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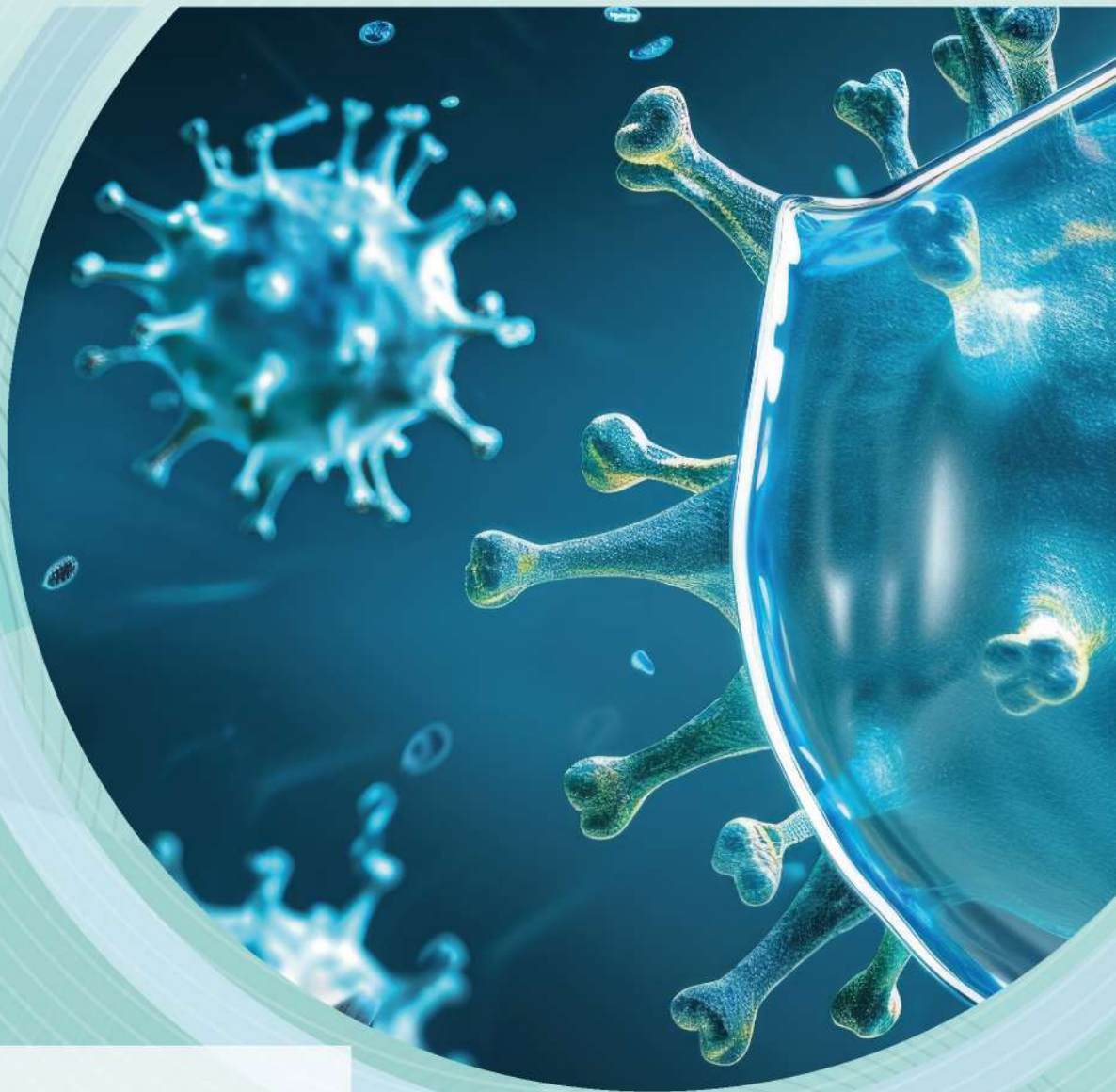
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NIDO2026

The XVIIth **International Nidovirus Symposium**



24 - 27 June 2026

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25 June 2026 (Thursday) 0845-0930

