IMAV: INTERNATIONAL MEETING ON ARBOVIRUSES AND THEIR VECTORS

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INVITED AND OFFERED TALKS

#IMAV2026

MICROBIOLOGY SOCIETY

Invited Speaker :

Recent advances in our understanding of vector-virus relationships

Stephen Higgs

Biosecurity Research Institute, Kansas State University, Manhattan, USA

Abstract

Despite over 100 years of research on mosquito-borne pathogens, our knowledge of vector-virus-vertebrate interactions remains incomplete. Over the last 25 years, many of the viruses transmitted by mosquitoes have been associated with increasing numbers of human cases and expanded geographic ranges. Examples include the 1999 introduction and establishment of West Nile virus in the Americas, the almost global spread of chikungunya and Zika viruses during the early-mid 2000s, and the 2022 introduction of Japanese encephalitis virus into Australia. Since there are relatively few approved efficacious vaccines to protect people from infection with vector-borne viruses, both traditional and recently-developed methods to reduce the incidence of vector-borne diseases, continue to focus on controlling vector populations. A better understanding of the determinants and influencing factors involved in mosquito species-specific susceptibility to infection with viruses, and viral genetic determinants of infectivity for mosquitoes, could provide new opportunities for disease control. With references to published experimental data, the presentation will review and discuss some of the recent discoveries in the fields of vector biology and virology that are advancing our knowledge and understanding of the infection, dissemination, and transmission processes. Gaps in our knowledge of the virus-vector relationship, that remain to be filled will also be discussed.

Understanding and targeting the taste system of mosquitoes

Lisa Baik[1], Gaëlle Talross[2], Stephanie Reis[2], Sydney Gray[2], Himani Pattisam[2], Taylor Peterson[2], James Nidetz[2], Felix Hol[3], John Carlson[2]

[1]University of California Davis, Davis, USA. [2]Yale University, New Haven, USA. [3]Radboud University Medical Center, Nijmegen,, Netherlands

Abstract

The taste system controls many insect behaviors but is greatly understudied in mosquitoes. Little is known about how tastants are encoded in mosquitoes or how they regulate critical behaviors. Here we examine how taste stimuli are encoded in mosquitoes and how these cues regulate biting, feeding, and egg laying. We identify three functional classes of taste sensilla with an expansive coding capacity. Complex cues, including human sweat, nectar, and egg-laying site water, elicit distinct response profiles from the neuronal repertoire. Certain bitter compounds suppress physiological and behavioral responses to sugar, suggesting their use as potent stop signals against appetitive cues. We identify key tastants on human skin and in sweat that synergistically promote biting behaviors. Lastly, we also identify a new class of compounds that effectively deter biting behaviors. Our study sheds light on key features of the taste system that suggest new ways of manipulating chemosensory function and innovating methods of vector control.

Genetic control of mosquitoes and arboviruses

Luke Alphey

University of York, York, United Kingdom

Abstract

Genetic control methods are being developed for several major insect pest species, based on genetic modification of the insect pest followed - after considerable laboratory, regulatory and community-engagement work - by release of modified insects into target population(s). Modifications may aim to reduce the numerical size of the pest population ("population suppression") or to reduce the vector competence of insects carrying the novel genetic trait ("refractory insects"). This talk focuses on the latter, **how to reduce the ability of *Aedes aegypti* to transmit key viruses such as dengue virus, Zika virus and Chikungunya virus**.

A further practical consideration is how to get such a heritable modification into a wild vector population at a high enough allele frequency to have a significant effect on vectorial capacity, and hence potentially an epidemiological impact. While mass-release of modified insects may work well in some cases, especially for some small or accessible target populations, in many cases more self-sustaining genetic systems - gene drives - are likely essential. CRISPR/Cas9-based artificial selfish DNA elements ("gene drives") have recently been described that might be capable of spreading through an entire species or species complex - though current designs would likely be neutralised by mutation, e.g. target site mutation, before reaching every individual. At York we are aiming to develop "local" gene drives that will sustainably modify a target population but not invade other populations of the same species. Some of the principles, progress and some limitations will be discussed.

Flavivirus immune history as a determinant of future viral infection outcomes

Ashley St. John

Duke-NUS Medical School, Singapore, Singapore

Abstract

Flavivirus exposure is increasingly common, and pre-existing flavivirus immunity can shape the outcome of subsequent infections. This is particularly well established for dengue virus (DENV), where prior infection can alter the risk and severity of later disease. Our studies show that these effects extend beyond antibody specificity and involve imprinting of the broader immune program. During primary DENV infection, NKT-cell-dependent Th1/Th2 polarization influences the functional quality of the developing antibody response and, consequently, protection or enhancement during subsequent dengue infection, linking acute immune programming to the long-term functions of antibody memory. Beyond heterologous DENV exposure, immunity generated by other flavivirus encounters can also modify disease. In a highly Japanese encephalitis virus (JEV)-immune population in Nepal, intermediate levels of JEV-neutralizing IgG antibodies were associated with increased dengue severity, emphasizing the ability of Flavivirus cross-reactive immunity to shape disease outcomes and the importance of maintaining robust JEV vaccine-induced protection in the face of rising dengue epidemics. A further example of how prior flavivirus exposure shapes host immunity emerged from a study of febrile patients in Sri Lanka, where DENV-specific IgM was detected in DENV-immune individuals without virologic evidence of acute dengue. Among patients with confirmed COVID-19, this serologic profile identified individuals with a primed immune state characterized by distinct transcriptional responses and was associated with faster symptom resolution and lower mortality. Together, these studies provide insight into how immune programming during primary infection, durable flavivirus-specific memory, and ongoing immune conditioning in dengue-hyperendemic settings can shape outcomes during subsequent viral infections.

Offered ARES Speaker :

Optimizing West Nile Virus Diagnosis: Molecular and Serological Insights from a Large Scale Outbreak

Vicki Indenbaum[1], Oran Erster[1], Danit Sofer[1], Ravit Koren[1], Shiri Katz Likvornik[1], Osnat Halpern[1], Enosh Tomer[1], Yaniv Lustig[1,2]

[1]Central Virology Laboratory, Ramat Gan, Israel. [2]School of Public Health, Tel Aviv University, Tel Aviv, Israel

Abstract

In 2024, Israel reported its largest West Nile Virus (WNV) outbreak to date, with 934 confirmed cases and 73 deaths. Current diagnostic practice predominantly relies on serological detection of IgM antibodies as the first-line approach, with molecular testing considered complementary; however, cross-reactivity with co-circulating flaviviruses and prolonged IgM persistence complicate accurate case confirmation. Leveraging the extensive sample collection during this outbreak, we evaluated and compared the diagnostic performance of molecular and serological assays across multiple sample types. During the outbreak, the Israeli National Laboratory for Zoonotic Viruses received more than 2,000 samples from 919 WNV-positive cases, including whole blood (WB), serum, urine, and cerebrospinal fluid (CSF), examined by qRT-PCR and ELISA. WNV RNA detection rates were highest in WB (91%), followed by serum (82%), urine (71%), and CSF (53%), with WB remaining positive up to 52 days post-symptom onset, substantially exceeding the detection window of all other sample types. Of note, while WB RNA detection rates were similar between hospitalized patients and health maintenance organization (HMO) patients (91% vs. 92%), IgM seropositivity in serum was significantly lower in HMO-derived samples (69%) compared to hospital samples (91%), likely reflecting earlier disease stage at the time of community testing. These data suggest that for a substantial proportion of community cases, serological diagnosis alone is insufficient and molecular testing on WB is the more reliable approach, with important implications for timely outbreak surveillance. In a complementary and independent validation study on a well-characterized cohort of confirmed acute and past WNV cases, IgG avidity testing at an optimized cut-off of 35% achieved a negative predictive value of 96%, offering a rapid single-sample alternative to the labor-intensive neutralization assay currently required for diagnostic confirmation. Collectively, these findings support reassessing current WNV diagnostic algorithms, prioritizing WB-based molecular testing as the primary modality, complemented by IgG avidity as a serological tool in cases where molecular testing is no longer informative due to late presentation after symptom onset, together improving diagnostic accuracy and public health outbreak response.

