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POSTER ABSTRACT BOOK

#IMAV2026

MICROBIOLOGY SOCIETY

P001

Single-cell transcriptomics of Dicer-2-deficient Aedes aegypti midguts upon dengue virus infection

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Abstract

Dengue virus is a growing public health concern, transmitted to humans by *Aedes aegypti* mosquitoes. Following the bite, the bloodmeal is digested in the midgut, where the virus first infects mosquito cells. Among immune responses, RNA interference, wherein the endonuclease Dicer-2 to cleave double-stranded viral RNA, into small interfering RNAs within the Ago2/RISC complex. In a prior study (Merkling et al., 2023), we demonstrated that Dicer-2 absence led several arboviruses, including dengue virus, to replicate more in the midgut and disseminate faster to secondary organs. Here, we ask which midgut cell populations depend on Dicer-2 for an effective RNAi response and how its activity varies across niches.

Employing single-cell RNA sequencing, we profiled individual midgut cell transcriptomes from wild-type and *Dicer-2* mutant mosquitoes 48 h after an infectious or a control mock bloodmeal. Following quality filtering and data integration, we clustered and annotated cell populations, yielding 16 625 single-cell transcriptomes (9 422 from wild-type and 7 203 from *Dicer-2*-deficient mosquitoes).

Our analysis identified major midgut cell types including enterocytes, enteroendocrine cells, stem cells and hemocytes, with heterogeneous Dicer-2 expression modulated by infection. For example, 55% of enterocytes expressed *Dicer-2* after dengue virus exposure, compared to 40% in the absence of virus. This indicates a dynamic and cell-type specific expression of *Dicer-2*.

Collectively, these findings present a cell-type specific, condition-dependent transcriptional landscape of Dicer-2 expression in the mosquito midgut, providing a foundation for identifying downstream RNAi effectors that govern antiviral immunity.

P002

Beyond the usual suspects: *Eretmapodites subsimplicipes* joins the Zika vectors' list

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Abstract

Identifying vector species is crucial for controlling vector-borne diseases. Although widespread in rural areas of Africa, Madagascar, and the Comoros archipelago, the mosquito species *Eretmapodites subsimplicipes* remains poorly studied. We investigated its potential role in Zika virus (ZIKV) transmission dynamics by assessing its vector competence (ability to transmit the virus) and modelling its vectorial capacity (VC, potential to sustain transmission).

Two colonies of *Er. subsimplicipes* and two control colonies, *Aedes aegypti* and *Aedes albopictus*, originating from the Comoros archipelago were orally exposed to two African ZIKV strains (Dakar_1984 and Senegal_2011). Vector competence was evaluated by detecting ZIKV in mosquito saliva at 15 and 21 days post-infection (dpi), using plaque forming unit (PFU) assays. VC was modelled under rural transmission settings characterized by high abundance and strong anthropophily of *Er. subsimplicipes.*

Both *Er. subsimplicipes* colonies were susceptible to ZIKV infections, transmission rates reached 29% and 13% at 15 dpi, and 25% and 33% at 21 dpi. Higher transmission rates were recorded in *Ae. aegypti* (83%) and *Ae. albopictus* (88%) at 21 dpi. Using these data, VC modelling highlighted mosquito survival as a key factor. While the VC of *Er. subsimplicipes* was lower under a reduced survival assumption (0.002 *vs*. 2.98), it became higher than that of both *Aedes* species when survival rates were assumed to be equivalent (5.80 *vs*. 2.98) or greater (16.60 *vs*. 2.98).

Eretmapodites subsimplicipes is a newly identified competent vector of ZIKV and may significantly contribute to transmission in regions where it is abundant.

P003

REIMS-based detection of dengue infection in mosquito vectors

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Abstract

Background
Mosquito surveillance relies on multiple specialised assays to determine infection status, species identity, age and insecticide resistance, increasing logistical complexity and processing time. Rapid Evaporative Ionisation Mass Spectrometry (REIMS) generates metabolomic spectra without sample preparation and, when coupled with supervised machine learning, may enable extraction of key vectoral traits from a single analysis. REIMS has previously detected species, age and larvicide resistance status with high accuracy and recently detected *Plasmodium falciparum* infection in *Anopheles coluzzii* with 79% sensitivity, highlighting its potential as a surveillance tool.

Methods
Laboratory-reared *Aedes aegypti* were infected with DENV2 via infectious blood meal at 2 days-old, and infection was validated using RT-qPCR of mosquito legs. The abdomen and head/thorax of 284 infected mosquitoes were analysed separately by REIMS and compared with uninfected controls (n=197). Spectral data were binned, dimensionally reduced and classified using linear discriminant analysis (LDA) and random forest (RF) with repeated cross validation and 20 replicated model runs.

Results
REIMS classification of DENV2 infection status (n=481) achieved 65.98% (±1.21) accuracy using abdomen spectra and 64.74% (±1.23) using the head/thorax spectra with LDA. Sensitivity for detecting infected mosquitoes was 64.05% and 66.62%, respectively.

Conclusion
Accuracy for DENV2 detection remains below operational thresholds, highlighting key challenges in arbovirus detection despite REIMS potential for parasitic infections. This may reflect smaller metabolic changes and potential effects of Trizol storage used for inactivation. Ongoing work is assessing Trizol influence and combining head/thorax and abdomen spectra to improve classification performance.

P004

After the Bite: Mapping Early Host Responses to West Nile Virus Infection at the Virus-Host-Vector Interface

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Abstract

West Nile virus (WNV) is an emerging arbovirus in Europe, with recent detections in horses in the Netherlands signalling an increasing risk to animal and human health. The skin acts as the first barrier to WNV infection following vector feeding. A unique local microenvironment is created following the bite of a mosquito with interactions between virus, host cells, and vector saliva, yet these early events and how they might influence subsequent virus spread remains poorly characterised. This project explores the interactions and relative contributions of WNV and vector saliva in shaping early responses at the bite site, and how these may influence downstream infection outcomes including disease severity and transmission risk.

Using single-cell RNA sequencing of mouse skin 24 hours post-exposure to bites from naive or WNV-infected mosquitoes, we will resolve early gene expression changes across cell populations and differentiate host responses to mosquito bite alone, WNV infection, and their combined effects. We anticipate heterogeneous, cell population-specific transcriptional responses at the skin, including distinct signatures driven by mosquito salivary factors alone and by combined salivary and viral exposure, involving modulation of vascular permeability, immune cell recruitment and inflammatory and antiviral pathways that may modulate early infection outcomes. Together, these findings will provide insight into how vector-derived factors and viral infection jointly shape host responses at the skin and will highlight early host-response pathways to inform intervention strategies against mosquito-borne diseases. In future work, these findings will be validated using ex vivo and in vitro models derived from natural host species.

P005

Flavivirus Exposure History Influences Susceptibility to Spondweni Virus, a Prototype for Emerging Arboviruses

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Abstract

The antigenic similarity among flaviviruses allows antibodies elicited by one virus to cross-react with others, potentially enhancing, attenuating, or minimally affecting subsequent infections. These outcomes partly depend on the order in which the host encounters specific viruses. We previously showed that pre-existing immunity to Zika virus (ZIKV) protects rhesus macaques against Spondweni virus (SPOV)—ZIKV's closest known relative—whereas pre-existing SPOV immunity does not protect against ZIKV. The basis for this immune asymmetry and the mechanisms by which pre-existing immunity influences subsequent flavivirus infection remain poorly understood. We are now exploring how flavivirus exposure history impacts the outcome of SPOV challenge; specifically, how previous sequential infections with dengue virus (DENV) 2, DENV3, and/or ZIKV, acquired in different orders, affect macaques' susceptibility to SPOV. We found that pre-existing immunity to DENV3 or ZIKV does not protect against DENV2 infection, and that pre-existing DENV2 immunity does not protect against ZIKV infection. Notably, 10/10 macaques with pre-existing ZIKV immunity—whether acquired before or after DENV2 infection—resisted SPOV challenge, indicating that DENV2 infection does not diminish the protective effect of ZIKV immunity. Conversely, 4/5 macaques previously infected with DENV3 followed by DENV2 became infected with SPOV, although peak viral load occurred significantly later than in naïve macaques. We also found that DENV3-immune and ZIKV-immune macaques had significantly shorter viremia following secondary DENV2 infection. Currently, we are profiling neutralizing antibody responses. These studies will inform vaccine development and help assess risks posed by emerging flaviviruses in populations with prior exposure to related viruses.

P006

Exploring barriers for alternative vectors of tick-borne flaviviruses

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Abstract

In literature, various studies suggest that mosquito-borne flaviviruses are capable of using alternative vectors than their name implies. Whether this might also be true for tick-borne flaviviruses remains unexplored. With tick-borne encephalitis virus (TBEV) and Usutu virus (USUV) as models for tick- and mosquito-borne viruses, respectively, we studied whether genetic barriers in the virus or physical barriers inside the mosquito might hamper successful infection, dissemination, or transmission. Therefore, we infected *Culex pipiens* mosquitoes with TBEV or USUV via an infectious bloodmeal or injection and tested at different timepoints post infection whether virus was present in bodies, legs, and saliva. In addition, we studied the replication kinetics of wild type USUV and TBEV on different mosquito cells and attempted to construct chimeras of these viruses to study the replication kinetics on tick and mosquito cells.

We identified some infectious TBEV particles at different timepoints post infection on several mosquito cell lines, including Chao ball, CxT and C6/36 cells, but not on HSU cells. TBEV could not infect *Culex pipiens* mosquitoes via a bloodmeal or injection. Interestingly, infectious virus was detectable until 2 days post infection. We successfully made a chimera with TBEV prM-E proteins and a USUV background, but the reverse was not viable. We will investigate growth kinetics with this chimera on tick and mosquito-cells. Ultimately, this study will help to understand what makes some flaviviruses tick-borne and others mosquito-borne, thereby creating a better fundamental understanding of the transmission cycles of these viruses.

P007

Identification and Characterization of Pirahy Virus (PIRAV): A Novel Potential Emerging Neurotropic Arbovirus

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Abstract

Brazil faces the co-circulation of multiple arboviruses, including dengue, Chikungunya, and Oropouche, alongside emerging pathogens. However, fewer than 25% of dengue-like cases are laboratory-confirmed, suggesting the presence of undetected viruses and underscoring the need for enhanced surveillance. We report the identification and characterization of Pirahy virus (PIRAV), a novel alphavirus isolated from *Trichoprosopon* and *Wyeomyia* mosquito pools in Brazil. Phylogenetic analysis places PIRAV basal to the Venezuelan Equine Encephalitis virus complex, with low amino acid identity (≤50%) relative to known alphaviruses. Genomic analyses indicate signatures of dual adaptation to vertebrate and invertebrate hosts. *In vitro*, PIRAV infects both mosquito and mammalian cells, including primary human cells, with restricted replication in immunocompetent systems. Infection triggers RIG-I–mediated sensing and activation of type I and III interferon responses. *In vivo*, PIRAV displays neurotropism, with viral replication effectively controlled by interferon signaling. Vector competence assays show that *Aedes aegypti* mosquitoes are susceptible to infection and harbor virus in saliva at 14 days post-infection, supporting transmission potential. We further resolved the structure of mature PIRAV particles by cryo-electron microscopy at 4.84 Å resolution, providing a framework for antiviral targeting. These findings identify PIRAV as a novel neurotropic alphavirus with features consistent with emergence. Continued surveillance and functional studies are critical to assess its public health relevance and potential as a model for viral emergence and vaccine development.

Keywords: Alphavirus; Emerging viruses; PIRAV; Vector competence; Neurotropism; interferon

P008

How Do Blood Meal Sources Shape Virome Structure of the Invasive Mosquito *Anopheles stephensi*?

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Abstract

Mosquito-borne viral diseases represent a major and growing threat to global public health, but our ability to predict and control their transmission remains limited. One promising avenue lies in the study of the mosquito virome. Increasing evidence suggests that certain members of the virome can modulate arbovirus infection, replication, and transmission within mosquito hosts. In parallel, numerous studies have shown that mosquito viromes are shaped by several ecological and environmental factors.

Blood feeding is a key ecological and physiological event in the mosquito life cycle and is known to affect mosquito fitness, immunity, and microbial communities, particularly the bacteriome. However, the extent to which blood meal source influences mosquito viral communities remains poorly understood.

Here, we investigated the virome of a laboratory colony of the invasive mosquito *Anopheles stephensi*, one of the main vectors of malaria in Southern Asia. In our study, more than 500 adult females were fed with either human or chicken blood and subsequently pooled in groups of 20 individuals. Then, viral communities were characterized using a metagenomic sequencing approach. This analysis allowed us to describe the viral diversity present in laboratory-reared *An. stephensi* and to assess how different blood meal sources can shape virome composition and persistence in mosquitoes.

Our results provide new insights into how blood-feeding ecology influences mosquito viral communities under controlled conditions. Understanding these interactions is essential for improving the ecological relevance of laboratory-based vector competence studies and for refining models of arbovirus transmission risk.

P009

Evaluation of candidate vaccines to disrupt swine transmission of Japanese encephalitis virus

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Abstract

The Unites States (U.S.) pork industry contributes billions of dollars annually to the national economy and supplies approximately 11% of the global pork market, making herd health a critical priority. Domestic swine remain vulnerable to several endemic and exotic arthropod-borne viral diseases, yet no U.S. Department of Agriculture (USDA) approved arboviral vaccines are available for use in swine. In contrast, multiple licensed arboviral vaccines are available for horses. In this study, we evaluated the safety and immunogenicity of commercially available equine arbovirus vaccines administered to domestic swine for potential use if JEV were to enter the U.S.

Three-week-old piglets received either a full or half dose of one of the approved equine WNV vaccines following manufacturers recommended schedules: primary dose with a booster 21 days later. A mock vaccinated control group was included. Piglets were monitored daily for adverse reactions and weighed twice weekly to assess growth. Serum samples collected on Day 0 and twice weekly from Day 7 through Day 35 post vaccination were analyzed for development of vaccine induced immune responses.

Vaccinated piglet serum showed JEV neutralizing antibody titers above 1:10 indicating potential protection against JEV infection. All vaccines evaluated elicited generation of cross-neutralizing antibodies. Mock vaccinated piglets showed JEV neutralizing antibody titers below the 1:10 threshold indicating no potential protection against JEV infection.

The study provides evidence that equine arbovirus vaccines can induce detectable immune responses in swine without significant side effects and may be used to respond to an introduction of JEV into the U.S.

P010

Convalescent pooled plasma confers protection against Chikungunya virus challenge in a non-human primate model

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Abstract

The World Health Organization (WHO) Expert Committee on Biological Standardization established the 1st WHO International Standard (IS) for anti-chikungunya virus (CHIKV) neutralising IgG (Paul-Ehrlich-Institut code: 1502/19) in 2022. This high order calibrant enables harmonisation of neutralising antibody measurements across laboratories in a common unitage, the International Unit (IU). To augment its biological relevance and value, we assessed whether 1502/19 confers protection against CHIKV challenge in a cynomolgus macaque model. Neutralisation potency underpins regulatory evaluation of CHIKV vaccines and defining a protective threshold in IU supports licensure and informs immunisation strategies.

Animals received neat or diluted 1502/19 prior to intradermal challenge with 10^5 infectious units of CHIKV strain LR2006-OPY1. Control animals developed viremia, fever, and *de novo* anti-CHIKV IgM and IgG responses following challenge and a titratable reduction of disease was observed in animals that received 1502/19. Neutralisation titres were measured using a CHIKV glycoprotein pseudotyped vesicular stomatitis virus assay and expressed relative to 1502/19 to ensure comparability. Naïve animals generated robust neutralising responses, whereas macaques given undiluted 1502/19 showed no clinical signs and no *de novo* antibody production. Intermediate protection was observed with 1:10 and 1:100 dilutions.

Collectively, these findings support a correlate of protection, with titres exceeding 10 IU/mL conferring protection in this model. This work strengthens the utility of 1502/19 as a universal calibrant and provides a framework for defining protective thresholds for novel CHIKV vaccines. This study facilitates future studies to determine the level and nature of protective correlates generated by new vaccines against CHIKV.

P011

Mosquito saliva, virus infections and the impact of *Wolbachia*

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Abstract

Mosquito-borne viral diseases have a profound effect on human health globally and the viruses that cause them are made up of a large genetically distinct group of viruses. This diversity makes it difficult to create treatments and vaccines that successfully target this group. Understanding and targeting transmission has proven to be an effective approach for developing novel control strategies. One effective strategy in reducing virus transmission has been the introduction of *Wolbachia*; as has been demonstrated for several medically relevant arbovirus families. Arbovirus transmission is a complex process encompassing a mix of virus-vector-host interactions, with mosquito saliva playing a crucial part by enhancing virus infection in the mammalian skin via vasodilation and an influx of immune cells. Introduced *Wolbachia* symbionts are abundant within the salivary glands in *Ae. Aegypti,* but their effect on mosquito saliva and subsequent virus transmission has not been examined. By conducting a transcriptomic analysis of mosquito salivary glands we demonstrate that key salivary gland gene expression is modulated in *Wolbachia* carrying mosquitoes. In addition, there are differences exhibited between salivary glands acquired from *Ae. aegypti* carrying different strains of *Wolbachia*. These changes in expression affects virus transmissibility to mammals by modulating key skin responses during early virus infection. Using a mouse model closely mimicking natural infection we study the impact these changes in saliva composition have on arbovirus infection.

