategies that have the potential to prevent infection, alter disease outcome and risk-stratify patients.

Poster Number: 25

Topic: Virus-host interactions and evolution

Gut antiviral immunity is down-regulated after blood-feeding in the mosquito *Aedes aegypti*

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Abstract

The female mosquito *Aedes aegypti* needs a blood meal on a vertebrate host to reproduce and is therefore a vector of arboviruses such as Zika (ZIKV) or chikungunya (CHIKV) viruses. However, mosquitoes possess different immune pathways to fight against pathogens. But until now, tissues expressing these pathways are still unknown. Moreover, a blood meal triggers important changes in female gene expression but the impact of blood-feeding on immune gene expression in tissues has not been addressed yet. For this reason, the lab analysed immune gene expression in the different tissues from the female mosquito before and after blood-feeding: immune pathways are expressed differently in all the tissues and surprisingly, they are specifically down-regulated in the gut after a blood meal. This finding is interesting since the gut is the first barrier to be overcome by an arbovirus during a blood meal and it could explain the high susceptibility of *Ae. aegypti* to arboviruses. By following the dynamic of immune gene expression after blood-feeding, we show that the repression occurs from 6 to 30 hours post blood meal, possible ideal window of time for an arbovirus to infect the first midgut cells. To investigate this, we are now testing different hypotheses that could explain this specific-gut immune gene repression with the aim to inhibit it and test whether this repression could explain the high susceptibility of *Ae. aegypti* to arboviruses.

Poster Number: 26

Topic: Virus-host interactions and evolution

Interrogation of the CHIKV nsP-host interactome in human and mosquito cells.

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Abstract

Chikungunya virus replicates in cells of both its vertebrate host and insect vector. To identify cellular pathways that the virus engages to allow optimal replication in these evolutionary distinct organisms we performed AP-MS to identify interaction partners of the viral non-structural proteins (nsPs) in both human and mosquito cells. CHIKV nsPs were C- or N-terminally Strep-tagged and transiently expressed in either HEK293T or C6/36 cells. Mass spectrometric analysis of on-bead-digestions of affinity purifications coupled to MiST analysis allowed sensitive and reproducible identification of a significant number of cellular protein interaction partners of nsP1, -3 and -4. The retrieval of well-established nsP3 interactors, G3BP and Bin1, in both human and mosquito cells validated our approach. Separate nsPs were associated with both shared and unique interaction partners, the latter belonging to different cellular pathways. Comparison of high-confidence interactors of nsP3 in human and mosquito cells identified 25 proteins that associate with nsP3 in both organisms. Functional classification of these shared nsP3 interactors using GO annotation showed engagement of cell-cell adhesion-, Hippo signaling-, ribosomal function- and innate immune signaling pathways by nsP3 in both human and mosquito cells. Interaction of members of each functional group with nsP3 were validated in AP-WB experiments. We will present data on the establishment of the virus-host interactome and the identification of a 'core' interactome of CHIKV in human and mosquito cells. We will further present data on the functional relevance of nsP3-host protein interactions in disrupting epithelial integrity, downstream hippo signaling and suppression of innate immune signaling.

Poster Number: 27

Topic: Virus-host interactions and evolution

Characterisation of the Zika virus induced small RNA response in *Aedes aegypti* cells

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Abstract

Investigations into interactions between arboviruses and their mosquito vectors provide important insights into the strategies involved in the control of virus replication. Among such control mechanisms is RNA interference (RNAi) which is pivotal in limiting arbovirus infection. More specifically, two different RNAi pathways are involved in antiviral responses: the Piwi-interacting RNA (piRNA) and exogenous short interfering RNA (exo-siRNA) pathways, characterised by virus-derived small RNAs of different length (25-29 and 21 nucleotides, respectively). The exo-siRNA pathway is considered to be a key mosquito antiviral response mechanism. We found that in *Aedes aegypti*-derived cells, Zika virus (ZIKV)-specific siRNAs were produced and loaded into the exo-siRNA pathway effector Ago2; although, surprisingly the knockdown of Ago2 did not enhance virus replication. However, enhanced ZIKV replication was observed in a Dcr2 knockout cell line suggesting that the exo-siRNA pathway is still involved in the antiviral response. ZIKV-specific piRNA-sized small RNAs were also detected. However, these lacked the characteristic piRNA ping-pong signature motif and were bound by Ago3 and not Piwi5 or Piwi6. Silencing of PIWI proteins indicated that knockdown of Ago3, Piwi5 or Piwi6 did not enhance ZIKV replication and only the Piwi4 displayed antiviral activity. We also report that expression of ZIKV capsid (C) protein amplified the replication of a reporter alphavirus; although, unlike the previously characterised yellow fever virus C protein, it does not act to inhibit the exo-siRNA pathway. Our findings elucidate ZIKV-mosquito RNAi interactions which may be important to understand its rapid spread.

Poster Number: 28

Topic: Virus-host interactions and evolution

Protection against mosquito-borne virus infection by topical innate immune agonists

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Abstract

Emerging and re-emerging arthropod borne virus (arbovirus) infections such as those caused by dengue virus, chikungunya virus and Zika virus have profound impacts on human health. Despite this, there are currently no licensed anti-viral drugs and few vaccines available for many of these virus infections. Using an *in vivo* model of arbovirus infection that unique incorporates relevant aspects of natural infection including; immunocompetent mice, *Aedes aegypti* mosquitoes and Semliki forest virus, we investigate key aspects of the mammalian innate immune response to arboviruses at cutaneous bite sites. We show that targeting these innate immune pathways at skin inoculation sites provide novel and efficiacious therapy against these infections. We show that innate immune augmentation, using topical immune agonists at the mosquito bite, can modulate the systemic course and clinical outcome of arbovirus infection. Topcial treatment reduced viral RNA at the bite site and decreased dissemination to remote tissues, as well as a significant decrease in mortality. Using a combination of in vivo and relevant in vitro models, we show that these protective effects of the immune agonsist were dependent on a functioning type I interferon response. A process that was orchestrated by both cutaneous stromal cells and leukocytes; including an influx of plasmacytoid dendritic cells. Together, our data shows that treating mosquito bite sites with innate immune agonists provides a novel treatment modality for limiting the development of these diseases.

Poster Number: 29

Topic: Virus-host interactions and evolution

Mechanisms by which mosquito saliva enhances arbovirus infection.

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Abstract

Arboviruses constitute a major public health problem. These viruses continue to emerge and re-emerge as recently demonstrated by the Zika epidemic and its spread to a wide range of new geographic locations. When mosquitoes bite they cause tissue trauma and deposit a mixture of saliva, microbiota and arbovirus if infected. Arbovirus infection of mammals is enhanced by the presence of a mosquito bite, in comparison to virus being experimentally administered by needle inoculation in the absence of a bite. Inflammatory responses to bites appear to be a key factor in this enhancement. However, the experimental inclusion of mosquito saliva with virus inoculum, in the absence of bite trauma, also has the ability to enhance viral infections; how this is achieved however remains unclear. Firstly, to determine if mosquito microbiota contributes to bite inflammation *Aedes aegypti* mosquitoes were treated with antibiotics and the degree of inflammation elicited by their bites compared. The ability of these bites to enhance virus infection was also determined. In addition, we have investigated if mosquito saliva directly makes key dermal cells that are the target of arbovirus infection (fibroblasts and macrophages), more permissive to virus infection and whether this triggers inflammatory cytokine expression. With these results, we are providing important new insights into how mosquito saliva enhances infection, which could aid in the development of anti-viral treatments by targeting factors within the mosquito bite.

Poster Number: 30

Topic: Virus-host interactions and evolution

Influence of *Wolbachia pipientis* on flavivirus infection in *Culex pipiens* Linnaeus (Diptera: Culicidae)

Mar Fernández de Marco[1], Luis M Hernández-Triana[1], Karen Mansfield[1], Leigh Thorne[1], Denise Marston[1], Sarah Lumley[2], Anthony R Fooks[1,3], Nicholas Johnson[1,4]

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Abstract

There are more than 100 genetically distinct *Wolbachia* strains in natural populations of *Cx. pipiens*, and *Wolbachia*-mediated antiviral interference has been demonstrated for RNA virus families including the family Flaviviridae. The observed effects on virus infection can vary widely. Usutu virus (USUV) is an emergent virus in Europe affecting avian species and is mostly transmitted by *Culex* mosquitoes. The aim of this study was to investigate if *Wolbachia* infection influenced the vector competency of *Culex pipiens* typical form for USUV.

We generated a Caldbeck *Culex pipiens* typical form free of *Wolbachia pipientis* by treating a naturally infected lab colony with Rifampicin and Oxytetracycline. All mosquitoes were reared and maintained at 25°C. A total of 120 *Wolbachia* positive (Wol+) mosquitoes and 80 *Wolbachia* negative (Wol-) were provided with a blood meal containing USUV (2.0×10^6 PFU/ml), and sampled at different time points. Only one specimen of *Cx. pipiens* Wol+ was positive for Usutu in saliva at 14dpi, while USUV was not detected in Wol- mosquitoes. Moreover, the infection of females Wol-, in comparison to uninfected females, showed a higher mortality after 72 hours post infection, increasing to more than 70% at 7 dpi.

This study suggests that at the virus dose and temperature used, UK *Culex pipiens* typical form shows limited susceptibility to infection with USUV, and the absence of *Wolbachia* in the *Cx. pipiens* caused an increase in mortality following infection with USUV.

Poster Number: 31

Topic: Virus-host interactions and evolution

Disruption of RNA structure with the 5'UTR of Chikungunya virus attenuates viral genome replication

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Abstract

Chikungunya virus (CHIKV) is a single-stranded positive sense RNA virus, within the alphavirus genus, presenting significant morbidity to patients and with no currently available vaccine or anti-viral therapy. CHIKV transmission occurs through *Aedes* infected mosquitoes, with its increasing geographical distribution partly reflecting expanding host vector range.

Using a combination of thermodynamic, phylogenetic and biochemical SHAPE mapping methods, we previously identified a novel RNA stem-loop structure within the 5' untranslated region (5'UTR) of the CHIKV genome, adjacent to the *nsp1* start codon. Using a subgenomic replicon system and full-length CHIKV infectious virus, we investigated the role of this RNA structure in CHIKV replication and translation.

Our studies demonstrate that nucleotide substitutions that disrupt base pairing with the heteroduplex of the stem-loop, in either the context of full-length infectious virus or sub-genomic replicon systems, significantly attenuate CHIKV replication - in both Huh7 mammalian hepatoma and *Aedes albopictus* C6/36 cell lines. These and comparative investigations, using a replication deficient sub-genomic reporter system, encoding a GND>GAA substitution in the polymerase active site, suggest that the stem-loop plays a role in genome replication, rather than initiation of translation or packaging.

Studies using compensatory mutants, which restore heteroduplex base-pairing but change nucleotide sequence are ongoing. These will allow us to differentiate RNA structure dependent effects from those involving nucleotide sequence dependent mechanisms.

Poster Number: 32

Topic: Virus-host interactions and evolution

Tissue tropism of bluetongue virus in *Culicoides* midges

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Abstract

Arthropod-borne viruses (arboviruses) cause diseases of significant consequence to human and animal health. The aspect of the lifecycle that distinguishes an arbovirus from other viral groups, is the requirement for replication in an arthropod vector, which is poorly understood. *Culicoides* midges (family: Ceratopogonidae) transmit several arboviruses of economic importance including bluetongue virus (BTV), a double-stranded RNA virus within the genus *Orbivirus* (family: *Reoviridae*). We present data, using a range of techniques, to describe BTV replication and dissemination in a model species, *C. sonorensis*.

We present information on the order of tissue and organ infection during the extrinsic incubation period as well as the extent of infection of different tissues. We examine the impact of changes in viral dose and strain on replication.

Our data show novel and key differences in the progression of BTV infections in *Culicoides* with the standard model of arbovirus-mosquito infection.

Poster Number: 33

Topic: Virus-host interactions and evolution

Vector competence of *Culex pipiens* infected with Spanish Culex Flavivirus for Rift Valley Fever Virus

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Abstract

Rift Valley Fever (RVF) is a mosquito – borne zoonotic disease caused by a phlebovirus (*Bunyaviridae* family), the Rift Valley Fever Virus (RVFV), that affects mainly domestic ruminants and humans. Spanish Culex Flavivirus (sCxFV) is an Insect – Specific Flavivirus (ISFV) that has been found naturally in sylvan mosquito populations. The influence of insect – specific CxFV on *Culex pipiens* vector competence for RVFV is unknown. The present study evaluated the vector competence for RVFV of one *Culex pipiens* population of Northeast Spain (Catalonia) co – infected with a CxFV previously isolated from a *Cx. pipiens* population from Huelva (Spain). This is the first study that asses the co – infection of a CxFV with a mosquito-borne phlebovirus (RVFV). Negative CxFV females and CxFV intrathoracic – inoculated females exposed to an artificial infectious RVFV blood – meal were analyzed. Infection, dissemination and transmission rates and transmission efficiency were estimated. RVFV was isolated from saliva of both *Cx. pipiens* females CxFV positive and CxFV negative without significant differences. The results indicated that the CxFV present in Spanish mosquito populations may not affect the vector competence of *Cx. pipiens* and the population tested is competent to transmit RVFV. The results of the present study increase the knowledge of RVFV epidemiology and contributes for better designed surveillance and control programs for RVF.