Genomic Epidemiology of West Nile Virus over recent years in France

Raphaelle Klitting, Laura Pezzi, Nazli Ayhan, Gilda Grard, Guillaume Durand, Xavier de Lamballerie, Albin Fontaine

Unité des Virus Emergents, Marseille, France

Abstract

West Nile virus (WNV), a mosquito-borne orthoflavivirus, represents an increasing public health threat in Europe. In 2025, the virus was detected in four distinct regions of metropolitan France including in the Paris area. Using virus genomic data collected from the human, animal and entomological compartments we characterize the spatial and temporal dynamics of WNV circulation in France from 2022 to 2025 and determine the origin of the virus lineages responsible for the 2025 emergence in Paris. Based on 52 sequences from WNV-positive samples (6 human, 21 veterinary, and 25 entomological samples), we find that WNV-L2 was the only lineage detected in France between 2022 and 2025. In particular, we identify the emergence of WNV-L2 in the Occitanie region —a large area in the south of France bordering Spain and the Mediterranean sea. By applying phylodynamics to WNV L2 sequences from France and Europe, we date the emergence of WNV in Occitanie and link it to recent virus sublineages from Nouvelle-Aquitaine, a region along the Atlantic coast. Further, we show that WNV L2 strains identified in the Provence-Alpes-Côte d'Azur region —situated only 100 km east of Occitanie— represent a distinct sublineage, likely recently introduced from a neighboring region of Italy. Finally, we find that the virus strains from the Paris area all grouped together and also originated from the WNV-L2 clade from the Nouvelle-Aquitaine region. These findings mark a turning point in the epidemiology of West Nile virus in Europe, highlighting the urgent need to reinforce integrated surveillance systems across all regions —not only where the virus has historically circulated or recently emerged, but more broadly to anticipate and respond to future spread.

Urban land cover drives fine-scale heterogeneity and extensive local amplification of West Nile virus

Corinna Patzina-Mehling[1], Anne Kopp[1], Yea-Seul Lee[1], Maximillian X.L. Birkl[1], Sophia Ebers[1], Selina L. Graff[1], Uli Beisel[2], Conny Landgraf[3], Florian Ganz[3], Aimara Planillo[3,4], Stephanie Kramer-Schadt[3], Sandra Junglen[1]

[1]Charité – Universitätsmedizin Berlin, Berlin, Germany. [2]Freie Universität Berlin, Berlin, Germany. [3]Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany. [4]Doñana Biological Station (EBD-CSIC), Seville, Spain

Abstract

Climate change can intensify mosquito-borne disease risks through rising temperatures and more frequent extreme weather events. To mitigate climate change, cities are implementing nature-based solutions like rainwater management. West Nile virus (WNV), an emerging human pathogen in Europe, is primarily transmitted between birds and mosquitoes. Key aspects of its amplification, particularly in urban habitats and in the context of climate adaptation strategies, remain insufficiently understood.

To examine how urban land cover influences WNV amplification, mosquitoes were systematically collected in 2023 and 2024 in Berlin, Germany, at five urban microhabitats: a sponge city site (S), a residential courtyard (RB), a park-like residential area (RA), a park-like cemetery (C) and a natural conservation area (N). Mosquitoes were morphologically identified and tested for WNV infection by qPCR. Viral genomes were sequenced, and blood-fed specimens were analysed for vertebrate host DNA.

13,627 and 10,463 mosquitoes were collected in 2023 and 2024, respectively. The highest mosquito abundance was observed at N, followed by C and RA. We identified fine-scale heterogeneity in WNV infection risk. In 2023, the highest minimum infection rates (MIRs) were observed at C (20.77) and RA (14.60). In contrast, MIRs at N remained low (up to 4.03). In 2024, similar infection patterns were again detected, likely shaped by habitat characteristics and avian host community structure.

Urban land cover, habitat characteristics and avian host community composition critically shape WNV infection risk at fine spatial scales. Integrating biodiversity restoration into nature-based solutions may support sustainable, climate-resilient urban planning while reducing vector-borne disease risk.

Offered Talk :

Transmission risk of recent African Zika virus strains by African *Aedes aegypti* mosquitoes

Adrien THIESSON, Stéphanie DABO, Alicia LECUYER, Louis LAMBRECHTS

Institut Pasteur, Université Paris Cité, CNRS UMR2000, Insect-Virus Interactions Unit, Paris, France

Abstract

Zika virus (ZIKV) is an orthoflavivirus primarily transmitted among humans by ***Aedes* (*Ae.*) *aegypti* mosquitoes.**Phylogenetically, ZIKV comprises **two main lineages**, referred to as the Asian and African lineages. Although all ZIKV outbreaks reported to date involved Asian lineage strains, experimental studies have shown that African lineage strains have a **higher transmissibility in mosquitoes**, indicating a significant epidemic potential. However, this has only been demonstrated in the globally invasive subspecies *Ae. aegypti aegypti* (*Aaa*), known to be more competent for ZIKV transmission than the **native African subspecies**. Whether African populations can **efficiently transmit African ZIKV** strains remains to be evaluated. Here, we used a combination of experimental and epidemiological modelling approaches to characterise the transmissibility and epidemic potential of two recent African ZIKV strains in several field-derived *Ae. aegypti* populations from West Africa. First, we confirmed that ZIKV **susceptibility** positively correlated with the proportion of ***Aaa* genetic ancestry**, regardless of the ZIKV strain involved. Next, we found that the **transmission dynamics** in mosquitoes depended on the **specific combination** of mosquito population and ZIKV strain. Finally, we implemented an agent-based model that integrates empirical data to quantify the **epidemic potential** of two distinct mosquito populations. Our epidemic simulations demonstrated **significant differences** in the potential for large outbreaks between the two populations. Together, these findings indicate that the risk of local ZIKV transmission in Africa **varies significantly** between mosquito populations, underscoring the importance of **conducting localised assessments** of *Ae. aegypti* genetic heterogeneity to effectively manage and mitigate the risk of outbreaks.

