XXII Jornada de Virologia – Virology Meeting 2023

Organització: Secció de Virologia de la SCB

Sala Prat de la Riba (Carre del Carme 47, Barcelona) / Virtual

27 d'octubre de 2023

Coordinadores de la Secció i responsables de la coordinació de la Jornada:
Sílvia Bofill-Mas i Susana Guix

Comitè científic:
Ana Angulo
Jordi Argilaguet
Núria Busquets
Nuria Izquierdo-Useros
Juan José López-Moya
Elisa Martró
Sofia Pérez del Pulgar
Josep Quer
Dolors Vaqué

Amb el suport de:

XXII Jornada de Virologia – Virology Meeting 2023

9:30h WELCOME Sílvia Bofill-Mas & Susana Guix

9:35-11:15h SESSION I - Viral Dynamics and Immune Interventions: Insights into Quasispecies, Infection Control and Therapeutic Approaches

9:35-10:05h OPENING LECTURE Chair: Sílvia Bofill-Mas

Carles Maluquer de Motes (University of Surrey) **Evasió de la resposta innata per (monkey)pox virus** (20'+10')

10:05-11:20h ORAL PRESENTATIONS I Chairs: Jordi Argilaguet & Sofía Pérez del Pulgar

(*) PhD students

O1. **Harnessing the innate immune response through NOD1 agonists prevents SARS-CoV-2 infection in human lung epithelial cells** (8'+4')

Roger Badia, IrsiCaixa AIDS Research Institute, Health Research Institute Germans Trias i Pujol (IGTP), Hospital Universitari Germans Trias i Pujol

O2. **HBV suppression by nucleos(t)ide analogues reduces the immunotherapeutic target PD-1 on liver-resident T cells** (8'+4')

(*) Mireia Garcia Lopez, Hospital Clínic, University of Barcelona, IDIBAPS, CIBERehd

O3. **Plitidepsin is a host-directed antiviral that transiently inhibits protein translation of distant viruses while shaping a protective proteostatic cellular response** (8'+4')

(*) Elisa Molina Molina, IrsiCaixa AIDS Research Institute, Can Ruti Campus, UAB

O4. **Massive endocytosis mechanisms are involved in CD169-mediated uptake of HIV-1 by dendritic cells** (8'+4')

(*) Fernando Laguía-Nueda, IrsiCaixa AIDS Research Institute

O5. **How quasispecies studies could help in viral infections: negative effect of early ribavirin discontinuation in a chronically infected HEV patient** (8'+4')

(*) Sergi Colomer Castell, Vall d'Hebron Institut de Recerca VHIR, CIBERehd, UAB

O6. **HDV quasispecies conservation and genetic evolution in presence of viral load fluctuation** (8'+4')

Maria Francesca Cortese, Fundació Hospital Universitari Vall d'Hebron, CIBERehd

11:20-11:30h SPONSOR PRESENTATIONS

11:30-12:00h COFFEE BREAK

12:00-14:00h SESSION II. Exploring Viral Ecology, Environmental, Animal and Plant virology

12:00-12:30h KEYNOTE LECTURE Chair: Dolors Vaqué

Guillermo Domínguez Huerta (Centro Oceanográfico de Málaga, IEO-CSIC) **Diversity and ecology of global ocean RNA viruses** (20'+10')

12:30–14:00h ORAL PRESENTATIONS II Chairs: Juan José López-Moya & Josep Quer

(*) PhD students

O7. **The combined use of machine learning techniques and time series analysis reveals changes in the marine viral abundance off the Catalan coast** (8'+4')

(*) Xabier Lopez-Alforja, Institut de Ciències del Mar (ICM-CSIC), Barcelona

O8. **Wastewater surveillance of enteroviruses, with special attention to poliovirus in Catalonia, Spain (2022-2023)** (8'+4')

(*) David Garcia-Pedemonte, Enteric Virus Laboratory, Research Institute of Nutrition and Food Safety (INSA-UB), University of Barcelona

O9. **Wastewater-based epidemiology applied at the building-level reveals distinct virome profiles based on the age of the contributing individuals** (8'+4')

(*) Cristina Mejías-Molina, Laboratory of Viruses Contaminants of Water and Food, The Water Research Institute (IdRA), University of Barcelona

O10. **Revealing occupational exposure to viral pathogens: Insights from Next-Generation Sequencing of aerosol and surface samples in a wastewater treatment plant and a swine farm** (8'+4')

(*) Marta Itarte, Laboratory of Viruses Contaminants of Water and Food, The Water Research Institute (IdRA), University of Barcelona

O11. **Elucidating the onset of immunity in pigs vaccinated with the attenuated Ba71ΔCD2 African swine fever virus** (8'+4')

(*) David Marín-Moraleda, Centre de Recerca en Sanitat Animal (CReSA, IRTA), OIE Collaborating Centre for the Research and Control of Emerging and Re-Emerging Swine Diseases in Europe (IRTA-CReSA)

O12. **Virus-derived small RNAs from *Tomato yellow leaf curl virus* (TYLCV)-infected plants can affect its whitefly vector** (8'+4')

(*) Irene Ontiveros, Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Institute for Mediterranean and Subtropical Horticulture La Mayora (IHSM), CSIC-UMA

O13. **Field-captured *Culex theileri* is a competent vector for West Nile virus** (8'+4')

(*) Albert Burgas-Pau, Centre de Recerca en Sanitat Animal (CReSA, IRTA)

14:00-15:00h LUNCH

15:00-17:30h SESSION III. Innovations in Virology

15:00-16:15h ORAL PRESENTATIONS III Chairs: Ana Angulo & Núria Busquets

(*) PhD students

O14. **A bioinformatics pipeline to discover and track potential pandemic viral species from metagenomic samples** (8'+4')

(*) Maria Tarradas-Alemany, Computational Genomics Lab, University of Barcelona (UB-IBUB)

O15. **Assessing the impact of Zika virus neurotropism on flight performance in *Aedes albopictus:* an automatic smart-trap approach for arbovirus surveillance** (8'+4')

Jaume Gardela, IRTA, Centre de Recerca en Sanitat Animal (CReSA)

O16. **Performance of dried blood spot samples at HBV-DNA detection with a commercial assay, both in the laboratory and in the field for the community screening of migrants** (8'+4')