Poster Number: 34

Topic: Virus-host interactions and evolution

Characterisation of the Role of the Deubiquitylase USP45 in Chikungunya Virus Infection

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Abstract

Chikungunya virus (CHIKV) is the cause of the extremely debilitating disease, Chikungunya fever, characterised by severe joint pain which can last for months or years. As a mosquito-borne alphavirus, CHIKV has been generating increasing concern as this re-emerging pathogen spreads worldwide. There are currently no vaccines or anti-virals available for CHIKV. With current outbreaks in the America's escalating, identifying novel drug targets is of increasing importance. As obligate parasites, viruses depend on host-cell machinery to replicate. Identifying cellular pathways which are hijacked by viruses may provide the opportunity to develop therapeutics targeting host-factors. Ubiquitylation has been shown to be a key pathway targeted by viruses and the reverse reaction, deubiquitylation, has been generating interest for therapeutic intervention. We have been investigating the interaction of CHIKV with host deubiquitylases (DUBs) a family of ~100 enzymes. An initial siRNA screen using the model alphavirus Semliki Forest Virus (SFV) identified the DUB USP45 as playing a role in alphavirus infection, with an increase in cell viability post SFV infection observed after depletion of USP45. This work has been extended to CHIKV using a USP45 knockout cell line. USP45-/- cells are less susceptible to CHIKV infection as reflected by a reduction in both viral RNA production and viral plaque formation compared to wild-type cells. To date little is known about the role of USP45. Our data imply a role of USP45 during CHIKV infection. Further analysis is on-going and suggests USP45 could be a potential target for anti-virals against CHIKV infection.

Poster Number: 35

Topic: Virus-host interactions and evolution

Mutational and proteomic analysis of Rift Valley fever virus nucleoprotein reveals new information on viral protein interactions

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Abstract

Rift Valley fever virus (RVFV, *Phlebovirus, Bunyaviridae*) is an important pathogen of both humans and livestock. RVFV transmission by mosquitoes across sub-Saharan Africa and the Arabian Peninsula has a significant impact on the socio-economics of these areas. RVFV nucleocapsid (N) protein has a number of identified functions, the most important of which are the ability to encapsidate viral RNA, to form higher-order multimeric structures and to be crucial for successful virus replication. Alignment of phlebovirus N sequences revealed conserved
residues with as yet undetermined function, some of which are surface-exposed based on the crystal structure. These residues were selected for mutational analysis and the ability of the mutants to multimerise and promote RVFV replication in a minigenome assay was assessed. The residue Y30 showed an increased activity within the minigenome assay despite reduced RNA binding capacity. This indicates that the nucleocapsid may have a previously undocumented relationship between particular residues and the formation of functional ribonucleoprotein complexes. Furthermore, analysis of additional residues indicates there are currently unknown protein-protein interactions with other viral or host proteins, which are essential for successful virus replication in mammalian host cells. Additionally, proteomics analysis has uncovered novel N protein interactions that may be essential for viral replication. Understanding the fundamental biology, followed by further characterisation of these interactions will aid future development of new intervention strategies.

Poster Number: 36

Topic: Virus-host interactions and evolution

Endosomal ionic balance dictates Bunyavirus entry

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Abstract

In order to multiply and cause disease a virus must transport its genome from outside the cell into the cytosol, most commonly achieved through the endocytic network. Endosomes transport virus particles to specific cellular destinations and viruses exploit the changing environment of maturing endocytic vesicles as triggers to mediate genome release.

Previously we demonstrated that several bunyaviruses, which comprise the largest family of negative sense RNA viruses, require the activity of cellular two pore domain K^+ (K_{2P}) channels to cause productive infection. Specifically, we demonstrated a surprising role for K^+ channels during virus endosomal trafficking. Here, we used Bunyamwera virus (BUNV) as a tool to decipher why K^+ channels are required for viral progression through the endosomal system. To achieve this, we generated dual-labelled BUNV that allows single virions to be tracked in cells, in the presence of K^+ channel modulation.

Our data indicates that BUNV traffics through endosomes containing high [K^+] and that these K^+ ions influence the infectivity of virions. We further show that K^+ channel inhibition can alter the distribution of K^+ across the endosomal system and arrest virus trafficking in endosomes. These studies reveal why K^+ channels facilitate bunyavirus infection which may represent a mechanism conserved across virus families, highlighting the potential of K^+ channels as druggable anti-viral targets. Our current work seeks to identify an endosomal role for K_{2P} channels by identifying the specific member(s) required by BUNV, implicating their functionality in endosomal K^+ balance.

Poster Number: 37

Topic: Virus-host interactions and evolution

Rift Valley fever virus P78 glycoprotein as a mosquito specific virulence factor

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Abstract

Rift Valley fever is an important zoonotic disease in Africa causing a huge economic and public health burden by affecting humans and livestock. Rift Valley fever virus (RVFV) is transmitted to its host via the bite of an infected mosquito causing a variety of symptoms ranging from influenza-like to lethal haemorrhagic fever and encephalitis. In the mosquito vector RVFV causes asymptomatic lifelong persistent infections. Persistence and transovarial transmission of the virus in the mosquito vector are important factors for maintaining RVFV in nature, especially during inter-epidemic episodes.

As a member of the *Bunyaviridae* family RVFV has a tri-segmented genome. The medium (M) segment encodes a polyprotein that is cleaved into the two major glycoproteins Gn and Gc, which form spikes on the viral envelope and two additional proteins, NSm and P78. The 78 kDa glycoprotein P78 is a stable fusion product between the viral glycoprotein Gn and the cytosolic NSm protein. P78 has been found to be a minor component of virions released from mosquito cells and contributes to virus dissemination within the mosquito vector. Using reverse genetics to generate P78 knockout viruses, we analysed the impact of P78 expression on virus replication in mosquito cells. We demonstrate that P78 modulates virus production during acute and persistent infection. Furthermore, we characterised the subcellular localisation and demonstrated that P78 facilitates virus spread within a cell monolayer.

Poster Number: 38

Topic: Virus-host interactions and evolution

Infection by a Brazilian Zika virus isolate inhibits interferon signalling

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Abstract

Zika virus (ZIKV, *Flaviviridae*) is a mosquito-borne pathogen, which has rapidly expanded its geographical distribution during recent years. Previously considered to be of little importance, the latest outbreaks have been characterised by the increased prevalence of neurological conditions, such as Guillain-Barré syndrome and congenital ZIKV syndrome, which have placed a significant strain on affected communities. We have previously shown that isolates within both the Asian and African lineages are genetically very similar to each other. Hence, virus genetics are unlikely to explain the increased incidence of neurological syndromes associated with certain strains. It is therefore important to understand the response of the host's innate immune defences against ZIKV infection. We utilised a clinical ZIKV isolate from Brazil to demonstrate that RNA isolated from infected cells results in the induction of type I interferons (IFNs); IFNα and IFNβ. This response can be attributed to the action of RIG-I and, to a lesser degree, MDA5. This data is consistent with our findings that the subsequent antiviral response is antagonised by subgenomic flavivirus RNA (sfRNA) that acts to block downstream IFN induction. In addition, as type 3 IFNs are thought to be involved in the protection of the placenta against ZIKV infection, we generated STAT1 and IFNLR1 (interferon lambda receptor 1) deficient cell lines to understand the contribution that type 1 and type 3 IFNs have on host response to, and protection against, ZIKV infection, and how ZIKV sfRNA might affect these signalling pathways

Poster Number: 39

Topic: Virus-host interactions and evolution

High-throughput proteomic analysis of the secretome of Dengue virus infected and replicon containing cells.

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Abstract

Dengue virus (DENV) infection is known to result in alterations in the levels of cytokines and vasoactive mediators in the peripheral blood of infected individuals. However, little is known about the overall alterations which occur in the secretome of infected cells in response to DENV replication. This study aims to determine the effect of DENV replication on the secretome of human cells (HEK-293T), either during infection or using HEK293T cells stably expressing a DENV-2 sub-genomic replicon (HEK293T-DV-Rep). Conditions were first optimized for high-throughput quantitative mass spectrometry based secretome analysis, using Tandem-Mass-Tagging (TMT). The culture supernatants of HEK293T-DV-Rep cells and HEK293T cells, either infected with DENV-2 or mock infected, were harvested at selected points after cultivation/infection. The supernatants were concentrated, secreted proteins labelled by 10-plex TMT and analysed by LC-MS/MS. 5533 proteins were confidently identified, of which 87 and 8 commonly increased and decreased ≥ 2 fold in the DENV-2 infected HEK293T and HEK293T-DV-Rep cell secretomes, respectively, compared to mock infected cells. Bioinformatic analysis of proteins that were increased/decreased ≥ 2 fold in the secretomes were associated with the gene ontology terms “negative regulation of endopeptidase activity/complement pathway”. The most significant KEGG pathway associated with the proteins analyzed was “complement and coagulation cascades”. The results are being compared with a proteomic analysis of serum from patients with different grades of dengue disease severity, to identify proteins that may be relevant to pathogenesis. Alterations in selected relevant proteins identified by secretome analysis are being validated by immunoprecipitation analysis.

Poster Number: 40

Topic: Virus-host interactions and evolution

Mitochondria at the crossroad of cellular pathways targeted by the Rift valley fever virus NSm virulence factor

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Abstract

Rift valley fever virus (RVFV, *Phlebovirus*, *Phenuiviridae*) is endemic in Africa and the Arabian Peninsula. RVFV affects primarily ruminants with a high mortality rate in fetuses and newborns, which has a high impact on local economy [1]. In humans, symptoms include hepatitis and hemorrhagic fever. RVFV encodes several nonstructural proteins known to act as virulence factors in mammalian hosts (NSs and NSm) or to promote viral dissemination in arthropod vectors (NSm-GN) [2, 3]. The present study focuses on the functional characterization of the NSm protein.

NSm is a 14 kDa protein localized at the surface of mitochondria in infected cells [4]. Our team previously showed that a mutant virus lacking NSm expression (del-NSm) is attenuated in mice [3]. To further investigate the underlying mechanisms of pathogenicity, we used label-free quantitation by mass-spectrometry and compared the proteomes of primary macrophages infected by either wild-type or del-NSm viruses. The results show that NSm acts cooperatively with the NSs virulence factor and modulates the expression level of cellular proteins involved in cell-mediated immune response, oxidative phosphorylation and fatty acid metabolism. This points to a complex regulation of the mitochondrial platform by the viral NSm protein. We are currently investigating the upstream effectors targeted by NSm during the course of RVFV infection.

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[2] Ikegami T et al., Immunology and Pathogenesis of Viral Hemorrhagic Fevers, 2009
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[4] Terasaki K et al., Journal of Virology, 2013

Poster Number: 41

Topic: Virus-host interactions and evolution

Elucidation of the mechanism by which a mutation in the Dengue virus NS4B protein confers a persistent phenotype in cell culture.

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Abstract

Although members of the *Flaviviridae* family can cause persistent infections, there are few reports of persistent Dengue virus (DENV) infections in mammalian cells. We have isolated a DENV-2 mutant that can establish a persistent infection in a range of mammalian cultured cells and have shown by reverse genetic analysis that this phenotype is due to a single amino acid substitution in the DENV-2 NS4B protein, T66A. However, studies using the exogenously expressed mutant NS4B gene have failed to reveal the mechanism conferring the persistent phenotype.

To further address this question, we performed a comparative RNAseq transcriptomic analysis using HEK293T cells that were infected with DENV-2, persistently-infected, or stably expressing a wild type (DV2-Rep) or NS4B mutant replicon (DV2-Rep-NS4B$_{T66A}$). Bioinformatic analysis of the transcriptomic dataset revealed that the expression of a range of interferon-sensitive genes was significantly increased in the persistently infected and DV2-Rep-NS4B$_{T66A}$ cells compared to control, DENV-2 infected and DV2-Rep cells. Interestingly, genes involved in cholesterol and fatty acid biosynthesis were downregulated in the persistently infected cells compared to the control cells. By contrast, we found the genes *HMGCR, HMGCS1, IDI1, INSIG1, MSMO1, SCD* and *SQLE*, involved in lipid metabolism, were upregulated during DENV-2 infection. The expression of these genes is being further validated by qRT-PCR using RNA extracted from the persistently infected cells and cells cured of the persistent infection by drug treatment, to determine whether their altered expression is required for the establishment of the persistent infection or a consequence of the NS4B T66A mutation.

Poster Number: 42

Topic: Virus-host interactions and evolution

Dengue in the secretory pathway – defining the role of cellular proteins that are modulated by dengue infection.

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Abstract

The interactions between cellular and viral proteins that mediate secretion of the Dengue virus particle and the NS1 protein are still largely unknown. An increased understanding of these interactions may provide a basis of the future development of host-targeted antiviral interventions. Previous high-throughput quantitative proteomic analysis of DENV-2 infected cells has identified proteins involved in ER-Golgi trafficking that are either decreased during infection or recruited to the site of virus replication, suggesting that they may play a role in DENV replication or secretion.

DENV-2 infection of human A549 and HEK-293 cells caused a decrease in the expression of pre-enylated Rab-associated family member 2 (PRAF2) - an ER-associated protein. To investigate the mechanism by which DENV-2 reduces PRAF2 levels, proteasomal and lysosomal degradation pathways in cells either infected with DENV-2 or harbouring a DENV-2 replicon are being inhibited. In addition, high-throughput mass spectrometry based interactomics is being used to determine if DENV-2 infection of cells overexpressing PRAF2 alters the PRAF2 interactome, which may affect the secretory pathway.

A number of transmembrane emp24 domain-containing (TMED) protein family members were found to be recruited to a heavy membrane fraction containing the DENV-2 replication complex. siRNA knockdown of TMED9 reduced DENV-2 infection, suggesting involvement of TMED proteins in DENV-2 replication or secretion. To determine if this is a selective effect or other TMED proteins are involved, we are investigating the effects of depleting TMED2, TMED7, and TMED10 by siRNA silencing, in DENV-2 infected Huh-7 cells, both individually and concurrently.