Building a complex understanding of dengue microvascular dysfunction through novel in vitro 3D multicellular systems

Fatima Abukunna[1], Justin Duruanyanwu[2,1], Paola Campagnolo[1], Kevin Maringer[2]

[1]University of Surrey, Guildford, United Kingdom. [2]The Pirbright Institute, Pirbright, United Kingdom

Abstract

Dengue virus (DENV) is the most important arthropod-borne virus globally, causing dengue fever and life-threatening severe dengue typified by vascular leakage and shock. Dengue is a multifactorial disease, but despite ultimately impacting the cardiovascular system, mechanistically dengue microvascular dysfunction remains poorly understood. Most studies are performed on capillary-derived endothelial cells and do not consider complex multi-cellular *in vivo* interactions, or vessel-specific and tissue-specific pathology. We developed complex models of dengue microvascular dysfunction by incorporating human perivascular cells (pericytes) and capillary or lymphatic endothelial cells into co-culture models, including microvascular spheroids, vessel-like networks grown in a 3D matrix, and mono- and multi-vessel organs-on-a-chip incorporating circulatory flow. We found that pericytes are crucial for amplifying leakage induced by the DENV non-structural protein NS1, and that pericyte dysregulation may explain the delay between peak serum NS1 levels and the later development of life-threatening vascular dysfunction. NS1's effects were mediated through contact-mediated and contact-independent signalling between pericytes and endothelial cells. Importantly, NS1 disrupted microvascular spheroids that recapitulate *in vivo* multicellular interactions, and induced liver-specific microvascular pathology that may explain liver dysfunction in patients. Finally, we showed for the first time that DENV NS1 impairs lymphatic vessel function. By developing a more complete picture of the human microvasculature, we therefore show that dengue fluid accumulation is driven by both leakage from capillaries and impaired reabsorption by lymphatics. Furthermore, pericytes and lymphatics play a crucial role in amplifying dengue microvascular leakage and their dysfunction contributes to tissue-specific pathology, which may open new therapeutic and prognostic opportunities.

A 5’ UTR mutation in the M segment affects RVFV infection in mosquitoes

Daphné BAUDON, Barbara Viginier, Maxime Ratinier, Frédérick Arnaud, Vincent Raquin

EPHE, Université PSL, INRAE, Université Claud Bernard Lyon1, IVPC UMR754, Lyon, France

Abstract

Over the past three decades, one third of (re)emerging viral diseases were caused by arboviruses, which are pathogens transmitted from arthropod vectors to vertebrate hosts upon blood feeding. Rift Valley fever virus (RVFV) spread within a few decades from Eastern to whole Africa, Indian ocean (1990) and Arabian Peninsula (2000). RVFV strains harbor an overall low genetic diversity compare to other arboviruses, with some mutations having a major impact on RVFV infection. Two RVFV strains, MRU25010-30 and MRU2687-3 isolated from infected animals during the 2010-2013 outbreak in Mauritania, displayed a relatively similar genetic make-up but a marked difference in host virulence. In mice, MRU25010-30 triggered a faster death compare to MRU2687-3 (Chabert et al., 2024). Reverse genetics (RG) showed that MRU25010-30 higher replication rate was associated with two point mutations in the 5’ UTR and the envelope protein Gn encoding gene of the M segment, respectively. My PhD project focuses on the viral determinants governing mosquito vector competence for RVFV. To this end, *Culex quinquefasciatus* and *Aedes aegypti* mosquitoes were exposed to an infectious blood meal spiked with selected RG RVFV strains, including RG MRU25010-30 with the 5’ UTR or Gn mutations mentioned above. Our results indicate that, in addition to host infection, the 5' UTR point mutation within the M segment also plays a key role in vector infection. These data highlight that mosquito infection and ultimately vector competence is strongly influenced by the genotype of RVFV circulating field strains.

Does viral persistence shape virus–virus interactions in mosquitoes?

Mine Altinli[1,2,3], Bianca Weber[2], Jorn Brink[2], Derin Topaktas[2], Bernhard Zibrat[2], Esther Schnettler[2,3,4]

[1]Friedrich-Loeffler-Institut, Greifswald, Germany. [2]Bernhard-Nocht Institute for Tropical Medicine, Hamburg, Germany. [3]German Centre for infection Research (DZIF), Hamburg, Germany. [4]Faculty of Mathematics, Informatics and Natural Sciences, University Hamburg, Hamburg, Germany

Abstract

Mosquitoes host a wide diversity of insect-specific viruses alongside medically important arboviruses. While insect-specific viruses have been proposed as potential modulators of arbovirus transmission, the mechanisms underlying these interactions remain poorly understood, particularly in the context of persistent infections (longterm infection without clearance) that characterise many mosquito–virus associations.

Here, we investigated interactions between insect-specific alphaviruses and arboviruses in mosquito cell lines. Insect-specific alphaviruses reduced the replication of related arboviruses only when they were persistently infecting cells. No strong interference was detected in sequential or simultaneous co-infection settings. To explore potential underlying mechanisms, we compared acute and persistent insect-specific virus infections. Differences were observed at multiple levels, including changes in host transcriptomic profiles and microRNA expression. In addition, small RNA sequencing revealed antiviral RNAi responses targeting ISVs, with differences between acute and persistent conditions.

Overall, our results indicate differences in host responses associated with persistent insect-specific virus infections, which may influence subsequent arbovirus infections. Further work will be needed to determine how these responses contribute to interference phenotypes and whether they can be leveraged to understand better or modulate arbovirus transmission.

SFTSV NSs protein is a novel tick antiviral RNAi response suppressor

Mazigh Fares[1], Melanie McFarlane[1], Rhys Parry[2], Rozeena Arif[1], Andrew Clarke[1], Wael Kamel[1], Kelsey Davies[1], Riona Datta-Savage[3], Ulrich Schwarz-Linek[3], Lesley Bell-Sakyi[4], Marine Petit[5], Esther Schnettler[6], Alfredo Castello[1], Alain Kohl[7], Benjamin Brennan[1]

[1]Medical Research Council–University of Glasgow Centre for Virus Research, University of Glasgow, Glasgow, United Kingdom. [2]The University of Queensland, St. Lucia, Australia. [3]University of St. Andrews, St. Andrews, United Kingdom. [4]University of Liverpool, Liverpool, United Kingdom. [5]University of Surrey, Guildford, United Kingdom. [6]Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany. [7]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Abstract

Severe fever with thrombocytopenia syndrome virus (SFTSV) is an emerging tick-borne phenuivirus causing high mortality in humans. While the non-structural protein NSs is dispensable for replication in interferon-deficient mammalian cells, we demonstrate for the first time that NSs is essential for viral replication in tick cells.

SFTSV infection triggers canonical Dicer-2-mediated antiviral RNA interference (RNAi) in tick cells, producing virus-derived small interfering RNAs (siRNAs) that target viral transcripts for degradation. We show that NSs functions as a viral suppressor of RNAi by selectively engaging and depleting single-stranded RNAs derived from 22-nucleotide siRNAs, likely limiting their incorporation into RNA- induced silencing complexes (RISC). Complementation with a heterologous RNAi suppressor (p19 protein) partially rescues replication of NSs-deficient virus, validating the RNAi suppressive function of NSs. These findings reveal that successful tick-borne viral replication requires host-specific immune evasion strategies and establish NSs-mediated RNAi suppression as essential for SFTSV persistence in arthropod vectors.