P012

Expanded geographic distribution and genetic diversity of Inkoo and Chatanga viruses in Finland

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Abstract

Background: Inkoo virus (INKV) and Chatanga virus (CHATV) are California serogroup orthobunyaviruses endemic in northern Europe. Despite high human seroprevalences, clinical diagnoses remain rare, and both their genetic diversity and disease burden are poorly understood.

Methods: Mosquitoes collected in Finland during 2018–2021 were screened for INKV and CHATV RNA. Positive samples were subjected to virus isolation in cell culture and genomic sequencing. In addition, two unpublished CHATV strains were included in the complete genome analyses.

Results: INKV and CHATV were detected in mosquitoes from eastern Finland, a known endemic region. CHATV was also detected in western Finland, an area not previously recognised as part of its distribution range. Genomic sequencing is ongoing; however, preliminary analyses indicate that the western CHATV strain is distinct from the eastern strains. One INKV RNA–positive mosquito sample from eastern Finland induced cytopathic effects in cell culture and, if confirmed, would represent the first INKV isolate obtained in Finland since the 1970s.

Conclusions: These findings expand the known geographic distribution of CHATV indicating that human exposure may be occurring unrecognised in western Finland. The results also provide new insights into the genetic diversity of both viruses. Given that California serogroup orthobunyavirus infections can cause severe central nervous system disease, increased clinician awareness, targeted summer-season testing, and updated seroprevalence studies would be needed for understanding the risk areas and disease burden caused by orthobunyaviruses in Finland and wider in Europe.

P013

Development of a novel pseudotyped-virus screening platform to characterise host factors involved in arboviruses cell entry.

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Abstract

Ceudovitox, is an innovative, new screening platform, allowing the characterisation of host entry factors of arboviruses with outbreak potential. Through using pseudotyped viruses (PV) expressing the chikungunya virus (CHIKV) envelope protein and the herpes simplex virus-1 thymidine kinase gene, cells susceptible to CHIKV entry could be selectively killed after ganciclovir treatment. Utilisation of these "killer" PVs in the context of a CRISPR-Cas9 (GeCKO) library gene-knockout cells permitted selection of cells refractory to CHIKV virus entry, without the need for high containment facilities. Deep sequencing of the selected cells and their comparison to controls using the MAGeCK algorithm, identified a number of significant hits (p-value <0.05 and FDR <0.25) highlighting both known and novel gene networks involved in CHIKV viral entry. Significant hits were then fed into a STRING analysis to find significant gene clusters potential involved in CHIKV entry, which highlighted matrix metalloproteinase (MMPs) as potential targets. Follow-up validation testing, then found that broad-spectrum MMP inhibitors inhibited CHIKV-PV entry, with Marimastat producing an IC50 of 2.2 µM. Specific inhibition of multiple MMPs through RNAi was seen to significantly reduce entry of CHIKV PVs. This trend was again seen in live virus assays, with both GM6001 and Marimastat reducing entry of a live Chikungunya virus, harbouring the ZsGreen marker. These results show that our rapid and robust screening platform can identify new therapeutic targets, thereby fortifying global preparations against epidemics and pandemics and making a significant contribution to the "100 days" mission.

P014

Investigating the role of nsP4 in alphavirus replication in *Aedes aegypti* mosquitoes

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Abstract

Alphaviruses are RNA arboviruses that pose significant health and socioeconomic burdens, yet alphavirus replication dynamics and the factors governing replicase-template specificity remain poorly understood. Elucidating the complexities of alphavirus replication will help inform the development of antiviral therapeutics that target their replication machinery, introducing more precise, virus-driven methods to reduce the impact of mosquito-borne viruses.

This research focuses on the replication dynamics of alphaviruses, with particular focus on the viral RNA-dependent-RNA polymerase, nsP4, a crucial component of alphavirus replication. As one of four non-structural proteins comprising the viral replication complex, nsP4, alongside P123, works together to coordinate synthesis of the viral RNA template. nsP4 is a tightly transcriptionally regulated and highly unstable protein, whose constraint plays an essential role in co-ordinating the rate of virus replication. Utilising a stabilised version of nsP4, we explore the role that rapid degradation and inherent instability of nsP4 plays in governing virus replication.

Selective recognition of the RNA template is essential for efficient alphavirus replication, however the contributing role of nsP4 in this interaction remains unclear. We explore the template specificity of individual nsP4s and the ability of an RNA template to be replicated by heterologous alphavirus replicases, to determine whether nsP4 itself is the driver of this selectivity. Furthermore, we investigate the specificity with which alphavirus nsP4 can form functional replication complexes with heterologous P123 proteins. Together, this research explores the factors governing nsP4 replicative function in mosquitoes and addresses important gaps in our fundamental understanding of alphavirus replication dynamics.

P015

Elucidating the functional relationship of the insect-specific Taï Forest Alphavirus with pathogenic alphaviruses and their mosquito vectors

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Abstract

Alphaviruses are a genus of arboviruses containing several medically important viruses, including Chikungunya and Mayaro virus, commonly vectored by *Aedes aegypti* mosquitoes. Closely related to pathogenic alphaviruses is the Taï Forest Alphavirus (TALV), an insect-specific virus (ISV) isolated from *Culex decens*. ISVs are of particular interest due to their potential interactions with pathogenic viruses during co-infection of important arboviral vectors.

This study characterises the functional relationship of TALV with pathogenic alphaviruses whilst elucidating TALV's host range and host range restrictions. Using a trans-replication assay, this study explored the cross-compatibility of both TALV replicase and template with the templates and replicases of ten other alphaviruses, including Chikungunya and Mayaro, in both insect and mammalian cell lines. Furthermore, we have generated a TALV reverse genetics system to aid understanding of the replication dynamics of TALV within *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes. We use a fluorescent-tagged virus to explore infection routes via sugar feeding, blood feeding and intrathoracic injection, and dissemination patterns throughout the mosquito. Our findings will increase knowledge on the close relationships and interactions between insect-specific and pathogenic alphaviruses and develop an understanding on the restrictions placed on ISV replication in important disease vectors.

P016

Diversity, abundance, and blood feeding preferences of mosquitoes at Belgian pig farms

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Abstract

Pig farms are important in the epidemiology of Japanese Encephalitis virus (JEV), as pigs act as amplifying hosts. Belgium is a major pig-producing region in Europe, so information on the presence of potential JEV vectors and their feeding preferences is essential to assess the JEV transmission risk following introduction. Mosquitoes were collected indoors and outdoors on 16 pig farms (7 conventional and 9 biological) during early, mid-, and late summer of 2025 using Mosquito Magnet® traps. Female mosquitoes were morphologically identified to the species level, and COI-barcoding of the blood meal was performed to identify the host. A total of 1,969 female mosquitoes representing 21 species were identified. Abundance was significantly higher outdoors than indoors on conventional farms, whereas mosquitoes were more abundant indoors on biological farms, although not significantly. Mosquito diversity was significantly higher outdoors across both farm types, and seasonal shifts in species composition were observed. During early and mid-summer, *Anopheles maculipennis* and *Coquillettidia richiardii* were the dominant species on conventional farms, while *Culiseta annulata* and *Culex pipiens* predominated on biological farms. During late summer, *Culex pipiens* was most abundant on both farm types. This species has previously been found JEV-positive in the field and proven competent under laboratory conditions. Blood meal analysis showed that the collected mosquitoes mostly had fed on humans or pigs. These results indicate that local transmission after a JEV introduction in Belgium is possible as JEV-competent mosquitoes are present on pig farms and feed on both the amplifying (pigs) and incidental (human) hosts.

P017

Vector competence of Belgian *Culex pipiens* mosquitoes for West Nile virus under different temperature conditions

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Abstract

Following the first detection of West Nile virus (WNV) circulation in wild birds in Belgium in 2025, assessing the transmission potential of local mosquito populations has become essential. To support risk assessments, we determined the vector competence of Belgian *Culex pipiens* mosquitoes, considered an important WNV vector. Field-collected mosquitoes were offered a WNV-spiked (Israel 98, lineage 1) blood meal. Blood-fed females were kept for 14 days at (i) a constant 25°C, (ii) a 25/15°C day-night gradient reflecting current Belgian summers, or (iii) a 25/20°C day-night gradient simulating a climate change scenario. After the 14-day incubation period, abdomens, head-wings-legs, and saliva were collected and tested in RT-qPCR to determine infection rate (IR), dissemination rate (DR), and transmission rate (TR). Observed viral loads in the abdomen and IRs (25.5%, 32.7%, and 34.3%) did not significantly differ between temperature conditions, suggesting limited impact of temperature on virus replication in the midgut. Interestingly, the DR at 25/15°C (28.6%) was statistically higher than the DR at 25/20°C (10.4%), indicating that WNV more easily passes the midgut barrier under the temperature condition reflecting current Belgian summers. The entry of WNV into the salivary glands did not seem to be altered by temperature, as no statistically significant differences were observed between the obtained TRs (3.9%, 8.2%, and 1.5% at 25°C, 25/15°C, and 25/20°C, respectively). These results demonstrate that *Culex pipiens* is a competent WNV vector at all three tested temperature conditions and could play an important role in WNV transmission in Belgium upon (re)introduction or continued circulation.

P018

Experimental BTV-3 and BTV-8 infection of *Culicoides sonorensis* biting midges

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Abstract

Background:
The bluetongue virus (BTV) is the causative agent of a major infectious disease in livestock, transmitted between ruminant hosts by *Culicoides* biting midges. The first recorded outbreak in Central Europe was caused by serotype BTV-8, leading to a major epidemic. In 2023, serotype BTV-3 emerged in the Netherlands and spread rapidly to neighbouring countries. Compared to the BTV-8 outbreak in 2006, the BTV-3 epizootic has a more severe clinical course and spreads faster.

Methods:
To explore the possible causes of the different epidemiologies, we compared the replication properties of BTV-8 and BTV-3 in a laboratory colony of biting midges, *Culicoides sonorensis*. The midges were orally infected with BTV-3 or BTV-8 via an infectious blood meal. Engorged females were either processed immediately or incubated for six days. They were then individually processed, and viral genome copies were quantified using BTV-specific RT-qPCR. Replication was assessed using a cut-off derived from day-0 viral loads. Two independent infection trials were performed.

Results:
Overall, 4.39% of the midges infected with BTV-3 replicated the virus, whereas a smaller proportion of midges infected with BTV-8 replicated the virus (3.44%). Moreover, oral infection with BTV-3 resulted in a significantly higher viral load in infected midges with demonstrated replication than infection with BTV-8.

Conclusions:
The significantly higher BTV genome load observed in midges with BTV-3 replication than in midges with BTV-8 replication may be a factor contributing to the observed faster progression of the BTV-3 outbreak in comparison to the BTV-8 outbreak in 2006/2007.

P019

AaVago3 enhances *Aedes aegypti* tolerance to Zika virus via GRP5 activation

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Abstract

Successful transmission of arthropod - borne viruses (arboviruses) requires vector mosquitoes to tolerate high viral loads without significant fitness costs. Although canonical antiviral pathways such as RNA interference restrict viral replication, the mechanisms that directly sustain mosquito tolerance remain poorly understood. Here, we show that *Aedes aegypti Vago-like protein 3* (*AaVago3*) is a key determinant of tolerance during Zika virus (ZIKV) infection. Silencing *AaVago3* reduced mosquito survival after ZIKV infection via injection without altering the viral replication, whereas injection of purified recombinant AaVago3 protein restored survival. Transcriptome analysis identified *glycine - rich protein 5 - like* (*GRP5*) as a novel downstream effector activated by *AaVago3*, and silencing *GRP5* recapitulated the fitness loss of *AaVago*3 - silenced mosquitoes. Mechanistically, *AaVago3* promotes nuclear translocation of the Toll pathway transcription factor *REL1A*, which in turn activates *GRP5*. In an *AaVago3* - deficient *A. aegypti* strain, we observed increased mortality and impaired blood - feeding behavior following oral ZIKV infection. Together, these results reveal an *AaVago3* - *REL1A* - *GRP5* axis that maintains mosquito tolerance to arbovirus infection, enabling *A. aegypti* to sustain arbovirus transmission.

P020

Characterisation of RNA species packaged into tick-borne encephalitis virus virions

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Abstract

The tick-borne encephalitis virus (TBEV; *Orthoflavivirus encephalitidis*, *Flaviviridae*) is a Eurasia-wide causative agent of tick-borne encephalitis (TBE), a severe neurological disease which affects thousands of patients annually. Despite being a serious pathogen, certain aspects of the viral life cycle remain poorly understood, such as virion assembly and the specificity of the viral RNA (vRNA) packaging. Viral replication and packaging are tightly interconnected processes, and the TBEV capsid protein (TBEV C) not only forms nucleocapsid, but also plays multiple roles in the TBE pathogenesis. Despite TBEV C being a strong, non-specific nucleic acid binder, vRNA incorporation is believed to be a specific event even in the absence of a clearly defined packaging signal. However, the overall composition of RNA packaged within virions has not been characterised. To elucidate the nature of the RNA species packaged into newly formed virions, TBEV particles were purified using a two-step approach of precipitation followed by ultracentrifugation on the tartrate gradient. Encapsidated RNA species were analysed using strand-specific library preparation and NovaSeq sequencing with separate libraries targeting small RNAs and longer RNA subpopulations. The vast majority of mapped reads were of viral origin, while less than a per cent corresponded to host (human) RNA. Our findings provide new insights into the molecular mechanisms of TBEV RNA packaging in host cells and highlight factors that may influence virion composition.

P021

Infectious pit stops: arbovirus prevalence and wild bird reservoirs at wintering and stop-over sites in the Gambia

Helena Broes

Abstract

Birds serve as reservoirs for several arboviruses and may facilitate their long-distance dispersal. Migratory 'pit stops', wintering and stop-over sites where birds congregate and interact, are potential hotspots for arbovirus exchange. The Gambia, a region with a high avian density, located along a major migratory bottleneck, remains understudied for Culex-borne arboviruses.

Birds were captured February 2023, prior to spring migration, and in November 2023 and 2025, after autumn migration. Oropharyngeal and cloacal swabs, serum, and feathers were collected. Swabs were screened for WNV, USUV, SINV using RT-PCR, and viral sequences were determined when possible. Serum was tested for USUV and WNV prior exposure via an enzyme-linked immunosorbent assay, with positive sample confirmation by WNV- and USUV-specific neutralisation tests. Negative samples were assessed for SINV using a plaque reduction neutralisation test.

A total of 674 birds (85 species) were sampled in February 2023, 253 birds (78 species) in November 2023, and 583 birds (90 species) in November 2025. Only February 2023 samples have been analysed to date. USUV prevalence was 0.6% (4/662), detected in Yellow fronted Tinkerbirds, a Yellow-crowned Bishop, and a Common Nightingale. WNV or SINV RNA was not detected. Serology identified WNV exposure in twelve birds, including Hooded Vultures. A Black-headed Weaver tested seropositive for USUV. Five more birds were positive for unspecified orthoflavivirus antibodies. SINV seroprevalence was 9,9% (23/232), mostly found in African Jacana.

USUV detection in migratory birds before migration suggests that wintering and stop-over sites can facilitate virus exchange and may contribute to long-distance spread.

P022

Elucidating Protein Sialylation in Ticks Using Bioorthogonal Chemistry

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Abstract

Sialic acids (Sias) are a group of saccharides that are mostly found in Vertebrates and serve in self/non-self recognition and in protein-protein and cell-cell interactions. In lower Eukaryotes, Sia, or sialylation of proteins, was found only in a few Arthropods at early developmental stages or in the neural system.

Here, we confirm the tick *Ixodes ricinus*' ability to produce sialylated glycoproteins by confirming the presence of the required genes for sialic acid biosynthesis using N-acetylneuraminate lyase, the presence and expression of sialyltransferase genes and activity of sialyltransferases. Using bioorthogonal chemistry in combination with Click chemistry, we show that ticks and tick cells produce sialylated glycoproteins.

P023

Neuroinvasion, behaviour and gene expression: strain-specific effects of West Nile virus in *Culex pipiens* form *pipiens*

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Abstract

West Nile virus (WNV) transmission depends on mosquito behavioural traits that determine host contact and dispersal. However, it remains unclear whether different WNV strains induce distinct behavioural and molecular changes in their mosquito vectors. We investigated the effects of WNV infection on female *Culex pipiens* form *pipiens* using representative lineage 1 and lineage 2 strains. Mosquitoes were intrathoracically infected and monitored over 14 days post-infection for flight activity and assessed for blood-feeding over two consecutive days. To investigate underlying mechanisms, viral antigen distribution was assessed by immunohistochemistry, with a focus on neural tissues. In parallel, transcriptomic analyses were performed to characterise infection-associated gene expression changes, comparing infected and uninfected mosquitoes as well as responses between viral lineages. WNV infection induced strain-specific behavioural changes. Mosquitoes infected with the lineage 2 strain showed increased flight activity and reduced blood-feeding compared with both uninfected controls and lineage 1-infected mosquitoes. Immunohistochemical analyses revealed the presence of viral antigen in key neural tissues, supporting a link between neuroinvasion and behavioural modulation. Transcriptomic analyses showed differential expression of genes involved in neuronal function, sensory perception and energy metabolism between infected and non-infected mosquitoes, as well as between WNV strains. Notably, genes linked to synapse formation, circadian and locomotor regulation, visual perception and chemical synaptic transmission were modulated, with distinct patterns observed between WNV lineages. Together, these findings indicate that WNV induces strain-specific behavioural and transcriptional responses in *Culex pipiens*, potentially driven by neurotropism and metabolic remodelling, with important implications for vector competence and transmission dynamics.