Poster Number: 43

Topic: Virus-host interactions and evolution

Insect-specific viruses as control measures for West Nile virus infection of animals and humans

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Abstract

The flavivirus genus includes arthropod-borne viruses (arboviruses) that are some of the globally most important pathogens causing disease in both human and animal populations. Examples of these are West Nile virus (WNV), Zika virus, dengue virus (DENV) and louping ill virus. Substantial arbovirus research, focusing on how these viruses cause disease in their vertebrate host(s), is proceeding but little is known when it comes to factors influencing vector competence. In recent years, it has been suggested that endogenous insect-specific viruses (ISVs) could influence the vector competence and that these viruses, similar to *Wolbachia*, could potentially be utilised in novel control strategies of arboviruses. However, in-depth knowledge regarding ISVs and how they influence their arthropod host is scare. Therefore, in this project, we aim to in detail elucidate the interaction between different ISVs and the mosquito as well as study if/how they influence the vector competence for WNV. We are currently collecting mosquitoes and carrying out viral metagenomic studies, in combination with viral isolation attempts, to characterize the virome of different mosquito species including *Culex* spp., the main vector of WNV. A selected number of the identified ISVs will be used for further biological investigations to determine their effect on the vectors cellular processes and its immune response. Thereafter their effect on the vector competence for WNV will be investigated. This project will provide fundamental knowledge of the effect insect-specific viruses have on their vector and how this could potentially affect the vector competence for arbovirus.

Poster Number: 44

Topic: Virus-host interactions and evolution

Analysis of tick-borne encephalitis virus-induced host responses in human cells of neuronal origin and interferon-mediated protection.

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Abstract

Tick-borne encephalitis virus (TBEV) is a member of the genus *Flavivirus.* It can cause serious infections in humans which may result in encephalitis/meningoencephalitis. Although several studies have described the involvement of specific genes in host response to TBEV infection in the central nervous system (CNS), the overall network remains poorly characterized. Therefore, we investigated the response of DAOY cells (human medulloblastoma cells derived from cerebral neurons) to TBEV (Neudoerfl strain, Western subtype) infection to characterize differentially expressed genes by transcriptome analysis. Our results reveal a wide panel of interferon-stimulated genes (ISGs) and pro-inflammatory cytokines, including type III but not type I (or II) IFNs, that are activated upon TBEV infection as well as a number of non-coding RNAs including long non-coding RNAs. To obtain a broader view of the pathways responsible for eliciting an antiviral state in DAOY cells we examined the effect of type I and III interferons and found that surprisingly only type I IFN pre-treatment inhibited TBEV production. The cellular response to TBEV showed only partial overlap with gene expression changes induced by IFN-β treatment –suggesting a virus-specific signature– and we identified a group of ISGs that were highly up-regulated following IFN-β treatment. Preliminary results indicate that the over-expression of two most up-regulated ISGs, IFI6 and IFI27, can decrease the cytopahtic effect of TBEV infection in DAOY cells. Further studies are in progress to verify this phenomenon in more detail.

Poster Number: 45

Topic: Virus-host interactions and evolution

Characterization of European Tick-Borne Flaviviruses: A Focus on Louping ill and Tick Borne Encephalitis Virus

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Abstract

Louping ill is a disease found predominantly in sheep and grouse that has been described in Britain for over 200 years. As such it is of significant economic concern to hill-sheep farmers and the estates where grouse are maintained for commercial shooting. It is caused by louping ill virus (LIV) a small, positive sense, single-stranded flavivirus. LIV is closely related to tick-borne encephalitis virus (TBEV), a significant human pathogen that is prevalent throughout Europe, Siberia, and the Far East. The mechanisms that underpin the highly variable phenotypes and differing host specificity of these viruses are poorly understood. Presently only three full LIV genome sequences are available on Genbank, compared to hundreds of TBEV sequences. Here, we describe the sequencing and pairwise comparison of over 18 LIV genomes derived from strains dating from 1930 to 2015. Utilizing these isolates we have performed phylogenetic analysis and molecular clock studies to determine the natural rate of evolution of the virus and its recent evolutionary history. Additionally, we have produced a model of the subgenomic flavivirus RNA (sfRNA) and we have assayed the ability of the viral non-structural genes and sfRNA to inhibit the interferon response. We have also investigated the growth of LIV and TBEV in human, chicken, and sheep cells at high and low MOI to further characterize the phenotypic differences between these viruses.

Poster Number: 46

Topic: Virus-host interactions and evolution

Zika virus induced apoptosis inhuman neuroblastoma cell line is independent of oxidative stress induction

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Abstract

Zika virus (ZIKV) is a highly neurotropic emerged arbovirus. ZIKV infection has been associated with severe neurological complications, such as microcephaly and Guillain-Barre Syndrome. Despite its importance, little is known about its pathogenesis mechanisms. Here, we aim to characterize specific molecular events involved in ZIKV induced cell death. To this, SH-SY5Y neuroblastoma cells were infected with ZIKV at different MOI, viral replication was analyzed by RT-qPCR and flow cytometry (FACS), at different times post-infection (pi). Our results evidenced an active replication of ZIKV in SH-SY5Y cells, with a viral load rapidly increasing between 1 and 2 days pi. Extensive apoptosis, quantified by Annexin-V/7-AAD staining on FACS, was observed starting at 2 days pi. Moreover, caspase 3/7 activation was observed following infection and the treatment with a pan-caspase inhibitor (Q-VD-OPh) partially inhibited ZIKV apoptosis, indicating that this process is partially dependent of caspase activation. Caspase-9 activation was also observed by immunoblot, suggesting the recruitment of an intrinsic pathway that triggers caspase-dependent apoptosis. Considering that oxidative stress is a major inductor of this pathway, we next analyzed ROS generation during infection. Surprisingly, no ROS production was detected in ZIKV infected cells and the treatment of infected cells with the antioxidant N-acetyl-L-cystein (NAC) had no effect on ZIKV induced cell death, as observed by Annexin-V/7-AAD staining. Altogether, these results provide new insights about possible cell death mechanisms induced during ZIKV infection in neurons, and suggest that this apoptotic process is independent of ROS generation.

Poster Number: 47

Topic: Virus-host interactions and evolution

Identification of native NS5 in orbivirus infected cells and complementary roles played by NS4 and NS5 of orbiviruses: identification of interactants and mechanisms by which these proteins act as positive regulators of virus replication

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Abstract

Genus *Orbivirus* (family *Reoviridae*) contains 22 species of arthropod-borne viruses. *Bluetongue virus* (BTV) is the 'type' species of the genus. Orbiviruses are non-enveloped and their genome is composed of 10 segments (Seg) of dsRNA. Two previously undescribed ORFs, in Seg-9 and Seg-10, were suspected to encode additional NS proteins. We previously identified NS4 as the 4th non-structural protein encoded by Seg-9 of both insect- and tick-borne orbiviruses. Here, we demonstrate the existence of NS5 in cells infected with representatives of *Culicoides*-borne, mosquito-borne and tick- borne orbiviruses. NS4 and NS5 were found to localise to the cytoplasm (lipid droplets: LD) and nucleus, particularly the nucleolus. The involvement in lipid pathways was confirmed using statins which impaired interactions with LDs, inhibited virus replication and helped identifying the branch of the pathway involved. We identified the full interactome of NS4 by a yeast two hybrid system using human and mouse cDNA libraries. Interactants were principally identified as anti-apoptotic factors or innate-immunity factors. Deletion/double-deletion mutants (NS4/NS5) were generated by reverse genetics and studied both in cell cultures and mice. We identified a complementarity between NS4 and NS5 in terms of interplay with pathways of innate-immunity, apoptosis, shut-off of host-cell protein synthesis and maturation of ribosomal RNA. NS4 is also suspected to be involved in early events of crossing species barrier from arthropods to mammals. The roles (mechanisms) of orbivirus NS4 and NS5 as positive regulators of virus replication are discussed.

Poster Number: 48

Topic: Virus-host interactions and evolution

Interactomic high-throughput mapping for Bluetongue virus in its natural hosts: towards the identification of new factors of pathogenicity and interspecies transmission.

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Abstract

Bluetongue virus (BTV) is an arbovirus responsible for bluetongue (BT), a non-contagious disease that affects a wide range of wild and domestic ruminants, inducing. It is transmitted by blood-feeding midges belonging to the *Culicoides* genus. BTV induces a large panel of clinical manifestations depending primarily on the infected host and the viral strains. Despite many studies, we still have little understanding of the molecular determinants of BTV virulence. We took advantage of the yeast two-hybrid (Y2H) method to map interactions between BTV and cellular proteins. As a starting point, this approach has been applied on serotype 8 of BTV (BTV-8), which has caused considerable economic losses between 2006 and 2010 and has recently re-emerged in France. Viral proteins were used as baits to screen two cDNA libraries originating from cattle and *Culicoides*. Therefore, 43 screens were performed and 1,478 positive yeast colonies were analyzed, allowing us to identify a hundred of new virus-host interactions. A preliminary global analysis of these interactions has uncovered many signal transduction factors involved in autophagy, apoptosis and the ubiquitin-proteasome system. All the cellular interactors are currently re-tested by several protein-protein interaction methods including GST-pulldown and GPCA (*Gaussia princeps* protein complementation assay) with the viral proteins encoded by BTV-8 and also other serotypes. Conserved protein interactions will be instrumental to design generic drugs against multiple serotypes. On the other hand, interactions that are highly specific of a particular virus would shed light on the molecular mechanisms responsible for its virulence/pathogenicity and transgression of cross-species barriers.

Poster Number: 49

Topic: Antivirals/vaccines

Current Applicable Findings Towards Designing Appropriate Transmission Blocking Vaccines (TBVs) against Mosquito Borne Viruses (MBVs)

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Abstract

Arboviruses use complex biological life cycles, involving a primary arthropod vector (including mosquitoes acting as the main vectors) and a primary vertebrate host responsible for some deadly diseases worldwide. Previously, it was thought that mosquito borne diseases threaten mostly undeveloped countries as well as Africa; but recently, pathogens like West Nile Virus, Zika Virus, Chikungunya virus and Dengue Virus are threatening all humans on the earth. The rapid rise in human infections caused by these viruses is attributed to rapid climate change and travel facilities. Most of arboviruses require adaptation to both vertebrates and mosquitos' midgut.A possible method of reducing emerging and re-emerging mosquito borne viruses is the application of altruistic Transmission Blocking Vaccines (TBVs) that prevent viral infection in the mosquito vector stage. Some successes have been achieved in the potential application of TBVs against mosquito borne viruses by identification of main virus receptors/molecules in mosquitoes' midgut/salivary glands by Next Generation Sequencing method.Analysis of similar genes that act as receptors of viruses in different mosquito species reveals main differences in peptide sequences; these differences may affect the capacity of mosquito species to be vectors as main viruses. Recent successes encourage investigators to apply these findings for designing TBVs. The aim of the present review is to provide an introduction for possible application and usage of the current and upcoming promising findings in designing and developing Transmission Blocking candidates for the control of mosquito borne viruses.

Poster Number: 50

Topic: Antivirals/vaccines

Cytokine transcripts Expression profile in peripheral blood mononuclear cells of sheep following vaccination of pentavalent bluetongue vaccine

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Abstract

Recent invasion of multiple bluetongue virus serotypes (BTV) in different regions of the world necessitates urgent development of efficient vaccine that aims numerous serotypes. In this experimental study, cytokine transcripts expression profile which is part of cell mediated immune response of binary ethylenimine (BEI) inactivated montanide adjuvanted pentavalent (BTV-1, 2, 10, 16 and 23) vaccine was evaluated in sheep against challenge with homologous serotypes in their respective group. Upon vaccination and challenge, it was found that both unvaccinated and vaccinated sheep peripheral blood mononuclear cells (PBMCs) exhibited expression of IFN-α, IL-2, IL-6, IL-12, IFN-γ and TNF-α transcripts. However, compared to unvaccinated ones, PBMCs of vaccinated sheep showed significant ($P < 0.05$) up regulation of these cytokines at certain point of time. There were no significant difference ($P > 0.05$) in cytokine induction, among BTV-1, 2, 10, 16 and 23 serotype challenges This finding with other study findings put forwarded that binary ethylenimine inactivated montanide adjuvanted pentavalent bluetongue vaccine has stimulated cell mediated immune response and most importantly reduced the severity of BTV-1, 2, 10, 16 and 23 infections following challenge in its respective group.

Poster Number: 51

Topic: Antivirals/vaccines

Targeting the host: *N*-8'-(2''-tetrahydrofuranyl)-octyl-deoxynojirimycin (2THO-DNJ) antiviral efficacy in dengue infection

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Abstract

Certain iminosugars with glucose stereochemistry have antiviral activity against particular enveloped viruses possessing *N*-linked glycoproteins. This antiviral activity is proposed to occur through competitive inhibition of endoplasmic reticulum α-glucosidases, leading to a reduction in functional viral glycoprotein folding and infectious virion production. Dengue virus (DENV) is susceptible to antiviral effects mediated by particular iminosugars. Here, the antiviral activity of the iminosugar *N*-8'-2''-tetrahydrofuranyl-octyl-deoxynojirimycin (2THO-DNJ, UV-12) against DENV is characterised using primary human monocyte-derived macrophages from healthy donors. 2THO-DNJ has potent antiviral activity against DENV, reducing infectious virus titres with an IC_{50} of 4.45 μM ± 2.15 μM, determined from 5 donors. In addition to virion infectivity, total virion secretion was analysed. This demonstrated that the antiviral effect of 2THO-DNJ is primarily mediated by a reduction in virion secretion rather than virion infectivity. Free oligosaccharide analysis and isothermal titration calorimetry support α-glucosidase inhibition as the mechanism of action of 2THO-DNJ. In the future, the galactostereochemistry compound, 2THO-deoxygalactonojirimycin, which is not expected to inhibit α-glucosidases, will be utilised in antiviral efficacy studies to discriminate between sugar head and tail group effects.