Lipidomic interactions at the skin interface that determine arbovirus transmission

Mihra Tavadia[1,2], Hacene Medkour[1,3], Julien Pompon[1]

[1]Research and Development Institute IRD Montpellier, MONTPELLIER, France. [2]University of Montpellier, Montpellier, France. [3]CNRS, MONTPELLIER, France

Abstract

Mosquitoes transmit pathogenic flaviviruses such as dengue (DENV), Zika (ZIKV), and West Nile (WNV) viruses. Current vector interventions remain incompletely effective and vaccines have safety concerns, emphasizing the need for novel strategies. Viruses exploit the host lipids for amplification. Bite-initiated skin infection is a bottleneck in transmission and therefore an interesting target for new interventions. My study aims at characterizing the lipid response in bitten skin to identify transmission-blocking targets. Applying lipidomics to an in-vivo mouse model of mosquito-mediated transmission, I first observed that bite upregulates ceramides in skin. Cutaneous ceramide upregulation was confirmed by targeted and kit-based quantification. Second, I used in-vitro and in-vivo approaches to identify the enzymes responsible for the ceramide upregulation. Third, I applied in-vitro gene silencing and ceramide supplementation to demonstrate that ceramides possess antiviral activity conserved across flaviviruses such as WNV, DENV, and ZIKV. Fourth, I showed that ceramides specifically alter viral attachment. Finally, I depleted the identified enzymes in the mouse skin to show that cutaneous ceramide upregulation reduces flaviviral transmission. This study elucidates the regulation of skin lipids, identifying ceramides as an antiviral lipid-based response which can be harnessed for topical transmission-blocking interventions.

Keywords: mosquito-borne viruses, flavivirus, ceramides, lipidomics, viral transmission

Discover Wolbachia Proteins that inhibit Zika virus infection

London Nemmers[1], Elodie Ghedin[2], Denis Voronin[2]

[1]NIAID, NIH, Bethesda, USA. [2]NIAID/NIH, Bethesda, USA

Abstract

Wolbachia is an endosymbiotic bacterium present in a broad range of arthropod hosts, including *Aedes* mosquitoes, that has been used in vector control strategies to reduce arbovirus transmission. Zika virus, a flavivirus transmitted by *Aedes* mosquitoes, can cause severe neurological complications in adults and microcephaly in newborns. While *Wolbachia* is known to restrict ZIKV within mosquitoes, the molecular mechanisms underlying its antiviral effects remain to be fully characterized. Previous studies have associated both ZIKV replication and *Wolbachia* density with autophagy. This study hypothesizes that *Wolbachia* proteins modulate host autophagy, thereby restricting ZIKV replication.

To investigate this, bacterial proteins associated with the host autophagosomal marker ATG8 were identified in *Aedes albopictus* (C6/36) cells. The impact of inhibiting these bacterial proteins on ZIKV replication was assessed by suppressing their expression in *Wolbachia*-positive cell lines using nucleic acid inhibitors. Inhibition of bacterial proteins, including outer membrane proteins, porins, and protein kinases, reduced *Wolbachia*-mediated protection and increased ZIKV infection in *Wolbachia*-positive cells.

To evaluate whether specific bacterial proteins confer cellular resistance to ZIKV in mammalian cell lines, *Wolbachia* genes of interest were transfected into VERO and HEK293T cells.

Expression of proteins in mammalian cells prior to viral infection resulted in a significant reduction in ZIKV. These findings indicate that autophagy-related *Wolbachia* proteins contribute to the restriction of ZIKV in mosquito cells and in mammalian cells. These results provide insight into the mechanisms of *Wolbachia*-mediated inhibition of arboviruses in *Aedes* mosquitoes and underscore the potential of *Wolbachia* proteins as preventive, therapeutic, or vector-control agents.

Differential pathogenesis of tick-borne flaviviruses in host-specific 3D vessel-on-a-chip models

Kirsten Bronsvoort, Timo Dijkstra, Barry Rockx

Wageningen Bioveterinary Research, WUR, Lelystad, Netherlands

Abstract

Although genetically related, the tick-borne orthoflaviviruses tick-borne encephalitis (TBEV) and louping ill (LIV) show distinct pathogenicity across their host species. In sheep, LIV is known to cause fatal encephalitis, while TBEV often remains asymptomatic, whereas in humans, both viruses can induce severe neurological disease. The molecular mechanisms of this species-dependent pathogenesis remain largely unknown. In this study, we explored the role of endothelial cells in orthoflavivirus pathogenesis. We established physiologically relevant perfused 3D vessel-on-a-chip (VoC) models representing the sheep (shVoC) and human (hVoC) vasculature, using primary endothelial cells. Next, we assessed infectivity and replication kinetics of TBEV and LIV in these models. Furthermore, using transcriptomic analysis of infected cells, we aimed to identify differentially expressed genes (DEGs) between virus infections within each host species. Consistent with field observations, results show that while LIV exhibits similar growth kinetics in VoCs of both species, TBEV growth is significantly reduced in shVoCs compared to hVoCs. Interestingly, for both viruses, transcriptome analysis revealed a minimal number of DEGs in hVoCs. On the other hand, while <50 DEGs were found in TBEV-infected shVoCs, >3500 DEGs were found in LIV-infected shVoCs. Upregulated genes were associated with cytokine signaling and interferon-mediated immune responses (e.g., CCL5, IL-8, ISG15, MX2), as well as vascular activation and leukocyte adhesion (e.g., ICAM-1, VEGF, E-selectin). In addition to providing an in-depth understanding of orthoflavivirus pathogenesis, the developed VoCs can serve as tools for quickly assessing the virulence of newly arising viruses and contribute to the 3Rs in animal research.

Ultraviolet exposure conditions skin to enhance arbovirus infection and mosquito probing

Ailish McCafferty-Brown[1], Amani Alhazmi[1], Yonca Keskek Turk[1], Emilie Pondeville[2], Clive McKimmie[3]

[1]University of Leeds, Leeds, United Kingdom. [2]MRC University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. [3]University of YOrk, York, United Kingdom

Abstract

Mosquito-borne viruses are inoculated into skin, yet how environmental exposures shape susceptibility remains unclear. We identify ultraviolet (UV) exposure as an unrecognised determinant of mosquito-borne virus infection. In mice, prior UV exposure increased Semliki Forest virus replication, viraemia and mortality, and also enhanced Zika virus infection. Early susceptibility was driven by recruited CCR2-dependent myeloid cells that were preferentially infected and amplified virus. This phase was transient: by one week, infection shifted toward proliferating fibroblasts within repairing skin, accompanied by a glycolytic tissue signature. In primary human dermal fibroblasts, viral replication was governed by metabolic state rather than proliferation alone, suggesting that repair-associated metabolic reprogramming underlies stromal permissiveness. Topical steroids partially alleviated UV-conditioned enhancement of virus susceptibility. UV also warmed skin and increased mosquito probing. Together, these findings establish UV exposure as a driver of conditioned states that determine arbovirus susceptibility, identifying sunlight as a modifiable determinant of vector-borne disease risk.

Insect-specific adaptation of Semliki Forest Virus reveals determinants of host restriction

Dennis Kenbeek, Wessel Willemsen, Derek van der Weijden, Eric de Gier, Casper Koops, Jelke Fros

Wageningen University and Research, Wageningen, Netherlands

Abstract

Semliki Forest virus (SFV) is widely used as a model alphavirus to study viral replication and pathogenesis. SFV is primarily transmitted by Aedes mosquitoes and can infect a broad range of vertebrate hosts, including humans. Here, we describe the discovery of an insect-specific SFV variant originating from a persistently infected Aedes albopictus cell line (U4.4). Next-generation sequencing revealed numerous mutations distributed throughout the viral genome. Some of these mutations occur in the structural proteins, providing insight into residues potentially involved in receptor binding, potentially influencing host and vector specificity. Unexpectedly, functional mutagenesis demonstrated that the primary determinants restricting replication to mosquito cells are located within the nonstructural proteins and exhibit strong temperature sensitivity. In particular, nsP3 emerged as a key determinant of host restriction. Although the precise role of nsP3 in the alphavirus replication cycle remains poorly understood, it is known to play critical roles in viral replication and in mediating virus–host protein–protein interactions. By comparing the interactomes of persistent and wild-type SFV nsP3 with known host factors, and by developing assays to identify novel mosquito-specific interactions, this work provides new insights into the molecular mechanisms governing alphavirus host and vector specificity.