P024

Dinucleotide composition differentially regulates alphavirus protein expression in vertebrate and mosquito cells

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Abstract

Arboviruses alternate between vertebrate hosts and mosquito vectors, requiring efficient replication in two evolutionarily distant systems. Host and vector transcriptomes exhibit distinct nucleotide usage biases, particularly in dinucleotide frequencies, which influence transcription, translation, RNA stability, and RNA–protein interactions, including antiviral responses. While these effects are increasingly recognized in vertebrates, the underlying mechanisms in arthropod vectors remain poorly understood. Here, we investigate how synonymous changes in dinucleotide composition differentially modulate protein expression in host and vector cells using alphavirus-derived self-amplifying RNAs.

Replicons derived from both Old World and New World alphaviruses were engineered to express luciferase reporters with systematically varied dinucleotide frequencies from the subgenomic RNA. In vertebrate cells, increased CpG dinucleotide frequency resulted in reduced luciferase expression, an effect fully dependent on the zinc-finger antiviral protein (ZAP). In contrast, mosquito cells exhibited enhanced luciferase expression from CpG-enriched replicons, indicating opposing selective pressures between host and vector environments. To distinguish between virus-specific and host- or vector-specific cellular processes, we also expressed luciferase outside the context of viral replication. This demonstrated that the CpG frequency additionally modulates gene expression in non-viral contexts.

Collectively, our findings reveal that dinucleotide composition differentially regulates protein expression in vertebrate and mosquito cells across both viral and non-viral transcripts. These insights highlight a key evolutionary constraint in arbovirus biology and inform rational design strategies for attenuated viruses and protein expression platforms based on both vertebrate and invertebrate systems.

P025

An ecological review of *Aedes detritus s.l.* and *Aedes rusticus* as potential West Nile virus vectors in the UK

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Abstract

West Nile virus (WNV) was detected in the United Kingdom in *Aedes vexans* in 2023, despite this species not previously being considered a principal WNV vector. This occurrence thus highlights the need to monitor other understudied WNV vectors in the UK. Here we assess the potential for two species- *Ae. detritus s.l.* and *Ae. rusticus*- to transmit WNV in the UK, combining a systematic review of vector competence and blood feeding patterns, with an ecological niche model of their current distribution. Our systematic review identified 18 relevant studies, revealing that, *Ae. detritus s.l.* is a competent vector for seven arboviruses and *Ae. rusticus* for two. Crucially, both species are competent for WNV. *Aedes detritus s.l.* has been observed to blood feed on humans, non-human mammals, and avian hosts. A Biomod2 ensemble model identified potential *Ae. detritus s.l.* habitats throughout coastal England, Wales, and Scotland. *Aedes rusticus* potential habitats are primarily in Eastern and Central England, and to a lesser extent in the Northwest and Northeast of England. Our results demonstrate *Ae. detritus s.l.* is a competent vector at UK realistic temperatures, that has the potential blood feeding pattern to play a role in WNV transmission, but that this might be restricted to coastal areas. Evidence for *Ae. rusticus* feeding patterns and vector competence was limited, though it has potential habitats that are widespread throughout England. Our results emphasize the need to include *Ae. detritus* s.l. in WNV preparedness plans and provide a basis for the surveillance of both species.

P026

Application of synthetic phage libraries to enhance species-specific surveillance and control of co-circulating flaviviruses

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Abstract

Background Cross-reactivity between co-circulating flaviviruses complicates serosurveillance and hampers effective disease control. **Materials and Methods** Panels of serum samples from individuals exposed to Zika (ZIKV) or dengue (DENV) or sheep exposed to louping ill virus (LIV) or tick-borne encephalitis virus (TBEV) were panned against synthetic peptide phage libraries to identify species-specific immunosignatures. The accuracy of immunosignatures identified by phage display was compared with commercially available ELISAs and ELISAs developed in-house using non-structural (NS1) flavivirus proteins expressed *in planta*. Furthermore, virus-like particles were panned against a synthetic ScFv phage display library to identify LIV-specific binders, which were expressed as ScFv–Fc fusions in mammalian cells. Binding of ScFv was confirmed by ELISA and biolayer interferometry and further characterization is ongoing. **Results** Applying the immunosignature approach using phage display to human serum samples exposed to Zika and/or dengue virus identified that pre-screening using commercially available ELISA wrongly categorized samples. ELISA using plant-expressed NS1 proteins different flaviviruses gave more specific results. ScFv to LIV were successfully expressed and their binding investigated. **Conclusions** Phage display can be applied to develop tests that can discriminate antibody responses to different flavivirus species. We also confirmed that plant-expressed NS1 proteins can be used in ELISA to distinguish antibodies to different flavivirus species. Synthetic phage libraries can also be used to generate flavivirus-specific antibody reagents.

P027

From fractions to function: a workflow for compartment-specific interactome profiling

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Abstract

Characterising protein interactomes within distinct subcellular compartments is essential for understanding context-dependent protein functions and cellular processes. However, conventional whole-cell interactome approaches often obscure spatially restricted interactions and fail to distinguish direct protein associations from indirect, nucleic acid-mediated complexes. Here, we present a workflow for compartment-specific interactome analysis combining subcellular fractionation, co-immunoprecipitation (Co-IP), and mass spectrometry (MS). Cells are separated into defined subcellular fractions, and Co-IP is performed, allowing identification of compartment-resolved interaction partners. To discriminate direct protein-protein interactions from RNA- or DNA-mediated associations, samples are processed in parallel with and without nuclease treatment. Candidate interactions identified by MS can be subsequently verified using reverse Co-IP and immunofluorescence-based co-localisation analysis. This strategy provides a robust framework for resolving spatially defined protein interaction networks and can be broadly applied to investigate multifunctional proteins whose roles vary across intracellular compartments. In our case, this approach was validated using the *Orthoflavivirus encephalitidis* (tick-borne encephalitis virus, TBEV; *Flaviviridae*) capsid protein, demonstrating its utility for analysing viral protein interactomes as well.

P028

Flooding the frontlines: Consequences of summer inundation on the ecology of mosquito-borne diseases

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Abstract

Introduction

The increased risk of flooding and associated mitigation measures including peak rainwater storage may change the risks of vector-borne disease outbreaks, particularly in summer. This study aims to assess the effects of temporary summer peak water storage on the risk of mosquito-borne virus (e.g. Usutu virus and West Nile virus) outbreaks, using the Eendragtspolder near Rotterdam, the Netherlands, as a field lab.

Methods

In August 2025, 2.5ha of wetland was flooded using temporary water barriers, to simulate a peak storage event. Data on weather, water, animals (birds, predators, rodents), vectors (mosquitoes), viruses, and visitors were collected before, during and after the inundation. Samples were tested using targeted and untargeted approaches for viruses (PCR, sequencing, serology) as well as eDNA techniques for host DNA including aquatic micro and macrofauna.

Preliminary results

A major increase in larval and mosquito counts was observed over the duration of the inundation. Large numbers of resting mosquitoes were trapped in the surrounding vegetation throughout the experiment. After inundation, an increase was observed in the number of carrion crows, Canadian geese, and common snipes in the area. Usutu virus was detected in seventeen mosquito pools and one bird. West Nile virus was detected in one mosquito pool. No virus was detected in rodents. On average, about 1200 humans visited the area daily.

Conclusion

Temporary inundation in summer causes significant fluctuations in population composition, increases and movements of mosquitoes, birds, and their predators, which may increase the risk of mosquito-borne virus outbreaks, such as West Nile virus.

P029

Host Blood Source Modulates O'nyong-nyong Virus Vector Competence and miRNA Responses in *Anopheles stephensi*

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Abstract

O'nyong-nyong virus (ONNV) is a neglected mosquito-borne alphavirus uniquely transmitted by *Anopheles* mosquitoes; however, the influence of host blood source on ONNV transmission dynamics and mosquito antiviral responses remains poorly understood. In this study, we investigate how different vertebrate blood sources modulate vector competence, vertical transmission potential, and miRNA-mediated responses in *Anopheles stephensi* during ONNV infection. Adult female mosquitoes are orally challenged with ONNV-containing blood meals derived from either human or chicken hosts, followed by comparative analyses of infection, dissemination, and transmission efficiency. ONNV RNA is quantified by RT-qPCR in the thorax and abdomen, head, wings, legs, and saliva of infected mosquitoes at 7, 14, and 21 days post-infection. Infectious virus in saliva is further evaluated using Vero B4 cell infection assays. In parallel, small RNA sequencing is performed to characterize blood meal–dependent miRNA expression profiles associated with ONNV infection. Differentially expressed miRNAs are integrated with predicted immune and metabolic target pathways to identify molecular signatures associated with altered vector competence. Additionally, the potential for vertical transmission is assessed through detection of ONNV in eggs, larvae, pupae, and F1 progeny derived from infected females. This study aims to provide novel insights into how host blood source shapes ONNV transmission biology and mosquito antiviral regulatory pathways. Understanding these interactions may reveal previously unrecognized ecological and molecular determinants influencing arbovirus–*Anopheles* vector interactions and transmission dynamics.

P030

Non-coding flavivirus RNA as key target to engineer live-attenuated vaccines that are 'safe-by-design'

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Abstract

Orthoflaviviruses, such as dengue, Zika and West Nile (WNV) pose significant public health threats due to their widespread transmission and potential to cause severe illness. All flaviviruses produce abundant quantities of small non-coding RNA required for induction of viral pathogenicity *in vivo* and efficient vector transmission. This so-called subgenomic flavivirus RNA (sfRNA), is the product of incomplete viral genomic RNA degradation by the cellular exoribonuclease XRN1 that stalls at conserved RNA structures in the 3' untranslated region (UTR). This research aims to engineer live-attenuated WNV vaccines, that provide live-long protection with a single shot, through random mutagenesis of three-dimensional RNA structures within the 3'UTR that are essential for sfRNA biogenesis. Using Golden Gate assembly a method was developed to achieve high efficiency cloning of mutagenic libraries of WNV infectious cDNA clones. Illumina sequencing was performed to follow the composition of the mutant library during virus replication. Individual viruses from the mutant library were phenotypically selected in a high-throughput manner, which was based on the ability of the mutant viruses to efficiently replicate yet fail to induce cytopathic effect in cell culture. Our selected WNV mutants were able to replicate efficiently, were genetically stable for at least 10 passages and were significantly less cytopathic compared to wildtype WNV *in vitro*. Additionally, the selected WNV mutants showed significantly reduced transmission by mosquitoes. These results show that we have developed promising live-attenuated vaccine candidates which are currently being validated in a mouse vaccination study using infected mosquitoes for WNV challenge.

P031

Analysis of Chikungunya Virus Non-structural Polyprotein Cleavage during Early Stages of Virus Replication

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Abstract

Chikungunya virus (CHIKV) is a medically significant alphavirus. Like other alphaviruses, CHIKV replication is hypothesized to be controlled by the precise sequential and timely cleavage of four non-structural proteins (nsPs). To study the requirements for and the function of the intermediate and mature processing products for CHIKV RNA synthesis, we generated CHIKV site-directed mutant replicons and infectious clones that have defects in processing the nsP1/2 (P1/234), nsP2/3 (P12/34), or both (P1/2/34) cleavage sites. In replicon assays, replication of all three mutants was impaired to varying degrees, and the formation of nsP3 granules was suppressed. Except for nsP4, the major products of P1/234 were P123+P12 and nsP3, those of P12/34 were nsP1 and P23, and P1/2/34 produced only P123. Confocal microscopy revealed that the subcellular localization of nsP3 in the mutants shifted from the cytoplasm to the plasma membrane, where it co-localized with dsRNA. We then rescued CHIKV nsP cleavage deficient viruses. Relative to WT, P1/234 and P1/2/34 produced viral titers that were reduced by 4.6 $\log_{10}$ and 3.7 $\log_{10}$, respectively. Plaque centre assays showed that P1/234 and P1/2/34 formed smaller plaques. Both plaque size and viral titers of P12/34 were similar to those of WT. Strand-specific qRT-PCR revealed that the −ve/+ve RNA ratio induced by P12/34 was 3.8-fold higher than that of WT. Taken together with dsRNA accumulation observed in P12/34 replicon, these results indicate that efficient +ve RNA synthesis in CHIKV requires cleavage between nsP2 and nsP3.

P032

Discovery of CHIKV spike protein–targeting nanobodies via an NGS library-mining approach.

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Abstract

Chikungunya virus (CHIKV) is a re-emerging mosquito-borne alphavirus that causes acute and chronic disease in humans. Its continued spread into new geographical regions, driven by expansion and adaptation of mosquito vectors, poses a significant global public health threat. Nanobodies, single-domain antibody fragments, represent promising diagnostic and therapeutic agents against viral infections. Here, we generated a nanobody library by immunizing a camelid using a DNA-launched replicon prime followed by a recombinant CHIKV spike protein boost. A bacteriophage display library was screened to identify nanobodies targeting the CHIKV spike complex. After a single round of bio-panning with recombinant E1 or full CHIKV spike proteins, basal and enriched libraries were analyzed by NGS, and enrichment values were calculated for individual nanobodies. Nanobodies were selected and their binding to the CHIKV spike was confirmed by flow cytometry using cells infected with CHIKV strains from the ECSA, WA, and Asian lineages. Neutralization activity against all three lineages was assessed by plaque reduction neutralization tests (PRNT). Nanobodies exhibiting the highest neutralization potency were converted into Fc-fusion constructs expressed in mammalian cells. In addition, neutralizing nanobodies recognizing distinct epitopes were site-specifically functionalized to generate homo- and heterodimeric constructs. Enhanced molecular size and avidity significantly improved neutralization potency. Dyl059-Fc, Dyl084-Fc, and Dy201-Fc showed the strongest neutralization against the WA lineage, with $PRNT_{50}$ values of 11.5 nM, 2.13 nM, and 14.3 nM, respectively. Moreover, three nanobodies demonstrated cross-reactivity with other Old World alphaviruses. Together, these nanobodies expand the CHIKV research toolkit and show potential for therapeutic and diagnostic development.

P033

Identifying the impact on NK-cell function of a *MICB* genetic polymorphism associated with severe dengue

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Abstract

Background:

Early NK-cell dysfunction discriminates patients progressing to severe dengue (SD) from mild dengue, but the mechanisms underlying immune dysfunction remain unclear. Genome-wide association studies (GWAS) identified a single nucleotide polymorphism (SNP) in *MICB* as a susceptibility locus for SD. MICB binds NKG2D expressed on NK/$CD8^+$ T-cells, triggering cytotoxicity. We investigated whether this *MICB* SNP impacts NK-cell responses.

Methods:

MICB SNP rs3132468 was introduced in K562 cells using CRISPR-Cas9. MICB mRNA and protein expression were assessed using RT-PCR, ELISA, and flow cytometry. Sensitivity of gene-edited K562 cells to NK-cell killing was assessed upon co-culture with NK-92 cells.

Samples from 192 Vietnamese dengue patients were genotyped for *MICB* rs3132468 using TaqMan. *Ex-vivo* analyses of patient PBMCs examined the contribution of rs3132468 to NK-cell function and its links to disease outcomes.

Results:

The “high-risk” variant of *MICB* rs3132468, associated with SD, leads to decreased MICB mRNA and protein expression in gene-edited K562 cells and renders cells less susceptible to NK-cell killing *in-vitro*. In patients, high-risk *MICB* rs3132468 expression is associated with SD with some features of NK/$CD8^+$ T-cell dysfunction.

Conclusion:

Our *in-vitro* findings reveal that *MICB* rs3132468 affects NK-cell cytotoxicity through decreased MICB expression. Consistent with the GWAS results, we demonstrate that *MICB* rs3132468 is associated with SD in Vietnamese patients. Furthermore, we show that *MICB* rs3132468 contributes to shaping NK/$CD8^+$ T-cell responses. Our data suggest that genetic polymorphisms may contribute to immune dysfunction leading to poor viral clearance and increased viremia/inflammation typical of SD.

P034

Mechanistic Insights into Capsid Protein-RNA Interactions in TBEV using Single-Domain Antibodies

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Abstract

Tick-borne encephalitis virus (TBEV), an *Orthoflavivirus* transmitted by *Ixodes* ticks, poses a significant public health threat, causing severe central nervous system infections with 10,000–13,000 annual cases worldwide and no specific antiviral treatments available beyond vaccination. The TBEV capsid protein (TBEV C), a 11 kDa dimer with four α-helices, plays a critical role in nucleocapsid formation by binding viral genomic RNA via its positively charged arginine-rich α4–α4′ region, though precise protein-RNA interaction mechanisms remain incompletely understood.