Poster Number: 52

Topic: Antivirals/vaccines

Understanding insecticide resistance mechanisms in *Aedes aegypti* dengue endemic areas of Saudi Arabia

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Abstract

In Saudi Arabia, dengue has been endemic since the 1990s and disease control programmes rely on using several insecticides to control mosquito (*Aedes aegypti*) populations. Insecticide resistance is a growing concern that has yet to be well characterised in Saudi Arabia. As resistance mechanisms remained unknown, this study aimed to 1) determine the prevalence of insecticide resistance in *Ae. aegypti* collected from Jeddah and Makkah and 2) investigate the role of target site mutations and metabolic resistance mechanisms. Three *kdr* gene mutations S989P, V1016G, F1534C were observed for the first time in Saudi Arabian mosquitoes. Microarray data was used to identify several differentially expressed genes. These *Aedes* mosquitoes are highly resistant to pyrethroids. Voltage-gated sodium channel (Vgsc) mutations are strongly associated with deltamethrin resistance and, though P450 enzymes seem to be the dominant metabolic mechanism, our data suggest a greater importance of target site mutations. We report the Vgsc mutations can be a valuable monitoring tool for monitoring developing insecticide resistance, however further investigations on other resistance mechanisms is still needed.

Poster Number: 53

Topic: Antivirals/vaccines

A Japanese encephalitis virus vaccine inducing antibodies strongly enhancing in vitro infection is protective in pigs

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Abstract

Japanese encephalitis virus (JEV) is responsible of zoonotic severe viral encephalitis transmitted by Culex mosquitoes. Although birds are reservoirs, pigs play a role as amplifying host, and are affected in particular through reproductive failure. Here we show that a lentiviral JEV vector, expressing JEV prM and E proteins (TRIP/JEV.prME), but not JEV infection induces strong antibody-dependent enhancement (ADE) activities for infection of macrophages. Such antibodies strongly promoted infection via Fc receptors. ADE was found at both neutralizing and non-neutralizing serum dilutions. Nevertheless, in vivo JEV challenge of pigs demonstrated comparable protection induced by the TRIP/JEV.prME vaccine or heterologous JEV infection. Thus, either ADE antibodies cause no harm in the presence of neutralizing antibodies or may even have protective effects in vivo in pigs. Additionally, we found that both pre-infected and vaccinated pigs were not fully protected as low levels of viral RNA were found in lymphoid and nervous system tissue in some animals. Strikingly, virus from the pre-infection persisted in the tonsils throughout the experiment. Finally, despite vaccination challenge viral RNA was detected in the oronasal swabs in all vaccinated pigs. These data on virus persistence and shedding are of relevance for JEV ecology and pig vaccination.

Poster Number: 54

Topic: Antivirals/vaccines

DENDRITIC CELLS, MACOPHAGES, AND FIBROBLASTS ARE THE INITIAL TARGETS FOR REPLICATION OF THE LIVE-ATTENUATED YELLOW FEVER VACCINE IN THE DERMIS OF MICE

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Abstract

The mosquito-borne yellow fever virus causes high fever, hemorrhage, and devastating liver destruction, with a case fatality rate of ~34% in the 2015/16 outbreak in Angola. The live-attenuated yellow fever virus vaccine (YFV-17D) has provided efficient protection for >80 years. Nevertheless, production in chicken eggs and the need of a cold chain hindered large-scale vaccination in the recent outbreaks. Further, many underlying mechanisms how YFV-17D induces long-lasting protection remain unknown. We here identified the first cellular targets for replication of the YFV-17D vaccine in the skin of mice following intradermal vaccination. Intradermal inoculation of (IFN-receptor deficient) AG129 mice with a mCherry reporter and analysis via flow cytometry revealed that fibroblasts and skin-resident macrophages as well as classical dendritic cells (the most efficient inducers of T-cell responses) are the initial targets of YFV-17D replication. Revealing the cellular tropism shows as to which cells produce viral antigens and may induce protective immunity. Further, we constructed a nanoluciferase-expressing YFV-17D reporter to determine virus dissemination via *in vivo* luminescence imaging. Intradermal inoculation led to localized replication in the inoculated mouse ear and draining lymph nodes. Our data provide crucial information on which dendritic cell subsets to target for optimal vaccination against yellow fever or other arboviruses. Also, our reporter virus constructs will allow to optimize the delivery of a novel DNA-launched YFV-17D vaccine platform that we are currently developing. Our novel vaccine technology allows to generate vaccines against various pathogens transport the vaccine independently of a cold chain, and could overcome acute vaccine shortages.

Poster Number: 55

Topic: Antivirals/vaccines

Antiviral effects of *N*N-DNJ, 2ThO-DNJ and E*O*O-DNJ on dengue-infected monocyte-derived dendritic cells

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Abstract

Dengue virus (DENV) infections range from asymptomatic illnesses to severe haemorrhagic disease and death. Iminosugars are monosaccharide mimics that competitively inhibit α-glucosidases in the host endoplasmic reticulum. *In vitro* studies have demonstrated antiviral effects of iminosugars against dengue in monocyte-derived macrophages. Dendritic cells (DC) are important targets for dengue infection and they play a significant role in the immune response against early dengue infection. Given the important role played by DC in innate immune defence against DENV, it is useful to investigate the antiviral effects of iminosugars in a DENV-infected DC model. We evaluated three deoxynojirimycin (DNJ) derivatives and a deoxygalactonojirimycin (DGJ) control in DENV-infected primary human monocyte-derived DC. *N*-(n-Nonyl)-deoxygalactonojirimycin (*N*N-DNJ), 8-tetrahydrofuranyl-octyl-DNJ (2ThO-DNJ/UV-12) and *N*- (8'-ethoxyoctyl)- deoxynojirimycin (E*O*O-DNJ) were used as DNJ derivatives. *N*-(n-Nonyl)- deoxygalactonojirimycin (*N*N-DGJ), which has galactose stereochemistry was used as the control. Immunofluorescence was used to detect % infection of DENV. Secretion of infectious virus was evaluated with plaque assays while total secreted virus was quantified with qRT-PCR. We demonstrated that iminosugar derivatives of DNJ but not DGJ elicit antiviral activity in DENV-infected monocyte-derived DC. Compounds *N*N-DNJ, E*O*O-DNJ and 2ThO-DNJ reduced the % of infected cells and inhibited the secretion of DENV in a dose dependent manner. In conclusion, we have demonstrated for the first time that the iminosugars *N*N-DNJ, E*O*O-DNJ and 2ThO-DNJ have antiviral effects on DENV-infected DC similar to those seen in DENV-infected macrophages.

Poster Number: 56

Topic: Antivirals/vaccines

Establishment of reverse genetics systems for Cache Valley and Kairi Virus; two emerging orthobunyaviruses of the Americas

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Abstract

Emerging bunyaviruses such as Cache Valley virus (CVV) and Kairi virus (KRIV) have the potential to re-assort and alter their vector, geographical range and virulence. Periodic outbreaks of CVV have resulted in the significant loss of lambs on North American farms. However, vaccines for bunyaviruses are either unavailable, ineffective or have unacceptable side effects. One approach is a vaccine based on the use of reverse genetics to develop attenuated viruses that elicit strong immune responses but cannot revert back to full virulence.

This study showed that CVV and KRIV have a wide *in-vitro* host range and that BHK-21 cells are a suitable host for virus propagation and virus titration. CVV and KRIV genomic segments were cloned and cDNAs used to rescue infectious virus in 3 and 5 plasmid rescue systems respectively. A mini-replicon system for both viruses also showed the production of ribonucleic acid protein complexes. The open reading frames of the NSs proteins for both viruses were disrupted by the introduction of stop codons and the removal of start codons. The recombinant viruses expressing non-functional NSs proteins induced more interferon (IFN) after infection of A549 cells when compared to the recombinant wild type virus, indicating that for both viruses, NSs functions as an IFN antagonist. The establishment of reverse genetic systems for CVV and KRIV will facilitate the development of a vaccine platform for orthobunyaviruses.

Poster Number: 57

Topic: Antivirals/vaccines

Long chain alkylated iminosugars are antiviral against Zika virus

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Abstract

The host endoplasmic reticulum protein-folding machinery is required by many viruses to correctly fold one or more of their glycoproteins. Iminosugars, which contain a nitrogen heterocycle, act as mimics of monosaccharides, and those which mimic glucose target the glucosidases which are key for entry into the glycoprotein folding cycle. By preventing viral glycoproteins from interacting with the protein folding machinery, iminosugars have a broad-spectrum an antiviral effect against a wide range of different viruses. For the first time we report the anti-Zika activity of 2 simple alykylated deoxynojirimycin (glucose mimic) iminosugars in cell culture.

Poster Number: 58

Topic: Antivirals/vaccines

Modulation of host cell chloride channels inhibits Chikungunya virus genome replication

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Abstract

Chikungunya virus (CHIKV) causes fever and debilitating joint pain, with frequent long-term health implications and cumulating fatalities. Recent outbreaks across the world highlight its global health impact. There are no specific antivirals/vaccines, therefore understanding CHIKV replication is essential to establish treatments/preventative measures. Cellular ion channels are druggable targets and are known to facilitate replication of other RNA viruses.

To determine if the activities of cellular chloride channels are required during CHIKV replication we applied broad-ranging inhibitors to Huh7 cells. Their effect on the CHIKV life cycle and genome replication was investigated by plaque assay and a luciferase-based sub-genomic CHIKV replicon assay, respectively. Knock down of individual chloride channels was mediated by siRNA.

The broad-spectrum chloride channel inhibitors NPPB and IAA-94 significantly lowered the titre of released CHIKV progeny at 24 hours post-infection suggesting that chloride channels are pro-viral factors. Time-of-addition studies with the inhibitors indicated that CHIKV requires cellular chloride channels post-entry. Replication of the sub-genomic replicon was restricted by chloride channel inhibition, implying that chloride channels are involved in genome replication. An siRNA knock down screen identified the chloride intracellular channels 1 and 4 to be required for the CHIKV life cycle. We hypothesise that the channels play a role in formation/maintenance of CHIKV membrane bound replication-complexes. Work to investigate this and the role of cellular chloride channels in further stages of the CHIKV life cycle is ongoing.

These findings advance our understanding of CHIKV replication in mammalian cells and identification drugs/druggable targets for treatment/prevention of CHIKV-induced disease.

Poster Number: 59

Topic: Antivirals/vaccines

Zika virus-like particle and subviral particle vaccines

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Abstract

Zika virus (ZIKV) is causing outbreaks of unprecedented scale in South America and the Caribbean. ZIKV can induce severe disease symptoms in humans, including congenital microcephaly and neurological complications. The severe symptoms, fast spread and long-term persistence of the virus are disruptive to the affected societies, and vaccines are therefore desperately needed. Vaccines against ZIKV need to be effective, scalable and of low cost. Ideally, a ZIKV vaccine has to be non-replicative to safely protect the mother and the unborn child. Here we report the development of two prototype ZIKV vaccines: virus-like particles (VLPs) and subviral particles (SVPs). These non-replicative, enveloped ZIKV VLPs and SVPs were produced using the scalable baculovirus-insect cell expression system. High-level secretion of ZIKV VLPs and SVPs into the culture fluid of insect cells was achieved, and particles with diameters ranging from 20 to 60 nm were observed after purification. The immunogenicity of the purified VLP and SVP vaccines was tested in mice. High levels of ZIKV-specific antibodies were detected in mouse serum upon vaccination. Challenge experiments with ZIKV to assess the ability of the VLP and SVP vaccines to confer protection in mice are currently ongoing.

Poster Number: 60

Topic: Antivirals/vaccines

A recombinant modified vaccinia virus Ankara expressing NS1 provides multiserotype protection against Bluetongue virus

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Abstract

Bluetongue is a hemorrhagic disease of ruminants caused by bluetongue virus (BTV). Twenty seven BTV serotypes have been identified within two well-defined topotypes (Western and Eastern). Vaccination is the most effective control measure to reduce virus circulation. We have engineered a multiserotype DIVA vaccine against BTV based on the non-structural protein NS1 expressed by recombinant modified vaccinia virus Ankara (MVA-NS1) and tested its efficacy against different BTV serotypes. NS1 is the most conserved protein across serotypes and the major inducer of cellular immune responses in mouse and sheep. MVA-NS1 was inoculated in IFNAR(-/-) mice following a prime-boost regimen and infected with lethal doses of five BTV serotypes or reassortants: BTV-1(ALG2006/01), BTV-4(SPA2004/02), BTV-8(BEL2006/01), BTV-4(MOR2009/09), and BTV-16(RSArrrr/16). Non-immunized animals succumbed to infection regardless of BTV serotype and showed increasing viremia levels. In contrast, animals immunized with MVA-NS1 were protected against their subsequent challenge in the absence of neutralizing antibodies. A reduction in the level of lymphocytes, monocytes and platelets together with an increase in the amount of neutrophils were observed in the non-immunized groups, while no changes were observed in the immunized mice. Cellular immune response analysed in the immunized mice showed an increase in the expression of IFNγ and CD107a (a marker of cytotoxic activity) in splenic CD8+ T cells upon specific re-stimulation. An experimental DIVA vaccine based on rMVA expressing the protein NS1 of BTV elicits strong and specific CD8+ T cell responses and protects IFNAR(-/-) mice against disparated and phylogenetically different BTV serotypes in the absence of neutralizing antibodies.

Poster Number: 61

Topic: Antivirals/vaccines

New protective vaccination strategies against orbiviruses based on avian reovirus microspheres and modified vaccinia virus Ankara.

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Abstract

Orbiviruses are the cause of important and emerging arboviral diseases of livestock, including bluetongue virus (BTV) and African horse sickness virus (AHSV), both transmitted by the same biting midge vector. The objective of this work was to develop reagents and strategies for vaccination against bluetongue virus (BTV) and African horse sickness virus (AHSV). Here we have generated and tested a novel immunization approach comprised of reovirus muNS protein microspheres tagged to BTV-4 or AHSV-4 antigens (MS-VP2/VP7/NS1) and modified vaccinia virus Ankara (MVA) as delivery antigen vector (MVA-VP2/VP7/NS1). IFNAR(-/-) mice immunized with MS-VP2/MS-VP7/MS-NS1 or MS-VP2/MS-NS1 without adjuvant in a homologous prime-boost vaccination regime protected against a homologous challenge with a lethal dose of BTV-4 or AHSV-4, respectively. Full protection was also observed when the animals were immunized with MS/rMVA expressing the same antigens and challenged with the homologous orbivirus. The booster with rMVA expressing BTV or AHSV antigens conferred a significant induction of neutralizing antibodies. Furthermore, this immunization strategy elicited strong CD8+ T cell responses and cytotoxic activity in immunized mice. In addition, the immunization strategy based on MS/rMVA elicited cross-protection against a heterologous lethal challenge with BTV-1. These new experimental strategies based on microspheres and rMVA are an attractive approach to generate new effective, safe and cross-protective marker vaccines against orbiviruses.

