Secció Virologia
12 Novembre 2024

Societat Catalana de BIOLOGIA

Institut d'Estudis Catalans

PROGRAMA I RESUMS

Coordinadores de la Secció de Virologia de la Societat Catalana de Biologia i coordinadores 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

Jurat Premi Millor Tesi Doctoral 2023-2024:
Yaiza M. Castillo de la Peña
Georgios Koutsoudakis
Jordana Muñoz
Ferran Tarres

Amb el suport de:

PROGRAMA

9:00h ACREDITACIONS

9:15h BENVINGUDA Sílvia Bofill-Mas i Susana Guix

9:30-11:00h SESSIÓ I – Virus de plantes, virologia ambiental i bioinformàtica

Chairs: J. López-Moya i D. Vaqué

9:30-10:00h XERRADA INAUGURAL

Reconfigurant virus de plantes com a eines biotecnològiques (20'+10')

José Antonio Darós, Institut de Biologia Molecular i Cel.lular de Plantes, CSIC-Universitat Politècnica de València.

10:00-11:20h PRESENTACIONS ORALS I

O1. **Evolutionary tuning of plant antiviral immunity (8'+2')**

Ignacio Rubio, Centre for Research in Agricultural Genomics (CRAG).

O2. **Niemann-Pick C1 como diana molecular para la resistencia al virus del mosaico del pepino (CMV) y otros virus en cultivo (8'+2')**

Irene Villar, Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB.

O3. **Developing a quantitative transient assay for evaluation of geminivirus Rep protein activity (8'+2')**

Alain Pitty, Institut Químic de Sarrià (IQS), School of Engineering, Universitat Ramon Llull, Barcelona, Spain.

O4. **Revealing Protist-Virus Interactions with Single-Cell Genomics (8'+2')**

Xabier López-Alforja*, Department of Marine Biology and Oceanography, Institut de Ciències del Mar (ICM-CSIC).

O5. **Detection of human viral pathogens in an urban aquifer in the Besòs Delta River (8'+2')**

Cristina Mejías-Molina*, Laboratori de virus contaminants d'aigua i aliments, Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, Universitat de Barcelona.

O6. **Type 2 Vaccine-Derived Poliovirus in Barcelona sewage: an environmental surveillance (8'+2')**

David García-Pedemonte*, Enteric Virus Laboratory, Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, Universitat de Barcelona. Research Institute of Nutrition and Food Safety (INSA-UB).

O7. **Improving Accuracy in Clinical Metagenomics: The Role of Contamination Controls in Bioinformatic Analysis (8'+2')**

Marta Ibañez-Lligoña*, Liver Diseases-Viral Hepatitis, Liver Unit, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Hospital Universitari.

11:20-11:30h PRESENTACIÓ DELS SPONSORS DE LA JORNADA

11:30-11:55h CAFÈ

11:55-13:45h SESSIÓ II. Patogènesi viral

Chairs: N. Izquierdo-Useros i J. Quer

11:55-13:15h PRESENTACIONS ORALS II

O8. **Elucidating Influenza A host tropism in animal-derived organoids (8'+2')**

Gerardo Ceada, IRTA Programa de Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA).

O9. **Recapitulating novel MLB Human Astrovirus -host interactions using human intestinal organoids (8'+2')**

Maria I Costafreda, Enteric Virus Laboratory, Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, Universitat de Barcelona.

O10. **Porcine nasal organoids for respiratory disease modelling (8'+2')**

Noelia Carmona Vicente, IRTA.

O11. **Cytotoxic T cells against African swine fever virus: a dual marker for protection and pathogenesis (8'+2')**

Jordana Múñoz-Basagoiti, Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, CReSA; IRTA, Programa de Sanitat Animal, CReSA; WOAH Collaborating Centre for the Research and Control of Emerging and Re-Emerging Swine Diseases in Europe (IRTA-CReSA).

O12. **Ultrastructure alterations induced by Zika virus on host mammalian cells (8'+2')**

Damià Garriga, ALBA Synchrotron Light Source.

O13. **MMS19 and IFIH1 Host Genetic Variants Associate with SARS-CoV-2 Infection in Elderly Residents of Long-Term Care Facilities (8'+2')**

Sandra Franco, IrsiCaixa Infectious Research Institute.

O14. **Point mutations at specific sites of the nsp12–nsp8 interface dramatically affect the RNA polymerization activity of SARS-CoV-2 (8'+2')**

Sergi Vázquez-Monteagudo*, Structural and Molecular Biology Department, Institut de Biologia Molecular de Barcelona, CSIC.

13:15-13:45h CONFERÈNCIA CONVIDADA

Nuevos avances en el conocimiento del virus de la gripe (20'+10')

Adolfo García-Sastre, Icahn School of Medicine at Mont Sinai, New York, USA.

13:45-14:45h DINAR

14:45-15:45h SESSIÓ III – Immunity, antivirals and vaccines

Chairs: A. Angulo i J. Argilaguet

14:45–15:45h PRESENTACIONS ORALS III

O15. **The Hidden Cost of Immunity: Acute Infections and Vaccine Responsiveness (8'+2')**

Paula Cebollada Rica*, Infection Biology Laboratory, Department of Medicine and Life Sciences, Universitat Pompeu Fabra (UPF).

O16. **Activation of macrophages hampers African swine fever virus replication: implications for live attenuated vaccines (8'+2')**

Aida Tort-Miró, Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA).

O17. **Hepatitis C, SARS-CoV-2 and West Nile infection and antiviral treatments for emerging virus monitored by multimodal imaging (8'+2')**

Ana Pérez-Berna, Mistral beamline, ALBA Synchrotron.