This project utilizes newly generated single-domain antibodies (sdAbs, also known as nanobodies) derived from camelid naïve phage display libraries. Their specificity to TBEV C were confirmed using ELISA and Western blot. Epitope mapping and characterisation of binding sites of sdAbs and TBEV C were performed using Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS). The blocking activity of selected sdAbs was assessed using electrophoretic mobility shift assays (EMSA).

Three high-affinity anti-TBEV C sdAbs were successfully produced. The blocking activity of sdAbs were tested using EMSA. These findings would elucidate key mechanistic details of TBEV C-RNA binding, highlighting sdAbs as versatile tools for dissecting orthoflaviviral nucleocapsid formation and potential therapeutic inhibitors. By mapping precise interaction sites, this work advances antiviral strategies against TBEV and related *Orthoflaviviruses*, addressing critical gaps in replication mechanisms.

P035

Identifying virus-host interactions for Oropouche virus through genome-wide CRISPR screening

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Abstract

Oropouche fever, caused by the Oropouche arbovirus (OroV), is an emerging threat in Latin America, transmitted by the biting midge *Culicoides paraensis.* Alarmingly, OroV has recently spread beyond endemic regions, caused fatalities and severe pregnancy complications, including miscarriages and microcephaly. Despite this, OroV biology remains poorly characterized, with only a few host dependency factors identified to date and no countermeasures available. We therefore aim to identify novel host factors involved in OroV replication to enhance our fundamental knowledge, understand host adaptation and uncover targetable steps in the viral life cycle.

To achieve this, we will perform two genome-wide CRISPR screens, a strategy not previously applied to OroV. A combinatorial CRISPR-KO screen with the Inzolia library will uncover single and paralogous proviral genes, a pioneering approach, while a CRISPRa screen using the Calabrese library will identify antiviral host factors. Following evaluation of 12 cell lines, Huh7, HepG2, U-87 and the patient-derived CME037 were selected as screening candidates. Efficient transduction (~25%) and near-complete EnCas12 knockout efficiency were confirmed in HepG2 and U-87 cells. Current work focuses on optimizing screening conditions, with the CRISPR-KO screen in U-87 cells planned in the near future.

Subsequent validation, with cross-testing across cell lines and (related) viruses to define shared host dependencies, followed by mechanistic studies, will characterize key host factors in OroV infection. This work will deliver the first systematic dissection of OroV host dependencies, providing a clear framework for other emerging viruses and enabling identification of host-directed antiviral targets to improve future outbreak preparedness.

P036

Arbovirus antibody landscapes in remote riverside communities in the Pantanal of Brazil – the use of multiplex serology

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Abstract

Background
The geographic range of arboviruses is expanding under the influence of climate and environmental change, increasing public health risks in endemic and newly affected regions. The Brazilian wetland Pantanal has experienced significant climatic shifts in recent years, potentially reshaping host, vector and arbovirus transmission dynamics. However, arbovirus circulation patterns in this remote region remain poorly characterized.

Methods
We assessed arbovirus antibodies in sera of riverside Pantanal communities across four expeditions in two regions (2023-2025) using a multiplex protein microarray. IgG and IgM binding-antibodies were detected against flaviviruses (dengue (DENV-1-4), Zika (ZIKV), Saint Louis encephalitis (SLEV), West Nile (WNV), and yellow fever (YFV) virus), alphaviruses (chikungunya (CHIKV), Venezuelan (VEEV), eastern (EEEV) and western equine encephalitis (WEEV), and Mayaro (MAYV) virus), and orthobunyavirus (Oropouche virus (OROV)).

Results
1656 individuals were sampled (South, n=698; North, n=958). Increasing age ($p<0.001$) and residence in semi-urban communities were associated with higher IgG mean titers across multiple flaviviruses, whereas CHIKV IgG titers increased with age but were significantly higher in rural communities of North Pantanal ($p<0.001$). Accounting for antibody cross-reactivity, monotypic signals and/or probable recent specific-infections were observed for all studied viruses with differences across communities, (type of) region and time.

P036 (continued)

Conclusion

The different antibody-landscapes observed between regions may indicate differences in arbovirus transmission cycles (urban, sylvatic, epizootic), and monotypic IgM-signals and probable recent specific-exposures suggest co-circulation of all studied arboviruses in the Pantanal (2023-2025). Together, this highlights the need for sustained region-specific surveillance as well as strengthening preparedness and intervention strategies in the Pantanal.

P037

Investigations into the host-restriction mechanisms of insect-specific alphaviruses.

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Abstract

Alphaviruses (*Togaviridae*) such as Chikungunya virus are a genus of arthropod-borne virus that continue to emerge globally, causing debilitating disease in humans and animals. Despite their burden to public health, diagnostics and vaccines against alphaviruses are limited. A subset of the genus termed “insect-specific alphaviruses” (ISAs) infect only mosquitoes, but the exact mechanisms behind their host-restriction are yet to be fully elucidated.

The Australian ISA, Yada Yada virus (YYV) has been utilised to construct recombinant viruses for application as potential vaccine candidates and diagnostic tools. Chimeric alphaviruses with the replication machinery of YYV and expressing the structural protein genes of various pathogenic alphaviruses have been constructed using the reverse-genetics method, circular polymerase extension reaction (CPER). These chimeric viruses replicate to high titres in mosquito cell culture and retain the insect-specificity of the YYV genetic backbone.

To investigate ISA host-restriction, a panel of chimeric viruses between YYV and the vertebrate-infecting, Sindbis virus (SINV) was produced. Using this range of unique chimeric viruses *in vitro*, we evaluated the roles of physiological conditions and host-cell immune responses. We demonstrated that YYV host-restriction is multifactorial, with inhibition occurring pre- and post-cellular entry. These key data underpin the safety of a recombinant alphavirus platform, using YYV as a backbone, facilitating applications in the vaccines and diagnostics sectors, while also providing important insights into alphavirus evolution.

P038

Antiviral sugar baits to reduce arbovirus transmission: a proof-of-concept study.

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Abstract

Approximately 4 billion people are at risk of *Aedes*-borne arboviral diseases, yet antiviral therapies are lacking. We have previously demonstrated that the NS4B inhibitor JNJ-A07 reduced DENV transmission when ingested by mosquitoes during blood feeding. In contrast, ingestion of the active metabolite of molnupiravir (MPV), β-D-N^4-hydroxycytidine (NHC), did not decrease chikungunya virus (CHIKV) transmission. Here, we explored whether these antiviral compounds can be delivered and have antiviral efficacy through the natural sugar-feeding behavior of mosquitoes using attractive sugar-targeted baits (ASTBs).

First, the effects of antiviral-containing ASTBs on *Ae. aegypti* mosquitoes' fitness traits (longevity and fecundity) were assessed. Mosquito attraction to ASTBs was then evaluated to exclude compound-induced repellency. Finally, female mosquitoes received antiviral-containing sugar meals ad libitum for 2 days before and 7 days after oral infection with CHIKV (MPV, NHC) or DENV-2 (JNJ-A07). Vector competence was assessed by determining infection, dissemination, and transmission rates.

MPV- and NHC-containing ASTBs (100 µM) significantly reduced female mosquito longevity, while fecundity was only reduced in MPV-exposed mosquitoes. Neither MPV nor NHC showed antiviral activity against CHIKV, as infection and dissemination rates were comparable to vehicle controls. In contrast, JNJ-A07-containing ASTBs (20 µM) significantly reduced DENV-2 RNA loads in exposed mosquitoes by 2.9 $\log_{10}$. Although MPV and NHC failed to inhibit CHIKV in this set-up, the efficacy of JNJ-A07 demonstrates that the administration of antiviral compounds via sugar baits could be a promising avenue to target arbovirus transmission.

P039

Deciphering tick-born encephalitis virus (TBEV) RNA–Host Protein Interactions: toward the identification of novel proviral and antiviral functions

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Abstract

Tick-borne arboviruses, particularly tick-borne encephalitis virus (TBEV), represent a growing public health concern in Europe. Indeed, the geographic distribution of such arboviruses is evolving, notably in relation to climate shifts, thereby placing new populations at risk. To successfully replicate and evade the host cell's immune responses, viruses hijack the cellular machinery by interacting with host proteins. While interactions involving viral proteins are well documented, those engaging viral RNAs are far less understood.

To address this knowledge gap, our team recently identified 211 human proteins that interact with TBEV genomic RNA using ChIRP-MS approach, an innovative technique allowing identification of RNA binding proteins *in cellulo*, during viral infection. For a subset of these, we characterized their functional role in viral replication through RNA interference, revealing both pro- and antiviral functions. Among the latter, WDR4, a component of the tRNA methyltransferase complex, is currently under deeper investigation. The antiviral activity of WDR4 was first confirmed in an orthogonal manner; that is, by analysis of TBEV replication in a WDR4 knock-out human cell line generated by CRISPR-Cas9. The antiviral effect of WDR4 also extends to the replication of other orthoflaviviruses and alphaviruses. To understand how WDR4 restricts TBEV replication, our ongoing study aims to elucidate the molecular mechanisms underlying this antiviral effect and to characterize the interaction between WDR4 and TBEV RNA.

These results uncover previously unknown host–viral RNA interactions that are critical for the TBEV life cycle, and may provide leads for the development of broad-spectrum therapeutic strategies against emerging arboviruses.

P040

Chikungunya virus capsid protein interactome

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Abstract

Alphaviruses are spread by mosquitoes in (sub)tropical regions and pose a significant medical and economic burden, highlighting the need to develop novel antiviral drugs. Understanding the interactome of viral proteins in human host and insect vector cells can reveal potential cellular pro- and antiviral factors, which could, in turn, serve as drug targets.

In this study, we explored the interaction partners of the Chikungunya virus (CHIKV) capsid protein (CP), which plays a key role in virus replication, virion assembly, and virus-cell interactions. A quantitative, label-free proteomics analysis was conducted to investigate CP interactors in two different types of cells: human host cells and mosquito vector cells. This approach enabled us to identify common proteins and pathways essential for viral infection in two evolutionarily distant species.

Several host and vector factors were identified, including many homologous human and mosquito proteins. The impact of selected interactors on CHIKV pathogenesis was assessed in cells by first conducting gene silencing, followed by infection with a nanoluciferase-expressing virus. Through this screening, multiple proteins were found to be proviral, demonstrating that CHIKV CP plays a broader role in infection beyond genome packaging.

Future research will involve the expression of capsid proteins from various alphaviruses to investigate their interactomes in cells, with the aim of identifying similarities across different members of this viral genus.

P041

Thermal variation across life stages shapes invasion success and arbovirus transmission in *Aedes koreicus*

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Abstract

Over recent decades, global environmental change has reshaped mosquito distributions, promoting the spread of invasive species across Europe. *Aedes koreicus*, first detected in Belgium in 2008 and in Italy in 2011, is now established across a wide altitudinal range and diverse climatic conditions, including cooler environments. Its expansion is likely linked to enhanced cold tolerance relative to other *Aedes* species, with important implications for seasonality and population dynamics.

Temperature is a key driver of mosquito life-history traits, influencing development, survival, reproduction, and transmission potential. Here, we investigated stage-specific thermal responses of *Ae. koreicus* across five ecologically relevant temperature regimes. We quantified variation in key life-history traits across developmental stages and reconstructed thermal performance curves to assess their contribution to overall fitness.

We further integrated these physiological data with vector competence assays for dengue (DENV) and chikungunya (CHIKV) viruses under both species-specific optimal temperatures and elevated conditions representative of other *Aedes* vectors. Our results reveal significant stage-dependent thermal variation and demonstrate that *Ae. koreicus* can support infection and transmission of both DENV and CHIKV. Notably, temperature effects were virus-specific, leading to differential impacts on infection and transmission dynamics.

Overall, our findings suggest that climate-driven shifts may favour cold-adapted invasive mosquitoes, enabling population growth and extended seasonal activity. This could expand the temporal and ecological window for arbovirus transmission beyond traditional thermal limits. These results highlight the need for revised frameworks to assess vector-borne disease risk under ongoing environmental change.

P042

Development of a One Health Metagenomic Workflow for West Nile Virus Diagnosis

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Abstract

Background. West Nile Virus (WNV) is a mosquito-borne virus whose geographical distribution has rapidly expanded during the last decades. Piedmont, a region in northern Italy, is characterized by extensive rice fields leading to proliferation of WNV vectors. Aim of this project is to develop a metagenomics workflow based on Next Generation Sequencing (NGS) to monitor WNV infections in Piedmont.

Methods. 17 urine and 2 plasma samples were collected from suspected positive patients at the AOU “Maggiore della Carità” (Novara, Piedmont). Samples were concentrated with centrifuge devices and then subjected to viral RNA extraction by using commercial kits. The obtained RNA was used for a first-level screening with Real-Time PCR. Subsequently, cDNA libraries will be prepared by using a target-enrichment approach, followed by NGS and data analysis.

Results. Viral RNA was successfully extracted from all samples, as confirmed by spectrophotometric analysis, with a mean concentration of 11,87±8,7 ng/µl. Real-Time PCR screening identified the presence of WNV RNA in a subset of 11 urine and 1 plasma samples, with Ct values ranging from 18,21 to 37,42. The remaining WNV-negative samples were further considered as negative controls for sequencing analysis. Library preparation for NGS is currently ongoing.

Conclusions. The results achieved with this project will lay the basis for the introduction of NGS analysis in the context of arbovirus surveillance in Piedmont. In the second phase of the project, NGS metagenomics analyses will be included into a multidisciplinary approach that will involve integration of data from entomological and public health surveillance.

P043

Vector competence and vertical transmission of three o'nyong-nyong virus strains in the invasive urban malaria mosquito *Anopheles stephens*i is temperature dependent

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Abstract

O'nyong-nyong virus (ONNV) is an arthritogenic arbovirus responsible for sporadic outbreaks across sub-Saharan Africa. Primarily transmitted by *Anopheles (An.) gambiae* and *An. funestus*, recent studies show that *An. stephensi* is a competent vector. Given its expansion in Africa and tolerance to diverse temperatures, it is important to understand how this affects ONNV vector competence and vertical transmission.

This study investigated the effects of temperature and viral strain on the vector competence of *An. stephensi* for ONNV and vertical transmission. Mosquitoes were fed blood meals containing one of the three ONNV strains, (Ibh10964, SG650 and DakAr234) and maintained at 23.5°C, 28°C, or 32°C.

Vector competence was assessed at 7 and 14 days post-infection using RT-qPCR of thorax and abdomen, and on CPE positive cells infected with mosquito saliva. Vertical transmission was assessed by screening progeny across life stages and testing saliva from F1 females.

All stains were transmitted at 28°C, at both time points with peak transmission at 7 days. At 23.5°C, Ibh10964 and SG650 were transmitted, whereas DakAr234 showed infection without transmission. At 32°C, only Ibh10964 established infection, without transmission.

Vertical transmission occurred for all strains at 28°C, with highest efficiency for Ibh10964. At 23.5°C, vertical transmission was detected only for Ibh10964, and none was observed at 32°C.

Summarising ONNV transmission by *An. stephensi* was influenced by temperature and viral strain, with optimal transmission at 28°C. The capacity for vertical transmission highlights that ONNV can be maintained in the environment.

P044

Training for prevention and control of vector-borne diseases: an interdisciplinary graduate course integrating vector-borne virus biology and outbreak communication

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Abstract

Vector-borne viruses represent a growing global health challenge, requiring effective strategies for prevention, control, and outbreak response. However, structured training in arbovirology remains underrepresented in many virus-focused graduate programs. In particular, opportunities to integrate vector biology, ecological drivers, and public health perspectives within a One Health framework—essential for informed prevention and control strategies—are limited. At the same time, outbreaks highlight the need for scientists who can effectively communicate complex transmission dynamics to diverse audiences.

We developed and implemented an interdisciplinary training module for MD and PhD trainees within the integrated Research Training Group of the Collaborative Research Centre 1648 “Emerging and Re-Emerging Viruses”. The course combines foundational teaching on selected arboviruses, vector biology, and host–vector–environment interactions with an interactive simulation of a hypothetical mosquito-borne disease outbreak. The training explicitly links biological mechanisms to prevention and control measures, including vector control strategies and public health interventions. In a structured role-play press conference, participants assumed the roles of scientists, clinicians, public health officials, journalists, and members of the public, simulating real-world communication during an outbreak.

Nine trainees from diverse backgrounds participated. The simulation facilitated cross-disciplinary understanding of vector-borne disease dynamics and highlighted challenges in communicating evidence-based prevention and control strategies.

This pilot course demonstrates a feasible framework for integrating vector biology, communication, and prevention into graduate training, supporting preparedness for future outbreaks.