Inhibition of Alphaviruses by Combinations of Directly Acting and Host-Targeting Antivirals

Jessica Wagoner[1], Sam Verwimp[2], Jennifer Hyde[1], Judith White[3], Joshua Schiffer[4], Leen Delang[2], Stephen Polyak[1]

[1]University of Washington, Seattle, USA. [2]KU Leuven, Leuven, Belgium. [3]University of Virginia, Charlottesville, USA. [4]Fred Hutch Cancer Center, Seattle, USA

Abstract

Alphaviruses such as Venezuelan equine encephalitis virus (VEEV) are associated with a significant burden of disease, disability, death, and medical expenditures. No oral antiviral drugs are available for this virus family. We have shown that combinations of oral nucleoside analogs approved for other virus families confer synergistic suppression of alphaviruses. However, not all nucleoside analogs were effective against VEEV. Here, we explored additional directly acting antiviral (DAA) drug combinations for their efficacy against VEEV in vitro. We also tested thirteen drugs approved for non-viral indications that have been shown potential as host targeting antiviral (HTA) drugs. We confirm the strong antiviral potency of ML-336, a previously described oral DAA that is thought to inhibit VEEV non-structural protein-protein interactions to suppress viral RNA synthesis. Combining ML-336 with nucleoside analogs favipiravir (FAV) or molnupiravir (MPV) confers synergistic suppression of VEEV TC83 in human skin fibroblasts and liver cells. Of thirteen HTA drugs screened, only the pyrimidine biosynthesis inhibitor brequinar (BRQ) suppressed VEEV TC83 in human skin fibroblasts. BRQ inhibition of VEEV in these cells was variable and this variability was not abated by synchronizing the cell cycle through serum starvation or by synchronizing infection by using high multiplicities of infection. BRQ did not inhibit VEEV TC83 in human liver cell cultures, but BRQ did suppress Sindbis virus (SINV) in these cells. The results emphasize the challenge and cell type variability associated with putative HTA drugs and further support the potential of all-DAA and DAA + HTA drug combinations against emerging and re-emerging alphaviruses.

First-in-human study of EVT894, a monoclonal antibody against chikungunya virus exhibiting a long plasma half-life

Hugh Watson[1,2], Kristie Miller[3], Akber Safder[4], Shruti Raja[5], Xavier Boulenc[6], Marie Mandron[6], Meg Gordon[7], Maryam Jahromi[7], Sara Woodson[7]

[1]Quayside Research, Lyon, France. [2]Aarhus University, Aarhus, Denmark. [3]Trialmed, Austin TX, USA. [4]Trialmed, Orlando FL, USA. [5]Duke Early Phase Research Unit, Durham NC, USA. [6]Evotec ID, Lyon, France. [7]NIAID, Bethesda MD, USA

Abstract

Background: Outbreaks of chikungunya virus (CHIKV) infection continue to emerge in new regions, but currently there are no antiviral drugs for treatment or protection of susceptible individuals during a CHIKV outbreak. Following positive results in treatment and prophylaxis preclinical models, a fully human IgG1 anti-CHIKV monoclonal antibody (mAb), EVT894, was entered into clinical development.

Methods: Healthy adults were enrolled in a randomized, double-blind, placebo-controlled, single dose escalation study (NCT04441905). Five dose levels of intravenous EVT894 were evaluated for safety, pharmacokinetics and immunogenicity. Doses administered were 0.3, 1, 3, 10 and 20 mg/kg. Each dose-cohort consisted of 8 subjects, randomized to EVT894 (n=6) or placebo (n=2), with a follow-up of 150 days for each subject.

Results: No serious adverse event (AE), death or dose-limiting toxicity was reported during the study. AEs considered related to treatment were reported in 3 of 10 subjects on placebo and 2 of 30 subjects on EVT894, all mild in severity with no dose-related trend. Across the dose-levels tested, EVT894 half-life ranged from 56 to 99 days. Exposure increased with dose: Cmax was dose-proportional in this dose range, while AUC was slightly supra-proportional between the two first doses. No anti-drug antibodies were detected after EVT894 infusion.

Conclusion: A single IV dose of EVT894 was generally safe and non-immunogenic in healthy adult participants in this study. The pharmacokinetic profile shows a mAb with a long half-life and potential for use both as treatment in acute CHIKV infection and as short to medium-term prophylaxis.

A Novel Small-Molecule Inhibitor of Yellow Fever Virus Targeting NS4B

Alina Soto[1], Shannan-Leigh Macleod[1], Kristien Minner[1], Rana Abdelnabi[1], Joyce Van Bree[2], Nanci Dos Santos Ferreira[1], Ahmed Mohamed Elsayed Mohamed[3], Irina V. Il'ina[4], Oksana S. Patrusheva[4], Yuri V. Vyatkin[5], Ekaterina P. Vasyuchenko[6], Johan Neyts[1], Joana Rocha-Pereira[1], Suzanne Kaptein[1], Konstantin P. Volcho[4], Nariman F. Salakhutdinov[4], Leen Delang[1]

[1]KU Leuven, Leuven, Belgium. [2]Wageningen University, Wageningen, Netherlands. [3]The Pirbright Institute, Woking, United Kingdom. [4]Novosibirsk Institute of Organic Chemistry, Novosibirsk, Russian Federation. [5]NOVEL Software Systems Ltd, Novosibirsk, Russian Federation. [6]Lomonosov Moscow State University, Moscow, Russian Federation

Abstract

Yellow fever virus (YFV), a mosquito-borne pathogen responsible for acute haemorrhagic fever, remains a significant health threat due to recurrent outbreaks, high disease burden, vaccine shortages, and the lack of antiviral treatments. Here, we report the identification of YFV inhibitors targeting the NS4B protein. Among a series of heterocyclic compounds containing hexahydro-2*H*-4,6-(epoxymethano)chromene moieties, compound I7-20-1 exhibited the potent antiviral activity in Huh7 cells (EC_{50} = 3.4 µM, CC_{50} = 60 µM) resulting in a ~3 log reduction in viral titres. No antiviral activity was observed against other flaviviruses, alphaviruses, or enteroviruses, indicating YFV-specific activity.

Time-of-addition experiments showed that I7-20-1 was most effective when added up to 4 hours post-infection, indicating that it acts during an early stage of the viral replication cycle. Resistance selection identified a I84T substitution in the NS4B viral protein, located within transmembrane domain 2. Reverse engineering confirmed the resistant phenotype (>9-fold resistance). Notably, cross-resistance studies with previously described YFV NS4B inhibitors, showed that the I84T mutant showed cross-resistance to CCG-4088, but not to BDAA. Molecular dynamics analysis applied to AlphaFold-generated proteins suggests that the I84T mutation affects NS4B structural conformation, reducing ligand-binding specificity compared to wild type.

Ongoing studies aim to elucidate the molecular mechanism of action, including whether viral RNA replication is directly affected, and to evaluate the activity of I7-20-1 in YFV-infected human liver organoids. In summary, we identified a new class of YFV inhibitors targeting a novel NS4B binding site, with a resistance profile distinct from the BDAA inhibitor.