Poster Number: 62

Topic: Antivirals/vaccines

Construction of Zika virus codon-deoptimized vaccine candidate

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Abstract

Zika virus (ZIKV) is a recently re-emerged mosquito-transmitted human pathogen belonging to the family *Flaviviridae*. The infection is either asymptomatic or has very mild nature, manifesting by fever, rash, headache and joint pain. However, epidemics of ZIKV infection has been causally linked to several neurological defects and fetal malformations in children of women infected during pregnancy. In the absence of specific antivirals, the development of a safe and effective vaccine against Zika virus represents a public health priority. Here, we describe an approach for construction of vaccine candidate using partial codon-deoptimization of ZIKV genome.

A part of Asian strain ZIKV genome encoding for NS3 (DO) or NS1-2A-2B-NS3 (full-DO) proteins has been replaced by synthetic fragments with numerous synonymous nucleotide changes thus preserving the amino acid sequence of viral polyprotein. Both deoptimized viruses were rescued from *in vitro* transcribed genome RNA by transfection of Vero E6 cells. ZIKV-full-DO was later propagated and detected by western blot only in insect C6/36 cells; viral proteins were not detectable in Vero cells, neither the virus was able to cause any cytopathic effect. Therefore, this variant was over-attenuated. Meanwhile, ZIKV-DO with codon-deoptimized NS3 region was successfully propagated in Vero cells although showing significantly slower growth rates, lower protein expression level and less cytopathic effect compared to the wild type virus. Further *in vivo* studies are needed to confirm the attenuated nature and protective properties of the developed codon-deoptimized vaccine candidate.

Poster Number: 63

Topic: Antivirals/vaccines

Development of dengue virus replicon cells expressing secretory luciferase

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Abstract

Dengue virus (DENV) belongs to the family *Flaviviridae* and has a single-stranded positive-sense RNA genome. A single long open reading frame of the viral RNA encodes a polyprotein that is processed by cellular and viral proteases into three structural and seven non-structural (NS) proteins. Previously, we have established a transient reporter replicon assay system (Kato et al., JJID, 2014). In this study, we aimed to develop a stable reporter replicon cells that enables consistent viral RNA replication in cell culture. An infectious molecular clone of DENV-1, D1(02-20)/pMW119, was used to construct the replicon cells. Major segments of genes encoding the structural proteins were deleted. The deleted sequence was replaced with a fragment containing a fusion of the gene encoding *Gaussia* luciferase (Gluc), FMDV2A cleavage site, neomycin-resistance gene, and EMCV internal ribosome entry site (IRES). After transfection of the replicon RNA into Huh7 cells, transfected cells were selected by geneticin treatment. Isolated replicon cells exhibited high levels of luciferase activity in the culture supernatant and expression of NS1, NS3 and NS5 proteins in the cells. Also, NS1, NS3 proteins and dsRNA could be detected in the replicon cells by immunofluorescence analysis. Furthermore, the luciferase activity was significantly reduced by anti-DENV inhibitors, ribavirin and bromocriptine in a dose-dependent manner. Taken together, the DENV subgenomic replicon cells expressing a secretory luciferase gene will be useful for high-throughput screening of anti-DENV compounds and analysis of the replication mechanism of the DENV RNA.

Poster Number: 64

Topic: Antivirals/vaccines

BRAZILIAN NATURAL COMPOUNDS AND DERIVATIVE SYNTHETIC ANALOGUES EFFICIENTLY INHIBIT CHIKV AND ZIKV INFECTION

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Abstract

Diseases caused by arboviruses involve complex cycles between vertebrate and hematophagous arthropod vectors and represent important causes of outbreaks and epidemics. Among these diseases, Chikungunya and Zika fevers have been receiving attention by the Brazilian government and public health authorities worldwide. There is no specific antiviral against chikungunya virus (CHIKV) and Zika virus (ZIKV) and current treatment is palliative. The efforts to develop innovative and specific drugs against these viruses are challenged by the high viral mutation rate and the need to develop drugs which can impair the virus with low damage to the host cell. In this context, natural and synthetic compounds are attractive candidates in the search for new therapeutic approaches, as numerous modern drugs have been developed from natural prototypes. Therefore, this study aims to investigate the antiviral effects of a panel of compounds isolated from Brazilian natural sources, and synthetic analogues based on natural scaffolds. Cells were infected with CHIKV or ZIKV and immediately treated with compounds at maximum non-toxic concentration for 16 and 72 hours, respectively. Initial screening of 100 compounds was performed and 12 compounds demonstrated anti-CHIKV or -ZIKV activity. Most interestingly, a synthetic alkaloid reduced both CHIKV and ZIKV infection by 70%, and a natural compound blocked both viruses to a maximum of 97%. These data are the first description of Brazilian natural compounds possessing anti-CHIKV and -ZIKV activities and further analyses are being performed in order to investigate the mode of action of those compounds.

Poster Number: 65

Topic: Antivirals/vaccines

Bunyavirus VSVΔG Pseudoviruses for Detection of Neutralising Antibodies

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Abstract

Crimean-Congo hemorrhagic fever (CCHF) is a severe tick-borne disease with case fatality rate of 5-40%. Principally vectored by *Hyalomma* ticks CCHF is endemic in parts of Africa, Asia and Southern and Eastern Europe, with increasing/ongoing outbreaks in Turkey, Kosovo, Greece, Iran, Tajikistan and Pakistan, including new introductions to India and recently Spain. The aetiological agent of CCHF, CCHF *orthonairovirus (CCHFV)*, a member of the *Nairovirus* family and Bunyavirales order is a tripartite negative sense RNA enveloped virus, which due to the severity of disease, lack of treatment options and potential for human to human transmission is classified by ACDP as a Hazard group 4 agent. Research into anti-CCHF therapeutics and vaccines is consequently heavily restricted due to the requirement for work to be carried out in containment level (CL) 4 laboratories. The development and implementation of non-infectious minigenome and pseudovirus assays is therefore vital, as they enable the efficacy of potential antivirals and vaccine candidates to be assessed at low containment, expediting research into this pathogen. Here we present data on the optimisation of VSVΔG GFP and luciferase based CCHFV pseudovirus assays for the detection of neutralising antibodies. Highlighting protocols that will allow assay qualification for use in vaccine trials and comparing data to PRNT assays (plaque reduction neutralisation tests) that were carried out at CL 4 using wild type CCHFV. Moreover, the versatility of the VSVΔG is system is shown with the production of pseudoviruses bearing the glycoproteins of *Rift Valley fever phlebovirus*, *Heartland phlebovirus*, and *SFTS phlebovirus*.

Poster Number: 66

Topic: Antivirals/vaccines

ACTIVITY OF TOXINS DERIVATES FROM BRAZILIAN SNAKES AGAINST CHIKV AND ZIKV INFECTION

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Abstract

Snake venoms contain a mixture of bioactive compounds that possess numerous metabolic activities. Toxins isolated from snake venoms have been widely studied with respect to their applications, including antiviral properties. In this context, there are no antiviral therapies available for the medically important arboviruses Chikungunya virus (CHIKV) and Zika virus (ZIKV). Therefore, this study aimed to investigate the antiviral effects of toxins isolated from Brazilian snakes against CHIKV and ZIKV. Cells were infected with CHIKV or ZIKV and immediately treated with compounds at maximum non-toxic concentration for 16 (CHIKV) or 72 hours (ZIKV). Screening of an initial panel of 20 toxins identified 4 toxins that inhibited ZIKV infection by up to 99%. More interestingly,1 toxin inhibit up to 97% both ZIKV and CHIKV. The possible interactions between virus particles and toxins were analyzed by Fourier transform infrared (FTIR) spectroscopy. Initial analysis indicated an interaction of ZIKV with one toxin, causing a change in the chemical composition, which is reflected as a difference in the spectroscopic signatures as revealed by the FTIR method. FTIR analysis do not demonstrate the peak at 1415 cm^{-1} (assigned to ammonium group) when ZIKV and one of the toxins were mixtures, indicating a possible interaction between virus and toxin. These data are the first description of toxins from Brazilian snakes possessing activity against CHIKV and ZIKV infection and further analyses are being performed in order to investigate the mode of action of those compounds.

Poster Number: 67

Topic: Vector biology & ecology

New paradigm for dengue surveillance

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Abstract

Dengue is a serious public health problem in Southeast Asia and cases are increasing yearly. *Aedes aegypti* is the primary vector of dengue as well as other viral diseases like chikungunya, Zika and yellow fever. House to house larval surveys has been the hallmark for *Aedes* surveillance for decades. Currently, with unplanned urbanisation, increasing migrant population and rapid air travel cases of dengue have become a huge problem beyond Southeast Asia. Existing vector control measures are reactive and not effective to control dengue epidemics in a timely and targeted way. We suggest a proactive method based on adult surveillance and detection of the virus in the mosquito using a simple technique. A gravid ovipositing sticky (GOS) trap has been developed which cost less than 1USD. Adult mosquitoes trapped were checked for virus using the NS1 antigen test kit and it was found that cases of dengue will occur lag of 1 week after infected mosquitoes were obtained. Thus, control measure should be instituted when positive mosquitoes are obtained and not wait for cases to be reported. Asymptomatic cases can be infective to mosquitoes and this could be one reason why cases of dengue are on the increase. Thus, a new paradigm for surveillance based on adult mosquitoes is an important tool. The outbreak response may be more efficient when timely vector control measures are implemented after the immediate detection of an infected mosquito from the GOS trap.

Poster Number: 68

Topic: Vector biology & ecology

Oxitec: Mosquito Vector Control Technology

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Abstract

Aedes aegypti mosquitoes are the primary vectors of Zika, chikungunya and dengue. As this mosquito is anthropophilic, preferring to inhabit urban environments, control of wild *Ae. aegypti* populations is critical. At Oxitec, we have genetically engineered a self-limiting *Ae. aegypti* strain (OX513A). Following male only releases, the progeny from matings with wild females inherit the self-limiting gene and die before reaching adulthood. Therefore, sustained releases of Oxitec male mosquitoes result in suppression of wild populations. Our OX513A self-limiting strain has proven its efficacy in numerous field trial sites and programmatic releases around the world, with >80% suppression of wild *Ae. aegypti* populations. Although insecticide use can be effective when applied in areas where *Ae. aegypti* populations have not developed resistance, it is not species-specific and can be damaging to the environment. Oxitec's self-limiting approach targets a single species, is non-toxic and does not persist in the environment. Oxitec mosquitoes can also be effectively monitored in the field as they contain a fluorescent marker.

Poster Number: 69

Topic: Vector biology & ecology

Resistance Status to Deltamethrin and Genetic Variability in *Aedes aegypti* form Jambi

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Abstract

Mosquito control is the most efficient method to protect humans from the dengue virus infection. However the overuse of insecticide may lead to the emergence ofinsecticide resistance in mosquito population. This study aimed to examine resistance status against Deltamethrin, kdr mutations and population structure in *Ae. aegypti* population from Jambi, where Deltamethrin had never been used. Larvae and pupae were collected from three regencies in Jambi (Muaro Jambi, Kota Jambi and Batang Hari). Samples were reared to adulthood and their resistance status against deltamethrin 0.05% was determined by standard WHO susceptibility bioassays. Deltamethrin-resistant and susceptible mosquitoes were then genotyped for the Val1016Gly mutation. Following the WHO protocol, bioassays implemented with mosquito surprisingly showed resistance to Deltamethrin in mosquito populations from Muaro Jambi (43.75% ± 2), Kota Jambi (21.25% ± 1) and Batang Hari (15.63% ± 2). The V1016G mutation analysis of 60 genotyped samples indicates that 7.1% of the specimens were homozygous for the mutant allele (1016Gly) and 50% were heterozygous (Val1016Gly). Genetic variability analysis found that haplotypes from this study suggest connections with Mexican and Brazilian populations. These results confirmed that a continuous monitoring and managing program of *Ae. aegypti* resistance in Jambi Province is required.

Poster Number: 70

Topic: Vector biology & ecology

Apical infection of porcine respiratory epithelial cells in air-liquid interface cultures by Japanese encephalitis virus

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Abstract

Japanese encephalitis virus (JEV), mainly distributed through East-Southeast Asia and Australasia, is responsible for a zoonotic viral encephalitis associated with fatal infections in humans. Culex mosquitoes act as main vectors in the JEV enzootic cycle, with pigs being an amplifying host. Recently we have observed efficient direct oro-nasal JEV transmission and infection as well as oronasal virus shedding in absence of viremia. Those findings suggest that JEV is able to infect cells from the porcine respiratory mucosa. In this work, we demonstrated JEV infection and replication in porcine nasal epithelium of cultured explants. In addition, air-liquid interface cultures of nasal epithelium were susceptible to JEV genotype 1 and 3 strains. Viral shedding at was found between 24 and 72 hours post-infection, at both apical and basal sides of the cultures We also found that JEV infection induced an increase of epithelial cell death, and induction of chemokines attracting monocytes. These results demonstrate that nasal epithelial cells are susceptible to JEV and could play an important role in non-vector mediated viral transmission between animals.

Poster Number: 71

Topic: Vector biology & ecology

Culex torrentium mosquitoes from Germany are not infected with *Wolbachia*

Mayke Leggewie[1], Ralf Krumkamp[1,2], Marlis Badusche[1], Anna Heitmann[1], Stephanie Jansen[1], Jonas Schmidt-Chanasit[1,2], Egbert Tannich[1,2], Stefanie C. Becker[1,3]

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Abstract

Wolbachia are intracellular bacteria that are known to infect a wide range of arthropods, including several mosquito species. The bacteria can induce a plethora of phenotypes in their host, such as the reproductive phenotype cytoplasmic incompatibility or resistance against infection with arboviruses. The latter is especially relevant when assessing the vector competence of mosquito species for emerging arboviruses.