(*) Anna Not, Germans Trias i Pujol Research Institute (IGTP), Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona (UAB)

O17. ***In vitro* platform to detect replication-competent monkeypox virus in swabs from asymptomatic and mildly symptomatic individuals** (8'+4')

Daniel Perez-Zsolt, IrsiCaixa AIDS Research Institute, Hospital Germans Trias i Pujol

O18. **Development of an animal organoid biobank to study epizootic and zoonotic viruses** (8'+4')

Ferran Tarrés-Freixas, Centre de Recerca en Sanitat Animal (CReSA, IRTA), Universitat de Vic

O19. **3D remodeling of HCV and SARS-CoV2- infected cells and therapies revealed by cryo-SXT** (8'+4')

Ana Pérez-Berna, ALBA Synchrotron, CoCID European Consortium

16:15-17:30h ROUND TABLE Chair: Nuria Izquierdo-Useros

Nous avenços en la cura i control d'infeccions virals encapçalats per investigadors/es que treballen en territoris IEC

Maria Salgado Bernal (AIDS Research Institute IrsiCaixa, IGTP) **¿Podemos curar el VIH?: Casos de curación mediante trasplante alogénico**

Núria Climent (Fundació de Recerca Clínic Barcelona-Institut d'Investigacions Biomèdiques August Pi i Sunyer FRCB-IDIBAPS, Centro de Investigación Biomédica en Red de Enfermedades Infecciosas CIBERINFEC) **Cèl·lules Natural Killer i cura funcional del VIH?: Control excepcional del VIH després d'aturar el tractament en "La Pacient de Barcelona"**

Toni Prenafeta (HIPRA) **BIMERVAX®, una nova vacuna contra SARS-CoV-2**

17:30-17:40h BREAK

17:40-18:10h CLOSING LECTURE: Premi Millor Tesi Doctoral Virologia SCB 2022-23. Chair: Susana Guix

Jordi Rodon (IRTA, Centre de Recerca en Sanitat Animal CReSA, IRTA) **Insights into MERS-CoV disease resistance in the camelid reservoir and strategies to prevent zoonotic spillover** (20'+10')

18:10-18:20h AWARDS AND CLOSURE

ORAL PRESENTATIONS ABSTRACTS

O1. **Harnessing the innate immune response through NOD1 agonists prevents SARS-CoV-2 infection in human lung epithelial cells**

Edurne Garcia-Vidal[1], Ignasi Calba[1,2], Eva Riveira-Muñoz[1], Elisabet García[1], Bonaventura Clotet[1,3,4], Pere Serra-Mitjà[5], Cecilia Cabrera[1,2], Ester Ballana[1,2,*] and Roger Badia[1,2,*]

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The lung is a particularly vulnerable organ susceptible to the infections caused by respiratory viruses such as the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The difficulty to exert a directed antiviral activity at the lung mucosal tract, is one of the major roadblocks for the management of respiratory infections. Therefore, the improvement of the capacity of the respiratory mucosa tract to trigger potent immune response at early stages of the infection for an early viral clearance, becomes a potential intervention alternative for respiratory infections.

In this study, we performed a screening of immunomodulators to boost innate immune response in lung epithelial and myeloid cells. First, we identified the nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs) family amongst the different PRRs subfamilies and pathways tested. NOD1 (TriDAP) and dual NOD1/2 (M-TriDAP) agonists increased the percentage of l IL8+ cells (3.3-fold change), through the activation of the NF-κB and interferon response pathways. In lung epithelial cells, the response to NOD1 and dual NOD1/2 agonists was 2-fold-higher compared to LPS control. Remarkably, a weaker response to NOD1 agonists was observed in PBMCs, suggesting a tissue specific activity of NOD1 in lung epithelial cells without triggering a global systemic activation. The specificity of the NOD agonist activation pathway was confirmed by gene silencing of NOD1 (siRNA) and the selective NOD1 and dual NOD1/2 inhibitors in lung epithelial cells. Finally, the activation triggered by NOD1 agonist Tri-DAP and dual NOD1/2 agonists M-TriDAP, promotes an antiviral environment that prevents SARS-CoV-2 replication in lung epithelial cells (57% of infection inhibition).

This work provides the biological basis for the development of host-directed therapies based on the NLR pathway to face the challenge of viral respiratory infections.

Keywords: NOD-like receptor, innate immunity, respiratory mucosa, viral respiratory infections, SARS-CoV-2.

O2. **HBV suppression by nucleos(t)ide analogues reduces the immunotherapeutic target PD-1 on liver-resident T cells**

Mireia García-López[1], Sabela Lens[1], Laura J Pallett[2], Sergio Rodríguez-Tajes[1], Thais Leonel[1], Ernest Belmonte[3], Ester García-Pras[1], Maria Saez-Palma[1], Zoe Mariño[1], Anna Pocurull[1], Concepción Bartres[1], Ariadna Rando-Segura[4], Francisco Rodríguez-Frías[4], Jonah Li[5,6], Adam J Gehring[5,6], Mala K Maini[2], Xavier Forns[1], Sofía Pérez-del-Pulgar[1]

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Background and Aims: PD-1 is known to be upregulated on global and virus-specific T cells in the HBV-infected liver. However, factors driving its expression in the liver and potential modulation by antivirals have not been well-defined. Our aim was to investigate the impact of viral suppression on PD-1 expression on intrahepatic versus circulating lymphocyte populations from chronic hepatitis B (CHB) patients.

Methods: 22 CHB patients, 9 of them under nucleos(t)ide analogs (NUCs), had paired blood, liver fine needle aspirations (FNAs) and biopsies. A subset had a follow-up FNA after treatment initiation (n=4) or discontinuation (n=4). Intrahepatic (iHBV-DNA and cccDNA) and serum (HBV-DNA, HBsAg, HBcrAg and cirB-RNA) viral markers were quantified. Flow cytometry was used for immunophenotyping PBMCs and intrahepatic leukocytes from FNAs. An independent liver FNA scRNAseq dataset was used to further characterize the phenotype and function of tissue-resident memory T cells (TRM).