O18. **Novel antiviral strategy: targeting eEF1A successfully blocks cap-dependent & IRES-dependent viral replication (8'+2')**

Eloi Franco Trepat, IRSICaixa, Germans Trias i Pujol Research Institute (IGTP), Universitat Autònoma de Barcelona (UAB).

O19. **Novel platforms based on Dendritic Cells and Vero E6 cells for testing clinically approved drugs to inhibit mpox virus infection (8'+2')**

Rytis Boreika, IrsiCaixa.

15:45-17:00h SESSIÓ IV – Zoonosis Virals

Chair: S. Bofill-Mas

15:45-17:00h **TAULA RODONA**

Vigilància des de la perspectiva OneHealth per prevenir properes pandèmies

Óscar Cabezón, Grup de Recerca en Medicina de la Conservació de la Fauna Salvatge, UAB.

Carme Chacón, Subdirectora de Seguretat Alimentària i Protecció de la Salut, Generalitat de Catalunya.

Natàlia Majó, Directora del Centre de Recerca en Salut Animal (CReSA).

Diana Pou, Unitat de Medicina Tropical i Salut Internacional, Hospital Vall d'Hebron.

17:00-17:15h DESCANS

17:15-17:45h PREMI MILLOR TESI DOCTORAL EN VIROLOGIA 2023-2024.

Chairs: S. Guix i F. Tarrés-Freixas.

L'exposició de virus presents en l'ambient: descripció del viroma i avaluació de risc (20'+10')

Marta Itarte, Laboratori de virus contaminants d'aigua i aliments, Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, Universitat de Barcelona.

17:45h PREMI MILLOR PRESENTACIÓ I COMIAT. S. Guix

(*) Estudiants de doctorat que opten al premi a millor presentació.

ORAL PRESENTATION ABSTRACTS

O1. Evolutionary tuning of plant antiviral immunity

Luis Villar-Martín[1], Tamara Jiménez-Góngora[1], Eric Moner-Ros[1], Paloma Álvarez[1], Ignacio Rubio-Somoza[1]

[1] Centre for Research in Agricultural Genomics-CRAG

Viruses are obligate intracellular parasites that can virtually infect any living organism constituting a major threat for their survival. Accordingly, animals and plants have developed sophisticated molecular mechanisms to cope with viral infections. Comparative studies including animal lineages that have diverged at different evolutionary times, have proven essential in reconstructing the evolution of their antiviral immune programs. In plants, most of our current knowledge about their interaction with viruses come from vascular plants, limiting similar comparative approaches. As a first step to study the evolution of plant-virus interactions we have characterized the molecular interplay between a virus and the liverwort Marchantia polymorpha leveraging transcriptomic assays along with cell biology and biochemistry approaches. Besides the absence of a differentiated vascular system, which is central for plant-virus interactions, Marchantia lacks core elements on antiviral defense present in vascular plants. Additionally, we have compared cell-type specific transcriptomes in Marchantia and the vascular plant Arabidopsis thaliana to ascertain the dynamics of evolutionary tunning of basal antiviral defenses. We will discuss our findings in this communication.

O2. Niemann-Pick C1 como diana molecular para la resistencia al Virus del Mosaico del Pepino (CMV) y otros virus en cultivos

Irene Villar[1,3], and Ana Montserrat Martín-Hernández[1,2]

1) Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, C/ Vall Moronta, Edifici CRAG, Bellaterra (Cerdanyola del Vallés), 08193 Barcelona, Spain. 2) IRTA (Institut de Recerca i Tecnologia Agroalimentàries), Caldes de Montbui, Barcelona, Spain. 3) Universidad de Zaragoza, Zaragoza, Spain* Corresponding author.

En el contexto actual del cambio climático, estamos observando un aumento en la aparición de nuevas cepas virales que amenazan la producción agrícola a nivel mundial. A medida que las enfermedades virales en los cultivos se vuelven más frecuentes y severas, la necesidad de desarrollar plantas resistentes capaces de enfrentar estos desafíos es cada vez más urgente. Sin embargo, los enfoques actuales presentan limitaciones importantes, ya que a menudo son específicos para un solo tipo de virus y, con el tiempo, pierden eficacia ante la evolución de nuevas variantes virales. Por lo tanto, identificar nuevas dianas moleculares de amplio espectro, aplicables a múltiples especies, es crucial para el futuro de la agricultura, la economía y la sociedad. En esta charla, presentaré nuestra investigación dirigida a proporcionar una solución efectiva a este creciente problema fitopatológico. Nos centramos en la proteína Niemann-Pick C1 (NPC1), un transportador de esteroles transmembrana que desempeña un papel crucial en la entrada de virus en mamíferos, como el ébola, Zika y SARS-CoV-2. Partiendo de esta base, exploramos la función de NPC1 en las plantas. Nuestros resultados revelan que NPC1 es esencial para la propagación sistémica del Virus del Mosaico del Pepino (CMV) en melón, facilitando el movimiento del virus. A través de la modificación de la expresión de los genes NPC1, hemos conseguido bloquear la propagación del CMV, reduciendo significativamente la infección. Además, hemos conseguido demostrar su relación con el movimiento, no solo del CMV, sino también de otros virus.
En resumen, nuestros resultados posicionan a NPC1 como una diana molecular clave para desarrollar resistencia antiviral en plantas, ofreciendo una solución robusta y potencialmente aplicable a otros cultivos.