MERS: Knowledge gaps

First Author: Peiris Malik, The University of Hong Kong

Zoonotic coronaviruses have caused repeated pandemics over the past 300 years and pose significant future pandemic threats. MERS coronaviruses (MERS-CoV) have caused repeated large outbreaks in humans, with up to four generations of human-to-human transmission. We identified human-adaptive amino acid substitution (nsp6 L232F) which repeatedly emerges following zoonotic transmission in MERS-CoV clade B viruses. This mutation is associated with increased viral replication competence in human ex-vivo respiratory tissue cultures and in lungs of experimentally infected hDPP4 knockin mice. Although zoonotic MERS cases have apparently declined in the Arabian Peninsula in recent years, the reason for this decline is unclear. Early case detection and improved infection prevention & control, genetic changes in recent clade B viruses or cross-reactive population immunity arising SARS-CoV-2 infection or vaccination are possible explanations for this decline. While mutations in MERS-CoV spike in some recent MERS-CoV is associated with reduced replication competence in humans, this is not uniformly observed in recent MERS-CoV. Prior SARS-CoV-2 vaccination and infection is associated with cross-reactive antibody to spike S2 (but not S1) domain. Thus, prior SARS-CoV-2 infection or vaccination may have affected population susceptibility to MERS-CoV, a possibility that deserves further investigation. Although zoonotic MERS-CoV so far has only been reported in the Arabian Peninsula, the vast majority of MERS-CoV infected dromedary camels are found in Africa (>75% of the global dromedary population) and in Central Asia, where no zoonotic disease has been reported. MERS-CoV across Africa belong to MERS-CoV clade C, which has lower replication competence in ex vivo cultures of the human respiratory tract and hDPP4 knockin mice, than clade B viruses currently dominant in the Arabian Peninsula. This may indicate a virus with lower human pathogenicity in Africa. However, specific serological and T cell responses suggest that unsuspected human MERS-CoV infection continues to occur in Africa. Thus, pandemic MERS may well emerge outside the Arabian Peninsula, in a setting of low awareness of MERS as a human disease. There is also a recent report of clade B MERS-CoV detection in camels in Egypt. There is no genotypic or phenotypic information on the MERS-CoV circulating in dromedary camels in Central Asia. Greater awareness and research efforts of MERS-CoV threats beyond the Arabian Peninsula is critically important.

26 June 2026 (Friday) 0845-0930

On a journey with coronavirus replication organelles: how far have we come?

First Author: Helena Maier, The Pirbright Institute

Coronaviruses are a significant family of pathogens causing disease in humans and animals resulting in important economic and health impacts. As a family of viruses with a proven ability to cross the species barrier with potentially devastating effects, it is critical to identify control measures likely to function across the whole family, including currently unknown viruses. To facilitate this, a deep understanding of critical and conserved steps of the coronavirus life cycle is needed.

Replication of coronaviruses is associated with the rearrangement of cellular membranes to form the viral replication organelle. This membranous compartment provides a protected environment for viral RNA synthesis, and its formation is considered essential for virus replication. In addition, the morphology of the replication organelle is now recognised to be highly conserved across all four coronavirus genera comprising both double membrane vesicles and double membrane spherules. Over the last two decades, our collective understanding of replication organelles has developed significantly. Despite this, numerous important questions still remain around, for example, how replication organelles form, precisely how they function and the details of the involvement of viral and cellular proteins in these processes. In my presentation I will describe our past and present contributions to understanding coronavirus replication organelles.



24 June 2026 (Wednesday) 0930-0955

Phenotypic evolution of circulating MERS-CoV in the Arabian Peninsula

First Author: Christian Drosten, Charité-Universitätsmedizin Berlin

Co-Author(s): Jordi Rodon Aldrufeu, Esam Azhar, Marylyn Addo, Marcel Müller

BACKGROUND OBJECTIVES:

MERS-CoV is a Merbecovirus acquired by humans from dromedary camels. The main region of endemicity is the Arabian Peninsula. Since 2015, the circulating virus population is dominated by a novel phylogenetic clade, termed the “novel recombinant clade” (NRC) or genetic lineage 5 (descending from lineages 3 and 4). We have intensely compared isolates from lineages 3, 4 and 5 for phenotypic changes and found enhanced cytokine resilience and suppressed cytokine secretion to be the most likely explanations for enhanced fitness of Lineage 5 over the parental lineages.

METHODS:

After a notification gap over the COVID-19 period, we studied circulating MERS-CoV in Saudi Arabia by sampling camels from across the country. To study phenotypic evolution, we generated full-length cDNA clones from representatives of each sublineage and chimerized the spike proteins of each sublineage into reference MERS-CoV-backbones to study the contribution of spike evolution in isolation. Using a standardized setup for replication kinetic tracing by live cell imaging, we assessed neutralizing antibody immune escape against serum from human participants of a phase Ia MERS-CoV MVA vectored vaccine trial conducted in Germany.