Self-adjuvanted nanoparticle vaccines against Japanese Encephalitis virus

Linda van Oosten[1], Fabian Guadalupe Martinez[2], Adam Sander[3,4], Adam Chambers[5], Stephen Adeniyi[5], Juul Steeghs[6], Linda King[6], Robert Possee[5], Daniel Rawle[7], Monique van Oers[1], Wilson Nguyen[7], Gorben Pijlman[1]

[1]Wageningen University, Wageningen, Netherlands. [2]University of Copenhagen, København, Denmark. [3]University of Copenhagen, Copenhagen, Denmark. [4]AdaptVac, Copenhagen, Denmark. [5]Oxford Expression Technologies, Oxford, United Kingdom. [6]Oxford Brookes University, Oxford, United Kingdom. [7]QIMR Berghofer Medical Research Institute, Brisbane, Australia

Abstract

Novel vaccine platforms are urgently needed to produce efficacious, safe, low-cost, and rapidly adaptable ('plug-and-play') vaccines to face the threat of zoonotic viral diseases in livestock. Protein nanoparticle vaccines are an optimal vaccine format because of their high efficacy and intrinsic safety. In this project, we developed a nanoparticle vaccine against Japanese Encephalitis virus (JEV), a zoonotic mosquito-borne virus that causes severe disease in livestock. The two-component nanoparticle vaccine technology involves expression of viral antigens in insect cells in the well-established baculovirus expression system, combined with antigen presentation on phage nanoparticles. The vaccine antigens were derived from the immunodominant JEV envelope (E) protein, and included the E protein domain III (EDIII) and the E protein locked in its native-like dimer conformation (E-dimer). The antigens were efficiently expressed in insect cells and were coupled onto self-adjuvanting protein nanoparticles by Tag/Catcher conjugation. These JEV vaccine candidates were subsequently evaluated in vaccination studies in relevant mouse models, where the E-dimer nanoparticle vaccine induced superior neutralizing responses. These results support further development of nanoparticle vaccines to combat JEV outbreaks.

Identification of an entry factor for chikungunya virus in mosquitoes using a pro-apoptotic CRISPR screening strategy

Anja de Bruin[1], Enzo Mameli[2], Lathika Valliyott[3], Andres Merits[4], Leen Delang[5], Nicholas Ariotti[3], Jonathan Abraham[6], Norbert Perrimon[2], Gisa Gerold[1]

[1]Institute of Virology, Medical University of Innsbruck, Innsbruck, Austria. [2]Department of Genetics, Blavatnik Institute, Harvard Medical School, Boston, USA. [3]The University of Queensland, Institute for Molecular Bioscience, Brisbane, Australia. [4]University of Tartu, Institute of Bioengineering, Tartu, Estonia. [5]Department of Microbiology, Immunology and Transplantation, Rega Institute, KU Leuven, Leuven, Belgium. [6]Department of Microbiology, Blavatnik Institute, Harvard Medical School, Boston, USA

Abstract

Chikungunya virus (CHIKV) is a re-emerging mosquito-borne alphavirus, which causes debilitating arthritis-like symptoms in humans. While determinants of CHIKV host cell entry into mammalian cells are known, their equivalents in mosquito cells remain largely elusive. To identify CHIKV entry factors in *Aedes* mosquito cells, we engineered a virus encoding the pro-apoptotic *Drosophila* Reaper gene, which was subsequently used to perform a near-genome wide CRISPR knockout screen. CHIKV-Reaper induced cell death in C6/36 cells upon infection and displayed normal replication kinetics in mammalian cells, while being slightly attenuated in C6/36 cells. Using the C6/36 cell-based CRISPR screening platform, we identified three cell surface proteins and multiple enzymes involved in their biosynthesis pathway. Using gene silencing in *Aedes aegypti* and *Aedes albopictus* cells, we confirmed that one of the three proteins, an immunoglobulin-like domain-containing membrane protein, is a CHIKV host factor. In line with these findings, transcomplementation of refractory mammalian cells with one of the host factors rendered cells susceptible to both West African and East Central South African CHIKV lineages. Moreover, transcomplementation rendered cells susceptible to other arthritogenic alphaviruses including Semliki Forest virus (SFV) and Ross River virus (RRV), while the encephalitic alphavirus Venezuelan equine encephalitis virus (VEEV) could not enter transcomplemented cells. Altogether, we identified a receptor for CHIKV in mosquitoes and demonstrate that recombinant pro-apoptotic arboviruses efficiently enable CRISPR knockout screens in insect cells.

Apolipoprotein E drives inflammation resolution pathways essential for recovery from Japanese encephalitis

Abigail Cox[1,2], Wilson Nguyen[1], Bing Tang[1], Kexin Yan[1], Ashwini Raghavendra[1], Rebekah Ziegman[1], Gunter Hartel[1], Quan Nguyen[1,2], Mark Hodson[1,2], Andreas Suhrbier[1,2,3,4], Cameron Bishop[1], Daniel Rawle[1,2,3,4]

[1]QIMR Berghofer, Brisbane, Australia. [2]The University of Queensland, Brisbane, Australia. [3]GVN Center of Excellence, Brisbane, Australia. [4]Centre for Tropical Health and Emerging Diseases, Brisbane, Australia

Abstract

Japanese encephalitis virus (JEV) is the leading cause of viral encephalitis in the Asia-Pacific region, causing an estimated 70,000-100,000 clinical cases annually, with a fatality rate of 20-30%, and frequent long-term neurological sequelae among survivors. Recovery from Japanese Encephalitis (JE) requires not only effective viral clearance but also timely resolution of the immune response and restoration of immune homeostasis. However, the mechanisms governing this transition remain unknown.

We identified apolipoprotein E (ApoE) as a key regulator of inflammation resolution during recovery from JE. Using a mouse model encompassing asymptomatic, symptomatic, and lethal disease trajectories, combined with single-cell spatial transcriptomics and RNA sequencing, we show that ApoE is strongly upregulated in microglia, myeloid cells, and T cells in symptomatic survivors, coinciding with the adoption of anti-inflammatory phenotypes. In contrast, immune cells from mice with lethal disease fail to induce ApoE and remain persistently pro-inflammatory. Moreover, ApoE-deficient mice mount dysregulated immune responses, deteriorate rapidly, and are unable to recover after symptom onset.

In humans, ApoE exists as three major isoforms (ε2, ε3, and ε4) with distinct neurobiological properties. Analysis of cerebrospinal fluid (CSF) from patients with acute encephalitis syndrome in central Laos reveals that ApoE ε2 carriers exhibit significantly lower CSF neutrophil proportions and shorter hospital stays, consistent with reported neuroprotective effects of this isoform. Together, these findings identify ApoE as a critical regulator of neuroinflammation resolution following JEV infection, with ApoE isoform-specific effects influencing recovery. ApoE-dependent resolution pathways therefore represent promising therapeutic targets for pathological encephalitis.

Chimeric viral platform enables high-resolution cryo-EM analysis, antigenic characterisation and vaccine application for diverse pathogenic tick-borne orthoflaviviruses

Mahali Morgan[1], Summa Bibby[1], Devina Paramitha[1], Cormac Lucas[2], Rhys Parry[1], Ke Chen Ng[1], Connor Scott[1], Stacey Cheung[1], Naphak Modhiran[1], Helen Lazear[2], Roy Hall[1], Jody Hobson-Peters[1], Daniel Watterson[1], Natalee Newton[1]

[1]The University of Queensland, St Lucia, Australia. [2]The University of North Carolina, Chapel Hill, USA

Abstract

Tick-borne orthoflaviviruses (TBF), including tick-borne encephalitis virus (TBEV) and Powassan virus (POWV), remain a global concern with high case fatality rates, long-term disabling sequela, and continued emergence. Structural analysis of TBFs by cryogenic electron microscopy (cryo-EM) single particle analysis, a critical method to understanding virion structure and antibody interactions, requires large quantities of infectious virus and appropriate biocontainment levels – difficult for BSL3-4 TBFs. We sought to investigate a new system to safely produce authentic TBFs virions through an insect-specific flavivirus (ISF) chimeric platform.