O3. Developing a quantitative transient assay for evaluation of geminivirus Rep protein activity

Alain Pitti Djida[1,2], Tarik Ruiz[2], Araceli G. Castillo[3], Eduardo R. Bejarano[3], Pablo Leivar[1], Roger Estrada[1], Jordi Teixidó[1], Ana Montserrat Martín[2], Juan José López-Moya[2]

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Replication of viruses frequently depends on essential and specific activities provided by viral proteins that complement hijacked cellular functions. This is particularly evident in the case of the multifunctional Rep protein found in many viruses with circular single-stranded DNA genomes, such as circoviruses and plant-infecting geminiviruses, all belonging to the group denominated CRESS DNA viruses (circular Rep-encoding single-stranded DNA), where Rep initiates the rolling circle replication and plays its crucial roles through specific recognition of an intergenic region (IR) within the viral ssDNA genome. To identify compounds that could effectively inhibit the activity of Rep, we have selected the begomovirus Tomato yellow leaf curl Sardinia virus (TYLCSV) as model organism, and we have developed a transient expression assay in the presence of different virus-derived RNA silencing suppressors (RSSs) to facilitate the production of the Rep protein in Nicotiana benthamiana leaves. The assay serves to quantify Rep activity by transiently co-expressing Rep with RSS and a reporter construct, denominated 2IR-GFP, that contains a specific IR sequence flanking the GFP gene. In the presence of Rep, the reporter construct produces episomal molecules boosting GFP expression. We confirmed that the system served to evaluate TYLCSV Rep activity by assessing GFP expression in the presence of different RSSs. Also, the specificity of GFP expression was confirmed for the homologous TYLCSV Rep through comparative analysis using Rep and 2IR-GFP constructs derived from a different geminivirus in reciprocal combinations. Regarding RSSs, we observed certain leaky expression of GFP in some cases, with optimal results achieved using the RSS derived from a begomovirus. This experimental system could be potentially used for a future screening of Rep inhibitors, and it could contribute to select compounds with potential antiviral activity against replication of CRESS DNA viruses.
[Funding provided by grant PID2022-139376OB-C33]

O4. Revealing Protist-Virus Interactions with Single-Cell Genomics

Xabier López-Alforja[1], Felipe H. Coutinho[1], Thomas Hackl[2], Guillem Marimon[1], David López-Escardó[3], Manuel Martínez García[4], Ramon Massana[1], & Dolors Vaqué[1]

(1) Department of Marine Biology and Oceanography, Institut de Ciències del Mar (ICM-CSIC), Barcelona, Spain
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(3) Oceanographic Observatory Banyuls-sur-mer, CNRS, France
(4) Department of Fisiologia, Genetica y Microbiologia, Universidad de Alicante (UA), Alicante, Spain

Protists, or unicellular eukaryotes, play vital roles in marine ecosystems, functioning as primary producers, predators, decomposers, and parasites. Viruses that infect these microeukaryotes are critical in regulating their abundance and diversity through lytic activity, which releases dissolved and particulate organic material that supports other microorganisms. Despite their recognized ecological significance in the ocean, knowledge on giant virus and native host interactions remains limited, and a comprehensive understanding of their life cycles and ecological impact is still lacking. Traditionally, studies on eukaryotic viruses have relied on host culture experiments or metagenomic approaches that generate Metagenome-Assembled Genomes (MAGs). While these surveys provide valuable insights, they often fail to reveal specific virus-host relationships at the single-cell level. Our study presents a more attractive approach using Single Amplified Genomes (SAGs) to investigate the interactions between viruses and marine protists. In Blanes Bay, we analyzed approximately 400 SAGs representing various unicellular marine eukaryote lineages, using metrics such as contig coverage and GC content to identify infections by giant viruses from the Nucleocytoplasmic Large DNA Virus (NCLDV) group. As a result, we identified 42 virus-host interactions, linked to viral orders such as Pandoravirales, Imitervirales, and Algavirales. Notably, our research highlights a previously unreported diversity of marine stramenopiles associated with these viruses. This single-cell approach provides unprecedented insights into virus-host dynamics within coastal marine ecosystems, shedding new light on the diversity and ecological roles of giant viruses and their protist hosts.

O5. Detection of human viral pathogens in an urban aquifer in the Besòs Delta River

Cristina Mejías-Molina [a,b], Ignasi Estarlich-Landajo [a] , Sandra Martínez-Puchol [a,c], Sílvia Bofill-Mas [a,b], Marta Rusiñol [a,b]

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Approximately half of the world's population use groundwater as a freshwater resource. Aquifers are susceptible to microbial contamination through multiples sources. Although viruses are reported as the main cause of water-related outbreaks, monitoring or understanding how virus behave in urban groundwaters is still poor. The study site is located within the aquifer of the Besòs River Delta (Catalonia, Spain) with groundwater recharge originated from rainfall and runoff infiltration, sewer network exfiltration, water supply network losses and the Besòs river water, which has a flow that mostly derives from the effluents discharged by the 27 wastewater treatment plants situated within the river. The aquifer's groundwater is currently used for garden irrigation and street cleaning. However, due to the actual water scarcity issues, some initiatives focusing on the use of groundwater as a drinking water source, enhance the importance of understanding the pollution sources impacting the quality of urban groundwater

This study was conducted during a 7-month surveillance campaign. The study aimed to assess the presence of 8 viral pathogens using qPCR (Human adenovirus (HAdV), Norovirus I (NoV GI) and II (NoVGII), rotavirus (RoV), influenza A virus (IAV), SARS-CoV-2, Enterovirus (EV) and hepatitis E virus (HEV) and a process control (Pepper mild mottle virus, (PMMoV)) and explore groundwater virome diversity using a time integrated approach (based on passive samplers) and target enrichment sequencing.