P045

Potent broad-spectrum Alphavirus inhibition mediated by MAYV and SFV nsP2 proteins

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Abstract

Alphaviruses are mosquito-borne positive-sense RNA viruses that include significant human pathogens that cause arthritis and encephalitis. Although the global distribution of several alphaviruses has expanded and they continue to pose a growing public health threat, broadly effective antiviral strategies remain limited. One potential strategy for limiting alphavirus transmission is by mimicking superinfection exclusion (SIE), where a primary infection restricts subsequent infection by a homologous or closely related virus. Previous studies suggest that alphavirus nonstructural protein 2 (nsP2), a viral protease required for processing the nonstructural polyprotein and replication complex formation, may be a key determinant of SIE. However, the specificity and mechanistic basis of nsP2-mediated antiviral activity remains poorly understood. Here, we investigate the ability of nsP2 proteins from a range of alphaviruses to mediate broad-spectrum inhibition of related alphaviruses in mosquito cells. Based on the conservation of alphavirus nsP2 cleavage sites, we hypothesised that selected nsP2 proteases may cleave nonstructural polyproteins from heterologous alphaviruses and thereby disrupt viral replication. We transiently expressed a panel of alphavirus nsP2 proteins and evaluated their antiviral activity using a trans-replicase system. This screen identified four candidates, WT MAYV nsP2, WT SFV nsP2, and their corresponding helicase mutants, that broadly inhibit replication of arthritogenic alphaviruses. Viral infection experiments demonstrated that these candidates reduced alphavirus infection rates in mosquito cells. Together, these findings support nsP2 as a functional mediator of alphavirus SIE and identify potential broad-spectrum anti-alphavirus effectors, which could serve as the basis for future transmission-blocking strategies within mosquito vectors.

P046

Single cell analysis of infected human brain organoids revealed preferential tropism of Japanese encephalitis virus (JEV) to immature cell populations and identified cell-specific antiviral responses

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Abstract

Japanese encephalitis virus (JEV) is a neurotropic mosquito-borne *Orthoflavivirus* capable of causing severe encephalitis leading to death of the infected individuals. Despite the substantial burden of the infection, the mechanisms underlying JEV neuropathogenesis remain poorly understood. To date, JEV neural infection is primarily studied in mouse models despite the potential species-specific differences in virus-host interactions. Human brain organoids represent an emerging model of viral infection with a potential to reveal the mechanisms of JEV encephalitis in human neural context.

Herein, we have established a human brain organoid (hBO) model to study JEV infection at single-cell resolution using virus-inclusive scRNA-Seq. 60-days old cortical hBOs were infected with 10^5 FFU of $JEV_{Nakayama}$ and dissociated by papain treatment at 2dpi. scRNA-Seq was performed using 10xGenomics Chromium Fixed RNA platform with custom probes for virus detection. The scRNA-Seq data was analysed using Seurat.

We found that JEV infection in hBOs predominantly targeted neural progenitor cells and immature neurons, with only a small number of astrocytes being infected. Notably, mature neurons were not infected. The analysis of the antiviral signalling demonstrated that only a small number of infected glial cells produced interferon. Infected and uninfected neurons did not produce interferon and developed little to no interferon-stimulated gene expression, indicating a weak response to interferon. In contrast, astrocytes and immature neural cells mounted a profound antiviral response. Together, these findings demonstrate cell-type-specific differences in viral tropism and antiviral response in human neural tissue and establish hBOs as an interferon-competent human model of JEV neuropathogenesis.

P047

Translational repression of viral RNAs supports persistent arbovirus infection in mosquitoes

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Abstract

Arboviruses induce acute lytic infection in human cells but establish persistent infection in their mosquito vectors, a strategy that is essential for sustained viral transmission. How mosquito cells maintain continuous viral production without compromising host viability remains unanswered. As arbovirus replication in human cells relies on remodeling of the host translational machinery, we investigated how translation is regulated during persistent infection in mosquito cells using chikungunya virus (CHIKV) as a model. Temporal analysis of viral translation in RNAi-competent and RNAi-deficient *Aedes albopictus* cells revealed that persistence is associated with repression of viral RNA translation. Further, we showed that, in contrast to human cells, CHIKV infection neither induces nuclear relocalization of the viral protein nsP2 to drive global host mRNA depletion, nor reshapes the tRNA landscape to compensate for suboptimal viral codon usage. These results indicate that persistence is characterized by a balanced host-virus translation, which enables sustained viral production without major disruption to host gene expression. Notably, similar repression of viral RNA translation was observed during Zika virus infection of mosquito cells, and this translation repression was also seen in vivo in CHIKV-infected *Aedes aegypti* mosquitoes, indicating that translational control represents a conserved, RNAi-independent mechanism underlying arbovirus persistence.

P048

Identification and role of skin microRNAs regulated during West Nile virus infection in the course of mosquito transmission

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Abstract

Flaviviruses transmitted through mosquito bites represent a global public health burden, with limited effective interventions available. Early events occurring at the mosquito bite site determine viral transmission and disease progression. While microRNAs influence viral infection, there is a dearth of knowledge regarding the cutaneous miRNA response to mosquito bites. My objective is to characterize the miRNA response following an infectious bite to develop novel interventions.

First, by combining a mosquito-to-mouse transmission model of West Nile virus (WNV) infection with small RNA sequencing, I identified 23 miRNAs regulated at the bite site 6 hours post-bite. These regulations were confirmed with stem-loop RT-qPCR. Second, I used in vitro skin cell models to determine how these miRNAs are regulated by testing the effects of infection and mosquito saliva, separately and in combination, and by deciphering the biogenesis pathways involved. Third, I conducted a mid-throughput functional assay using gain-of-function miRNA mimics in fibroblasts and keratinocytes, revealing the pro-viral function of several miRNAs during flavivirus infection. The function of these miRNAs was further confirmed using loss-of-function assays with anti-miRs.

Overall, we identified a profile of host-derived miRNAs regulated at 6 hours post-WNV infection in mosquito-to-mouse models and validated in vitro. These results suggest that miRNAs contribute to the early antiviral response triggered by the bite of an infected mosquito.

P049

Midgut bacterial microbiota–virus–vector interactions in *Culex pipiens* exposed to two lineages of West Nile virus

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Abstract

Culex pipiens is a major vector of West Nile virus (WNV) in Europe, and larval rearing water is a key source shaping its microbiome. This study assessed how two different water types influence the vector competence of *Cx. pipiens* for two WNV lineages (1 and 2) circulating in Europe and explored virus-mosquito- midgut bacterial microbiota interactions.

Field-collected *Cx. pipiens* larvae were reared in either laboratory or stream water and ten-to-12 days old females were fed with chicken blood doped with WNV (7 $\log_{10}$ $TCID_{50}$/ml). Vector competence was evaluated at 3, 7-, 14-, 21- and 28-days post-exposure (dpe), while midgut bacterial microbiota composition was characterized by 16S rRNA sequencing according to rearing water type and viral exposure/infection status.

Despite differences in bacterial microbiota alpha diversity between water types, *Culex pipiens* showed similar vector competence for WNV lineages 1 and 2 under both rearing conditions. Transmission efficiency from 14 dpe onwards ranged from 2.94% to 30% in WNV-1 and from 2.33% to 13.51% in WNV-2. Gene sequencing of midguts revealed that *Elizabethkingia, Serratia*, *Wolbachia*, *Phytobacter* and *Cutibacterium* were the most abundant bacterial genera in *Cx. pipiens* females. Notably, the midgut bacterial microbiota differed between exposed or infected mosquitoes and the unexposed control group, evidencing virus induced shifts in midgut bacterial composition.

Our results highlight the complex interactions among midgut microbiome, WNV and *Cx. pipiens* vectors. Improved understanding of these interactions may pave the way for better arbovirus transmission control measures.

P050

Highly Multiplex Protein and Antibody Profiling for Differential Diagnosis and Prognostic Biomarker Discovery in Arbovirus Infections

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Abstract

We have explored affinity proteomics for the analysis of the circulating proteome for many years in large scale, with in-house generated antigens and antibodies for bead-based assays, but also recently with very extensive pan-disease protein profiling in blood utilizing Olink Explore HT and Somascan assays (1,2).

During the pandemic, we converted our autoantibody profiling setup to a high-throughput multiplex SARS-CoV-2 serology assay (3) and followed up with analysis of autoantibodies associated to post-COVID (4). We have extended the serology assay to provide a platform for multi-disease serological studies including a wide range of antigens representing various respiratory and infectious diseases. We are now targeting arbovirus infections such as Dengue, Zika, West Nile, Yellow fever, Chikungunya, Oropouche, Mayaro with the analysis of large sample sets from Brazil, Singapore and Uganda, as well as samples from infected travellers coming to Sweden.

The aim is to enable broad and large-scale pan-disease seroprevalence studies with high specificity and sensitivity and with thousands of samples analysed on hundreds of antigens. The combination of high-throughput and highly multiplex profiling of circulating proteins, autoantibodies and virus targeting antibodies has great potential to be utilized within an arboviruses pan-disease set-up with the aim to explore signatures of diagnostic and prognostic values.

(1) Bueno Alvez (2025) Science

(2) Bergström (2026) Nature Communications

(3) Hober (2021) Clinical Translational Immunology

(4) Jernbom (2024) Nature Communications

P051

Not All Dengue Viruses Are Equal: RNAi Responses Differ Across DENV Serotypes

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Abstract

Dengue virus (DENV) and Zika virus (ZIKV) are major arthropod-borne orthoflaviviruses transmitted by *Aedes aegypti* (*Ae.aegypti*) that cause disease in humans. Both viruses can infect the same mosquito. This underscores the importance of understanding how the mosquito's innate antiviral immune response shapes infection and co-infection. Two RNA interference (RNAi) pathways are the primary innate antiviral defence in mosquitoes. This involves the short-interfering RNA (siRNA) pathway, with two key proteins Argonaute 2 (Ago2) and Dicer 2 (Dcr2), as well as the PIWI-interacting RNA (piRNA) pathway. Studies indicate that antiviral RNAi responses to mosquito-borne orthoflaviviruses vary between viruses.

Here, we analyse the mosquito antiviral RNAi response to DENV serotypes and ZIKV, in single and co-infections, to compare across serotypes and reveal the dynamics of RNAi in vector-derived cells.

To investigate the role of the RNAi pathways during single and co-infections with the different DENV serotypes and ZIKV, we used either siRNA pathway-deficient *Ae. aegypti* cell lines (Ago2-deficient and Dcr2-deficient) or dsRNA-mediated silencing of key piRNA pathway proteins.

In the siRNA pathway, loss of Dcr2 leads to enhanced replication of specific DENV serotypes and ZIKV in single infections. Loss of Ago2 also showed a serotype-specific role. In the piRNA pathway, PIWI4 showed antiviral activity against DENV-2 and ZIKV, whereas other piRNA pathway components had pro-viral effects for DENV-1 only.

Taken together, our results show that the antiviral RNAi pathways in mosquitoes display virus- and serotype-specific activities. These findings highlight RNAi as a highly virus-specific and dynamic component of the mosquito innate immune response.

P052

Vertebrate ZAP Targets dsRNA Arboviruses, Providing a basis for Live Attenuated Vaccine Design

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Abstract

Zinc-finger antiviral protein (ZAP) is a vertebrate-specific host restriction factor, providing a compelling basis for vaccine design. While ZAP has been shown to restrict ssRNA arboviruses, such as Zika virus (ZIKV) and Sindbis virus (SINV), in mammalian cells by recognizing CpG-enriched regions, its role in dsRNA arboviruses remains unclear.

Using CRISPR/Cas9-mediated knockout and rescue experiments, we demonstrate that ZAP exerts broad antiviral activity against bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV), both of which are transmitted by *Culicoides* midges and cause severe hemorrhagic diseases in ruminants. Employing BTV as a model, we show that ZAP inhibits viral protein synthesis at the step of translation elongation, as evidenced by dual-luciferase reporter assays combined with surface sensing of translation (SUnSET)-based ribosome speed of elongation (SunRiSE) analysis.

To identify ZAP-recognition sites on the viral genome, we performed CLIP-seq, revealing preferential binding to the negative-sense of viral RNAs, and promoting their degradation. Furthermore, we demonstrate that BTV segment 5, characterized by a low CpG content, encodes non-structual protein 1 (NS1) that antagonizes ZAP by impairing its RNA-binding ability. Notably, synonymous CpG enrichment in segment 5 significantly attenuated viral replication in mammalian cells and *in vivo*, while having minimal impact in insect cells.

Together, these findings uncover a dynamic interplay between ZAP and dsRNA arboviruses, providing a promising strategy for ruminant protection against vector-borne orbiviruses.

P053

Deep mutational scanning of West Nile Virus capsid protein reveals translational and host constraints in mosquitoes.

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Abstract

West Nile virus (WNV) alternates between mosquito vectors and vertebrate hosts, imposing evolutionary trade-offs on viral coding sequences. The capsid (C) gene is especially constrained because it encodes a functional structural protein while preserving embedded RNA elements, including the capsid hairpin and 5′ cyclisation sequence. We used deep mutational scanning (DMS) to define nucleotide and amino acid-level constraints in the $WNV_{NSW2011}$ capsid gene that shape host-specific fitness and translational regulation.

A capsid DMS library was generated by Circular Polymerase Extension Reaction, recovering a P_0 virus population containing 98.9% of all possible amino acid variants. The library was passaged in mammalian and mosquito cells at an MOI of 0.1. Mutations mapping to embedded RNA structural elements were strongly depleted after recovery, consistent with overlapping protein- and RNA-level constraints.

Enrichment analysis revealed host-specific tolerances at the capsid N-terminus. Substitutions at the initiating methionine were tolerated in mammalian cells but strongly depleted in mosquito cells. Recombinant viruses showed that non-methionine mutations promote leaky scanning, producing a truncated capsid isoform from a downstream start site. Western blotting confirmed that mammalian-derived virions incorporated both full-length and truncated capsid proteins, whereas translation was severely reduced in mosquito cells, resulting in marked attenuation. Replication was partially restored in mosquito cells at 37 °C, implicating temperature-dependent effects on translation or local RNA structure. Structural modelling further identified additional host-biased substitutions mapping to the capsid dimer and C-prME interfaces.

Together, these findings show that WNV host range is constrained by coupled translational, RNA-structural and protein-interface bottlenecks within the capsid.

P054

Testing the “out of arachnids” theory of vector-borne orthoflavivirus emergence.

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Abstract

Orthoflaviviruses are positive-sense RNA viruses that include major vector-borne human pathogens. Recent phylogenomics suggest their origins extend beyond 100 million years, with chelicerate-associated viruses branching basal to tick- and mosquito-borne lineages. We tested this “out of arachnids” hypothesis by mining 10,230 Chelicerata transcriptomes from the NCBI Sequence Read Archive, performing *de novo* assembly, viral screening and phylogenetic analysis of divergent orthoflavivirus-like sequences.

We identified seven novel chelicerate-associated orthoflaviviruses from spiders, horseshoe crabs and ticks. Phylogenetic analyses resolved these viruses within distinct lineages basal to recognised tick-borne orthoflaviviruses, supporting a model in which vector-borne orthoflavivirus evolution was shaped by transitions through aquatic chelicerates, terrestrial arachnids and blood-feeding acarids. Xinyang flavivirus (XiFV) occupied a basal position relative to recognised vector-borne orthoflaviviruses, while the remaining chelicerate-associated viruses fell outside recognised vector-borne lineages but were more closely related to them than to classical insect-specific orthoflaviviruses. Comparative genomic analysis revealed contrasting host-associated signatures: XiFV resembled classical insect-specific orthoflaviviruses in CpG and UpA dinucleotide composition and lacked a canonical premembrane furin cleavage site, consistent with altered prM processing and immature virion enrichment. In contrast, basal arachnid-associated orthoflaviviruses displayed CpG and UpA profiles closer to vertebrate-infecting tick/mosquito orthoflaviviruses. For functional studies, recombinant viruses were generated using predicted chelicerate orthoflavivirus prM/E regions in the Binjari virus platform, with XiFV-BinJV validating the predicted immature-virion phenotype.

Together, these findings support an “out of arachnids” origin for vector-borne orthoflaviviruses. They place medically important lineages within a deep chelicerate context shaped by ancient host associations, blood-feeding transitions, and structural adaptations.

P055

Structural basis for innate immune suppression by Rift Valley fever phlebovirus

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Abstract

Rift Valley fever phlebovirus (RVFV) non-structural small protein (NSs) oligomerises into filaments within the nuclei of infected cells. These filaments associate with FBXO3 to form a transient ubiquitin E3 ligase which targets TFIIH p62 for proteasomal degradation, leading to downregulation of host gene transcription, including that of proteins which lead to a type I IFN response. Despite this central role of NSs in dampening host immune responses, structural characterisation of RVFV NSs remains limited. Thus, we aim to create a system by which to view these NSs filaments *in situ* using cryo-electron tomography (cryo-ET). We optimised the transfection of A549 lung epithelial cells with a pCMV vector encoding the NSs gene with a C-terminal tetracysteine (Cys_4) tag, which, unlike larger tags, did not affect filament formation. The Cys_4 tag allows for staining with FlAsH dye to enable confocal microscopy. FlAsH staining at 36 hours post transfection revealed long NSs filaments, in the absence of any other viral gene products. This transfection was repeated onto gold grids and correlative light and electron microscopy (CLEM) was used to identify filaments. This method is repeatable and will allow us to use focused ion beam (FIB) milling of areas on grids where filaments are present, in preparation for cryo-ET imaging. An *in situ* structural study will reveal how NSs interacts with nuclear proteins in its native biological context, thus aiding further biochemical studies into what drives these interactions.