RESULTS:

We found that all circulating virus still pertains to lineage 5, with subdifferentiation into at least five sublineages that are panmictic across the country and show inter-sublineage recombination. We found clear signs of phenotypic differentiation, including the formation of a spike protein group with high fitness on par with the 2015 lineage 5 virus, as well as a group with decreased spike-dependent intrinsic fitness. The latter but not the first group of viruses shows signs of neutralizing antibody immune escape (antigenic change), with ca. 2 to 4-fold neutralizing antibody titer reductions in different subjects, depending on the test virus used. In contrast, spike receptor binding domain mutations selected during the 2015 outbreak in South Korea did not show any signs of antibody immune escape.

CONCLUSIONS:

This is the first instance of finding MERS-CoV to escape antibody immune escape. While selective pressure is exerted on the virus in camels, the resulting antigenic change may affect the neutralizing epitopes in a way that cross-affects human neutralizing antibody activity. As the affected strains show concomitant reduction in intrinsic fitness, this suggests a fitness-reducing effect (fitness cost) that might be outbalanced by the effective fitness gain through neutralizing immune escape.



24 June 2026 (Wednesday) 1125-1150

Coronaviruses: Past and present cross-species, zoonotic and “Oh Deer” Reverse zoonotic transmission

First Author: Linda Saif, The Ohio State University

Zoonotic coronaviruses (CoVs) have emerged repeatedly from multiple animal hosts to infect humans. Historically the four endemic human CoVs emerged as zoonoses through projected intermediate livestock hosts (camelids, cattle) via bat or rodent reservoir hosts. Although our understanding of interspecies and zoonotic transmission of CoVs has advanced, the origin, pathway to emergence and sustained transmission, and the underlying evolutionary mechanisms remain unclear. Recombinants play a major but complex role. Examples from alphaCoV-1 species include a recent spillover to humans of a canine alphaCoV (CCoV HuPn2018) with a recombinant mosaic Spike (S) from cats, dogs and pigs and the feline alphaCoV FC23 associated with fatal feline infectious peritonitis, with a recombinant S from the lethal pantropic canine alphaCoV and a large deletion in the S NTD. Although host cell receptors are critical for CoV entry and infection, the role of sialic acids (SA) as co-receptors, are understudied. Besides FC23, other alphaCoV S NTD deletion mutants (e.g. TGEV/PRCV), with loss of SA binding, display altered host or tissue tropisms and virulence. Likewise, the functions of many accessory proteins are unknown, although many are mutated (deletions or lost) upon changes in tissue or host tropism, as evident for TGEV/PRCV (ORF3ab), CCoV/Hu CCoV (ORF7b), etc. During the past 2 decades, 3 novel BetaCoVs causing severe acute respiratory syndrome and fatalities in humans have emerged (SARS CoV, MERS CoV and SARS-CoV-2). Bat CoVs are the projected ancestral progenitors. Intermediate hosts include livestock (ungulates-camels) and wildlife in wet markets. A common feature is their broad host range. Unique aspects of SARS-CoV-2 are its high transmissibility in humans, its extensive reverse zoonosis from humans to animals and the tremendous range of susceptible species. Especially concerning is the circulation of SARS-CoV-2 in free-range wildlife (white-tailed deer), posing threats of secondary spillover of ancestral or new variants to humans. The evolutionary trajectory in deer or other susceptible animals is unknown and necessitates continued surveillance. Similarly fatal MERS-CoV infections continue to spillover from camels. Also SARS CoV and SARS-CoV-2-related viruses that bind human ACE2 receptors persist in bats. HuCCoV and human porcine detacoV (HuPDCoV) illustrate recent spillovers to humans, but with unsustained human-to-human transmission. Monitoring is critical to confirm potential adaptation of these emerging zoonotic CoVs to humans. Finally One Health targeted surveillance to detect spillover of zoonotic CoVs at high-risk

human-animal interfaces is essential to implement control measures. Research to address these and other critical knowledge gaps may help to prevent the emergence of CoV Disease X and avert another devastating pandemic.