The results of qPCR showed human faecal contamination in nearly the 50% of the groundwater samples and occasional detections of EV (December and January), NoV GI (October and January) and NoV GII (October). Target enrichment sequencing enabled the detection of 21 viral families. The highest number of assignments belonged to viruses infecting animals such some members of the Circoviridae and Parvoviridae families infecting bats, birds, rabbits and cattle. Human pathogens were detected with a low relative abundance of reads belonging to Adenoviridae, Caliciviridae, Herpesviridae, Picornaviridae, Polyomaviridae and Reoviridae families. Also, members of the Baculoviridae, Dicistroviridae, Ifliviridae and Iridioviridae families infecting cockroaches, butterflies, flies, vespa and mosquitoes were detected in groundwater samples.

The study of viral occurrence in groundwater can provide data that is useful for understanding the microbiological pollution of this water source, as well as its origins, which could be critical before using this water for various purposes.

O6. Type 2 Vaccine-Derived Poliovirus in Barcelona sewage: an environmental surveillance

David García-Pedemonte[1], Albert Carcereny[1], Albert Blanco1, Maria I Costafreda[1], Jacobo Mendioroz[2], Margarita Palau[3], Belén Galofré[4], Miquel Paraira[4], Rosa M Pintó[1], Susana Guix[1], Albert Bosch[1]

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[4] Aigües de Barcelona, Barcelona, Spain.

Poliovirus is the causative agent of poliomyelitis, an infectious disease that can lead to flaccid paralysis and, in extreme cases, death. Poliovirus has been at the verge of eradication for many years now with only two countries remaining endemic for wild-type poliovirus. Nevertheless, many circulating vaccine-derived poliovirus (cVDPV) strains are reported in countries with low vaccine coverage and where oral poliovirus vaccines are being administrated. In 2022, free-polio regions such as New York, London and Israel detected cVDPV in their sewage, and one case of acute flaccid paralysis was diagnosed in New York.

In Catalonia, two imported cases have been detected in clinical respiratory samples in the last few years. In 2019, VDPV types 1 and 3 infections were notified in an asymptomatic person with an immunodeficiency, of which only the VDPV3 was detected in sewage samples. In February 2024, a Sabin-like type 1 PV was identified in a 2-year-old. This case was not detected in sewage.

Sewage samples from two wastewater treatment plants in the metropolitan area of Barcelona covering the entire city's population were bimonthly analysed since 2018 on a regular basis. An amplicon-based deep sequencing method using the Oxford Nanopore technology to determine a 400-bp region of the VP1 gene was used with the aim to perform an enterovirus diversity surveillance.

On September 16, 2024, a cVDPV-2 was detected in a sewage sample, accounting for a 4.89% of the total sample reads. For further confirmation, a 1000-bp fragment covering the entire VP1 gene and a 4000-bp fragment covering a region from the 5'UTR to the 2B gene were sequenced, confirming that a VDPV-2 related to a Nigerian strain described beforehand was circulating. After the initial detection, an enhanced wastewater surveillance was performed, and sewers from different parts of the metropolitan area of Barcelona were weekly monitored for a month. No more positive samples were detected.

This is the first time a cVDPV-2 has been reported in a sewage sample in Spain and Europe, thus highlighting the use of wastewater surveillance as a complementary sanitation tool. Detection of cVDPV-2 in a new region highlights the interconnectedness of global health and underscores the critical need for maintaining high vaccination rates.

O7. Improving Accuracy in Clinical Metagenomics: The Role of Contamination Controls in Bioinformatic Analysis

Marta Ibañez-Lligoña(1,2), Sergi Colomer-Castell(1,3,4), Carolina Campos(1,3,4), Arnau Llauradó (5,6,7), Daniel Sánchez-Tejerina(2,5,6,7), Ariadna Rando-Segura(2,8), Cristina Andrés(8), Mar Riveiro-Barciela(1,3), Maria Buti(1,3), Amanda Cano(9,10), Agustin Ruiz(9,10), Esther Del Barco(11,12), Tomàs Pumarola(8,13), Andrés Antón(8,13), Maria Goya(11,12), Raúl Juntas-Morales(2,5,6,7), Josep Quer(1,2,3,4,*).

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(11) Maternal-Fetal Medicine Unit, Department of Obstetrics, Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain.
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Background: Accurate disease diagnosis is essential for e2ective treatment, as diagnostic errors can lead to delays, ine2iciency, increased mortality, and higher healthcare costs. Metagenomics, a branch of next-generation sequencing (NGS), allows the genetic identification of both known and unknown genomes across a wide range of clinical samples. However, this technique can also amplify contamination present in the samples, making it crucial to track and remain mindful of potential contaminants throughout the process to avoid misleading results, specifically in the clinical setting. A well-designed bioinformatic pipeline is also necessary to handle the large amount of data generated.
This study aimed to analyze metagenomic data from various clinical samples and to validate the technique's e2icacy while minimizing the risk of contamination through the addition of technical controls.
Methods: RNA and DNA were extracted from plasma/serum, stool, cerebrospinal fluid, and amniotic fluid samples using the QIAamp MinElute Virus Spin kit (QIAGEN). Both negative controls and positive samples (from HEV-infected patients) were included.
Libraries were prepared using the TruSeq Stranded Total RNA kit (Illumina) and sequenced on the NextSeq2000 platform. Bioinformatic analysis included quality control to remove contaminants and duplicates using tools like Trimmomatic and Bowtie2. Short reads and contigs assembled with MEGAHIT were taxonomically classified using Kraken2 with the RefSeq database.
Results: The full HEV genome was successfully recovered from one plasma and stool sample. The stool sample generated over 5000 contigs, allowing the identification of additional viral and bacterial genomes, including Cosavirus E (Picornaviridae). However, negative controls also yielded bacterial and viral sequences, some of which overlapped with those in clinical samples, underscoring the importance of tracking contamination sources closely and di2erentiating them from genuine findings.
Conclusion: Metagenomics is a powerful tool for recovering whole viral genomes and identifying additional microbial entities within clinical samples. However, contamination can be amplified alongside genuine sequences, making it crucial to include controls that help di2erentiate between contaminants and true pathogens. Further research is needed to optimize the technique for accurate diagnosis and infection monitoring.