Here, we developed a qPCR assay that enables high-throughput screening of *Culex* mosquito samples for *Wolbachia* infection. Using this assay, the *Wolbachia* infection status of the two most common *Culex* species in Germany, namely *Culex pipiens* biotype *pipiens* and *Culex torrentium* was assessed. About 93 % of all tested *Culex pipiens* biotype *pipiens* individuals were positive for *Wolbachia*, whereas none of the *Culex torrentium* samples was found to be infected with the bacteria. Furthermore, we explored other applications of the qPCR assay by assessing a potential link between the level of *Wolbachia* and WNV infection in German *Culex pipiens* biotype *pipiens* mosquitoes. We found no causative relationship between the two variables, indicating that a *Wolbachia*-induced antiviral phenotype in this mosquito population is not exclusively due to the general level of bacterial infection.

Poster Number: 72

Topic: Vector biology & ecology

Deltamethrin Resistance and Its Underlying Mechanism in *Aedes aegypti* Population from Yogyakarta Province

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Abstract

Insecticides resistance has spread rapidly among dengue vectors, and requires an effective vector control strategies. This study aimed to examine the susceptibility to Deltamethrin and the biochemical and molecular mechanism involved in resistance in *Ae. aegypti* population from Yogyakarta, where Deltamethrin had never been used. Three populations (Sleman, Gunung Kidul and Bantul) of *Ae. aegypti* were collected from public residential areas and bioassays were conducted according to WHO guidelines to determine their susceptibility to deltamethrin 0.05%. The mixed-function oxidases activity (MFO) as well as the presence of V1016G and F1534C mutation and its frequency were evaluated. Following the WHO protocol, bioassays implemented with mosquito surprisingly showed resistance to Deltamethrin in mosquito populations from Sleman (50.63% ± 2), Gunung Kidul (73.75% ± 2) and Bantul (78.13% ± 3). Biochemical assays showed low level elevated enzyme activity of MFO. The V1016G mutation analysis of 60 genotyped samples indicates that 36.7% of the specimens were homozygous for the mutant allele (1016Gly) and 23.3% were heterozygous (Val1016Gly). F1534C mutations were also found, but with a lower frequency (3.33% of the specimens were heterozygous) than the V1016G mutation, indicating that V1016G mutation can more contribute to the insensitivity of Voltage Gated Sodium Channel (VGSC) than F1534C. This study highligthted the presence of deltamethrin resistance in *Ae. aegypti* population in Yogyakarta which were never exposed to deltamethrin. Knowledge of resistance status and underlying mechanisms helps in making rational decisions in selection of appropriate and effective insecticides in the event of a dengue outbreak.

Poster Number: 73

Topic: Vector biology & ecology

The WIN Initiative: A Global Network to Combat Insecticide Resistance in Arbovirus Vectors

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Abstract

Arbovirus transmitted by Aedes mosquitoes, such as dengue, zika, chikungunya and yellow fever have been re-emerging all over the world. Vector control, mainly by the use of insecticides, play a key role in disease prevention but the use of the same chemicals for decades, together with the dissemination of vectors resulted in the global spread of insecticide resistance. To implement insecticide resistance management, it is important to identify countries/regions where resistance is present and could challenge vector control interventions. Supported by the WHO Special Programme for Research and Training in Tropical Diseases (TDR) and the Department of Neglected Tropical Diseases (NTDs) since 2016, the Worldwide Insecticide resistance Network, WIN (http://win-network.ird.fr/) aims to track insecticide resistance worldwide and guide the WHO and National Authorities in decision-making on insecticide resistance management and the deployment of alternative or complimentary vector control tools. This presentation will aim at providing an overview of the global distribution and mechanisms of insecticide resistance in Aedes mosquitoes, review the strength and weakness of alternative tools for arbovirus vector control and provide some guidance to improve the management of insecticide resistance in arbovirus vectors.

Poster Number: 74

Topic: Vector biology & ecology

Differential capacity of Zika virus transmission for *Aedes aegypti* from New Caledonia

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Abstract

Zika virus (ZIKV) is a re-emerging *Flavivirus* transmitted to human by infected *Aedes* mosquitoes. ZIKV is divided into two phylogenetic lineages: African and Asian. The recent ZIKV outbreak, due to the Asian lineage, started in 2013 in the Pacific (French Polynesia, New Caledonia…) and then spread to Americas. The aim of this study is to assess the ability of *Aedes aegypti* from New Caledonia (NC) to transmit different strains of ZIKV belonging to both lineages.

Seven ZIKV strains, of which four isolates fit in the Asian lineage, were selected for the study. These last ones were isolated form patients in NC (2014-2015) and in French Guiana (2016), while the three others were isolated in Africa between 1947 and 2002.Vector competence experiments were performed with F2 generation of *Ae. aegypti* from NC. Vector competence indices was estimated at 6, 9, 14 and 21 days post-infection (dpi) for each viral strains.

In contrast with homogeneous infection rates, the transmission efficiency between ZIKV strains was significantly different (ranging from 6.7% to 70% at 14 dpi). The capacity of ZIKV transmission by *Ae. aegypti* from NC is better for the viral isolates belonging to the African lineage than for the Asian ones.

This study confirms the role of *Ae. aegypti* in ZIKV transmission and demonstrate the capacity of different ZIKV strains to infect and replicate in NC *Ae. aegypti*. It also highlights the importance of other factors like vector density, human susceptibly or environmental conditions in ZIKV transmission in NC and in the Pacific.

Poster Number: 75

Topic: Vector biology & ecology

VERTICAL TRANSMISSION OF DENGUE VIRUS ON FIELD MOSQUITOES IN BANYUMAS REGENCY, CENTRAL JAVA, INDONESIA

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Abstract

The role of vertical transmission on the dengue virus (DENV) transmission is still under research. Some researcher believed that vertical transmission play an important role on DENV persistence in nature. However, still a few reports showed vertical transmission on the field mosquitoes. This study aims to detect the vertical transmision on field mosquitoes in Banyumas Regency, Central Java, Indonesia using immunohistochemistry (IHC) assay and Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR). 100 houses were chosen in each of three dengue endemic villages, resulting in a total of 300 houses for ovitrap installations. Eggs were collected from ovistrips and grown in a rearing room to adulthood. Caputs of Fillial 1 from adults mosquitoes were assesed using IHC assay, while thorax part were assesed using RT-PCR. Based on IHC assay, all of the studied villages showed the occurence of vertical transmission with percentage around 13-27%. *Ae. aegypti* showed higher percentage of vertical transmission than *Ae. albopictus.* There is a discrepancy results based on RT-PCR assay since there is no positive band detected on electrophoresis gel indicated negative of dengue virus. The discrepancy results between IHC and RT-PCR is an interesting facts to be explored, due to the specificity and sensitivity of IHC. Even though there is no positive band on electrophoresis gel from RT PCR assay showed that negative result of DENV detection on the studied samples, with respect to positive results confirmation based on IHC assay, it should be an awareness to the health officer to prevent and control DENV tranmission.

Poster Number: 76

Topic: Vector biology & ecology

RNA interference activity in cells of the predatory elephant mosquito, *Toxorhynchites amboinensis*, against Semliki Forest virus.

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Abstract

Mosquitoes of the *Toxorhynchites* genus (Diptera, Culicidae) are autogenous and do not require a blood meal for egg production. Instead, the larvae of several species have been documented as feeding on the larvae of medically important mosquitoes, such as *Aedes aegypti*, the main vector for many arboviruses; including Zika, dengue and chikungunya viruses. As a result, species of this mosquito have been historically recognised as an agent for the biological control of mosquitoes which contribute to disease transmission. However, it is not understood if these non-vector mosquitoes have developed an antiviral response against infections that may be acquired through the ingestion of vertically-infected larvae. In order to investigate the antiviral capacity of these mosquitoes, we identified orthologues of mosquito RNA interference (RNAi) genes and performed RNAi assays in the *Toxorhynchites amboinensis* derived cell line, TRA-171. Following infection with Semliki Forest virus (SFV) it was observed that this cell line possesses an RNA silencing response and generates small RNAs of 21 nucleotides in length, consistent with the cleavage of long double-stranded RNA by Dicer-2. Furthermore, these small RNAs are functional and are capable of mediating SFV infection, in addition to silencing plasmid gene expression.

Poster Number: 77

Topic: Vector biology & ecology

Limitations on estimating the competence of natural mosquito populations from laboratory infection studies.

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Abstract

In recent years there has been an emergence (and re-emergence) of many mosquito-borne viruses of public and animal importance (e.g. Zika). Research into these viruses and their vectors has increased substantially and became both national and international priority. However, work with virus-infected mosquitoes in high containment often demands substantial deviations from the environmental conditions typically encountered by mosquitoes in their natural habitat, such as temperature, food availability and larval density. These deviations often vary from one laboratory to the other. Consequently, the full extent to which laboratory-acquired data on within-vector virus dissemination and vector competence are representative of those observed in the field is not fully understood.

The aim of this work was to investigate the effects of a variety of environmental conditions on the ability of mosquitoes to transmit a range of arboviruses. Laboratory-reared *Culex pipiens* and *Aedes aegypti* were orally infected using sheep blood spiked with several arboviruses at a range of doses. Infected mosquitoes were incubated at a range of temperatures with 70% humidity, 16:8 light:dark photoperiod and with sucrose ad libitum for up to days. The presence of virus in the saliva and whole body was assessed by virus isolation and plaque assay.

This proof-of-concept work will permit us to better understand the conditions needed to develop standard infection protocols that can reliably reproduce field infection of mosquitoes, and in turn better understand the risk of virus transmission in a given environment. This work will be continued as part of the Infravec2 Horizon2020 project.

Poster Number: 78

Topic: Vector biology & ecology

Lipid droplets and prostaglandins are key players in *Aedes aegypti* immune response

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Abstract

In mammals, lipid droplets (LDs) play key roles in the modulation of immune response. Nothing is known about the role of LDs and prostaglandins in mediation of insects' immune response. We have shown that Aedes aegypti Aag2 cells and midgut epithelia accumulate LDs when challenged with bacteria and viruses. This LD increase correlates with prostaglandin production by Aag2 and midgut cells. Impairment of prostaglandin production through treatment with Acetylsalicylic acid (ASA), decreases the expression of several components of mosquito immune pathways, turning Aag2 more permissive to infection. Similarly, in mosquitoes, the down-regulation of genes involved in immune response, by ASA, compromises the immune response, decreasing its survival after challenges. In this study we show for the first time that LDs and prostaglandins are key components of insect immunity. We are now working on the identification of the genes responsible for prostaglandin production and of the prostaglandin receptor in *Aedes*.

Poster Number: 79

Topic: Vector biology & ecology

Developing genetic manipulation methods for *Culex quinquefasciatus*

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Abstract

Culex quinquefasciatus is a major vector of filarial worms, West Nile Fever virus and other pathogens. It also transmits avian malaria and avian pox virus, and in doing so threatens the survival of unique Hawaiian bird species – Hawaii has no native species and the inadvertent introduction of this mosquito and subsequently the pathogens has led to their introduction into highly susceptible native species. We are attempting to develop novel, species-specific genetics-based control methods for this mosquito species, which may provide new possibilities for control. As a first step we are attempting to establish transposon-mediated germline transformation methods, based on earlier work by Meg Allen, and CRISPR/Cas9-based gene editing ("knock-out") and targeted integration ("knock-in") approaches. Our progress towards this will be described.

Poster Number: 80

Topic: Vector biology & ecology

Diversity, abundance and behaviour of potential Zika virus vectors in two urban foci of transmission in Ecuador

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Abstract

In the Americas, Chikungunya, Dengue, and Zika virus are mainly transmitted by *Aedes aegypti* mosquitoes. The large outbreak of Zika in South America last year prompted the need to enhance and improve vector surveillance and control. However, these efforts are hampered by a lack of detailed, current understanding of *Ae. aegypti* ecology within major transmission settings; particularly those outside of Brazil. To address this gap, this study aimed to characterize the local diversity, abundance, behaviour and arbovirus infection rates in potential mosquito vectors within two cities of Ecuador where a high number of Zika cases were reported in the recent epidemic. Starting in September 2016, an 8-month longitudinal mosquito surveillance programme was set up in urban and suburban areas of Portoviejo and Quinindé cities. Collections made by BG-sentinel traps and Prokopack aspirators indicated that *Culex quinquefasciatus* (61.41%) and *Aedes aegypti* (32.61%) dominated the mosquito community. Investigation of the timing of human exposure to *Ae. aegypti* biting is currently underway using a novel, exposure-free sampling method, as is molecular analysis of mosquito samples to estimate viral infection rates. These data will be used to estimate the degree of human exposure to arbovirus infection in both settings, and test whether this can explain local variation in reported human cases. These findings will help identify where and when humans are most at risk of exposure, and guide the selection and deployment of appropriate vector control measures to reduce disease transmission at the local level.

Poster Number: 81

Topic: Vector biology & ecology

Engineering reduced-vector-competence traits into *Aedes aegypti*

Sanjay Basu[1], Katharina von Wyschetzki[1], Barry Atkinson[1], Jessica Mavica[1], Kai Rausalu[2], Age Utt[2], John Fazakerley[3], Indra Vythilingam[4], Jamal I-Ching Sam[4], Rennos Fragkoudis[1], Andres Merits[2], Luke Alphey[1]

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Abstract

Aedes aegypti is a major vector species known to transmit a range of viruses responsible for a significant global disease burden including dengue and chikungunya. Few of these arboviral diseases have vaccines or licensed drugs available. As mosquito synthetic biology becomes more developed, a range of novel genetics-based approaches are being pursued. We are attempting to engineer *Aedes aegypti* to reduce its transmission ability with particular focus on chikungunya virus, as part of a Wellcome-MRC Newton Award-funded consortium. We are developing in parallel three different molecular approaches to reducing vector competence: (1) expression of a specific hairpin RNA, building on successful demonstration of this for DENV2 by Ken Olson's group (2) initiation of a super-infection exclusion mechanism such that the engineered cell acts as if already infected and therefore refractory to further infection (3) building genetic viral sensors that detect the presence of the virus, which can then be linked to the expression of a lethal effector. These different approaches and our progress towards them will be discussed.