25 June 2026 (Thursday) 0930-0955

Neurological PASC in humans and mice

First Author: Stanley Perlman, The University of Iowa

PASC (Post Acute Sequelae of COVID-19) has become more prominent as numbers of cases of acute severe COVID-19 and associated death have decreased. Here, we discuss a mouse model of PASC, in which mice are infected with a mouse-adapted SARS-CoV-2 that causes sublethal or lethal disease. We found prolonged inflammatory and pathological changes in the brain, as well as behavioral changes. In specific, neurons that express tyrosine hydroxylase, the precursor to dopamine, were diminished in numbers in the olfactory bulb and substantia nigra. Similar results were found in the brains of deceased COVID-19 patients. These regions are impacted in patients with Parkinson's Disease, raising the possibility that SARS-CoV-2 infection could lead to neurodegenerative disease. While virus infects supporting cells (sustentacular cells) in the nasal cavity, olfactory sensory neurons are not infected. Therefore, a key question is how olfactory neuron dysfunction develops in the absence of direct virus infection. We have tentatively identified a key role for IFN signaling in many of the changes that we observe. Treatment with antiviral therapy and corticosteroids at the time of infection or shortly thereafter, but not antiviral therapy alone ameliorated the behavioral defects. We also detected long term changes in the vasculature and nasal cavity. We also detected findings consistent with LASC in the olfactory system and vasculature. Together, these results suggest that a prolonged inflammatory response is critical in PASC development.



25 June 2026 (Thursday) 1125-1150

Coronaviruses Suppress Innate Immune Response by Targeting Nucleo-cytoplasmic Trafficking system

First Author: Ying Liao, Shanghai Veterinary Research Institute, CAAS

Co-Author(s): Wenxiang Xue

A hallmark of coronavirus infection is its ability to evade host immune defenses, a process intricately linked to the nuclear entry of transcription factors essential for initiating antiviral gene expression. Central to this immune evasion strategy is the manipulation of the nucleo-cytoplasmic trafficking system, which serves as an effective target for viruses to modulate the expression of immune-related genes. However, the molecular mechanisms underlying coronavirus targeting of the nucleo-cytoplasmic transport system remain to be further elucidated. To investigate how does coronaviruses manipulate host cell nucleocytoplasmic trafficking to evade innate immunity, we employed a multi-pronged experimental approach combining virology, molecular biology, cell biology, and proteomics. We discovered that infection with infectious bronchitis virus (IBV) dynamically impedes the nuclear translocation of multiple transcription factors, including IRF3, STAT1 / STAT2, NF- κ B p65, and p38 MAPK, leading to compromised transcriptional induction of key antiviral genes such as IFN β , IFITM3, and IL-8. Further examination revealed that during IBV infection, components of the nuclear pore complex (NPC), particularly FG-Nups (including NUP62, NUP153, NUP42, and TPR), undergo cytosolic dispersion from the nuclear envelope. Notably, NUP62 becomes phosphorylated, and NUP42 exhibits a mobility shift, collectively suggesting disruption of nucleocytoplasmic trafficking. Through screening, we identified the IBV nucleocapsid protein (N) as the agent responsible for the cytoplasmic redistribution of FG-Nups, which subsequently hinders transcription factor nuclear entry and suppresses antiviral gene expression. Interactome analysis further revealed that the IBV N protein interacts with the scaffold protein RACK1, facilitating the recruitment of activated protein kinase C α (p-PKC α) to RACK1 and relocating the RACK1-p-PKC α complex to the cytoplasm. This N-mediated redistribution enhances the phosphorylation of NUP62 and promotes NPC disassembly, suggesting that the N protein facilitates RACK1-p-PKC α kinase activity toward NUP62. The presence of both RACK1 and PKC α / β proved essential for NUP62 phosphorylation and cytoplasmic dispersion, suppression of antiviral cytokine expression, and efficient viral replication. Importantly, these observations are conserved across pan-coronavirus N proteins. Together, these findings unveil a novel, highly effective, and evolutionarily conserved mechanism by which coronaviruses regulate nucleocytoplasmic trafficking to antagonize host innate immune responses.

26 June 2026 (Friday) 1125-1150

Airborne transmission of SARS-CoV-2

First Author: Kanta Subbarao, Université Laval

A major factor in the epidemiological success of respiratory pathogens including SARS-CoV-2, is their ability to spread through the air. The virus, host and the environment contribute to determinants of transmissibility. Understanding airborne transmission is important for mitigating spread in healthcare, aged care and other indoor settings. We have evaluated airborne transmission of SARS-CoV-2 using a multi-disciplinary approach, in a variety of settings including a virology laboratory, animal models, hospitals and outpatient settings. Lessons learned in these scenarios will be discussed.