O87. Elucidating Influenza A host tropism in animal-derived organoids

Gerardo Ceada[1,2,3], Ferran Tarrés-Freixas[1,2,4], Nuria Navarro[1,2], Marta Pérez-Simó[1,2], Noelia Carmona-Vicente[1,2], Laura Bonillo-Lopez[1,2], Alejandro Moreno[1,2], Carlos López[1,2], FarmBank Consortium[1,2,5,6,7], Natàlia Majó[2,8], Joaquim Segalés[2,8], Karl Kochanowski[1,2], Júlia Vergara-Alert[1,2]

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Animals play a crucial role in the transmission of high-risk pathogens and zoonotic diseases. However, the current gold standard to determine the susceptibility of an animal species to a given pathogen, namely experimental infection experiments, is often limited to animals that can be readily accessed, thus excluding many wildlife species. In this context, organoids offer a promising approach for studying infectious diseases because of their physiological relevance and capacity for long term maintenance. While mouse and human derived organoids are extensively used in biomedical research, organoids from livestock and wildlife species remain largely unexplored. To address this gap, we aimed to generate a comprehensive repository of organoids from various animal species to serve as experimental models for infectious disease research.

We collected tissue samples from the respiratory tracts from livestock and wildlife animals both free ranging and captive) in Catalonia, Spain Animal species included in this work were pig, chicken, mink, buzzard, alpaca, red panda, gazella, monkey, roe deer and wolf. Tissues underwent enzymatic digestion to isolate epithelial cells which were then embedded in matrigel to generate organoids. These organoids were seeded as monolayers on Matrigel coated plates, providing access to the apical surface of the cells. By infecting these organoid monolayers with two different influenza A virus strains, we identified striking differences in susceptibility across the tested ani m al species. Importantly, these differences recapitulate the known host tropism pattern for these influenza A strains.

Overall, our results highlight the power of organoids as platforms to assess pathogen's tropism. The comprehensive biobank of organoids derived from livestock and wildlife animals reported here serves as a valuable resource for advancing in the detection, prevention, and treatment of current and emerging infectious diseases.

O9. Recapitulating novel MLB Human Astrovirus -host interactions using human intestinal organoids

Maria I Costafreda (1,2), Alba Arrebola (1,2), Cristina Fuentes (1,2), Albert Carcereny (1,2), Albert Bosch (1,2), Rosa M Pintó (1,2) and Susana Guix (1,2)

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The gastrointestinal (GI) tract is the entry point of numerous pathogens. While classic human astroviruses (HAstV) are an important cause of gastroenteritis in children, the elderly and immunocompromised, novel HAstVs have also been associated with extra-intestinal infections affecting the central nervous system. This work aims at implementing novel human organoid models to unveil the viral entry process and characterize the virus egress as naked and/or quasi-enveloped particles, which may affect virus dissemination to other organs.
We used Human Intestinal Enteroids (HIEs), as these systems can recapitulate many physiological features of the intestine. HIEs obtained from the adult jejunum (J2 line) were maintained in Matrigel and complete Intesticult medium, and seeded as undifferentiated or differentiated 2D-monolayers for infection. Differentiation status was assessed by expression of specific gene markers. Infections were performed apically with MLB1 and MLB2 HAstV stocks and replication was analyzed by RTqPCR and immunofluorescence assays. Released viral particles were characterized by iodixanol density gradients to estimate the abundance of naked and quasi-enveloped virions.
J2 HIEs support replication of MLB1 and MLB2 HAstVs, after infection of cells through the apical compartment, regardless of the cell differentiation status, without causing significant cytopathic effect nor loss of epithelial barrier permeability. MLB1 and MLB2 strains show different release kinetics at the basolateral compartment and different proportions of naked and quasi-enveloped particles. This study shows that MLB1 and MLB2 may have different pathogenic determinants and highlights that HIEs provide an improved model to unveil viral pathogenesis mechanisms to better understand which factors promote virus entry and extraintestinal dissemination.
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O10. Porcine nasal organoids for respiratory disease modelling

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Background. The nasal epithelium is the first barrier in direct contact with the external environment providing protection from viral, bacterial and fungal pathogens, as well as pollutants in the air. This tissue is also responsible for mucociliary clearance. Interactions between the nasal epithelium, commensal nasal microbiota, and respiratory pathogens play a key role in respiratory infections. Currently there is a lack of realistic and scalable experimental models mimicking the in vivo conditions to study such interactions. To tackle this issue, we have successfully established and characterized the first Porcine Nasal Organoid (PNO) system from nasal tissue of pigs as well as from cytological brushes.