Results: Tissue-resident memory CD8 T cells were strikingly enriched for PD-1 expression, which correlated with both iHBV-DNA and cccDNA. These associations were not reflected in circulating T cells. PD-1 expression intensity on CD8 TRM and intrahepatic B cells was lower in NUC-treated than in naive patients, changes that were again not detectable in the circulation. Analysis of follow-up FNAs showed that viral load rebound induced by NUC discontinuation had the potential to drive re-expression of high levels of PD-1 on CD8 TRM. On the other hand, therapy initiation and subsequent viral suppression reversed these changes. scRNAseq results extended the profiling of these PD-1+CD8 TRM which had an exhausted phenotype but retained functionality.

Conclusions: Our results reveal a close association between viral replication and global liver-resident T cell PD-1 expression, with a reduction after prolonged NUC therapy and re-expression following treatment withdrawal. The mechanism by which HBV load associates with global (rather than just HBV-specific) T cell PD-1 remains to be explored.

O3. **Plitidepsin is a host-directed antiviral that transiently inhibits protein translation of distant viruses while shaping a protective proteostatic cellular response**

Elisa Molina Molina[*,1], Daniel Perez-Zsolt[*,1], Joan Josep Bech-Serra [2], Roger Badia[1,3], Eva Riveira-Muñoz[1], Edurne Garcia-Vidal[1,], Martin Sachse[4,5], Marçal Gallemí[1], Jordana Muñoz-Basagoiti[1], Sandra Franco[1], Sara Y. Fernández-Sánchez[4], Dalia Raïch-Regué[3], Cristina Lorca-Oró [6,7], Raquel Tenorio[4], Isabel Fernández de Castro[4], Jorge Carrillo[1,3,8], Julià Blanco[1,3,8,9], Alejandro Losada[10], Pablo Aviles[10], Carmen Cuevas[10], Júlia Vergara-Alert[6,7], Joaquim Segalés[6,11], Miguel Ángel Martínez[1], Roger Paredes[1,9], Bonaventura Clotet[1,8,9], Cristina Risco[4], Ester Ballana[1,3], Carolina de la Torre[2], Nuria Izquierdo-Useros[1,3,8].

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Background: Different viruses employ similar pathways for replication that reveal key intracellular hot spots to target with host-directed therapies and achieve a broad-spectrum antiviral activity. Plitidepsin is a clinically approved antitumoral agent that blocks the elongation factor eEF1A required for protein translation. This drug impairs SARS-CoV-2 replication and shows a favorable safety profile in COVID-19 patients. Yet, the precise antiviral mechanism of action of plitidepsin remains unknown.

Methods: Here we used a deep quantitative proteomic assay to measure the impact of plitidepsin on the proteome of SARS-CoV-2-infected Vero E6 cells. This was complemented with transmission electron microscopy assays, which unraveled the subcellular and morphological changes associated to plitidepsin treatment. In addition, we performed functional *in vitro* assays to dissect the antiviral activity of plitidepsin against SARS-CoV-2 and other viruses, including Zika virus, Hepatitis C virus replicon, Herpes simplex virus, and HIV-1.

Results: We found that plitidepsin inhibited the synthesis of all SARS-CoV-2 proteins in a dose dependent manner. These included the viral R1AB polyproteins, which yield the non-structural proteins involved in double membrane vesicle (DMV) formation needed for viral replication. As a consequence, plitidepsin treatment led to a reduction in both DMVs and the morphogenesis of new viruses, having a greater impact on the viral proteome than on cellular proteins. Less than 14 % of the cellular proteome was significantly affected by plitidepsin, which induced the up-regulation of key molecules associated with protein biosynthesis, such as the translation initiation factors eIF4A2 and eIF2S3. Plitidepsin at 50 mM induced a compensatory state that rescued the translation of cellular proteins. This proteostatic response explains how cells preserve the cellular proteome after treatment with a translation inhibitor such as plitidepsin. These results also indicated that plitidepsin could inhibit other RNA-dependent and non-integrated DNA viruses, as we confirmed *in vitro* testing Zika virus, Hepatitis C virus replicon, and Herpes simplex virus. However, the compensatory

proteostasis induced by plitidepsin also explains why this drug failed to inhibit DNA integrated proviruses such as HIV-1.

Conclusions: Unraveling the mechanism of action of host-directed therapies like plitidepsin is imperative to define the indications and range of antiviral activity. This knowledge will be key to develop broad-spectrum treatments and have them ready to deploy when future pandemic viruses break through.

O4. **Massive endocytosis mechanisms are involved in CD169-mediated uptake of HIV-1 by dendritic cells**

Fernando Laguía-Nueda[1], Jakub Chojnacki[1,2,3], Itziar Erkizia[1], Elena Rebollo[6], Maria Isabel Geli[6], Carlos Enrich[7,8], Javier Martínez-Picado[1,2,4,5], Patricia Resa-Infante[1,4]

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Background: Myeloid cells, such as monocytes, macrophages, and dendritic cells (DCs), are main sentinels of the immune system against invading viruses. However, HIV-1 can take advantage of this to disseminate throughout the body. This process relies on the recognition of gangliosides on the viral envelope by the CD169/Siglec-1 receptor expressed on the cell surface of activated myeloid cells. This interaction triggers the internalization of HIV-1 in a structure named Viral Containing Compartment (VCC), where viral particles remain infectious. Although VCC formation process is largely unknown, previous data suggest that it is independent of clathrin and requires cholesterol in the cell membrane, as well as transient deregulation of cortical actin. Therefore, we hypothesized that HIV-1 exploits the machinery used during Massive Endocytosis (MEND) to enter dendritic cells after binding to CD169.

Methods: Live cell confocal imaging of DCs was conducted to obtain a 3D reconstruction of VCC formation and measure its dimensions and dynamics. Actin and cholesterol staining was performed in fixed and live cells to assess their roles during this process. PI3K inhibitors and protein palmytoilation inhibitors were used to evaluate their effect in the uptake of Viral Like Particles (VLP) by confocal microscopy. Images were processed with ImageJ and data analysis with GraphPad.

Results: HIV-1 VLP uptake dynamics and VCC dimensions do not match with classical endocytic pathways, but VCC size (2.9 ± 0.7 µm diameter and 20 ± 9.9 µm3 volume) indicates massive plasma membrane invagination. Although actin was not observed by phalloidin staining in the early steps of VCC formation, it is essential for VCC maintenance. PI3K and protein palmitoylation inhibition arrested the process before VLP entry, reducing the rate of VCC formation in DCs from 70% to 40% and 20%, respectively. Strikingly, cholesterol coalescence determined VCC formation as the cholesterol probe signal was doubled in the VLP uptake regions, as observed by live cell imaging.