P056

Molecular characterisation of two 2023/2024 reassortants of Oropouche virus

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Abstract

Oropouche virus (OROV) is an *Orthobunyavirus* transmitted primarily by *Culicoides* biting midges in Central and South America. During 2023–2024, OROV incidence markedly increased across the Americas, with the first fatal infections reported. The molecular basis for this apparent rise in pathogenicity and transmission remains unclear.

Here, we investigated the molecular and vector-associated determinants of OROV infection by comparing a historical strain (BeAn19991) with two reassortant isolates (BC89/AM0088 and BC90/AM0059) associated with recent outbreaks. The interferon antagonistic activity of each strain's non-structural protein (NSs) was assessed using transient reporter assays in mammalian cells, showing strong suppression of the RIG-I pathway, no suppression of the JAK/STAT pathway, and inhibition of host cell protein synthesis. Ongoing work aims to further explore the interactions between NSs and the host immune response.

To examine replication dynamics in cells derived from putative midge vectors, *Culicoides sonorensis*(American) and *Culicoides nubeculosus* (European) cell lines were inoculated with each OROV strain, showing a higher level of replication in the American midge cell line KC, compared to the European midge cell line CNE/LULS44. Ongoing optimisation of transfection protocols aims to enhance expression studies in midge cells and small RNA sequencing aims to explore the antiviral RNA interference response to OROV in midge cell lines.

Together, these studies will provide new insights into the molecular and cellular features underpinning OROV immune evasion and vector adaptation. Our findings lay the groundwork for defining how reassortment and host–vector interactions may influence OROV emergence, transmission potential, and pathogenicity.

P057

ANALYSIS OF THE *IXODES RICINUS* TICK VIROME IN SLOVENIA

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Abstract

Slovenia has been recognised as an endemic area for tick-borne encephalitis (TBE) for over 70 years, with one of the highest incidences of TBE in Europe. The country is also endemic for Lyme borreliosis and anaplasmosis, and *Babesia sp*. parasites and *Francisella tularensis* are occasionally detected. Several studies in recent years have indicated that *I. ricinus* ticks harbour previously undiscovered and potentially pathogenic viruses in Europe.

This study aimed to characterise the RNA virome of I. ricinus in Slovenia across different developmental stages (larvae, nymphs, adults) and geographical locations. We collected 1,056 ticks using the flagging method in seven statistical regions. Collected ticks were homogenised and pooled. RNA was extracted and transcribed into double-stranded cDNA using the SISPA method. Sequencing libraries were analysed through a two-stage bioinformatic pipeline involving read classification and de novo contig assembly.

The most abundant viral families identified were Nairoviridae, Phenuiviridae, and Partitiviridae. Recovered RdRP sequences from the families *Nairoviridae* and *Phenuiviridae* were further analysed. Analysis shoved low diversity of nairoviruses, with similarity to sequences of the *Norwavirus* genus detected in ticks in Central and Northern Europe and Russia. For the *Phenuiviridae* family analysis showed that most sequences were similar to the *Ixovirus* genus, though Uukuvirus and unclassified phleboviruses were also detected. Combined bioinformatic analysis confirmed the presence of tick-borne encephalitis virus, Uukuniemi virus, Pustyn virus, Norway nairovirus 1, and Grotenhout virus.

These findings emphasise the need for continued surveillance of tick-associated viruses to improve our understanding of their diversity, distribution, and potential public health relevance.

P058

High-throughput screening reveals cell-type-dependent antiviral activity and dispensable p38 signalling during Chikungunya virus infection

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Abstract

Chikungunya virus (CHIKV) continues to cause large outbreaks with substantial morbidity, yet no approved antiviral therapies are available. Here, we describe development of robust sub-genomic replicon and infectious clone live-cell, fluorescence-based screening platforms for CHIKV. These platforms enabled identification of small molecule inhibitors in both immortalised and primary cell cultures for systematic interrogation of CHIKV replication kinetics and antiviral activities.

During assay validation, we evaluated the well-characterised antiviral ribavirin. Surprisingly, we observed striking cell-type-dependent effect. Ribavirin potently inhibited CHIKV replication in immortalised cells but failed to suppress viral replication in primary cells, highlighting important differences in antiviral responsiveness and underscoring the limitations of screening exclusively in transformed systems. These findings emphasise the necessity of incorporating primary cells into antiviral discovery pipelines for arboviruses.

In parallel, we investigated opposing results for two different inhibitors targeting p38 MAPK signalling. CHIKV infection induced robust phosphorylation of p38, confirmed by western blot, consistent with activation of stress-responsive pathways. While SB202190 restricted viral replication, SB203580 had no antiviral effect despite inhibiting p38 signalling, suggesting potential off target effects. RNAi-mediated knockdown of p38 failed to impair infection, confirming that p38 MAPK activation was a downstream consequence of infection rather than a functional requirement for CHIKV replication.

Together, this study establishes a versatile screening framework for CHIKV and reveals that both antiviral efficacy and host-pathway relevance can differ profoundly between cell types. These data argue for greater use of primary cells in arbovirus research and refine our understanding of host signalling pathways activated during CHIKV infection.

P059

Taking the tube: tracking Chikungunya virus nsP3 puncta in cell-to-cell transit

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Abstract

Chikungunya virus (CHIKV) can spread between cells through actin-based intercellular nanotubes, a process proposed to facilitate immune evasion and persistence. Previous studies have shown that viral structural proteins are required for productive cell-to-cell transmission; however, the spatial organisation of viral replication complexes, and their relationship to structural protein function remain incompletely defined. Here, we extend existing models by directly visualising CHIKV replication structures during cell-to-cell spread in physiologically relevant primary human cells.

To enable live-cell imaging, CHIKV nsP3 was tagged with mNeonGreen, allowing visualisation of nsP3-positive puncta associated with viral replication complexes. In dermal fibroblasts and skeletal muscle myoblasts, puncta were observed trafficking within intercellular nanotubes connecting infected and neighbouring uninfected cells. Using dual-fluorescent sub-genomic replicons expressing nsP3-mNeonGreen together with ORF2-mScarlet-I3, we show that although nsP3 puncta efficiently localised to nanotubes, productive cell-to-cell transmission required the presence of viral structural proteins, consistent with prior reports.

Mechanistic refinement revealed a specific role for capsid function in this process. Insertion of mScarlet-I3 at the N-terminus of capsid abolished cell-to-cell spread, indicating a requirement for intact capsid-RNA interactions or capsid assembly rather than structural protein expression alone. In addition, host RNA-binding proteins, including G3BP1, co-localised with nsP3 puncta within dsRNA spherule-coated nanotubes, consistent with their established interaction with nsP3 and a scaffolding role in replication complex organisation.

Together, these findings provide direct visual evidence that integrates replication complex dynamics with established structural protein requirements, advancing mechanistic understanding of CHIKV cell-to-cell transmission via nanotubes in primary human target cells.

P060

Generation of TBEV Replicon-Bearing Stable Cell Lines

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Abstract

Flaviviruses are re-emerging arboviruses that pose a significant global health threat, yet effective antiviral therapies remain unavailable. Research and drug development are further complicated by the requirement to handle most flaviviruses under biosafety level 3 (BSL3) conditions. Subgenomic replicons offer a safe and efficient alternative for studying flaviviral RNA replication and screening antiviral compounds under lower biosafety settings.

This study aimed to generate stable cell lines bearing flaviviral replicons that can be maintained under BSL2 conditions. Replicons derived from Tick-borne encephalitis virus (TBEV) were engineered to encode fluorescent or luminescent reporters (mCherry, oxGFP, NanoLuc) with in-frame encoded puromycin N-acetyltransferase (PAC) selection marker. BHK-21 cells were transfected with *in vitro* transcribed replicon RNAs and subjected to puromycin selection to establish stable cell lines.

The resulting cell lines demonstrated persistent expression of TBEV non-structural proteins (confirmed by western blot analysis), reporter signals (fluorescent- mCherry, oxGFP/luminescent- NanoLuc), and puromycin resistance, confirming ongoing RNA replication of the reporter-replicons.

These replicon-based stable cell lines provide robust tools for studying overall flaviviral replication and enable high-throughput screening of antiviral inhibitors in BSL2 settings, facilitating the development of novel therapeutic strategies.

P061

Formulation of reference reagents for nucleic acid amplification test assays against high consequence pathogens

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Abstract

Nucleic Acid Amplification Testing (NAAT) is a highly sensitive molecular diagnostic method that detects the genetic material of pathogens, enabling rapid and accurate identification of causative agents. Since the isolation of Toscana virus (TOSV) in central Italy in 1971 and Usutu virus (USUV) in Austria in 2001, both arboviruses have demonstrated notable geographic expansion across Europe. Despite belonging to distinct viral families, the two viruses share several epidemiologically meaningful similarities; both exhibit marked seasonality driven by the activity of their respective vectors, both possess neuroinvasive potential and both remain substantially under-diagnosed, given their reliance on molecular methods with limited understanding of disease pathogenesis.

New molecular diagnostic assays have been developed resulting in a corollary requirement for TOSV and USUV reference materials and, at the MHRA Science Campus, we have produced inactivated NAAT reference material for these two arboviruses. Virus stocks were propagated in-house and inactivated by heat for the preparation of reference materials. Heat inactivation was validated for both pathogens by an absence of CPE upon incubation with permissive cells and an increase in virus-specific nucleic acid after 3 passages. The inactivated material was diluted and its performance in NAAT such as RT-qPCR was evaluated. The global availability of such reference materials will facilitate standardisation of molecular diagnostic assays for the two pathogens between and within laboratories.

P062

Characterisation of the 2024 epidemic Oropouche virus *in vitro* and assessment of vector competence in *Culex quinquefasciatus* and *Aedes aegypti*

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Abstract

Oropouche virus (OROV) is a segmented negative-sense RNA *Orthobunyavirus* that causes Oropouche fever in South and Central America. The 2024 epidemic, characterised by expansion beyond the Amazon basin, increasing imported cases in North America and Europe, and reports of severe disease, vertical transmission and congenital abnormalities, has heightened concerns over its epidemic potential.

We developed reverse genetics systems for two OROV strains (BC89 and BC90) isolated from Brazilian patients during the 2024 epidemic, enabling comparative analyses with the historic Brazilian BeAn19991 isolate (1955). Vector competence of *Culex quinquefasciatus* and *Aedes aegypti* was assessed following oral infection with BeAn19991 or BC90 by evaluating viral infection, dissemination and tissue tropism.

Comparative *in vitro* analyses revealed no major differences in replication kinetics or viral fitness between the historic isolate and the recent human-derived strains. Vector competence studies demonstrated low levels of midgut infection in *Cx. quinquefasciatus*, limiting dissemination of OROV BeAn19991. In contrast, *Ae. aegypti* exhibited higher midgut infection rates, with viral titres increasing between 6 and 12 days post-infection, indicating active replication within the midgut. OROV BeAn19991 also disseminated to peripheral tissues, including the head and salivary glands, demonstrating transmission potential, albeit at low levels. Consistent with the *in vitro* findings, the recent epidemic strains did not exhibit enhanced replication or dissemination compared with the historic strain following oral infection of mosquitoes.

These findings establish reverse genetics platforms for contemporary human OROV isolates and demonstrate that *Ae. aegypti* possesses limited vector competence for OROV transmission, whereas *Cx. quinquefasciatus* appears largely refractory.

P063

Mouse-to-mosquito transmission challenges differential transmissibility between Zika virus lineages

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Abstract

Zika virus (ZIKV) is a mosquito-borne pathogen that caused significant outbreaks associated with birth defects and neurological disorders. Phylogenetically, ZIKV is classified into African and Asian lineages, with the African lineage exhibiting higher transmissibility in experimental mosquito infections using artificial blood meals. However, only sporadic human cases have been associated with the African lineage. In contrast, the less transmissible Asian lineage has been responsible for large-scale outbreaks. This discrepancy hints at an inadequate ability to assess ZIKV transmission risk through traditional laboratory tests. To address this, we developed a mouse-to-mosquito transmission assay that mimics natural transmission conditions. Mosquitoes were periodically fed on mice previously inoculated subcutaneously with either an African or an Asian ZIKV strain. Following a 14-day incubation, mosquito saliva was collected to detect infectious viruses. African strain-infected mice exhibited approximately 1000 times higher plasma viral loads than Asian counterpart. Despite this difference, there was no detectable difference in the prevalence of infectious saliva and the period during which the virus is detected from mosquito saliva. Although the prevalence of infectious saliva increased in a dose-dependent manner following artificial infectious blood meals, there was no strong positive correlation between plasma viral load and the prevalence of infectious saliva during mouse-to-mosquito transmission. This suggests the potential influence of host-derived factors on ZIKV transmission dynamics and underscores the importance of considering these variables in risk assessments. We are conducting further investigations to elucidate how Asian strains achieve high transmissibility from hosts with low viremia.

P064

Umbre Virus Detection and Dissemination in Birds of Prey: Implications for One Health Surveillance of Arboviruses

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Abstract

Background:

Arboviruses circulate in ecological cycles involving vertebrate hosts and mosquito vectors, and wildlife species may serve as important sentinels for detecting viral circulation. Birds of prey are apex predators that can accumulate pathogens and have been shown to be infected with West Nile and Usutu virus. Here, we pilot metagenomics in birds of prey as a method for early detection of emerging zoonotic viruses.

Methods:

Metagenomic sequencing was performed on brain tissue from 23 owls with undetermined causes of death. Viral nucleic acids were enriched using a capture-based approach and sequenced on Illumina platform. Following identification of Umbre virus, a qPCR assay was developed to screen additional archived samples. Viral genomes were reconstructed using metagenomics, and ISH was performed to examine viral localization.

Results:

Metagenomic analysis detected Umbre virus, an Orthobunyavirus first isolated from Culex mosquitoes, in the brain of a Northern Hawk Owl that died at Blijdorp Zoo, Rotterdam in 2006. Umbre virus has previously been associated with encephalitis in three immunocompromised human patients. Histopathology of the owl tissue was consistent with encephalitis. Subsequent qPCR screening identified in total four Northern Hawk Owls from the same cage infected with Umbre virus. Genome reconstruction demonstrated 97-98% nucleotide similarity compared with human infected sequences. ISH results showed viral RNA localized predominantly around blood vessels and immune cells.

Conclusion:

These findings expand the geographical and known host range of Umbre virus. Wildlife surveillance combined with metagenomic approaches can provide a powerful tool for understanding arbovirus ecology and identifying potential transmission events.

P065

Discovering novel molecular determinants of arbovirus infection in the mosquito host

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Abstract

Mosquito-borne RNA viruses include major human pathogens such as Dengue, Zika, West Nile and Japanese encephalitis viruses. The successful transmission of these viruses by mosquitoes depends on their ability to infect and replicate within the mosquito midgut which in turn is determined by the availability of essential host factors in the midgut epithelial cells as well as the ability of the virus to evade host stress and antiviral responses. However, the host factors that facilitate or restrict viral replication in mosquito cells remain poorly understood. To address this, we employed an RNA-centric proteomics-based approach to identify mosquito proteins associated with Dengue virus (DENV) RNA in infected mosquito embryonic Aag2 cells. We identified approximately 25 DENV RNA-binding proteins, several of which overlapped with interactors previously identified in human cells, highlighting conserved features of the viral life cycle across species. Among these, AeSAFB, AeYBX1, AeDDX5, and AeRBM4 exhibited strong pro-viral effects and interacted with both viral RNA and viral proteins. Knockdown of these proteins significantly reduced viral RNA and protein abundances, as well as viral titers, across multiple DENV-2 strains. Subcellular fractionation experiments revealed that many of these proteins are enriched at endoplasmic reticulum (ER) membranes, the primary sites for DENV replication, suggesting roles in viral replication complex formation or function. Proteomic profiling of ER membranes also revealed enrichment of ubiquitin–proteasome pathway components and metabolic enzymes involved in alleviating ER stress and promoting cell survival, suggesting that infection-induced ER remodeling contributes to viral replication and persistence within the mosquito host.

P066

Mutations in the nsP1 Dodecamerization Surface Impair Semliki Forest Virus RNA Synthesis in Mosquito Cells

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Abstract

Positive-sense RNA (+ssRNA) viruses remodel host membranes to form replication organelles (ROs), a process largely driven by the oligomerization of virus-encoded membrane-binding proteins. In the case of Togaviruses, non-structural protein 1 (nsP1) assembles into dodecameric rings that serve as scaffolds bridging viral replication spherules with the host plasma membrane. However, the precise role and necessity of nsP1 oligomerization in viral replication remain incompletely understood. In this study, we employed structure-based computational modeling to design nsP1 mutants predicted to disrupt monomer-monomer interactions within the dodecamer. Molecular dynamics (MD) simulations further indicated that these mutations may impair the membrane-binding affinity of nsP1. Western blotting was performed under denatured reducing conditions and confocal microscopy was used to assess the expression levels and subcellular localization of mutant nsP1. They also appear mutant proteins were not to be stably retained at the plasma membrane, instead being mislocalised to endosomal compartments. Three-component trans-replicase assays and viral challenge experiments revealed that nsP1 dodecamerization-surface mutants are deficient in their ability to support viral replication. This study highlights the critical role of nsP1 dodecamerization in Togavirus replication and proposes a potential antiviral strategy targeting oligomeric protein structures in +RNA viruses.