Results. PNOs preserved the self-renewal of nasal-resident stem cells allowing to indefinitely expand the organoid culture while recapitulating the cell diversity of the native tissue, as we confirmed by PCR and IFA. Similar to nasal turbinates from pigs, PNOs were composed of proliferative cells, goblet cells (mucus production), basal cells, ciliated cells (cilia formation) and tight junctions, showing an in-vivo like stratification. To evaluate the potential of PNOs as a respiratory model we used the PNOs to study host-bacterial interactions using nasal colonizers of pigs. PNOs were inoculated with porcine commensal strains of *Moraxella pluranimalium, Rothia nasimurium* and the pathobiont *Glaesserella parasuis* for examining host-commensal-pathogen interactions. All strains adhered to the PNOs, although at different levels. Colonizers did not affect the integrity of PNO monolayers, whereas virulent *G. parasuis* disrupted the tight junctions and affected the production of mucus. Besides, *M. pluranimalium* and *G. parasuis* strains stimulated the production of proinflammatory cytokines, whereas *R. nasimurium* induced the production of IFNγ and diminished the proinflammatory effect of the other strains.

Conclusions. Overall, PNOs are a good proxy of the nasal mucosa and are significant and useful for understanding the role of host-microbe interactions at the nasal epithelium.

In addition, PNOs can be taken into consideration for studies to predict future transmissions and pandemics, as pigs are natural reservoirs for a wide variety of zoonotic pathogens. Finally, our study also supports the reduction of the number of animals used in experimental studies.

O11. Cytotoxic T cells against African swine fever virus: a dual marker for protection and pathogenesis

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African swine fever (ASF) is a lethal haemorrhagic disease of pigs currently causing a pandemic that results in a significant economic impact in the global porcine industry. Attempts to manage the disease are hampered by the absence of effective vaccines, with live attenuated vaccines (LAV) being the most advanced strategies despite the biosafety concerns associated. The rational development of safer vaccines is limited by the scarce knowledge on ASF immunity, including the lack of correlates of protection. Although LAV induce both strong adaptive humoral and cellular responses, the analyses of these responses conducted so far have not been able to predict the outcome of infection following a lethal challenge. In previous studies, we already shown that the LAV prototype BA71ΔCD2, developed in our lab, induces broad cytotoxic memory responses during the recall response in pigs vaccinated with an optimal dose. However, in another study we did associate a blood cytotoxic transcriptomic signature with ASF pathogenesis. In the present study, we aimed to better understand these findings by characterizing the cytotoxic cell subsets involved in these two immunological and virological contexts. First, we suboptimally vaccinated pigs with the LAV prototype BA71ΔCD2, consciously inducing partial protection before a lethal challenge. As expected, neither ASFV-specific antibody titters nor IFNγ-producing blood cells levels present before challenge associated with protection after challenge. In contrast, flow cytometry analysis of cytotoxic blood cells upon in vitro ASFV-specific stimulation showed that elevated levels of perforin-producing CD8αβ+ T cells significantly correlated with a protective status. Second, we analysed ex vivo the percentages of cytotoxic cells in blood and tissues from animals infected with the virulent Georgia 2007/1 ASFV strain. The results showed a high percentage of NK, CD4+CD8αβ+ and CD8αβ+ T cells in animals with overt ASF clinical signs and close to death from ASFV infection. Importantly, the levels of these cytotoxic cells positively correlated with viral loads, indicating their contribution to the ASFV-induced systemic uncontrolled immune response. Taken together, these results highlight the central role of cytotoxic responses in ASF immunity. Importantly, we show a dual role of CD8αβ+ T cells, demonstrating its importance as a correlate of protection in immunized pigs, while also being associated to virus-induced pathology during the late stages of a lethal infection. This study provides valuable insights for the development of rational vaccination strategies against ASF.

O12. Ultrastructure alterations induced by Zika virus on host mammalian cells

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Zika virus (ZIKV) is a mosquito-borne flavivirus that gained attention of the scientific and healthcare communities due to the epidemic that swept through South and Central America in 2014-2016, owing to the association of this virus with the occurrence of congenital deformities, particularly microcephaly in infants born to the infected mothers, as well as other severe neuropathies. [1-2]

ZIKV belongs to the Flaviviridae family and is closely related to the other flaviviruses, including dengue virus, West Nile virus, yellow fever virus and chikungunya virus. The virus has an enveloped, icosahedral capsid about 50 nm in diameter, harboring a monopartite, linear (+) ssRNA genome.

A previous study combining immunofluorescence microscopy with electron tomography showed ZIKV infection in human hepatoma and neuronal progenitor cells induces a drastic structural modification of the cellular architecture, leading to the formation of viral replication factories surrounded by a network of microtubules and intermediate filaments [3-4]. To obtain further details of the cellular context of these ultrastructure alterations, human glioblastoma U251 cells were grown on support grids and infected with Zika virus American strain. After vitrification by plunge freezing, infected cells were imaged by soft X-ray tomography in BL09-MISTRAL beamline, at ALBA synchrotron [4]. The acquired tomograms were used to generate 3D maps covering the whole cell volume, allowing not only for the identification of several virus-induced structures, such as formation of viral factories and budding sacks filled with newly formed particles, or the alteration of cell cytoskeleton, but also informing on the location and extent of such alterations in the context of the whole cell.