26 June 2026 (Friday) 1400-1425

Attenuation of PEDV virulence by an NSP3 Mac1 mutation with unchanged in vitro replication

First Author: Qiuhong Wang, The Ohio State University

Co-Author(s): Mingde Liu, Men Thi Hoai Duong, Bikash Aryal, Anthony R. Fehr, Lari Lehtiö

BACKGROUND OBJECTIVES:

The conserved macrodomain 1 (Mac1) domain within non-structural protein (NSP) 3 of coronaviruses (CoVs) can reverse ADP-ribosylation of proteins and contributes to viral virulence. While Mac1 has been characterized in several CoVs, its role in porcine epidemic diarrhea virus (PEDV) remains unknown.

METHODS:

We performed CoV sequence alignments, a literature review, and 3D structure prediction to identify conserved, potentially important residues in the PEDV NSP3 Mac1 domain. Mac1 proteins bearing D445A and N462A substitutions were expressed and assayed for ADP-ribose binding and hydrolysis activity. Recombinant viruses (icPC22A-nsp3-D445A and icPC22A-nsp3-N462A) were generated from the infectious clone of PEDV PC22A strain (icPC22A) and characterized in Vero, porcine kidney epithelial LLC-PK1, and porcine intestinal epithelial IPEC-J2 cells; the pathogenicity of icPC22A-nsp3-N462A was then evaluated in neonatal gnotobiotic pigs.

RESULTS:

Sequence alignment showed that N462 is highly conserved among porcine CoVs, whereas D445 is less conserved. N462A markedly reduced Mac1 ADP-ribose binding and hydrolysis, while D445A had minimal effect. Both virus mutants replicated as efficiently as icPC22A in Vero, LLC-PK1 and IPEC-J2 cells, yet icPC22A-nsp3-N462A exhibited reduced peak viral shedding and attenuated virulence in gnotobiotic neonatal piglets compared with icPC22A. These findings indicate that N462, but not D445, is critical for Mac1 ADP-ribose binding and hydrolysis; Although N462 is dispensable for replication in vitro, it modulates viral replication and pathogenesis in vivo. Type I and III interferon induction after infection and the effect of IFN β pretreatment on viral replication are currently under evaluation.

CONCLUSIONS:

Our findings identify Mac1 N462 as a rational target for live attenuated vaccine design and highlight Mac1 as a potential target for antiviral therapeutics against PEDV. This study provides the first in vitro and in vivo evidence for the function of key residues in the Mac1 domain of a porcine enteric CoV and represents the first in vivo characterization of Mac1 function of alpha-CoVs.

27 June 2026 (Saturday) 0930-0955

Discovery and Development of Coronaviral Inhibitors targeting Viral Methyltransferases

First Author: Deyin Guo, Guangzhou National Laboratory

Co-Author(s): Liu Cao, Tiefeng Xu, Sidi Yang, Hong Peng, Chun-Mei Li

The 5' cap structures of eukaryotic mRNAs are important for RNA stability and protein translation. Many viruses that replicate in the cytoplasm of eukaryotes have evolved guanine N7- methyltransferases (N7-MTase) and 2'-O-MTase to autonomously modify their mRNAs and carry a cap-1 structure (m7GpppNm) at the 5'; end, thereby facilitating viral replication and escaping innate immune recognition in host cells. Our previous studies showed that coronaviral non-structural protein (nsp) 14 functions as the N7-MTase and the effective 2'-O-MTase activity of coronaviruses (CoVs) is commonly relied on the complex formation of nsp16 and nsp10. Here, we will report successful development of specific inhibitors targeting CoV N7-MTase and 2'-O-MTase by AI-aided virtual and compound library-based phenotype screening and optimization. We found that several peptides and chemical compounds could broadly inhibit the MTase activities of CoVs in vitro and the viral replication in animal models. We further optimized the inhibitors systematically by using computer- aided drug design (CADD) and biochemical analysis. Most interestingly, a small molecule compound named as MI54 specifically bind and inhibit the 2'-O- methyltransferase of coronaviruses but not cellular methyltransferases. MI54 can potently suppress virus replication and pathogenesis in mouse infection models of different CoVs including SARS-CoV-2 and mouse hepatitis virus. Therefore, the current results indicate that coronaviral MTases can be used as potential targets in antiviral discovery and development.