P067

Efficient Replication and Broad Mosquito Tropism of Alphamesonivirus-1 (AMV1) in Aedes and Culex-derived Cell Lines

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Abstract

Insect-specific viruses (ISVs) are gaining attention for their role in the mosquito microbiome and their potential to modulate mosquito-borne viruses. *Alphamesonivirus-1* (AMV1), a member of the *Mesoniviridae* family, has been identified in several mosquito species. However, a detailed comparative analysis of its replication efficiency across different mosquito genera remains essential for evaluating its ecological impact and potential for superinfection exclusion (SIE).

In this study, we measured the replication kinetics of AMV1 in cells derived from *Aedes albopictus* (C6/36) and *Culex pipiens* (CPELULS56 & CPELULS50), both of which are AMV1-free. Cells were infected with various MOI's (0.1, 1 and 5) and assessed using RT-qPCR.

Our results showed that AMV1 replicates efficiently in different cell lines. In C6/36 cells, viral load increased rapidly at high MOIs. However, when using lower MOIs of 0.1 and 1, replication initiation was slower. In contrast, for CPELULS56 and CPELULS50, the viral load increased fast within 24 hours and reached a high plateau at about 10^9 genome copies/µL. Interestingly, the final viral load remained consistent across different MOIs. Baseline screening confirmed the absence of endogenous AMV1 in the cell lines, validating the observed kinetics resulting from a clean infection without background interference.

These findings demonstrate that AMV1 can reach high viral RNA levels in both *Aedes* and *Culex* cell lines. This virus is therefore an ideal candidate for further studies on SIE. In conclusion, this work provides a baseline for our future experiments on how AMV1 interferes with pathogenic viruses like West Nile Virus.

P068

MOZART: Model for Arbovirus Infection of the Skin

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Abstract

Mosquito-borne viruses such as Rift Valley fever virus (RVFV) and Chikungunya virus (CHIKV) cause significant global morbidity and mortality. Transmission via mosquito bite exacerbates disease severity, likely through immunomodulatory components of saliva. To facilitate mechanistic studies under physiologically relevant conditions and reduce dependence on animal models, we established a human skin explant system for mosquito-mediated arbovirus infection.

In the pilot “MOZART” project, we optimized *ex vivo* human skin explant cultures and inoculated them either by *Aedes aegypti* probing or by microinjection. Quantitative RT-PCR analysis revealed that RVFV RNA copy numbers remained stable over a 7-day period, whereas CHIKV exhibited a modest but consistent increase. Immunohistochemistry of explants, together with infection assays in primary human dermal fibroblasts and keratinocytes, confirmed that both viruses productively infect skin cells.

Building on these results, we have developed a standardized protocol for presenting *ex vivo* skin explants to infected mosquitoes. This platform enables precise dissection of early cutaneous host responses to natural arbovirus transmission and supports ethically responsible research into vector–host–pathogen interactions.

P069

Understanding the urban microclimate-ecosystem nexus to enable holistic climate adaptation in a changing climate (UMEX-HOPE):Implications of landscape structure diversity and climate change in urban-rural microclimates on vector-borne diseases

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Abstract

Urban environments are increasingly vulnerable to the impacts of climate change, with rising temperatures, declining air quality, and the expansion of habitats for vector-borne disease agents exacerbating public health risks. The UMEX-HOPE project, embedded within the Climate Future Labs initiative at the Lower Saxony Centre for Climate Research (ZKfN), adopts a One Health approach to investigate the complex interactions between urban microclimates, ecological systems, and human health. By focusing on the Hanover region and the rural Wedemark area in Lower Saxony, Germany, our sub-project within UMEX-HOPE examines how climate change and land-use transformations influence the distribution and transmission dynamics of mosquito-borne diseases. It combines spatial modeling of land cover, habitat fragmentation, and landscape connectivity with field-based surveillance to map potential breeding sites for native and invasive mosquito species. Species identification and population genetic analyses will reveal local biodiversity patterns and dispersal pathways, while controlled laboratory experiments will assess how temperature, humidity, and resource availability affect mosquito development and their capacity to transmit pathogens such as West Nile and Chikungunya viruses. Indoor sampling will further quantify human exposure risks at the household level. Integrating ecological, spatial, and epidemiological data, UMEX-HOPE will identify high-risk areas and inform adaptive public health strategies. A freely accessible, web-based dashboard will provide real-time risk assessments, supporting urban planners, public authorities, and citizens in making climate-resilient decisions. Ultimately, the project aims to strengthen urban resilience by advancing understanding of how urban planning, nature, and climate collectively shape human and ecosystem health in a changing world.

P070

Stage-resolved metabolomics reveals the methionine cycle as a key regulator of *Aedes aegypti* development and dengue virus susceptibility

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Abstract

Aedes aegypti mosquitoes are major vectors of arboviruses like dengue and Zika, posing significant global health challenges. Their life cycle includes aquatic egg, larval, and pupal stages, followed by a terrestrial adult phase, with larval conditions critically influencing adult traits. Environmental factors during this stage, such as nutrient availability, population density, and microbial exposure, affect adult size, reproduction, and vector competence, the mosquitoes' ability to acquire, support, and transmit pathogens. While the impact of environmental factors on the larval stage is well-studied, the understanding of the molecular, metabolic, or physiological processes key to this life stage remains incomplete.

We developed comprehensive metabolomic profiles across developmental stages and analyzed larval diet composition to assess how nutrition contributes to development. The larval diet provided abundant essential nutrients, including B vitamins critical for growth and metamorphosis. Stage-specific metabolic signatures were evident, with notable enrichment of S-adenosylmethionine (SAM) during larval development. Since SAM is the primary methyl donor for DNA and histone methylation, its accumulation indicates coordination between metabolic and epigenetic regulation.

Functional disruption of the methionine cycle in mosquito cells showed pathway robustness and regulatory cross-talk, but also revealed a vulnerability: silencing *adenosylhomocysteinase (ahcy)*, which hydrolyzes the by-product of SAM-dependent methylation, significantly increased dengue virus replication. Conversely, suppression of *SAM synthase (sams)*, which produces SAM from methionine, did not affect viral replication.

Overall, these findings identify the methionine cycle as a metabolic–epigenetic hub linking nutrition, development, and viral susceptibility, emphasizing the larval stage as a promising target for mosquito control strategies.

P071

Proximity interactomes of alphavirus replication proteins include novel pro-viral host factors

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Abstract

Mosquito-borne alphaviruses cause recurring epidemics. Alphavirus non-structural proteins nsP1-4 form membrane invaginations (spherules), acting as viral replication factories. We aim to identify the host factors involved in alphavirus replication. To characterize the micro-environment of replication factories, we generated a recombinant Semliki Forest virus wherein nsP3 and nsP2 were separately fused with a promiscuous biotin ligase that was enzymatically active during virus replication. At an early timepoint post-infection, biotinylated proteins were identified with mass spectrometry.

We found known nsP interactors, validating our approach. However, most of the identified proteins were novel in the context of alphavirus infection. siRNA screen confirmed host proteins that have a pro-viral role, which included chaperones, membrane trafficking proteins and cytoskeleton proteins. The novel proviral proteins included USP10, AHNAK, eIF4G1, SH3GL1, XAB2 and ANKRD17. The nsP2 interactome additionally contained multiple RNA binding proteins that were proviral. We established a link between the eukaryotic translation initiation complex and virus replication. Both the siRNA as well as the small molecule 4E1RCat directed against the canonical translation initiation factor eIF4G strongly decreased SFV replication [1]. Thus, this compound has potential to be a new broadly acting anti-viral candidate. Currently, we are establishing the roles of the other identified pro-viral proteins in the replication of alphaviruses. Identifying the key components in the life cycle of viruses will play an important role in the development of host-targeted antivirals; broadly applicable targets and pathways are likely to exist.

[1] Thiruvaiyaru et al (2025) Plos Pathog 21, e1013050

P072

Understanding the regulation of stress granule formation during bunyavirus infection

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Abstract

A key conundrum is how cells adapt to rapidly activate innate immune pathways upon sensing viruses, while limiting their activation under homeostasis. The formation of biomolecular condensates, such as stress granules (SGs) allows the cell to tightly regulate RNA metabolism and cellular signalling in response to stress, including viral RNAs. During infection in mammalian cells, different SGs or SG-like foci can assemble and are proposed to regulate antiviral signalling pathways. Some viruses also hijack phase separation to promote replication and thus condensates can play antiviral roles or promote replication. Understanding if, when and why condensates assemble during infection can reveal new fundamental layer of virus-host interactions.

Here we investigated the interplay between SGs and host responses during Bunyawera virus (BUNV) and Oropouche virus (OROV) infection in several cell models. Our results demonstrate that both BUNV and OROV activate eIF2a signalling and the associated host protein synthesis shut-down, triggering the assembly of SG-like foci during infection. These foci share characteristics of previously described RNAse-L bodies, with the non-structural protein, NSs playing a role in this process. Importantly, impairing the assembly of BUNV-induced foci reduced cell death, suggesting they contribute to cytotoxicity during infection. Furthermore, we show that OROV infection actively limits SG assembly during infection in placental and neuroblastoma cell lines, with preliminary evidence suggesting non-structural proteins contribute to this process. Overall, this reflects a complex interplay between viral sensing, condensate assembly, and the regulation of cellular functions, fine-tuned in a virus- and cell-specific manner, and defines novel infection biology rules.

P073

Characterisation of novel *Wolbachia*-transinfected *Aedes albopictus* lines for vector control in endemic and invasive contexts.

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Abstract

Wolbachia-infected *Aedes* mosquitoes are being increasingly used as a sustainable and environmentally friendly vector control intervention. Initially used in *Aedes aegypti*, the primary vector of dengue virus (DENV), *Wolbachia* can be used to suppress vector populations through the Incompatible Insect Technique – IIT, or the population can be replaced with one refractory to virus transmission through the *Wolbachia* Replacement Technique – WRT. Both methods utilise cytoplasmic incompatibility (CI), a reproductive manipulation that gives the *Wolbachia*-infected population a relative reproductive advantage. *Aedes albopictus* is an important vector of DENV in tropical and subtropical regions. In areas co-endemic for both *Ae. aegypti* and *Ae. albopictus*, the targeting of *Ae. aegypti* alone will leave the possibility for DENV transmission cycles to be maintained by *Ae. albopictus*. In addition, *Ae. albopictus* is invasive in temperate regions, such as Europe, and is rapidly spreading because of climate change. There are significant annual DENV and Chikungunya virus outbreaks across Europe, from imported cases circulating in local *Ae. albopictus* populations. Furthermore, incursions of invasive *Ae. albopictus* into the south of England presents the opportunity for the use of IIT to suppress populations before they become established. We report the generation of two novel stable *Wolbachia* transinfections in *Ae. albopictus*. Data to be presented include the maternal transmission of *Wolbachia* to offspring, the extent of CI induction, stability under high temperature rearing conditions, and the degree of Zika virus inhibition. Localisation of both *Wolbachia* strains in different germline and somatic tissues of *Ae. albopictus* will also be presented.

P074

Identifying avian hosts of West Nile and Usutu viruses in African and European transmission systems through systematic review and meta-analysis

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Abstract

West Nile virus (WNV) and Usutu virus (USUV) are emerging mosquito-borne viruses maintained in bird-mosquito transmission cycles, yet the avian host range remains incompletely characterized. We conducted a systematic review and meta-analysis to identify frequently infected bird species and associated mortality in African-European flyways.

Following PRISMA guidelines, we retrieved studies reporting WNV and USUV infections in free-ranging birds within Africa and Europe. We extracted serological and molecular testing results of live and dead birds to understand exposure and virus-associated mortality. To understand infection prevalences we fit phylogenetic multivariate generalized linear mixed models, using MCMCglmm, to species with >16 tested individuals. We separately analyzed results of serology and dead bird testing and estimated phylogenetic signal (Pagel's λ) to assess whether species-relatedness explains infection patterns.

We reviewed 186 studies conducted in Europe and 19 in Africa, WNV-data covered 25 orders, 74 families and 375 species (203 positive at least once). USUV-data covered 25 orders, 66 families and 272 species (104 positive at least once). WNV antibodies were elevated above average in *Accipitridae* and lowered in *Acrocephalidae*, no species had significantly higher mortality.*Turdus merula* and *Columba palumbus* showed significantly elevated USUV antibodies and mortality. *Cyanistes caeruleus, Strix aluxo, Asio otus,* and multiple *Turdidae* exhibited significantly increased mortality. *Falco tinnunuclus* had significantly fewer antibodies. Overall, phylogenetic signals were low, indicating that species-relatedness does not explain infection and mortality.

We present potential WNV and USUV reservoirs. Additionally, we describe that species-relatedness does not explain infection but hypothesize that instead behavior might be the driver.

P075

Characterising the antigenic cross-reactivity of arboviruses to aid vaccine assessment and development, and serosurveillance.

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Abstract

A central challenge in arbovirology is understanding cross-reactivity among viral species, which shapes vaccine design and complicates serological studies. Despite the need for effective vaccines and robust serosurveillance, epitope-level determinants of cross-reactive antibody recognition remain poorly defined.

To address this, we developed pseudotyped viruses (PV) and single-round infectious particles/replicons (SRIP) to study antibody cross-reactivity of West Nile virus (WNV), chikungunya virus (CHIKV), tick-borne encephalitis virus (TBEV) and usutu virus (USUV). We generated a panel of CHIK PV, covering 4 lineages: Asian Urban (AU), East Central South African (ECSA), Indian Ocean (IOL), and West African (WA), achieving titres ranging from $9.0x10^3$ - $3.9x10^6$ $TCID_{50}$/ml and $4.3x10^7$ - $5.8x10^8$ average RLU/ml. Similarly, we produced SRIP for lineages 1a (NY99 strain) of WNV with $9.0x10^3$ – $7.7x10^5$ $TCID_{50}$/ml and 2.5×10^7 - 2.0×10^8 average RLU/ml; additional lineages are in development.

Preliminary pseudovirus neutralisation assays demonstrated differential reactivity among CHIKV lineages. The Anti-Chikungunya Virus Immunoglobulin G International Standard neutralised ECSA PV and S27 prototype strains with reciprocal IC_{50} titres of 41,771 and 112,613, while cross-neutralisation of the WA and IOL lineages was observed with titres of 47,324 and 44,529, respectively. In contrast, the AU lineage exhibited reduced neutralisation sensitivity (reciprocal IC_{50} = 15,130). These early findings suggest lineage-dependent differences in epitope cross-reactivity.

This data will be considered alongside viral sequence and structural information to identify conserved regions targeted by broadly reactive antibody responses. By elucidating cross-reactive epitopes, this work will refine approaches to serosurveillance and inform design of arboviral vaccine antigens.

P076

Cytokines Elicit Pathogen-specific Immune Responses in *Aedes aegypti* Mosquitoes

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Abstract

Cytokines are essential immune regulators but are poorly studied in mosquito vectors. The *Vago-like gene* (*VLG*) family has been viewed as NF-κB-regulated analogues to vertebrate interferons that trigger the JAK-STAT pathway. However, recent studies in *Aedes aegypti* challenge this "antiviral dogma", showing minimal activation during orthoflavivirus infection with no significant impact on viral replication. These findings raise critical questions about the antiviral properties and regulatory mechanisms of these cytokines.

We investigated the transcriptomic modulation of *A. aegypti* VLGs by diverse stimuli, including alphavirus (Semliki Forest virus, chikungunya virus) infection, dsRNA mimic poly(I:C), and bacterial challenges. Interestingly, we identified a distinct functional bifurcation in the two *A. aegypti* orthologues. *VLG-1* exhibited modest induction by viral molecular patterns and alphavirus infection. While *VLG-1* demonstrated antiviral activity against alphaviruses, it lacked activity against orthoflaviviruses and bunyaviruses. In contrast, *VLG-2* showed broad responsiveness to both poly(I:C) and bacterial challenges but failed to exhibit antiviral activity against any tested viruses.

Our expression analyses of downstream immune-responsive genes in the JAK-STAT pathway revealed their induction by both viral and bacterial challenges, suggesting activation of this pathway, possibly through VLG-dependent/-independent mechanisms. Interestingly, genomic analysis of global *A. aegypti* populations revealed differential evolutionary selection of *VLG* variants, indicating that these cytokines may engage in separate "evolutionary arms races" with specific pathogens.

Our findings reveal that *VLGs* elicit pathogen-specific and pan-microbial responses. This understanding of the mosquito immunity highlights the need to decipher these molecular mechanisms to develop targeted transmission-blocking strategies against arboviral diseases.