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O13. MMS19 and IFIH1 Host Genetic Variants Associate with SARS-CoV-2 Infection in Elderly Residents of Long-Term Care Facilities

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The coronavirus disease 2019 (COVID-19) pandemic has significantly affected older adults. Identifying host COVID-19 susceptibility genes in elderly populations remains a challenge. Here, we aimed to identify host genetic factors influencing the susceptibility to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. We genotyped 12 single nucleotide polymorphisms (SNPs) previously associated with the innate immune response in a total of 97 elderly (age >65 years) residents of three long-term care facilities located in Barcelona. Individuals were PCR-tested during SARS-CoV-2 outbreaks between September and November 2020. SARS-CoV-2 PCR tests revealed infections in 81 residents. Importantly, the 16 uninfected residents remained SARS-CoV-2 seronegative until vaccination (January and February 2021). After adjusting for sex and age, we found that two SNPs were significantly associated with SARS-CoV-2 infection susceptibility: MMS19 nucleotide excision repair protein homolog (MMS19)/rs2236575 ($p = 0.029$) and interferon-induced helicase C domain-containing 1 (IFIH1)/rs1990760 ($p = 0.034$). No association with SARS-CoV-2 infection was found for the 10 additional genotyped SNPs, which included 4 SNPs on chromosome 12 in the gene encoding oligoadenylate synthetase (OAS). Our results indicate that MMS19/rs2236575_A and IFIH1/rs1990760_TC genetic variants were associated with resistance to SARS-CoV-2 infection in a cohort of institutionalized seniors.

O14. Point mutations at specific sites of the nsp12–nsp8 interface dramatically affect the RNA polymerization activity of SARS-CoV-2

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RNA viruses, like the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), exhibit a high degree of variability that difficult viral disease control. A previous study identified 41 amino acid substitutions in the non-structural protein 12 (nsp12), the main subunit of the RNA-dependent RNA polymerase (RdRp) complex which also contains the co-factors nsp8 and nsp7. Eight mutations were chosen for in vitro characterization, and some other mutations of the RdRp complex were designed based on the structures available on the Protein Data Bank (PDB).

Mutations were introduced into a pRSFDuet-1 plasmid coding for the RdRp complex (nsp12-nsp8-nsp7), which allowed the expression of the complex in E. coli. The recombinantly expressed complexes were purified in three consecutive steps. The polymerization activity of each mutant was studied with in vitro primer extension assays using different fluorescently-labelled Primer/Template (P/T) duplexes, reaction temperatures, and polymerization times. Furthermore, the ability of each of the mutated complexes to bind RNA was studied with the same P/T duplexes through Electrophoresis Mobility Shift Assays (EMSAs).

Substitutions close to the nsp12 active site (D499G, V560A and M668V) produce little differences in the RdRp activity compared to the wild-type (wt). Meanwhile, mutations involving the binding region of the nsp12 fingers domain with the closest nsp8 promote significant changes in the polymerase activity of the complex. This binding region contains a hydrophobic cluster involving residues L372, V373 and L527. Substitutions that weaken the hydrophobicity of this cluster (L372P, V373A or L527H) reduce the ability of nsp12 to elongate the RNA, specially L372P and L527H which greatly impact the hydrophobicity of the region. On the contrary, mutation L372F, which introduces a bulkier hydrophobic residue, displays an increase in activity. Mutations that were designed to disrupt this nsp12-nsp8 interaction (V330S, V341S and nsp8-R111A/D112A) have a considerable effect on polymerization rates, although not as dramatic as L372P, L527H or nsp8-Δ1-76. This last mutation deletes part of the RNA-binding region of nsp8 and causing the complex to show extremely low activity.

Our research reveals a contact interface involving a hydrophobic cluster in the nsp12 fingers subdomain that is critical for the modulation of the SARS-CoV-2 RdRp activity offering an alternative towards antiviral design by targeting this interaction.

O15. The Hidden Cost of Immunity: Acute Infections and Vaccine Responsiveness

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Acute viral infections typically resolve within weeks, but their impact on the host can persist long after the virus is cleared. While it's often assumed that recovered hosts develop virus-specific immune memory without other immunologic alterations, this is not always the case. Many viruses can cause long-term sequelae after the acute infection phase, known as post-acute infection syndromes (PAISs). The mechanisms underlying PAISs remain poorly understood, partly due to a lack of suitable model systems that connect physiological changes to post-infection outcomes. To investigate the immunological aspects of post-acute viral infection sequelae, we have used the acute LCMV-Docile infection mouse model.
Our results suggest that controlling an acute viral infection may come at the cost of temporary immunosuppression. This could leave the host vulnerable to subsequent infections, particularly those that rely on cellular immunity for control. This finding challenges the conventional understanding of post-infection immune status and highlights the complex, long-lasting effects that even resolved viral infections can have on host physiology and immune function.

O16. Activation of macrophages hampers African swine fever virus replication: implications for live attenuated vaccines

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African swine fever (ASF) is a devastating disease causing a worldwide pandemic that results in severe economic consequences for the porcine industry. Lack of efficient vaccines hampers its control, and the insufficient knowledge regarding the immunological mechanisms underlying protection hinders rational vaccine design. Although biosafety concerns hold back their implementation in the field, some live attenuated vaccines (LAV) have shown a high effectiveness against ASF. Besides their potential application in endemic countries, LAV are also an essential tool to gain knowledge on ASF protective immunity. We have previously demonstrated that vaccination of pigs with the LAV prototype BA71ΔCD2 induces ASF virus (ASFV)-specific memory T cells, which trigger an IFNγ-induced activation of macrophages during a recall response. To validate the physiological relevance of this early activation of macrophages in controlling AFSV replication, here we analyzed ASFV replication in porcine alveolar macrophages treated with different stimulatory compounds: LPS, IFNγ and the bacterium *Rothia nasimurium*. The results show that activated macrophages are less susceptible to ASFV infection and highlight the importance of a prompt innate immune response to reduce virus spread in immune pigs, indicating the antiviral potential of these immunostimulants. Moreover, treated macrophages also showed higher resistance to the porcine reproductive and respiratory syndrome virus (PRRSV), a relevant respiratory pathogen that replicates in macrophages as well. Importantly, macrophage activators also promoted the production of cytokines by cells infected with the LAV BA71ΔCD2, thus changing the immunological context induced by the vaccine and suggesting their potential role as adjuvants. To validate this hypothesis, pigs were intranasally vaccinated with the LAV BA71ΔCD2 combined or not with *R. nasimurium*. Results showed that *R. nasimurium* hampered the vaccine-induced ASFV-specific humoral and cellular responses, observing a higher mortality after a lethal ASFV challenge. The decrease of vaccine efficacy is probably a consequence of the reduction of available antigens due to a lower replication rate of the LAV virus in activated macrophages also in vivo. In conclusion, the present work helps to better characterize the role of innate immunity against ASFV infection, to identify novel antiviral compounds against two relevant porcine viruses targeting macrophages, and to evaluate the potential effects of the use of adjuvants for ASF vaccines based on live attenuated viruses.