Conclusions: These data suggest that MEND mechanisms participate in the internalization of viral particles and subsequent formation of VCC in DCs. This work provides new insights into the interaction of HIV-1 with myeloid cells revealing new therapeutical targets to hinder virus dissemination. Blocking VCC formation offers potential cross-protection against enveloped viral infections that use CD169 receptor, such as Ebolaviruses and other hemorrhagic fever viruses.

O5. **How Quasispecies Studies Could Help in Viral Infections: Negative Effect of Early Ribavirin Discontinuationin a Chronically Infected HEV Patient**

Sergi Colomer-Castell[1,2,3], Josep Gregori[1], Damir Garcia-Cehic[1,2], Mar Riveiro-Barciela[1,2,7], María Buti[1,2,7], Ariadna Rando-Segura[2,4], Judit Vico-Romero[1], Carolina Campos[1,2,3], Marta Ibañez-Lligoña[1,7], Caroline Melanie Adombie[1,5], María Francesca Cortese[2,6], David Tabernero[2,6], Juan Ignacio Esteban[1,2,7], Francisco Rodriguez-Frias[2,6], Josep Quer[1,2,3]

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Hepatitis E is a liver inflammation caused by the Hepatitis E Virus (HEV). According to WHO, approximately 20 million people get infected with HEV every year, out of which 3.3 million exhibit symptoms and 44,000 die due to hepatic failure. HEV is a major cause of acute viral hepatitis globally, particularly in low- and middle-income countries, and its incidence is on the rise in industrialized nations. Although HEV mostly triggers an acute infection, it becomes chronic when patients are inmunodepressed. HEV has a single-stranded RNA genome of around 7.2kb in length, consisting of three open reading frames although a fourth ORF has been described in genotype 1. The lack of polymerase proofreading activity makes HEV to incorporate different mutations in each replication cycle, being considered as a quasispecies virus.

In our study, several samples from a chronic HEV patient treated at different stages with ribavirin –mutagen- have been collected during the course of the infection. HEV RNA has been extracted and purified using spin columns, and a conserved fragment of ORF2 amplified using consecutive RT-PCR and Nested-PCR. Finally, amplified fragments have been sequenced using NGS MiSeq platform, obtaining a high deep coverage for every sample. A new method to analyze viral quasispecies relying on haplotype fitness has been designed by dividing the quasispecies in four fractions: the master haplotype, the master haplotype, rare haplotypes (RHL) at two levels (those present at 1%, but less than that of the master haplotype. Results showed that HEV quasispecies were much unstructured, being very complex at a nucleotide level. What is more, the treatment with ribavirin increased the proportion of RHL to master haplotype. However, at protein level (phenotype/functional) the pattern was the opposite, with high frequencies of dominant haplotype, meaning that most of the ribavirin-induced variability were synonymous mutations, leading to a final resistance to the drug. Taken all together, the study of quasispecies in HEV chronic disease has been shown useful to understand the virus response to a mutagenic drug, especially ribavirin resistance, with important clinical implications.

O6. **HDV quasispecies conservation and genetic evolution in presence of viral load fluctuation**

Beatriz Pacin-Ruiz[1,2], Adriana Palom[2,3], Josep Gregori[4], Selene Garcia-Garcia[1,2], David Tabernero[2,4], Ariadna Rando-Segura[1], Marta Vila[1], Mar Riveiro-Barciela[2,3], Maria Buti[2,3], Francisco Rodriguez-Frias[1,2,5], Maria Francesca Cortese[1,2]

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Background: Hepatitis delta virus (HDV) is known to be highly variable. More than 2log10 decline of the viremia is considered a surrogate endpoint of treatment response. The identification of viral factors that differentiate the patients experiencing or not spontaneous or treatment-associated viral load decline might help in clinics to improve patients' management and better define potential responders.

Method: HDV RNA was isolated from plasma of 7 patients with chronic hepatitis delta (CHD) at two timepoints. Patients who experienced a drop of more than 2log10 in the viral load at the last available follow-up sample was defined as fluctuating. Viral quasispecies (QS) in the *HDAG* were analyzed by next-generation sequencing by amplifying two overlapping amplicons (nucleotide [nt] 3'-end:912-1298 and 5'-end: 1234-1631). Gene and aminoacidic sequence conservation were studied calculating the information content (IC) and the Grantham distance, was isolated from plasma respectively, whereas QS evolution (index of commons- Cm, Yue–Clayton index,-Yc, genetic distance- Dxy and normalized genetic distance- Da) between the two patient's samples was compared between fluctuating and not patients.

Result: Although the nt conservation of the *HDAG* QS was irregular along the sequence, some highly conserved regions (with high average IC) were observed (nt 1185–1216, 1438–1464, 1468–1507, 1569–1595). At aminoacidic level, several substitutions were observed, however they all showed a limited evolutionary functional distance related to the consensus (Grantham distance <50). Fluctuating patients (n=3) presented similar viral load, follow-up time and percentage of editing that not-fluctuating (n=4). When analyzing QS evolution between the groups we observed that fluctuating patients presented a similar evolution in the 3' region, whereas at the 5'-end the QS presented a lower variability related to the not-fluctuating (Cm: 0.89±0.17 and 0.61±0.27; Yc: 0.81±0.19 and 0.29±0.23 respectively). Similarly, in this extreme, the distance between the QS at both FU samples was 4.5-fold lower in fluctuating patients in term of distance between the two patients' samples (Dxy), reaching more than 30-fold reduction when considering normalized distance (Da).

Conclusion: Despite the genetic variability of *HDAG* QS, the occurred amino acid changes seem to have a very low functional impact, which suggests that the structure and function of the protein may be conserved. The *HDAG* QS of the CHD patients presenting viral load fluctuations shows lower variability and evolution rate related to the not-fluctuating patients especially in the 5' extreme of the sequence, corresponding to the protein C-terminal domain, where most of the immune epitopes reside. These data suggest that, in persistent viremic patients, variants that provide replication or immune advantages may be selected, thus determining this high viral replication rate and HDV persistence.