Chimeric orthoflaviviruses were produced by exchanging the pre-membrane and envelope proteins of Binjari virus (an ISF) with the corresponding genes of the pathogenic flavivirus. They are unable to replicate in vertebrate cells, including human neuronal cell line. Over ten TBF chimeras, including mammalian (TBEV, POWV, Omsk haemorrhagic fever, Alkhurma, Kyasanur Forest disease, Langat (LGTV), Gadgets Gully (GGYV)) and seabird viruses (Saumarez Reef; SREV) were constructed.

Chimeric TBEV, POWV and LGTV were imaged by cryo-EM with high-resolution structures resolved by single particle analysis and asymmetric reconstruction and revealed structurally authentic virions. Multiple tick-borne orthoflavivirus neutralising monoclonal antibodies were recombinantly produced and used to validate these antigens in ELISA and neutralisation assays. The antigens were immunogenic in mice and neutralised wild-type viruses.

We demonstrate a safe and efficient platform to study structure and antibody interactions of TBFs in BSL2 conditions. Additionally, we have shown that this platform produces promising antigens for TBF vaccine applications.

Thermal proteome profiling reveals new insights into host factors G3BP and RNA Pol II in alphavirus infection

David Mentrup[1], Hanna Bley[1], Mykhailo Guzyk[1], Sainan Wang[2], Andres Merits[2], Pär Nordlund[1], Gerald McInerney[1]

[1]Karolinska Institutet, Stockholm, Sweden. [2]Tartu University, Tartu, Estonia

Abstract

We have developed protocols applying the cellular thermal shift assay coupled with mass spectrometry (MS-CETSA) to investigate cellular dynamics and identify viral host factors during the early stages of alphavirus infection. CETSA exploits the observation that proteins in intact cells undergo temperature-dependent unfolding and precipitation, similar to purified proteins. Following controlled heating, the remaining soluble fraction reflects the amount of folded protein, enabling generation of protein melting curves across the proteome.

MS-CETSA enabled detection of proteome-wide changes as early as 2 and 6 hours after infection with Semliki Forest virus in two distinct cell lines, yielding highly concordant results. These analyses revealed several novel aspects of alphavirus replication. We show that activation of the Akt pathway regulates the balance between genomic and subgenomic mRNA translation and modulates nonsense-mediated decay during infection. In addition, we demonstrate that alphavirus nsP3 preferentially interacts with G3BP2 over its paralogue G3BP1. Functional analyses further revealed that G3BP2 alone is sufficient to provide the full proviral activity required for efficient replication of SFV and chikungunya virus, whereas G3BP1 is not. Finally, our data provide evidence that the RNA polymerase II subunit Rpb8 (POLR2H) is targeted by nsP2 to destabilize the RNA polymerase II complex and suppress host transcription. Together, this work establishes MS-CETSA as a powerful platform for identifying early virus-induced changes in cellular protein behaviour at whole-proteome scale.

Defining monocyte/macrophage migration to and within the brain during arbovirus encephalitis

Fabian Schuette[1], Alistair Gamble[1], Elise Pitmon[1], Heather Mathie[1], Jun Fu[2], Adrian Francis Stewart[2], Eve Anderson[3], Douglas Strathdee[3], Gerry Graham[1], Marieke Pingen[4]

[1]University of Glasgow, Glasgow, United Kingdom. [2]Technische Universität Dresden, Dresden, Germany. [3]Cancer Research UK Scotland Institute, Glasgow, United Kingdom. [4]Cardiff University, Cardiff, United Kingdom

Abstract

Many arboviruses can cause viral encephalitis, leading to substantial morbidity and mortality. We previously demonstrated that targeting immune cell migration can improve outcome in a mouse model of lethal arboviral encephalitis. Yet, immune cell migration to and within the brain is incompletely understood.

The first and most abundant immune cells recruited to the encephalitic brain are monocytes, which play a role in antiviral defence but also neuropathologies. Their mobilisation from bone marrow (BM) depends primarily on the chemokine receptor CCR2, which is encoded within a locus with CCR1, CCR3 and CCR5. Notably, CCR5 deficiency in humans is associated with increased morbidity during viral encephalitis.

Using chemokine receptor reporter mice, we observed lower CCR2 expression by monocytes in the brain compared to BM and blood (46% vs 85-97%), whilst CCR5 expression was higher (28% vs 2-5%).

To determine the importance of CCR2 for monocyte migration to the encephalitic brain, we infected mice with Semliki Forest Virus and compared monocyte migration in mice lacking CCR1/2/3/5, or deficient for CCR1/3/5 but not CCR2. Full locus deletion reduced the presence of monocytes and their macrophage progeny in the encephalitic brain by 97%, a defect fully restored by re-establishing CCR2 alone.

RNAseq analysis of mixed glial/neuronal cell cultures treated with interferon-beta, a key antiviral cytokine, demonstrated all brain cell types expressed chemotactic cytokines involved in monocyte recruitment.

Conclusions. CCR2 expression is critical for monocyte migration to the inflamed brain. We hypothesise CCR5 is important for movement within the brain, ongoing studies are testing this.

Released from predation? How salinisation can potentially influence mosquito borne disease risk

Jordy van der Beek[1,2,3], Charlotte Linthout[4], Gualbert Oude Essink[5,6], Tessa Visser[4], Jacobus Biesmeijer[1,2], Marion Koopmans[3,7], Reina Sikkema[3,7], Constantianus Koenraadt[4], Maarten Schrama[1,2,3]

[1]Institute of Environmental Sciences, Leiden University, Leiden, Netherlands. [2]Naturalis Biodiversity Center, Leiden, Netherlands. [3]Pandemic and Disaster Preparedness Center, Delft, Rotterdam, Netherlands. [4]Laboratory of Entomology, Plant Sciences Group, Wageningen University, Wageningen, Netherlands. [5]Department of Physical Geography, Utrecht University, Utrecht, Netherlands. [6]Department of Groundwater and Water Security, Deltares, Utrecht, Netherlands. [7]Department of Viroscience, Erasmus University Medical Center, Rotterdam, Netherlands

Abstract

Background

A large share of the world's population lives in low elevation coastal areas, which are vulnerable to freshwater salinisation resulting from sea level rise. These changes in the environmental conditions affect local aquatic ecology, including disease vectors and their predators. We studied how projected changes in salinisation affect freshwater mosquitoes' life-history traits, their predators and their ability to transmit viruses.

Methods

We experimentally investigated the effects of salinity on larval survival and development, as well as the longevity, size, and Sindbis virus vector competence of adult *Culex pipiens* mosquitoes. In addition, salt tolerance of invertebrate predators of mosquito larvae was assessed by analysing a long-term dataset with field observations from a range of fresh to extremely salty habitats in the Netherlands.