Viral Replication

24 June 2026 (Wednesday) 1400-1425

Machinery of RNA virus replication and transcription——New Target, New Mechanism, Leading New Inhibitor

First Author: Zihe Rao, Tsinghua University

Virus Entry

24 June 2026 (Wednesday) 1555-1620

Receptor diversification in merbecovirus evolution

First Author: Huan Yan, Wuhan University

Immune Response to Virus Infection

26 June 2026 (Friday) 0930-0955

Coronavirus activation of interferon signaling pathways is critical for control of infection in the nasal epithelium

First Author: Susan Weiss, University of Pennsylvania

Zoonotic Transmission

27 June 2026 (Saturday) 1125-1150

Viral recombination as a multiscale pathway of conditional constraints

First Author: Simon John Anthony, University of California Davis

Abstract No.: 223

24 June 2026 (Wednesday) 0955-1010

The spread of RNA exonuclease domain within Nidovirales hints an evolution mechanism bridging RNA and DNA worlds

First Author: François Ferron, Laboratoire AFMB, CNRS-Aix Marseille University

Co-Author(s): Bhawna Sama, Veronique Fattorini, Claire Zimmerger, Barbara Selisko, Vincent Wilde

BACKGROUND OBJECTIVES:

DNA-based life is thought to have originated from ancestral coding RNA, evolving into primitive genomes of increasing complexity. Present RNA viruses represent the most evolved form of RNA genomes. Among these, Nidovirales is a vast and diverse order of positive-stranded RNA viruses with genome sizes ranging from 12 kb ('small') to >41 kb ('very large'), among the largest RNA genomes known to date. Like most RNA viruses, synthesis of small nidovirus RNA genomes is presumably error-prone, as they lack an RNA synthesis correction system. The 3'-to-5' RNA exonuclease gene appears in the nidovirus phylogenetic tree in a host-independent manner, with a positive correlation to genome size. The presence of an exonuclease domain is a hallmark of 'large' (>20 kb) RNA genomes, thought to rely on 3'-to-5' exonuclease activity for RNA mismatch repair, genetic stability maintenance, innate immunity suppression, and possibly genome size expansion.

METHODS:

Through sequence and structural modeling, we identified and mapped exonuclease signature sequences and folds across Nidovirales. The enzyme activities and substrate specificities of identified, prototypic nucleases was determined along the Nidovirus phylogenetic tree.

RESULTS:

Our analysis reveals three distinct exonuclease types, characterized by the presence of zero, one, or two zinc finger subdomains, reflecting a structural evolution linked to the acquisition of new structural motifs. The acquisition of zinc fingers in RNA exonucleases correlates with genome expansion in the 'large' RNA virus subgroup. Enzymatic assays of prototypic members from each exonuclease type confirmed 3'-to-5' exonuclease and proofreading activities.

CONCLUSIONS:

Strikingly, the zinc finger depleted nidovirus exonuclease represents a minimal domain, both in sequence and fold, that shares phylogenetic and structural homology with DNA PolIII the proofreading exonuclease of DNA organisms thus establishing a direct evolutionary bridge between RNA and DNA worlds.

ExoN domains are essential for genome evolution, enabling size expansion, stability, and maintenance in both RNA and DNA systems.



Abstract No.: 208

24 June 2026 (Wednesday) 1010-1025

Genetic diversity in horseshoe bat ACE2 and sarbecovirus Spike proteins mutually shape one another

First Author: Wenye Li, The University of Tokyo

Co-Author(s): Shoma Sato, Taichiro Takemura, Xiao Niu, Daniel Arnold, Maximillian Stanley Yo, Jarel Elgin Tolentino, Shigeru Fujita, Yusuke Kosugi, Nguyen Thi Thu Thuy, Le Thi Quynh Mai, Nguyen Thanh Luong, Hidetaka S. Oshima, Kanata Matsumoto, Kazuhiro Sawada, Hiroaki Akasaka, Wataru Shihoya, Jumpei Ito, Yunlong Cao, Futoshi Hasebe, Osamu Nureki, Vu Dinh Thong, Spyros Lytras, Kei Sato

BACKGROUND OBJECTIVES:

Angiotensin-converting enzyme 2 (ACE2) serves as the entry receptor for a broad spectrum of sarbecoviruses, including SARS-CoV-2. In nature, horseshoe bats (genus *Rhinolophus*) are naturally infected with sarbecoviruses. The polymorphism in *Rhinolophus* ACE2s is thought to be driven by virus-host co-evolution. Despite the extensive circulation of these viruses in many *Rhinolophus* bat species, the potential interactions between virus and receptor evolution remain poorly understood. We characterized new *Rhinolophus* ACE2 genotypes circulating in Vietnam and elucidated their interactions with different sarbecovirus Spike proteins. We analyzed the molecular mechanism of typical interactions and proposed a plausible co-evolutionary dynamic between sarbecovirus and host.

METHODS:

We collected the samples from *Rhinolophus affinis* (*R. affinis*) bats from multiple locations in Vietnam, extracted total RNA, and analyzed the sequences of ACE2 gene. The ACE2 sequences were used for molecular phylogenetic and evolutionary analyses. Concurrently, each ACE2 gene was subcloned into an expression vector, stably expressed in human HOS-TMPRSS2 cells, and infected with a panel of pseudotyped sarbecovirus Spikes. Genotypic and phenotypic datasets were subsequently integrated bioinformatically to investigate their correlations. We finally selected one representative sarbecovirus Spike to visualize how it plausibly evolved and interacted with ACE2 proteins at the molecular level through cryo-EM.

RESULTS:

In total, we analyzed ACE2 genes from 155 *R. affinis* and identified 15 genotypes of *R. affinis* ACE2. Phylogenetic analysis and pseudoviruses infectivity assays suggested that *R. affinis* ACE2 genotypes originating from distinct geographic regions confer divergent tropism profiles across a broad panel of sarbecoviruses. We detected site-specific positive selection on a residue in *R. affinis* ACE2. Through the mutagenesis assays and structural analysis, we demonstrated the functional impact of this amino acid residue and proposed an evolutionary trajectory between *R. affinis* ACE2 and *R. affinis*-infecting sarbecoviruses.

CONCLUSIONS:

R. affinis ACE2 polymorphism is geographically separated and associated with distinct sarbecovirus entry phenotypes. Focusing on the interaction between *R. affinis* ACE2 and one representative sarbecovirus sampled in the same host species, we identify mutations controlling the infectivity phenotype on both the receptor and the virus Spike protein. Hence, we present evidence of both host receptor polymorphism shaping virus evolution, but also ancestral sarbecoviruses likely having shaped the intra-species evolution of *Rhinolophus* bat ACE2 proteins. Our findings support that *Rhinolophus* bat ACE2s and sarbecovirus Spikes are bidirectionally shaped by each other's evolution and provide valuable insights into the risk of future zoonotic spillover events.



Abstract No.: 388

24 June 2026 (Wednesday) 1025-1035

DeepCoV: a DMS-informed protein language model predicts SARS-CoV-2 evolution dynamics with spatiotemporal resolution

*First Author: Fanchong Jian, Peking University**Co-Author(s): Xiaowei Luo, Jiejian Luo, Sijie Yang, Yunlong Cao*

BACKGROUND OBJECTIVES:

While the early identification of dominant SARS-CoV-2 variants is pivotal for public health, existing surveillance methods struggle to model the dynamic fitness landscape across varying spatiotemporal scales. A major challenge lies in capturing how viral transmission advantages diverge across regions and time, driven by distinct local immune histories. Furthermore, rich functional data from Deep Mutational Scanning (DMS) remains significantly underleveraged for evolutionary forecasting. To address this gap, we introduce DeepCoV (DMS-Empowered Evolution Prediction of CoronaVirus), a deep-learning model designed for the prospective identification of emerging variants with high prevalence potential at spatiotemporal resolution.

METHODS:

We designed DeepCoV, a deep-learning model that predicts the future prevalence of any SARS-CoV-2 Spike or RBD variant, leveraging three complementary data collected during months before the time of prediction: 1) Multiple sequence alignment (MSA) of viral antigen sequences including the variant for prediction and other co-circulating strains competing within the same environment. 2) The proportions of the above strains since 180 days before the day of prediction, capturing recent viral fitness evolution with the population-level immunity and selective pressure considered implicitly, and endowing the model with the ability to learn the spatiotemporal dynamics of variant circulation. 3) DMS-derived functional mutation phenotypes quantifying the impacts on virological

characteristics, thereby grounding the model in experimentally validated datasets and molecular mechanisms.