O7. **The combined use of machine learning techniques and time series analysis reveals changes in the marine viral abundance off the Catalan coast**

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Viruses play a fundamental role in controlling the abundance and composition of microbial communities, as well as in the biogeochemical cycles and productivity of marine ecosystems. Although the spatial distribution of viruses has been investigated, little is known about the temporal variations in viral communities. Furthermore, climate change and its effects on the environment can have consequences for the temporal dynamics of viruses and their potential hosts. To assess these effects over the past two decades, we collected samples from the sea surface at the Blanes Microbial Observatory (BBMO, Catalan Coast) to determine viral abundance and other biological and environmental variables. We applied time series analysis and machine learning methods to determine if environmental factors have changed during these 20 years and if they influence viral communities and their hosts. Our results indicate that at BBMO, during this period, increases in temperature, salinity, and water transparency were observed, while phosphate levels, chlorophyll concentration, and the abundance of bacteria and viruses decreased. This suggests a continuous transition of the ecosystem towards oligotrophy. In particular, the last decade has seen these changes and their influence on the decrease in viral abundance. Long-term studies like ours contribute to describing and understanding variations in viral dynamics in the oceans. The incorporation of the viral component into future ocean and climate models will improve the accuracy of climate change predictions.

O8. **Wastewater surveillance of enteroviruses, with special attention to poliovirus in Catalonia, Spain (2022-2023)**

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Wastewater surveillance provides a broader perspective on the pathogens circulating in a population. Due to the reports of poliovirus detection in 2022 in London and New York wastewater, and the vaccine-derived poliomyelitis (VDPV) case in New York, besides many other reports on VDPV cases in other parts of the world, serious public health concerns have been raised and prompted the development of direct detection methods based on NGS sequencing approaches.

The aim of our study was to monitor enterovirus species and polioviruses in samples from two wastewater treatment plants in the urban area of Barcelona within various periods of 2022 and 2023. Two amplicon Nanopore NGS direct-detection methods were used, a generic approach consisting in a semi-nested RT-PCR of a VP1 region of 400 bp, allowing the direct identification of all enterovirus serotypes, and a nested RT-PCR of the entire capsid region, followed by amplification of the full 1000-bp VP1, allowing a more specific detection of enterovirus C, including poliovirus. All amplicons were sequenced using Nanopore (MinION) technology and a bioinformatic pipeline based on the Vsearch software was designed, enabling to compare the sequences from an in-house enterovirus database.

The surveillance shows a high prevalence of Echovirus 11 throughout the duration of the study, with Echovirus 9, Coxsackievirus B5 and Coxsackievirus B2 being also present with minor frequencies. Four enteroviruses associated to polio-like flacid paralysis and other severe neural syndromes were detected in very low proportions: enterovirus -A71, D68, C99 and C109, these latter two belonging to Cluster C enteroviruses. Nevertheless, no poliovirus sequences were detected.

O9. **Wastewater-based epidemiology applied at the building-level reveals distinct virome profiles based on the age of the contributing individuals**

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Viruses infecting humans can be identified and analyzed in wastewater, as they are excreted through various means such as feces, urine, saliva and skin cells. Given that the occurrence and severity of viral infections can vary across different life stages, studying the virome in wastewater samples, contributed by various age groups, can yield valuable insights into the prevalence of viral infections within different age demographics. Furthermore, wastewater from diverse sources, combined within the sewer network, offers a consolidated perspective on the presence of viruses within a given population.

In our study, targeted enrichment sequencing was employed to characterize the human virome present in wastewater at a building-level scale. We achieved this through passive sampling of wastewater in schools, university settings, and nursing homes across two different cities in Catalonia. Additionally, wastewater from a third city, representing a population of over 1.5 million inhabitants was analyzed to examine the entire excreted human virome.

The virome extracted from the wastewater treatment represented the collective viral contributions of individuals of all ages and exhibited a list of viral family reads similar to those reported in previous studies with astroviruses and human bocaviruses as the more abundant viruses followed by human adenoviruses, polyomaviruses and papillomaviruses. There were significant differences in the distribution of viral families among the different types of buildings studied. Mamastrovirus 1 was predominant in school samples, salivirus and human polyomaviruses JC and BK in university settings while nursing homes showed a more balanced distribution of viral families presenting papillomavirus and picornaviruses and interestingly, some viruses linked to immunosuppression.

These results underscore the relevance of studying specific wastewater sources in shedding light on diverse viral excretion patterns in contrast to what is observed from larger wastewater treatments. In conclusion, this study shows the utility of building-level wastewater-based epidemiology as an effective tool for monitoring the presence viruses circulating within specific demographic groups.

O10. **Revealing occupational exposure to viral pathogens: Insights from Next-Generation Sequencing of aerosol and surface samples in a wastewater treatment plant and a swine farm**

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Occupational exposure to pathogens can pose significant health risks. This study aimed to identify the viruses to which workers in a wastewater treatment plant (WWTP) and a swine farm are exposed. Aerosol samples were collected using a Coriolis µ sampler, while surface samples were obtained through a long-term sampling approach using paper-based stickers. Both aerosol and surface samples from the WWTP and the swine farm were analyzed by quantitative polymerase chain reaction (qPCR) assays to detect and quantify Human Adenovirus (HAdV) and Porcine Adenovirus (PAdV), respectively. Furthermore, a target enrichment sequencing (TES) approach was employed to characterize the aerosol and surface samples, thereby enabling the study of vertebrate-infecting viruses within these environments and the identification of potential viruses to which workers might be exposed.