Results

Larvae survived up to ~45% ocean water salinity (9 g/l Cl^-), though life-history traits were only affected at near-lethal salinity. Even at 8 g/l Cl^-, effects were quite minimal: larvae exhibited higher mortality (24%), delayed development (17%), and adults were marginally smaller (5.5%), but lived thrice longer with unchanged vector competence. Across the same salinity gradient, predator diversity decreased markedly, losing approximately 90% of dragonflies and aquatic beetles, and 65% of aquatic bugs.

Conclusion

Limited effects of increasing salinity on mosquito survival combined with substantially impoverished predator communities (i.e. ecological release) potentially result in a rise in mosquito abundances in salinising coastal areas. This highlights that, besides changes in temperature and precipitation, salinisation may become a critical factor influencing mosquito-borne disease risk in coastal landscapes.

A tissue-resolved atlas of the *Aedes aegypti* mosquito proteome reveals local cellular signatures of infection and a novel host factor required for Zika virus transmission *in vivo*

Stefan Pfister[1,2], Anna Heitmann[2], Adrien Thiesson[3], Stephanie Jansen[2,4], Malte Kuehl[5,6], Charlotte Flory[1], Lucas Wilken[1], Natan Nagar[7], Victor G. Puelles[8,9,10,11], Louis Lambrechts[3], Jonas Schmidt-Chanasit[2,4], Pietro Scaturro[1,4,12,13]

[1]Leibniz Institute of Virology, Systems Arbovirology, Hamburg, Germany. [2]Bernhard Nocht Institute for Tropical Medicine, Arbovirus and Entomology, Hamburg, Germany. [3]Institut Pasteur, Université Paris Cité, CNRS UMR2000, Insect-Virus Interactions Unit, Paris, France. [4]University of Hamburg, Faculty of Mathematics, Informatics and Natural Sciences, Hamburg, Germany. [5]Aarhus University, Department of Clinical Medicine, Aarhus, Denmark. [6]Aarhus University Hospital, Department of Pathology, Aarhus, Denmark. [7]Leibniz Institute of Virology, Viral Data Science, Hamburg, Germany. [8]Aarhus University, Department of Clinical Medicine, Aarhus, Germany. [9]Aarhus University Hospital, Department of Pathology, Aarhus, Germany. [10]University Medical Center Hamburg-Eppendorf, III. Department of Medicine, Hamburg, Germany, Hamburg, Germany. [11]University Medical Center Hamburg-Eppendorf, Hamburg Center for Kidney Health (HCKH), Hamburg, Germany. [12]German Center for Infection Research, Hamburg-Borstel-Lübeck-Riems, Germany. [13]Centre for Structural Systems Biology (CSSB), Hamburg, Germany

Abstract

Despite the central role of *Aedes aegypti* mosquitoes as vectors in outbreaks of human pathogenic viruses such as dengue virus, chikungunya virus, and Zika virus (ZIKV), little is known about their spatial proteome organization. Previous proteomic studies mainly relied on whole-body or single-tissue analyses, limiting the resolution needed to uncover tissue-specific molecular responses governing vector competence and viral transmission.

Here, we present ProAedesDB, the first spatial proteomic atlas of naïve and ZIKV-infected *Ae. aegypti* mosquitoes. Using label-free quantitative mass spectrometry, we profiled more than 6,700 proteins across 13 anatomical compartments, including exoskeletal structures and internal organs such as salivary glands, midgut, and ovaries.

Our analysis identified distinct tissue-specific protein signatures, selective markers, and functional enrichments. Comparative profiling with the widely used Aag2 and Aag2-C3 cell lines revealed protein-based tissue analogies, informing the physiological relevance of *in vitro* studies. Importantly, analysis of ZIKV-infected mosquitoes uncovered infection-driven host responses. Through RNAi-mediated silencing *in vitro*, we functionally assessed more than 100 dysregulated mosquito proteins, identifying a novel group of potent host factors regulating ZIKV replication. Finally, *in vivo* genetic approaches revealed a previously unknown host protein essential for efficient ZIKV transmission across multiple *Ae. aegypti* colonies.

Taken together, these findings provide unprecedented insight into the tissue organization of the mosquito proteome and establish a molecular framework for studying vector biology and virus-host interactions at the organismal level. The dataset will be made publicly available through the ProAedesDB website, enabling discovery of molecular targets for vector control strategies.

The third wave: Repeated incursions of Schmallenberg virus into the United Kingdom

Ben Jones, Akbar Dastjerdi, Suzanna Bell, Rudolf Reichel, Vanessa Swinson, Nick Johnson

Animal and Plant Health Agency, Addlestone, United Kingdom

Abstract

Schmallenberg virus (SBV; genus *Orthobunyavirus*) emerged in mainland Europe in 2011 and causes febrile disease in adult ruminants following transmission by *Culicoides* midges. Infection *in utero* causes abortion and congenital malformations in neonatal sheep, cattle, and goats. The virus was first detected in the United Kingdom in 2012, followed by a second outbreak in 2016–2017. A third emergence of SBV subsequently occurred during 2023–2024, with extensive spread throughout England, Wales, and Scotland.

Here, we describe the dissemination of SBV during this most recent outbreak, from its initial detection in south-west England to widespread circulation across Great Britain within three months. Sequencing of SBV isolates from the 2023–2024 UK outbreak resulted in the assembly of one complete genome and near-complete M-segment sequences from a further four isolates, enabling comparisons between outbreaks. Phylogenetic analysis of the M segment, incorporating viruses from all three UK outbreaks together with representative mainland European SBV sequences, demonstrated distinct clustering of viruses from the two most recent UK outbreaks. These findings indicate repeated introductions of genetically distinct SBV lineages into the UK rather than re-emergence through cryptic endemic transmission of a single lineage.

Our findings demonstrate the cyclical reintroduction of SBV into the UK at approximately 4–6-year intervals, followed by rapid dissemination throughout the national livestock population. Together, this work demonstrates the continuing threat posed by repeated SBV incursions into the UK livestock population and emphasises the importance of sustained genomic surveillance and reconsideration of vaccination strategies to mitigate future outbreaks.

Anopheles mosquitoes as vectors for pathogenic alphaviruses

Jason Rasgon

Pennsylvania State University, University Park, USA

Abstract

Anopheles mosquitoes (>400 species and ~40 of interest as vectors of human disease) have a global distribution, are highly mobile, and thrive using human activities and movement to disperse. Due to the malaria-associated socioeconomic and health burdens they cause, Anopheles mosquitoes are almost exclusively studied as vectors of malaria parasites. However, when feeding on vertebrate blood, female Anopheles are exposed to pathogens and microorganisms present in the blood meal, including pathogenic arboviruses. While the dogma in the field is that Anopheles mosquitoes are not important players in arbovirus transmission compared to Aedes or Culex mosquitoes, the fact remains that pathogenic arboviruses have been isolated from wild Anopheles populations and detected by high-throughput parallel sequencing. As many Anopheline species distributions are currently expanding, they have significant potential to encounter and transmit, and drive invasion of arboviral pathogens. While the only arbovirus thought to be transmitted by Anopheles in the field is the alphavirus O'nyong nyong virus (ONNV), we have identified that multiple diverse Anopheles species are highly competent vectors for multiple diverse alphaviruses and, using Anopheles stephensi and Mayaro virus as a model, we have identified the mosquito virus midgut receptor. These results have broad impact in highlighting the neglected importance of Anopheles as drivers of global arbovirus infection and epidemics, and may lead to novel opportunities and targets for vector-borne disease control.

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