Poster Number: 82

Topic: Vector biology & ecology

Vector competence of British mosquitoes for Rift Valley fever virus

Sarah Lumley[1,2,3], Daniel Horton[2], Roger Hewson[1], Jolyon Medlock[1], Luis Hernández-Triana[3], Maria Del Mar Fernández de Marco[1], Anthony Fooks[3], Nicholas Johnson[3]

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Abstract

Rift Valley fever virus (RVFV) is a pathogen of both veterinary and public health importance causing widespread abortions and mortality in ruminants and acute febrile illness in humans. RVFV is endemic throughout Africa, maintained in a transmission cycle between its mosquito vector and mammalian hosts. In order to assess the risk of establishment in the UK an understanding of the competence of indigenous mosquitoes to support and transmit RVFV is required.

In this study we offered 440 *Aedes detritus*, collected as larvae in Cheshire, a blood meal containing RVFV strain ZH501 or Lunyo at a final concentration of ~10^6 PFU/mL. Mosquitoes were maintained at 20^{o}C or 25^{o}C and tested on days 0, 7 and 14 post-infection. The bodies, legs and saliva were separated for each mosquito and assayed to calculate the infection, dissemination and transmission potential respectively. Viral RNA was detected by qRT-PCR and plaque assays were performed on positive samples to confirm the presence of infectious viral particles. An average infection rate of 20%, dissemination 10% and transmission 1% were observed. Concluding there is limited transmission potential at this low viral dose in this species further limited at 25^{o}C due to reduced survival at the higher temperature.

Poster Number: 83

Topic: Vector biology & ecology

Learning the ACTGs of arbovirus transmission: genomic prediction of the existence and identity of arthropod vectors of RNA viruses

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Abstract

Current sequencing technologies can rapidly identify novel pathogens during outbreaks. However, determining the ecological origins of emerging pathogens and routes of transmission to humans and domestic animals still requires multi-disciplinary field and laboratory research. This can create long time lags between pathogen discovery and actions for prevention and control. We developed machine learning frameworks to infer the existence and broad-scale taxonomic identity of arthropod vectors of RNA viruses using phylogenetic and genomic traits extracted from viral genomes. Across 487 viruses from 11 families that include some of the most important modern emerging pathogens, our algorithms infer the presence of arthropod vectors with over 95% accuracy. Furthermore, separate algorithms trained on a smaller subset of 103 known arboviruses from 3 viral families distinguished midge, mosquito, sandfly and tick transmitted viruses with over 75% accuracy. Applying our trained models to the genomes of viruses with uncertain roles of vectors in transmission or with uncertain vector identities provided predictions that might help target vectors for future field surveillance and control. Real-time inference of viral vectors from increasingly available viral sequence data enlightens diagnostics and pre-emergence surveillance and may accelerate the deployment of effective interventions during the critical early phases of outbreaks, potentially impeding further epidemic spread.

Poster Number: 84

Topic: Vector biology & ecology

Synthetic miRNAs induce dual arboviral-resistance phenotypes in the vector mosquito, *Aedes aegypti*

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Abstract

Mosquito-borne arboviruses cause some of the world's most devastating diseases and are responsible for recent dengue, chikungunya and Zika pandemics. The mosquito *Aedes aegypti*, plays an important role in the transmission of all three of these viruses. The ineffectiveness of chemical control methods for of *Ae. aegypti* makes urgent the need for novel vector-based approaches for controlling these diseases. Genetically-engineered *Ae. aegypti* have been proposed as possible solutions for stopping arboviruses spreading within urban cycles. We developed an miRNA-based approach that results in a dual resistance phenotype in mosquitoes to dengue serotype 3 (DENV-3) and chikungunya (CHIKV) viruses. Ten artificial antiviral miRNAs capable of targeting ~97% of all published strains were designed based on derived consensus sequences of CHIKV and DENV-3. The antiviral miRNA constructs were placed under control of either an *Aedes PolyUbiquitin* (*PUb*) or *Carboxypeptidase A* (*AeCPA*) gene promoter triggering respectively expression ubiquitously in the transgenic mosquitoes or more locally in the midgut epithelial cells following a blood meal. Challenge experiments using viruses in blood meals showed subsequent reductions in transmission efficiency in the saliva of transgenic mosquitoes as a result of lowered infection rates and dissemination efficiency. Several components of mosquito fitness were examined and transgenic mosquitoes with the *PUb* promoter showed minor fitness costs at all developing stages whereas, those based on *AeCPA* exhibited a high fitness cost. Further development of these strains with gene editing tools could make them candidates for releases in population replacement strategies for sustainable control of multiple arbovirus diseases.

Poster Number: 85

Topic: Vector biology & ecology

What's new in the Tick Cell Biobank?

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Abstract

Tick cell lines are increasingly used in many fields of tick and tick-borne disease research, including isolation, propagation and study of arboviruses and endogenous viruses carried by ticks and other arthropods. The Tick Cell Biobank was established eight years ago to facilitate the development and uptake of these unique and valuable resources. As well as serving as a repository for existing and new ixodid and argasid tick cell lines, the Tick Cell Biobank supplies cell lines and training in their maintenance to scientists worldwide and generates novel cultures from tick species not already represented in the collection. Now part of the Institute of Infection and Global Health at the University of Liverpool, the Tick Cell Biobank has embarked on a new phase of activity particularly targeted at research on problems caused by ticks, other arthropods and the diseases they transmit in less-developed, lower- and middle-income countries. As well as genotypic and phenotypic characterisation of selected cell lines derived from tropical tick species and establishment of novel cell lines from other arthropod vectors such as midges, mites and sand flies, outposts of the Tick Cell Biobank will be set up in Malaysia, Kenya and Brazil to facilitate uptake and exploitation of cell lines and associated training by scientists in these and neighbouring countries. Thus the Tick Cell Biobank will continue to underpin global research into biology and control vector-borne viral pathogens with both existing and new collaborators.

Poster Number: 86

Topic: Emergence from A to Zika

Co-infection of *Aedes aegypti* with Zika and chikungunya virus allows simultaneous transmission in one mosquito bite

Giel Göertz[1], Chantal Vogels[2], Corinne Geertsema[1], Constantianus Koenraadt[2], Gorben Pijlman[1]

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Abstract

Zika virus (ZIKV) and chikungunya virus (CHIKV) are highly pathogenic arthropod-borne viruses that present a serious health threat to humans. Since 2015, both viruses circulate in the same geographical regions of the Americas where they are predominantly transmitted by *Aedes aegypti* mosquitoes. There is a growing number of case reports of ZIKV and CHIKV co-infections in humans, but it is uncertain whether these co-infections occurred via a single or multiple mosquito bites. Furthermore, it is unclear whether mosquito co-infections affect the vector competence for either virus. Therefore, we investigated whether mosquitoes can be co-infected with ZIKV and CHIKV, and if this has an effect on virus transmission. *Aedes aegypti* mosquitoes were infected via an infectious blood meal with ZIKV, CHIKV or co-infected. Saliva and bodies of (co-)infected mosquitoes were tested 14 days post infection for the presence of CHIKV/ZIKV using a dual-colour immunofluorescence assay. The results show that *Ae. aegypti* is competent for both viruses with transmission rates of up to 73 % for ZIKV and 21 % for CHIKV. Twelve percent of the co-infected mosquitoes became saliva-positive for both viruses, indicating that *Ae. aegypti* is capable of transmitting both ZIKV and CHIKV in a single bite. Importantly, co-infections did not influence the infection or transmission rates for either ZIKV or CHIKV, compared to single infections. Our results demonstrate that ZIKV and CHIKV can be transmitted through a single mosquito bite and that co-circulation of ZIKV and CHIKV in the Americas does not limit the transmission and geographical dissemination of either virus.

Poster Number: 87

Topic: Emergence from A to Zika

Detection of Zinkvirus in Iran

mohammad kazem Sharifi-yazdi

Tums, Tehran, Iran, Islamic Republic of

Abstract

Zika virus is a member of *Flaviviridae* viruses, which is transmitted by Aedes mosquitoes and this is the same mosquito that transmits other infections such as dengue fever and yellow fever . Zika virus was also found in the semen of a man at least two weeks after he was infected with Zika fever . In year 2015, the virus was reported in South American countries (Brazil and Colombia) and Africa. In addition, more than 13 countries in the Americas have reported sporadic cases of Zika virus infection . Today, this virus is circulating in Africa, South America and Asia. The name of this virus (Zika) comes from the Zika Forest in Africa (Uganda), where the virus was first isolated in 1947. Zika fever often causes no or only mild symptoms, similar to a mild form of Crimean-Congo Hemorrhagic Fever (CCHF), dengue fever and sometimes like respiratory viruses, influenza or corona viruses. There have been several cases of acute ZIKV infection in Iranian residents across the country confirmed by molecular or serological testing including sequence data.

Poster Number: 88

Topic: Emergence from A to Zika

Mapping of transcription termination within the S segment of SFTS phlebovirus facilitated the generation of NSs-deletant viruses.

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Abstract

SFTS phlebovirus (SFTSV) is an important emerging tick-borne pathogen that was first reported in China in 2009.

Using 3' RACE analysis of RNA isolated from SFTSV-infected cells we show that the Nucleocapsid (N) sub-genomic mRNA terminated past the intergenic region within the coding region of the positive sense Non-Structural (NSs) protein open reading frame (ORF). Utilizing our SFTSV minigenome system, Northern blotting and luciferase-based assays we identified specific transcription termination signals responsible for the correct synthesis of the N and NSs sub-genomic messenger RNAs.

C-terminal truncation mutants of the NSs ORF were generated and used in our reverse genetics system to assess the ability of recombinant viruses to rescue. We found that a 36nt region within the NSs ORF was essential for efficient N mRNA termination and hence crucial for virus rescue. The identification of this termination region allowed us to generate complete functional NSs-deletant viruses, viruses encoding eGFP in place of the NSs ORF and a virus which expresses a NSs-eGFP fusion. Recombinant viruses lacking the NSs ORF were attenuated and failed to efficiently antagonize the human interferon response in cell culture, with NSs-deletant viruses inducing high levels of IFN during infection.

Our data demonstrate that the NSs protein of SFTSV is dispensable for virus replication and that NSs protein is the major virally encoded interferon antagonist. Now the hurdle of producing an NSs-deletant virus has been overcome efforts can focus on the development of recombinant viruses that can be utilized as live attenuated vaccines against this emerging pathogen.

Poster Number: 89

Topic: Emergence from A to Zika

Localization of Zika virus capsid protein expression in HEK 293 cells

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Abstract

The Zika virus (ZIKV) capsid (ZIKV-C) is one of the viral structural proteins. During the virus life cycle, the capsid is located in the endoplasmic reticulum (ER) membrane via the transmembrane-anchored part (ancC). The nucleolar accumulation of the viral capsid protein is a common feature of most flaviviruses (e.g. Dengue- West Nile- and Japanese encephalitis virus). Accordingly, nuclear localization signals (NLS) were identified in all of these three viral capsid proteins. However, NLS has not been identified in new emerging flaviviruses such as ZIKV. Our aim was to characterize the localization pattern of ZIKV-C protein expression in human embryonic kidney (HEK) 293 cells.

To determine if ZIKV-C also localized and accumulated in the nucleus, we constructed a ZIKV-ancC plasmid, using a Brazilian ZIKV isolate, and fused it with the *Zoanthus* sp. green fluorescent protein (ZsGreen) to visualize the protein expression in the cell. After transfection of the plasmid into HEK 293 cells, anc-C was expressed not only in the nucleolus, but also localized to the membrane of intracellular cytoplasmic vesicles. These structures could be an ER membrane accumulation, lipid droplets, lysosomes, or autophagosomes. Currently, the investigation is ongoing to identify NLS in ZIKV-C and define the link between anc-C and the vesicles.

Our finding suggests that (1) ZIKV-C could have a role in the nucleolus and interacts with nucleolar proteins; (2) the presence of ZIKV-C in cytoplasmic vesicles might connect to ER reorganization, fatty acid synthesis, lysosome genesis, or autophagy pathway.

Poster Number: 90

Topic: Emergence from A to Zika

European Virus Archive goes Global (EVAg) support during the recent ZIKV emergence

cecile baronti, Christine Prat

UMR "Émergence des Pathologies Virales" (EPV: Aix-Marseille Univ – IRD 190 – Inserm 1207 – EHESP – IHU Méditerranée Infection), Marseille, France, Marseille, France

Abstract

The "European Virus Archive goes Global" (EVAg), is a non-profit European Union consortium grouping virology laboratories worldwide which isolate, characterize, certify and make available for the scientific community relevant clinical viral strains and derived products.The main mission of EVAg is the development and maintenance of a large active biological resource in order to facilitate their access to both academics and industries.Together with this background activity, the role of EVAg is also to mobilize specific task force in case of emergence in order to support public health response.

In 2015, Zika virus (ZIKV) has emerged for the first time in the Americas and has since been causing an unprecedented outbreak in several countries of South, Central, and North America. In February 2016, the World Health Organization (WHO) declared ZIKV as a Public Health Emergency of International Concern. In this context, the "European Virus Archive goes Global" coordinated response was to rapidly propose a set of products available via its online catalogue: (i) relevant panel of ZIKV clinical strains, (ii) molecular diagnostic tools including ready to use diagnosis kits (recommended by the WHO), synthetic encapsulated positive controls and external quality controls, (iii) and research tools such as reverse genetic systems and expression plasmids.