Results from qPCR assays revealed that HAdV and PAdV were widespread viruses in the WWTP and the swine farm, respectively. Specifically, HAdV was detected in 60% of WWTP aerosol samples, with a mean concentration value of 3,23E+01 GC/m^3, and 57.29% of WWTP surface samples, with a mean concentration value of 4,72E+01 GC/cm^2. On the other hand, PAdV was detected in 83.33% of swine farm aerosol samples, with a mean concentration value of 5,85E+01 GC/m^3, and in 86.49% of swine farm surface samples, with a mean concentration value of 2,87E+04 GC/cm^2. The TES approach enabled the detection of human and other vertebrate viruses in aerosol and surface samples from the WWTP, including viruses from families such as *Adenoviridae*, *Circoviridae*, *Orthoherpesviridae*, *Papillomaviridae* and *Parvoviridae*. Additionally, several families, such as *Anelloviridae*, *Astroviridae*, *Caliciviridae*, *Coronaviridae* and *Picornaviridae*, were also sequenced in aerosol samples, while *Herpesviridae*, *Polyomaviridae* and *Retroviridae* were exclusively found in surface samples. Similarly, in the swine farm, TES enabled the detection of vertebrate viruses in aerosol and surface samples, with most of them being porcine viruses belonging to families like *Adenoviridae*, *Astroviridae*, *Circoviridae*, *Herpesviridae*, *Papillomaviridae*, *Parvoviridae*, *Picornaviridae* and *Retroviridae*. Additional families, including *Anelloviridae*, *Genomoviridae* and *Tobaniviridae* were exclusively detected in aerosol samples, while *Polyomaviridae* and *Sedoreoviridae* members were only found in surface samples.

Overall, both aerosol and surface sampling strategies employed in this study provided essential information about HAdV and PAdV concentrations in aerosol and on surfaces within WWTP and swine farm facilities. This data could be valuable for conducting Quantitative Microbial Risk Assessment (QMRA) analysis. Additionally, these sampling strategies allowed the detection of sequences from other vertebrate-infecting viruses through NGS analysis using a TES approach. To the best of our knowledge, this is the first study to utilize a paper-based sticker strategy for surface sampling to perform NGS analysis. These findings provide valuable insights into workers' exposure to virus present in WWTP and swine farm environments, facilitating the implementation of measures to reduce potential risks.

O11. **Elucidating the onset of immunity in pigs vaccinated with the attenuated Ba71ΔCD2 African swine fever virus**

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African Swine Fever (ASF) is a highly contagious and deadly disease affecting domestic pigs and wild boar around the globe, thus representing a major worldwide threat to the pig industry. Neither vaccines nor treatments are commercially available against ASF virus (ASFV), being live attenuated viruses (LAV) the most advanced vaccine prototypes to induce protective immunity. Although biosafety concerns still hamper their implementation in the field, such vaccines are a very useful tool for acquiring knowledge about the immune components involved in protective immunity. We have previously demonstrated that the vaccine prototype Ba71ΔCD2 (Genotype I), developed in our laboratory, is able to confer cross-protection against the pandemic virus Georgia 2007/1 (Genotype II) in experimental studies. However, a remaining important question regarding vaccine efficacy is the elucidation of the onset of immunity. Thus, in the present study we aimed to define the specific time point post Ba71ΔCD2-vaccination in which animals are protected against severe disease. Groups of five pigs were intranasally vaccinated with Ba71ΔCD2 and infected with a lethal dose of the virulent virus Georgia 2007/1 at different early time points post-vaccination (12, 7 and 3 days). The results demonstrate that animals vaccinated 3 and 7 days before the challenge showed a delay in the onset of symptoms. However, only pigs challenged at 12 days post-vaccination, with presence of ASFV-specific antibodies, were able to control the disease with mild clinical signs and lower viral loads. These results suggest that the early immune response triggered by the Ba71ΔCD2 LAV might help to minimize the disease in its early stages, but that a robust protective immunity can only be expected with the onset of adaptive immunity. Transcriptomic studies are currently on-going to characterize the specific components underlaying the immune protection onset conferred against ASFV at early time points post-vaccination.

O12. **Virus-derived small RNAs from *Tomato yellow leaf curl virus* (TYLCV)-infected plants can affect its whitefly vector**

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Most plant viruses causing economically important diseases in agriculture relies on insect vectors for their transmission. Although there are some evidences that plant viruses can influence the interaction with their insect vectors, the underlying mechanisms still are relatively unknown.

We hypothesized that the small RNAs (sRNAs) present in virus infected plants might be transferred to their insect vectors during feeding, and modulate the virus-vector interaction through silencing of particular insect genes. To address this, sRNAs from tomato plants infected with the begomovirus *Tomato yellow leaf curl virus* (TYLCV) at 14 dpi were mapped to the genome of *Bemisia tabaci* (MED) to examine their potential to affect expression of whitefly genes. Several sRNAs that moderately accumulated in TYLCV-infected plants were found to potentially target genes in the insect, and among the selected matches three genes involved in neonicotinoid detoxification-related pathways were identified. When analysed by RT-qPCR, their expression levels were approximately 3-fold lower in TYLCV-viruliferous whiteflies than in non-viruliferous ones, suggesting that this downregulation might be caused indeed by the TYLCV-derived sRNAs (viRNAs), and this hypothesis was further corroborated through feeding whiteflies on synthetic viRNAs. Moreover, experiments with whiteflies feeding on artificial diets were performed to validate the hypothetical role of the selected genes in survival when the toxic substance nicotine was included in the diet. The significantly lower survival rate observed in our experimental setup suggested that the TYLCV-viruliferous condition of the whiteflies might be critical for their reduced capacity of nicotine detoxification. Our results strongly suggest a possible cross-kingdom communication through sRNA in the interplay between TYLCV, the tomato plant and the whitefly vector, shedding new light to better understand the complexities of plant-virus-vector interactions.

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O13. **Field-captured *Culex theileri* is a competent vector for West Nile virus**

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Background: *Culex theileri* (Theobald, 1903) is distributed in afrotropical, paleartic and oriental regions. It is a mainly mammophilic floodwater mosquito which has been involved in the transmission of West Nile virus (WNV, recently renamed as *Orthoflavivirus nilense* by the ICTV). This virus is a mosquito-borne orthoflavivirus that is kept in an enzootic cycle mainly between birds and *Culex* mosquitoes. Occasionally, it affects mammals including humans and equines causing encephalopathies. The main purpose of the present study was to evaluate the vector competence of a European field collected *Cx. theileri* population for circulating WNV lineages (1 and 2).

Material and Methods: *Cx. theileri* field-collected larvae from Sevilla province were reared in the laboratory under summer environmental conditions. To assess the vector competence for West Nile virus (WNV) transmission, 10-to-12 days old *Cx. theileri* females were fed with blood doped with WNV lineages 1 and 2 (7 log10 TCID50/mL). Fourteen- and 21-days postexposure females were sacrificed, and head, body and saliva were extracted to assess infection, dissemination and transmission rates and transmission efficiency.