P077

Dissecting the dengue virus NS1 protein entry process into human hepatocytes

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Abstract

Dengue fever is a mosquito-borne viral infection endemic in tropical and subtropical regions, recently expanding into more temperate areas. Clinical manifestations range from a mild, flu-like illness to severe hemorrhagic fever and hypovolemic shock. Currently, there is no fully effective vaccine against all four dengue virus (DENV) serotypes and no specific treatment. The nonstructural protein 1 (NS1) is a key virulence factor expressed during DENV infections. NS1 exists as a cell membrane-associated homodimer and a soluble hexamer secreted in the extracellular fluids of DENV-infected patients. This hexamer can rapidly associate with human high-density lipoprotein (HDL) particles, forming highly stable NS1–HDL complexes that circulate in plasma during the acute phase of the disease.

Our previous work demonstrated that secreted NS1 enhances DENV infectivity in pre-exposed hepatocytes and that the NS1–HDL complexes induce a strong pro-inflammatory response in human primary macrophages. However, the cellular receptors that mediate these biological effects remain unknown. In the present study, we investigated the initial steps of NS1 or NS1–HDL entry into host cells and their subsequent activation using biochemical and cellular approaches. We expressed and purified ectodomains of candidate receptors and tested direct binding by ELISA and biophysical techniques. In parallel, we investigated the use of putative cellular receptors on the surface of hepatocytes by flow cytometry.

Understanding how NS1–HDL complexes engage an interaction with host cell receptors will elucidate critical steps in dengue pathogenesis, and offer new perspectives for the development of novel therapeutic strategies against severe dengue.

P078

Hidden in plain sight: local network unveils mosquito and arbovirus dynamics in Lyon metropolis area

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Abstract

Mosquito-borne viruses (MBVs) are on the rise worldwide, buoyed by climate crisis, urbanization, globalization and change in land use (among others). Northern temperate areas remain largely underprepared to face mosquito related health stakes despite the current increase in MBVs incidence and dispersion, as exemplified by France. In 2022, the MASCARA (*Moustiques et arbovirus: Surveillance et action Collective en Auvergne-Rhône-Alpes*) network was built together with academics, citizens, national and local stakeholders to implement innovative actions at regional scale in order to mitigate MBVs emergence and spread. Mosquito longitudinal sampling, arbovirus xenomonitoring and more recently animal surveillance were performed around Lyon metropolis and in Auvergne-Rhône-Alpes region. These data unveil the dynamics of local mosquitoes species as well as (un)expected circulating arboviruses and host reservoirs. The resulting material (specimens & data) are currently used to fuel experimental vector competence assays and modeling efforts, but is also open for collaborative research projects. Transdisciplinary networks development should be promoted as they represent cost-effective, scalable and integrative tools to improve MBVs understanding, anticipation and prevention.

P079

Comparative transcriptomic analysis of individual mosquito midguts reveals potential novel host targets for West Nile virus inhibition

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Abstract

In order for West Nile virus (WNV) to be transmitted through a blood meal by *Culex pipiens* it first needs to infect the mosquito midgut. The molecular determinants governing this initial and early infection are currently unknown but critical for developing interventions to disrupt the viral life cycle. To identify these determinants we performed RNA-seq on individual *Cx. pipiens* midguts at 3 days post-exposure. Differential gene expression was analyzed by comparing three distinct groups: mock-infected midguts, WNV-exposed midguts that became infected, and WNV-exposed midguts that remained uninfected. Sequence reads were mapped against the *Cx. pipiens pallens* reference genome and supplemented with *de novo* assembly to ensure the capture of novel or divergent transcripts. The analysis identified three specific gene targets that showed consistent differential expression across both mapping and *de novo* workflows: *Sorting nexin-17* (Snx17), Sterol carrier protein 2 (SCP2), and a small unannotated protein with a *Chitin binding Peritrophin-A domain*, herafter called Chitin binding protein (CBP). While Snx17 expression increased with infection status, SCP2 and CBP were significantly upregulated specifically in the WNV-exposed midguts that remained uninfected, suggesting a role in viral restriction. To validate these candidates, we are employing small molecule inhibition of SCP2 and dsRNA-mediated RNA interference (RNAi) for all three targets to investigate their role in WNV infection of the midgut. These results highlight the utility of individual midgut transcriptomics in identifying subtle host-pathogen interactions to develop novel mosquito-based interventions to disrupt WNV transmission.

P080

Development of reverse genetics for LIV, a tick-borne flavivirus of sheep and red grouse

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Abstract

Ticks and tick-borne disease are spreading to newer areas due to climate and land use changes. It is therefore critical to enhance monitoring and surveillance tools for scientific and policy-making communities.

Louping ill virus (LIV, *Flaviviridae*) is transmitted by the hard tick *Ixodes ricinus* and causes encephalomyelitis in sheep and red grouse. It is endemic in the UK and Ireland but has been imported into other parts of Europe causing significant economic strain.

In this project, we aim to understand tick-borne flavivirus pathogenesis using reverse genetics and large-scale codon re-encoding to attenuate LIV, generating a more ideal control to compare pathogenesis with the wild type virus.

LIV (isolate SCO_INV14_1992_MK007541.1) was rescued 5 days after transfection in A549 IRF3 knockout cells with the attenuated virus carrying approximately 255 synonymous mutations in its NS5 protein. Both viruses show comparable growth kinetics at high and low MOIs, and we confirmed attenuation by measuring viral plaques with the attenuated virus producing significantly smaller plaques than the wild type.

With these *in vitro* infection parameters, we performed *in vivo* infection in BALB/c mice, comparing the viruses and monitoring pathogenesis. Predictably, the wild-type LIV infected mice showed significant weight loss, lower clinical scores and reduced survival in a dose-dependant manner. Conversely, the attenuated LIV-infected mice showed comparable weight change, survival and welfare to uninfected controls irrespective of dose.

From this we can identify host effectors that may serve as targets for alternative therapeutic strategies to prevent disease and/or improve outcomes.

P081

Individual-based Modelling of Larval Competition and Density Dependence in Aedes aegypti (Diptera: Culicidae)

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Abstract

Aedes aegypti (L.) is a major vector of arboviral diseases whose population dynamics are strongly influenced by larval-stage competition within small, resource-limited aquatic habitats. Many existing population models represent density dependence through prescribed mathematical functions without explicitly modelling the mechanisms of larval competition and food acquisition. Here, we developed an individual-based model for a domestic *Ae. aegypti* breeding site in which larval growth and reserve accumulation, movement, feeding and starvation are represented explicitly within a spatially structured environment. The model incorporates a reserve-dependent growth framework and examines alternative forms of local competition, including exploitative and interference competition, under varying temperature conditions, larval densities, food quantities and food-timing regimes. Rather than imposing density-dependent relationships *a priori*, the model allows adult–larval relationships to emerge from individual-level physiological and competitive interactions. Simulations produced multiple forms of density-dependent responses, including approximately linear, threshold collapse, saturating dynamics and persistent non-linear regulation, with outcomes varying according to competition mechanism and ecological conditions. Competition mechanism emerged as a major organising factor shaping population responses, while food availability, food timing, and temperature altered modified the onset and intensity of density-dependent effects. These findings demonstrate that qualitatively different forms of density dependence can emerge from distinct local interaction processes within the same biological system and highlight the importance of mechanistic representations of larval competition for studies of *Ae. aegypti* population regulation and vector control modelling.

P082

Insights into Arbovirus Ecology from Molecular and Serological Evidence in Wild Birds in West Africa

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Abstract

Wild birds are central to the ecology of many arboviruses, functioning both as reservoirs and dispersers of flaviviruses like West Nile virus (WNV) and Usutu virus (USUV), and alphaviruses like Sindbis virus (SINV), while also experiencing potential health impact. Limited arbovirus surveillance in many parts of W/Africa, constraints insight into their circulation and transmission dynamics. This study presents a longitudinal molecular and serological investigation of wild birds in Nigeria. Dry blood spots from 504 unique birds in 29 species were analysed using RT-qPCR, alongside 875 plasma samples from 810 individuals representing 60 species, tested via ELISA and virus neutralisation assays. Results revealed low but active SINV circulation (0.6%), detected exclusively in the African Thrush (*Turdus pelios*) and in the dry season. Genomic analysis indicated close similarity to other African strains, suggesting local persistence rather than introduction via migratory Palearctic birds. Serological analysis revealed that 21% of sampled birds carried flavivirus antibodies, and the highest species seroprevalence was observed in *T. pelios (30.2%).* WNV and USUV neutralising antibody prevalence reached 4.3% and 8.7%, respectively. The *T. pelios* emerged as a key reservoir for SINV, WNV, and USUV. Recaptured individuals showed seroconversion and declining antibody levels over time, indicating dynamic exposure patterns. Season and sex were significant predictors of seroprevalence, with higher prevalences in adult birds and the dry season. Notably, this study provides the first evidence of SINV in wild birds in Nigeria and highlights widespread flavivirus exposure, emphasizing the importance of sustained avian surveillance within a One Health framework.

P083

Improved Methodology for the Culture of Dengue 3 Virus in the Generation of Authenticated Virus Stock to Support Arbovirus Research and Public Health Preparedness

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Abstract

Background

The National Collection of Pathogenic Viruses (NCPV) preserves, authenticates, and distributes arboviruses to the scientific community. NCPV holds 11 strains of dengue virus covering all four serotypes. High titre dengue virus stocks have historically been difficult to produce. Enhanced methods have been developed to generate high titre dengue virus.

Methods

Dengue 3 virus was cultured on Vero E6 (ECACC 85020206) cell monolayers at concentration adjusted inoculums with increased incubation times until CPE was observed, without passage or infectious splits at 33 °C. The dengue virus stock was characterised and quality controlled using an optimised $TCID_{50}$, and Whole Genome Sequenced. Banks were also sterility tested by incubation in TSB and FTM broths and tested for the presence of mycoplasma by qPCR.

Results

A historical isolate of Dengue 3 virus was successfully grown showing clear and substantial CPE at 21 days post infection. The titre was 4.68×10^6 $TCID_{50}$/mL quantified by the optimised $TCID_{50}$ assay. This was a substantial improvement on previous batches. No mycoplasma or bacterial contamination was detected.

Conclusion

NCPV authenticated viruses, such as dengue 3 can support key activities such as diagnostic assay validation, antiviral and vaccine research and development, and vector competence and transmission studies. The use of standardised reference materials enables reproducible experimentation, meaningful inter-laboratory comparisons, and robust interpretation of results across multiple research settings.

P084

Seroprevalence of West Nile virus among blood donors in mainland France, 2021-2022

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Abstract

Background: The *Orthoflavivirus* West Nile virus (WNV) maintains an enzootic cycle between birds and ornithophilic *Culex* mosquitoes. Transmission to accidental dead-end hosts, such as humans, usually results in asymptomatic or paucisymptomatic manifestations. Severe neurological diseases occur mainly in the extreme ages of life and in immunocompromised individual. Despite evidence of WNV circulation in France since the 1960's, nationwide seroprevalence data remained unavailable.

Aim: To determine the seroprevalence of WNV among blood donors in mainland France in 2021-2022 and examine associated risk factors.

Methods: Sera from 44,490 French voluntary blood donors collected in 2021-2022 were screened for anti-WNV IgG by ELISA on pools of up to four samples, with sera from non-negative pools being individually confirmed. Additional ELISAs and Virus Neutralization Tests for various flaviviruses were used to assess non-negative results using a multi-parameter algorithm.

Results: We observed a low overall seroprevalence, similar in pools and individual samples (0.87% and 0.97%, respectively). Interestingly, prevalence was 1.13% in the Nouvelle-Aquitaine and 1.81% in the Ile-de-France regions, preceding the first local human cases reported in 2023 and 2025, respectively). Risk factors identified as being associated with WNV seropositivity were geographical (living in the south of France: Occitanie, Provence-Alpes-Côte d'Azur, Corsica) and related to ABO blood groups (O and A blood groups were associated with higher and lower WNV seroprevalences, respectively).

Conclusion: This study reveals the low, geographically variable, seroprevalence of WNV in France, and indicates that the virus likely circulated undetected in Nouvelle-Aquitaine and Île-de-France regions before posing a public health threat.

P085

Toward a better understanding of the mechanisms of co-infection by West Nile and Usutu viruses in the avian host.

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Abstract

West Nile virus (WNV) and Usutu virus (USUV), two neurotropic arboviruses, share an enzootic cycle involving avifauna and Culex spp. mosquitoes. Since 2018, co-infections have been reported in Europe in humans, birds, and mosquitoes, yet their impact on transmission dynamics and epidemiology remains poorly characterized. This study aims to elucidate the viral and cellular mechanisms involved in mono- and co-infections with WNV (lineage 2, France 2018) and USUV (Africa 3, France 2016) using different avian models: *in ovo* and *in vivo*.

We developed, for the first time, an *in ovo* model of mono- and co-infection via inoculation of the chorioallantoic membrane (CAM) of chicken embryos. Viral replication kinetics and dissemination were analyzed in the CAM, liver, heart, and brain at 24-, 48-, and 72-hours post-infection, cellular and immune responses were explored using RNA-seq. Results demonstrate viral interference depending on infection conditions. Across all organs, at 2 days post-infection, primary USUV infection completely inhibits WNV replication, whereas primary WNV infection significantly reduces USUV replication by 22%. Moreover, in simultaneous co-infection, WNV replication is significantly reduced by 32% at day 3, with no effect on USUV replication.

Sequential infections (USUV followed by WNV) conducted in four wild magpies revealed widespread tissue dissemination but marked inter-individual variability (viremia, viral loads, shedding, immune response), leading us to favor the *in ovo* model for initial transcriptomic analyses.

By highlighting competitive interactions between WNV and USUV in birds, this work provides key insights for better understanding eco-epidemiological risks associated with viral co-circulation and adapting surveillance strategies.

P086

Impacts of urban ecological restoration on mosquito-borne disease risk in a changing climate

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Abstract

Background: Climate change increases the risk of mosquito-borne diseases in cities through shifts in ambient temperature and altered rainfall. Urban areas are particularly vulnerable as urban heat islands and fragmented ecosystems accelerates mosquito development and enhance pathogen transmission. Cities increasingly implement Nature-based Solutions such as urban greening to increase climate resilience. However, their effects on arbovirus transmission remain poorly understood.

Methods: We compared five public green spaces along the Meuse river (tidal parks) in Rotterdam, in different stages of development, with urban control sites lacking major green-blue interventions. Between 2024-2026, we combined mosquito trapping, molecular arbovirus screening, traditional bird counts and passive acoustic monitoring of birds within a One Health framework to understand if potential arbovirus outbreak risks are exacerbated.

Results: Eight mosquito species were detected across study sites, with *Culex pipiens* as most prevalent species. Usutu virus (USUV) RNA was detected in mosquito pools during the 2024 and 2025 seasons, and phylogenetic analyses suggested circulation of multiple viral variants. Bird community composition differed markedly among sites. Completed and intermediate-stage parks supported higher avian and mosquito abundance, Shannon diversity, and evenness than non-intervened urban control. Frequently observed species included the Eurasian blackbird (*Turdus merula*), European robin (*Erithacus rubecula*), jackdaw (*Corvus monedula*), and carrion crow (*Corvus corone*), all recognized or suspected USUV or West Nile virus hosts.

Conclusions: Our findings suggest that urban ecological restoration can enhance biodiversity while reshaping host-vector interactions relevant to arbovirus transmission, highlighting the need to integrate public health considerations into the design of climate adaptation strategies.

P087

Genome-wide functional interrogation of Aedes mosquito host factors that regulate arboviral infection and vector competence.

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Abstract

Background

Mosquito-borne arboviruses, including Chikungunya (CHIKV), Dengue (DENV), and Zika (ZIKV), are expanding globally alongside their Aedes vectors, posing increasing public health risks. Current vaccines and therapeutics are largely virus-specific and incomplete, making vector control essential. However, the mosquito host pathways governing arboviral replication and transmission remain poorly defined.

Methods

We developed the first genome-wide functional genetic screening platform in mosquito cells, targeting 17,047 protein-coding genes in *Aedes albopictus*. The platform incorporated chemically modified siRNAs to enhance stability and knockdown efficiency. Hits from the primary screen were validated through siRNA deconvolution, CRISPR-Cas9 knockout, and overexpression analyses, followed by mechanistic and in vivo studies in mosquitoes.

Results

The screen identified thousands of host factors influencing CHIKV, DENV, and ZIKV infection. Validation confirmed 11 genes that modulate arboviral replication. Mechanistic studies revealed three key proviral factors: Gamma-glutamylcyclotransferase (GGCT), which enhances viral NS3 protease activity; Toll-like receptor 2 (TLR2), which promotes post-entry replication; and Furin-like protease 1 (FLP1), which mediates viral glycoprotein processing for maturation. Silencing these genes in vivo significantly reduced viral replication. Additionally, FLP1 and its paralogue FLP2 showed functional redundancy in infection, while FLP2 also contributed to mosquito reproduction via vitellogenin processing.

Conclusion

This study provides the first genome-scale map of mosquito host factors regulating arbovirus infection. It reveals how viruses exploit metabolic, immune, and proteolytic pathways, identifying potential targets for next-generation genetic vector control strategies to reduce arbovirus transmission.

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