O17. Hepatitis C, SARS-CoV-2 and West Nile infection and antiviral treatments for emerging virus monitored by multimodal imaging

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Recent advancements in virology have revealed a common feature among positive-strand viruses: the alteration of cellular membranes to form replication complexes. Despite variations in origin and structure, these membranous compartments, classified as double-membrane vesicles (DMVs) or invaginated vesicles (IVs), are observed across diverse viruses, including hepatitis C virus (HCV) and SARS coronavirus. Notably, West Nile virus (WNV) generates IVs, suggesting a conserved strategy across distant viruses.
In this study we have performed infrared microscopy, confocal immunofluorescence and correlative cryogenic light-soft X-ray tomography (CLXT) in the water window photon energy range to investigate in whole, unstained cells, the morphology of the membranous rearrangements induced by WN, HCV and SARS-CoV-2 infection and after antiviral treatments in near-native conditions. These infection alterations could be reverted by combination of different antiviral treatment and monitored the healing process by multimodal imaging techniques. In addition to providing structural insight into cellular aspects of viral 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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O18. Novel antiviral strategy: targeting eEF1A successfully blocks cap-dependent & IRES-dependent viral replication

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Plitidepsin, a small molecule originally approved as an antitumoral agent, targets the eukaryotic translation elongation factor 1A (eEF1A) blocking the protein synthesis machinery. Plitidepsin has shown antiviral activity in vitro & in vivo, as well as safety and efficacy in distinct clinical trials with COVID-19 infected individuals (NCT04382066, NCT04784559, NCT05705167). Here we aimed to study the molecular fingerprint of plitidepsin to understand the cellular changes induced by the treatment in the signaling landscape beyond eEF1A inhibition.

Transcriptome analysis revealed that plitidepsin treatment at 50 nM effectively inhibited all major SARS-CoV-2 viral transcripts, including the envelope (E), nucleocapsid (N), and spike (S). These effects were further confirmed via proteomics, where all viral proteins were downregulated while no major changes were observed in cellular proteins. Less than 13% of the host proteome was affected by plitidepsin, thus preserving cellular viability. In response to plitidepsin, cells upregulated EIF2AK3 and additional proteins that reduce cap-dependent translation. Yet, other proteins supported proteostasis via ribosome synthesis and cap-independent translation routes, which relied on eIF4G2, PABPC1, and readers such as IGF2BP2. We therefore identified an underlying cap-independent route preserved upon plitidepsin treatment. Translation mediated by internal ribosome entry site (IRES) was inhibited whereas translation mediated by N6-methyladenosine (m6A) was unaffected thanks to the IGF2BP2 m6A-reader activity. This unique molecular fingerprint enabled us to predict the potential activity of plitidepsin in front of different infections. Viruses such as HIV that are translated via m6A were unaffected by plitidepsin. In contrast, viruses that translate through IRES and cap-dependent pathways, including hepatitis C virus (HCV), respiratory syncytial virus (RSV), and Middle East respiratory syndrome coronavirus (MERS-CoV), were effectively inhibited by plitidepsin.

O19. Novel platforms based on Dendritic Cells and Vero E6 cells for testing clinically approved drugs to inhibit mpox virus infection

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Monkeypox virus (MPXV) is the causative agent of mpox, endemic in West and Central Africa and responsible for an international outbreak in 2022 which continues to this day. A new MPXV clade Ib recently led to a novel public emergency of international concern declared by the WHO. Currently, there is no antiviral treatment with proven efficacy for mpox, highlighting the need for efficient countermeasures for the prevention and treatment of already infected individuals. The development and characterisation of an in vitro cell model could substantially accelerate the identification of novel antivirals to effectively treat and control mpox, and further elucidate the effect of MPXV on immune cells. We first optimised MPXV infection on Vero E6 and human monocyte derived dendritic cell (DCs) using confocal microscopy and flow cytometry. The analysis indicated a higher percentage of immature DCs were positive for MPXV as compared to antigen-presenting mature DCs activated in the presence of LPS. Confocal analysis showed MPXV association with DC cell surface membranes and viral internalization and replication in the cytoplasm of the cells. We pulsed Vero E6 cells and DCs with different clinically approved drugs in combination with a 2022 MPXV clade IIb strain and measured the infection outcome by FACS. Interferons, cyclodextrins, intravenous immunoglobulins, EIPA and hydroxychloroquine showed no protection against MPXV. Viral replication in Vero E6 cells and DCs was effectively inhibited by the preclinical cathepsin inhibitor CA074, molnupiravir, metoprolol, and itraconazole. The identification of optimal cellular models for MPXV infection is essential in discovering novel antivirals and studying their effects on MPXV strains in vitro. In our study, clinically approved drugs showed protection against MPXV and should be further tested for their potential to serve as treatments for mpox disease and other Orthopoxviruses.