Results: *Cx. theileri* population was experimentally confirmed as a high competent vector for WNV (both lineages 1 and 2). The virus was able to infect and disseminate within *Cx. Theileri* mosquitoes, and they were able to transmit the virus as infectious virus was isolated from the saliva of disseminated individuals. Transmission efficiency was 50% for lineage 1 for both 14 and 21 dpe time-points, while it was 24% and 37,5% for lineage 2, respectively. There was barely any effect of midgut infection barrier for lineage 1 and a moderate effect for lineage 2. The main barrier which limited the virus infection within the mosquito was midgut escape barrier.

Conclusions: In the present study, *Cx. theileri* showed to be highly competent to transmit WNV. However, vector density and feeding pattern of *Cx. theileri* mosquitoes must be considered to estimate its vectorial capacity for WNV in the field.

Keywords: West Nile virus, *Culex theileri*, vector competence

O14. **A bioinformatics pipeline to discover and track potential pandemic viral species from metagenomic samples**

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From the dawn of Next Generation Sequencing (NGS) technologies, those strategies have become crucial in the study of microbial communities from environmental samples. However, there are still some challenges to overcome, either from biological and computational perspectives, to characterize their virome composition. Viral metagenomics has to deal with low quality sequences, possible sample biases (due to chemical inhibitors, degradation, etc), challenging data analysis, and more specifically the lack of standardized regions for classification, the arduous purification of enough biomass for sequencing, and the limited completeness of the available virus databases. In addition, most of the viral particles found in environmental samples correspond to bacteriophages, which further complicates the detection of specific taxons or viral families.

To overcome some of those issues a bioinformatic protocol is being implemented, to spot and characterize viral species with zoonotic potential in environmental samples processed using capture-based high throughput sequencing. The ongoing NextFlow pipeline includes sequenced reads clean-up, assembly, virome annotation, and viral discovery. A set of viral samples obtained from sewage and bat guano have been already analyzed with this pipeline; moreover, sequences obtained by whole-genome shotgun and probe-based viral capture approaches have been also considered to assess the performance of the capture kit and test the performance of the pipeline.

The results show an increased number of assigned viral contigs in the capture approach (using RVDB database), which also achieves higher coverage and similarity to reference sequences of potentially zoonotic viruses.

O15. **Assessing the impact of Zika virus neurotropism on flight performance in *Aedes albopictus:* an automatic smart-trap approach for arbovirus surveillance**

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Vector-borne diseases are a worldwide burden that is projected to rise due to climate change and globalization. Mosquito species of the genus *Aedes* transmit viruses such as dengue (recently renamed as *Orthoflavivirus dengue*) and Zika (ZIKV, recently renamed as *Orthoflavivirus zikaense*) viruses, which pose significant global health burdens. The Asian tiger mosquito (*Aedes albopictus*) is a competent vector for such arboviruses. *Ae. albopictus* was first detected in 2004 in Spain (Sant Cugat del Vallès). Since then, populations of the invasive species have been established in the Iberian Peninsula, increasing the risk of mosquito-borne diseases in this region. ZIKV is an emergent mosquito-borne virus that can cause congenital malformations and neurological disorders. At the same time, ZIKV targets mosquito neurons and induces changes in its behavior, which may impact the vectorial capacity of the infected mosquitoes. In this study, we tested an optical sensor, in combination with machine learning techniques, to detect potential alterations in the flight performance of ZIKV-infected *Ae. albopictus* due to virus replication in the mosquito's nervous system. Female *Ae. albopictus* females (n = 433) were intrathoracic inoculated with ZIKV (6.74 log10PFU/mL) or with culture media as a control group (n = 410). Non-infected and ZIKV-infected *Ae. albopictus* females were sequentially introduced in a cage containing the smart-trap system consisting of an optical sensor attached to a standard mosquito trap. The smart-trap system reported 63% of classification accuracy with power spectral density feature and deep neural network algorithm. To confirm the presence of ZIKV in the neuronal tissues, sections of mosquitoes were tested using immunohistochemical staining for ZIKV. ZIKV infection was present in 100% of the ZIKV-infected mosquitoes (n = 6). The central nervous system (head and thoracic ganglia) and the eyes (ommatidia) were positive for the stain, indicating a neurotropism of ZIKV. Additionally, the basal lamina of the salivary glands was positive for the stain. The saliva of 1 out of 7 mosquitoes with disseminated infection (14%) was positive to ZIKV at 11-12 days post-infection. Our preliminary data suggest that ZIKV neurotropism may affect the flight performance of ZIKV-infected mosquitoes, which could be detected by our smart-trap system with promising accuracy. This result opens new future vector biology research and potential applications of the smart-trap technology in mosquito and arbovirus surveillance.

O16. **Performance of dried blood spot samples at HBV-DNA detection with a commercial assay, both in the laboratory and in the field for the community screening of migrants**

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Background: Decentralized testing is often required to achieve viral hepatitis elimination in vulnerable populations, especially in those with poor access to the healthcare system. Hepatitis B virus (HBV) DNA testing is necessary to confirm diagnosis and guide treatment decisions. Dried blood spot (DBS) samples are widely used for decentralized hepatitis C virus testing in vulnerable populations, but less real-world evidence is available for HBV. We aimed to evaluate HBV-DNA testing with a commercial assay in DBS prepared in the laboratory and obtained in the field when screening migrants approached in the community.

Methods: In order to determine the lower limit of detection (LLoD), serial dilutions of an HBV standard were prepared in negative blood (viral loads comprised between 31 and 20,000 IU/mL). Mock DBS were prepared with 50 µl of blood per spot and 10 replicates per dilution, and let dry. Blood was eluted from DBS with lysis buffer and processed in the Abbott Alinity m HBV assay. Results were interpreted qualitatively (positive/negative) and the LLoD was determined by Probit regression analysis.
The detection of HBV-DNA in real fingerstick DBS samples was performed in participants of the HepBC*link* project, based on a community action screening migrants living in Barcelona. DBS were collected for those participants testing HBsAg positive by a rapid detection test (Determine HBsAg 2). Simplified access to care was provided through an international health unit (IHU), where participants received HBV serological and viral load testing through venipuncture.