EVAg has proven to be extremely efficient in supporting the international response during the ZIKV outbreak: more than 300 ZIKV related products were supplied worldwide and the consortium demonstrated also an active interplay with others European Union projects (ZikAlliance and EVDLabNet).

Poster Number: 91

Topic: Emergence from A to Zika

Transcriptomic analyses reveal differential gene expression of immune and cell death pathways in the brains of mice infected with West Nile virus and chikungunya virus

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Abstract

West Nile virus (WNV) and chikungunya virus (CHIKV) are arboviruses that are constantly (re)-emerging and expanding their territory. Both viruses often cause a mild form of disease, but severe forms of the disease can consist of neurological symptoms, most often observed in the elderly and young children, respectively, for which the mechanisms are poorly understood. To further elucidate the mechanisms responsible for end-stage WNV and CHIKV neuroinvasive disease, we used transcriptomics to compare the induction of effector pathways in the brain during the early and late stage of disease in young mice. In addition to the more commonly described cell death pathways such as apoptosis and autophagy, we also found evidence for the differential expression of pyroptosis and necroptosis cell death markers during both WNV and CHIKV neuroinvasive disease, while no evidence of cell dysfunction was observed. Interestingly, there was overlap when comparing immune markers involved in neuroinvasive disease to those seen in neurodegenerative diseases. Nonetheless, further validation studies are needed to determine the involvement of these effector pathways at the end stage of disease. Furthermore, evidence for a strong inflammatory response was found in mice infected with WNV and CHIKV. The transcriptomics profile measured in WNV and CHIKV neuroinvasive disease in our study showed strong overlap with the mRNA profile described in the literature for other viral neuroinvasive diseases. More studies are warranted to decipher the role of inflammation and cell death in viral neuroinvasive disease and whether common mechanisms are active in both neurodegenerative and brain infectious diseases.

Poster Number: 92

Topic: Emergence from A to Zika

The First Serological Evidence of West Nile Virus Transmission in Ethiopia

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Abstract

Introduction
West Nile virus (WNV) is a mosquito-borne virus that belongs to genus Flavivirus. Even though WNV infections can cause fatal neurological diseases, approximately 80% WNV infections are asymptomatic. Consequently, a surveillance system may substantially miss WNV infection cases. The surveillance system in Ethiopia has never reported or detected WNV infections. Here, we report serological evidence of WNV transmission in Ethiopia.

Methodology
Serum samples were collected from a total of 1643 study participants from ecologically distinct areas throughout the country and tested for flavi viruses namely; Yellow Fever, Dengue Fever, Zika and West Nile viruses specific IgG using ELISA as a screening test and Plaque Reduction Neutralization Test (PRNT) as a confirmatory test. Demographic and laboratory data was analyzed using excel spreadsheet.

Results
Of the total serum samples tested, 14 (0.91%) showed a confirmed PRNT IgG antibodies against WNV. Nine of the total positive cases (64.2%) were Females and the median age for the positive cases was 23.5; with the youngest study participant infected with the virus being a five year old girl and the oldest a 65 year old woman.

Conclusions and Recommendations
This is the first serological evidence showing WNV transmission in Ethiopia. Further studies are required to see if there is active infection in the areas with IgG positivity and understand the risk factors for infection and transmission. This study also warrants existing surveillance system in Ethiopia to consider WNV infection more closely and be able to detect and respond accordingly.

Poster Number: 93

Topic: Emergence from A to Zika

BTV-GLUE: a bioinformatic resource for bluetongue virus sequence data

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Abstract

Bluetongue virus (BTV) is an insect-borne virus within the Orbivirus genus of the Reoviridae family. BTV can be the cause of severe hemorrhagic disease (bluetongue) in domestic and wild ruminants. In the last two decades, BTV has expanded its geographical distribution worldwide.

The virus has 10 double-stranded RNA (dsRNA) segments encoding 7 structural and 5 non-structural proteins. Reassortment involving all genome segments has been well documented. Surveillance and vaccination programmes against bluetongue are complicated by the fact that there are at least 27 serotypes in circulation.

We have created BTV-GLUE, a new bioinformatic sequence data resource for bluetongue virus. We collated several thousand BTV sequences from the NCBI nucleotide database and added complementary contextual metadata alongside each sequence. BTV sequence data were integrated inside GLUE, a data-centric software package for capturing virus sequence data and organising it along evolutionary lines.

Each of BTV's 10 segments is allocated a separate phylogenetic data structure. Each sequence is held within a multiple sequence alignment specific to the segment and clade. Reference sequences with genome feature annotations are defined for each segment clade.

While BTV-GLUE can be used offline in a conventional bioinformatics context, some aspects of its functionality, including access to sequences, metadata and alignments, have also been made available via a public web server (http://btv.glue.cvr.ac.uk). BTV-GLUE will help the wider BTV community to study the ongoing evolution and epidemiology of the virus worldwide, whilst also allowing analysis of reassortment and other phenomena.

Poster Number: 94

Topic: Emergence from A to Zika

Experimental transmission of Zika virus by mosquitoes from central Europe

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Abstract

In 2015, Zika virus (ZIKV) emerged in Columbia and Brazil and spread rapidly across the American continent and the Caribbean, causing an epidemic associated with notable clinical cases of microcephaly and Guillain–Barré syndrome. Mosquitoes of the species *Aedes aegypti* and *Ae. albopictus* are considered primary and secondary vectors of ZIKV, respectively. Due to the rapid spread of *Ae. albopictus* in Europe, it is important to assess its vectorial capacity. Furthermore, other common mosquito species such as *Culex* spp. may also play a role in the transmission cycle of ZIKV. In addition, information is lacking on ZIKV vector competence of mosquitoes under reduced temperature conditions (< 20°C).

This study aimed to evaluate the vector competence of central European mosquito species for ZIKV. Therefore, we collected German populations of *Ae. albopictus*, *Culex pipiens pipiens* biotype *pipiens, Cx. pipiens pipiens* biotype *molestus*, and *Cx. torrentium,* and performed infection experiments with ZIKV at 18 °C or 27 °C. *Aedes aegypti* and Italian *Ae. albopictus* were used as positive controls.

As expected, *Ae. aegypti* showed relative high infection and transmission rates for ZIKV. None of the *Culex* taxa had vector competence for ZIKV. In contrast, *Ae. albopictus* from Italy and Germany showed transmission at 27°C. None of the tested *Aedes* populations were susceptible to ZIKV at 18°C, which may limit the spread of ZIKV in central Europe to short summer periods. However, to allow a comprehensive risk assessment of ZIKV transmission in Europe, infection studies at intermediate temperatures are needed.

Poster Number: 95

Topic: Emergence from A to Zika

Dengue infection among acute undifferentiated febrile illness (AUFI) patients in Bangkok, Thailand, 2013-2015

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Abstract

Dengue is the most important vector borne diseases and also the major cause of acute undifferentiated febrile illness (AUFI) in Thailand. This study aimed to determine the incidence of dengue infection among the case presenting as AUFI, and also factors to differentiate from other diseases. The hospital based surveillance study was done in the Hospital for Tropical Diseases (HTD), Bangkok, Thailand during July 2013 to April 2015. There were 188 cases of dengue cases (total AUFI 397 cases). Of those, 31 cases (16.5%) had co-infection with other diseases such as leptospirosis, rickettsiosis, influenza andbacteremia. The most common identified serotype was serotype 3 (69 cases, 48.6%), followed by serotype 4 (33 cases, 22.9%), serotype 1 (24 cases, 16.7%) and serotype 2 (10 cases, 7.0%). Interestingly, 6 cases were infected with more than one serotype at the same time. Nevertheless, there was no severe disease among cases infected with 2 serotypes. Data from multivariate analysis found that the clinical parameters at presentation using to distinguish dengue from other etiologies were history of dengue fever in neighborhood, history of vomiting, pethichiae, positive tourniquet test, Hct ≥40%, WBC ≤4500/µl, lymphocyte ≥ 30%, Atypical lymphocyte ≥8%, Plt ≤80,000/µl, and AST/ALT≥ 1. These results demonstrated that co-infection between dengue and other diseases were not uncommon in tropical area. Additionally, infection with 2 serotypes of dengue virus could be found in context of multiple serotypes circulation without evidence of severe diseases.

Poster Number: 96

Topic: Virus discovery

Diversity of viruses in mosquitoes and sandflies collected from Nepal

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Abstract

Introduction: Various arthropod-borne viruses cause diseases such as dengue, chikungunya and bluetongue in humans and animals in Nepal. However, so far no vector-based surveillance study has been conducted. Here, we sought to investigate the diversity of arboviruses in mosquitoes and sandflies.

Materials and methods: Mosquitoes (n=1,794) and sandflies (n=152) were collected in human houses and animal sheds in Nepal. Samples were tested for Flaviviruses, Alphaviruses, Orthobunyaviruses, Phleboviruses, Goukoviruses, Phasiviruses, Rhabdoviruses and Orbiviruses by generic RT-PCR. Virus isolations were done in C6/36 and Vero E6 cells.

Results: We identified 5 Flavi-like viruses, 1 Phlebovirus, 2 Goukovirus-like viruses, 2 Phasivirus-like viruses, 3 Rhabdo-like virus and novel strains of blue tongue virus (BTV) and Tibet orbivirus (TIBOV) in mosquitoes and sandflies based on phylogenetic analyses. The novel sandfly-borne phlebovirus which shared 76% pairwise nucleotide sequence similarity to Rift Valley fever virus was discovered. Out of 179 pools, 27% of them showed cytopathic effects in cell culture. Cell culture supernatants were tested negative by generic PCR assays suggesting the isolation of previously unknown viruses. Deep sequencing results revealed the discovery of novel reovirus, rhabdovirus and strains of BTV and TIBOV that replicated well in various invertebrate cells. However, BTV and TIBOV strains that were isolated from Anopheles mosquito also replicated in different vertebrate cells including livestock cells.

Conclusion: Our findings showed the presence of a great diversity of novel arthropod-associated viruses in Nepali mosquitoes and sandflies. Further investigation is needed to measure their impact on human and animal health.

Poster Number: 97

Topic: Virus discovery

Discovery of novel vector-borne viruses in the Zambezi valley of Mozambique

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Abstract

Arthropods carry a wide variety of viruses that can cause vector-borne infectious diseases and affect both human and veterinary public health. Although Mozambique can be considered a hotspot for emerging infectious diseases due to factors such as a rich vector population and a close vector/human/wildlife interface, the viral flora in arthropod vectors have not previously been investigated in this area. In this study, viral metagenomics was employed to analyze the viral communities in *Culex* and *Mansonia* mosquitoes, and in *Rhpicephalus* ticks in the Zambezia province of Mozambique. Viruses belonging to a number of different viral families such as, for example, *Flaviviridae, Rhabdoviridae* and *Iflaviridae* were detected in the mosquitoes, as were different unclassified RNA viruses. The complete genome of a flavivirus, tentatively named Cuacua virus, was obtained from *Mansonia* spp mosquitoes. Phylogenetic analysis of the NS5 amino acid sequence showed that it grouped with insect-specific viruses and was most closely related to Nakiwogo virus (85% of identity at amino acid level). The majority of the viral sequences from ticks showed closest relationship to the genus *Quaranjavirus* in the family *Orthomyxoviridae*, and the near full-length sequence of five segments was recovered (32-50% of identity at amino acid level). This study demonstrates that a large number of uncharacterized viruses circulate in the arthropod populations in Mozambique. Viral metagenomic studies, such as this, demonstrate the diversity of the virome associated with arthropod vectors and highlight the need for investigations into the potential role that these viruses may play in disease emergence and spread.

Poster Number: 98

Topic: Virus discovery

Triple-D Targets: The UK-Philippines Dengue Diagnostic and Drug Targets Research Consortium.

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Abstract

Dengue is the most important arthropod-borne human disease and endemic in the Philippines which has the 4th largest disease burden among Southeast Asian countries. DENV infection produces a variety of clinical presentations, but the factors contributing to differential disease severity are poorly understood. Our overall objective, by adopting a liquid biopsy approach and integrating clinical and molecular data, is to correlate the spectrum of disease severity with DENV genetic diversity, host transcriptomics and proteomic changes observed in the periphery. This should enable identification of candidate virological and/or host biomarkers to develop diagnostics that predict progression to severe disease.

We have developed pipelines to generate and analyse metagenomic and proteomic data from retrospectively-collected sera from patients with DENV and other febrile illnesses. Two bioinformatic pipelines, using *de novo* assembly and reference-based mapping, have been compared using the metagenomics data. Both approaches enabled assembly of full-length DENV genomes without PCR-based enrichment and established a broad range of viral sequences in clinical samples. All four DENV serotypes were identified, with a higher prevalence of DENV-4 detected compared to standard diagnostic methods. We also found co-infections of DENV serotypes, HIV/DENV co-infection and Chikungunya virus infection. Analysis of the proteomic dataset identified possible host markers that distinguish DENV-infected patients with distinct clinical presentations. The potential biomarkers will be verified using samples from a dedicated biobank, collected longitudinally, from patients with differing disease severities. Analysis of the cohort samples will also enable linkage of clinical, viral genomic and proteomic data to blood transcriptomic profiles.

Poster Number: 99

Topic: Virus discovery

Application of a portable sequencer to serotype and genotype dengue viruses.

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Abstract

Abstract:

The innovation of portable DNA sequencer can help to advance disease identification. By combining isothermal amplification, this could greatly help disease identification to genetic level directly in the field, where we collect the samples. Here, we report the development of a method to detect and genotype tropical disease pathogens, using dengue fever as a model. This easy-to-do technique needs only a water bath to amplify and sequence target viral genomes. Starting from a serum sample, the entire procedure could be finished in a single day. We describe the analysis of blood samples collected from 141 Indonesian patients to demonstrate the robustness of the method for identifying and serotyping dengue virus (DENV) with high sensitivity and specificity. The overall successful dtection rate was 79%, and a total of 58 SNVs were detected. We were also able to differentiate between DENV serotypes, with DENV1 being the most detected.

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