Results: The LLoD of the Alinity m HBV assay in DBS was 1115 IU/mL of blood (95% CI, 876-1773 IU/mL). A total of 768 migrants were screened and 30 (3.9%) were HBsAg positive (25 from Senegal, 3 from Pakistan and 2 from Romania); 20/30 (66.6%) were new diagnoses. Of the total, 10/30 (33.3%) were already diagnosed and linked to care. Among new diagnoses, 14/20 (70%) attended the visit at the IHU (linked to care), and all tested DNA-HBV positive through routine testing. Subsequently,

10/14 (71.4%) attended a visit with the hepatologist at the hospital, and none met antiviral treatment criteria.
DBS were obtained in 25/30 cases (83.3%) but, due to loss to follow up, routine HBV viral load testing at the IHU was only possible in 16 cases for results comparison. HBV-DNA was detected in 8/16 (50%) DBS samples; those participants had viral loads in plasma between 331 and 14,454 IU/mL. No amplification was detected in the rest of the DBS samples, corresponding to participants with viral loads in plasma between 33 and 2089 IU/mL. None of these 16 cases fulfilled current antiviral treatment criteria.

Conclusion: Given the LLoD of this assay in DBS, most cases fulfilling antiviral treatment criteria (viral load >2000 IU/mL of plasma among other parameters) would be identified. However, in this group of migrants, viral loads below this threshold were common (62.5%) and would not be detected by DBS testing. Therefore, DBS testing could complement rapid HBsAg testing for HBV screening in these migrant populations, but viral load testing through venipuncture is recommended upon linkage to care.

O17. **In vitro platform to detect replication-competent monkeypox virus in swabs from asymptomatic and mildly symptomatic individuals**

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Background: Mpox is a zoonotic infectious disease caused by monkeypox virus (MPXV), which is endemic to western and central Africa. However, the mpox outbreak ongoing since 2022 has affected more than 110 countries, with almost 90,000 diagnostics and at least 152 reported deaths. In nonendemic countries, MPXV transmission is consistent with a sexually transmitted infection. Gay, bisexual and other men who have sex with men (GBMSM) and trans women (TW) have been mainly affected during this outbreak. Some mpox patients are asymptomatic or mildly symptomatic, but whether these individuals can transmit the infection remains poorly understood. Here, we aimed to set up an *in vitro* assay for the detection of replicative-competent MPXV in these individuals.

Methods: Asymptomatic/mildly symptomatic mpox patients were identified among GBMSM and TW (n =113) through a self-sampling screening. Pharyngeal and anal swabs were tested for MPXV using a real-time PCR. Positive samples were inoculated in Vero E6 cells and cultured up to 14 days in the presence of antibiotics and antifungals. Cultures where checked every 2 days for the presence of cytopathic effect (CPE), which is indicative of viral replication. Supernatants of CPE^+ cultures were titrated in Vero E6 to measure infectivity using the Reed & Muench method. In addition, viral antigens were detected in infected cells following the staining with anti-vaccinia polyclonal antibodies, which was assessed by flow cytometry and confocal microscopy.

Results: Seven out of 113 asymptomatic/mildly symptomatic participants were diagnosed with mpox by PCR. Five tested positive in pharyngeal swabs, one in anal swab and one in both. Ct values ranged 24.85-38.06, with estimated viral loads ranging 2,674-8,532,000 copies/mL. Positive swabs were assayed *in vitro* for competent viral replication using Vero E6 cells, and CPE was observed in three

out of seven samples. Infectious titers of the recovered virus ranged between 10^2.8 and 10^4.8 $TCID_{50}$/mL. Viral infection was further confirmed by flow cytometry and confocal microscopy, which revealed the presence of cell-associated viral antigens in all three CPE^{+} cultures.

Conclusions: The *in vitro* platform set up here demonstrated the presence of viable MPXV in swabs from asymptomatic or mildly symptomatic patients with low viral loads. As these patients could potentially transmit MPXV, testing should not be restricted to the symptomatic population in the current and/or future outbreaks.

O18. **Development of an animal organoid biobank to study epizootic and zoonotic viruses**

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Organoids are advanced 3D cultures that mimic the architecture of the tissue they are derived from. These cultures are becoming an increasingly relevant alternative method to animal experimentation. IRTA-CReSA is currently developing an organoid biobank of farm and wild animals to study infectious diseases, specially focusing on pigs and poultry. This biobank can be useful to: i) characterise the landscape of potentially susceptible animal species against epizootic threats, ii) identify animal reservoirs and intermediate species for future emerging zoonotic diseases, iii) screen for novel antiviral drugs, and iv) study the pathophysiology and virus adaptation and evolution against antiviral therapies. We have successfully developed a pipeline to generate organoids from nasal turbinates, lungs, and intestines and demonstrated that these organoids are composed of a heterogeneous population of cells that recapitulate the tissue of origin. Initial results infecting organoids with zoonotic coronaviruses (SARS-CoV, MERS-CoV, and SARS-CoV-2) and an avian influenza virus (H1N1 LPAIV) highlight the relevance of these models. Our preliminary data suggest that organoids are useful tools to recapitulate viral infections and to provide a reliable experimental model to accelerate research in emerging pathogens.

O19. **3D remodeling of HCV and SARS-CoV2- infected cells and therapies revealed by cryo-SXT**

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A common feature among positive strand viruses is that they alter cellular membranes to generate replication complexes. Although the origin, nature and structure of these membranous compartments are not identical, they constitute a characteristic feature of these viruses and are observed in yeast, plants and higher eukaryote (+)-strand RNA viruses. In this study we have performed full-field cryo soft X-ray tomography (cryo-SXT) in the water window photon energy range to investigate in whole, unstained cells, the morphology of the membranous rearrangements induced by HCV and SARS-CoV-2 infection in near-native conditions.

These infection alterations in HCV could be reverted by combination of sofosbuvir/daclatasvir, which are clinically approved direct-acting antivirals (DAAs) for the treatment of chronic HCV infection. In addition to providing structural insight into cellular aspects of HCV pathogenesis our study illustrates how cryo-SXT is a powerful three-dimensional wide-field imaging tool for the assessment and understanding of complex cellular processes in a setting of near native whole hydrated cells. Our results also constitute a proof of concept for the use of cryo-SXT at ALBA synchrotron and at lab-scale soft X-ray microscope (SXM) as a platform that enables determining the potential impact of candidate compounds on the ultrastructure of the cell that may assist drug development at a preclinical level.

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