RESULTS:

Benchmarked against logistic regression-based methods and representative deep learning approaches in simulated retrospective surveillance scenarios, DeepCoV accurately forecast the dominance of recently circulating lineages a month in advance, achieving a 90% reduction in false discovery rate (FDR), compared to existing statistical approaches. DeepCoV captures temporal and geographic dynamics of variant spread and reconstructs their regional prevalence trajectories. It can also identify mutational hotspots of Omicron-derived backbones via *in silico* DMS analyses, revealing convergent evolution trends in multiple Omicron sublineages.

CONCLUSIONS:

In conclusion, by integrating comprehensive epidemiological and DMS datasets with deep-learning-based protein language models, DeepCoV unifies evolutionary, functional, and epidemiological insights to build a reliable platform for the identification and prediction of prevalent SARS-CoV-2 strains. The model could be retrained and utilized in other fast-evolving epidemic viruses, such as influenza and RSV, once corresponding DMS datasets become available. Collectively, these innovations establish DeepCoV as a powerful tool for global health preparedness, enabling proactive responses to emerging infectious threats and informing timely vaccine and surveillance strategies. ACE2 proteins. Our findings support that Rhinolophus bat ACE2s and sarbecovirus Spikes are bidirectionally shaped by each other's evolution and provide valuable insights into the risk of future zoonotic spillover events.



Abstract No.: 390

24 June 2026 (Wednesday) 1035-1045

High-throughput antibody DMS enables comprehensive epitope modeling and evolution prediction of SARS-CoV-2 and other fast-evolving viruses

*First Author: Fanchong Jian, Peking University**Co-Author(s): Sheng Long, Xiao Niu, Yunlong Cao*

BACKGROUND OBJECTIVES:

Amid the continuous co-evolution of SARS-CoV-2 and population immunity, closely monitoring the antigenic drift of the viral Spike glycoprotein remains crucial. Rapidly identifying mutations capable of escaping herd immunity is essential for the early detection of emerging variants. Previous studies have demonstrated that mapping immune pressure via deep mutational scanning (DMS) of neutralizing antibodies (NAbs) is highly effective for forecasting viral evolution. However, precisely mapping this pressure traditionally requires time-consuming and expensive wet-lab experiments for antigen-specific monoclonal antibody (mAb) isolation and characterization. Leveraging an extensive dataset of SARS-CoV-2 receptor-binding domain (RBD)-targeting antibodies, this study aims to build a computational framework to model RBD antibodies and viral evolution using DMS data. This framework is designed to be extensible to other fast-evolving respiratory viruses, including influenza and respiratory syncytial virus (RSV).

METHODS:

Building upon established high-throughput DMS protocols for characterizing antibody-antigen interactions, we developed a standardized bioinformatics pipeline for data processing, quality control (QC), and epitope group analysis. This pipeline features two key downstream applications: 1) evolution forecasting based on estimated antibody-induced immune pressure, and 2) predicting targeted epitopes directly from mAb Fv sequences using pre-trained protein language models (pLMs). Integrating these predictive models enables the identification of immune-escape mutations solely from antibody sequences, significantly reducing the reliance on additional wet-lab experiments.

RESULTS:

We compiled a comprehensive dataset of over 10,000 mAbs experimentally verified to bind the RBD of at least one SARS-CoV-2 variant. Within this collection, the escape mutation profiles of over 7,000 mAbs were successfully mapped via DMS. Our evolutionary modeling faithfully captures and explains known mutational hotspots on the SARS-CoV-2 RBD. Furthermore, our sequence-based epitope predictor achieves precision ranging from 0.25 to 0.83 across 14 epitope groups. While performance varies by group, it demonstrates substantial improvement over universal structural prediction models such as AlphaFold3.

CONCLUSIONS:

This study provides a robust bioinformatics pipeline for processing high-throughput antibody DMS data and modeling viral evolution. Although the accuracy of sequence-based epitope prediction varies across distinct epitope groups, the tool offers a highly efficient, scalable alternative to traditional structural models, and it is validated that this pipeline could faithfully recover the overall immune pressure landscape. Ultimately, this framework not only enhances our ability to forecast SARS-CoV-2 immune escape but also holds significant potential for broader application to other fast-evolving pathogens, such as influenza and RSV.



Abstract No.: 443

24 June 2026 (Wednesday) 1045-1055

Direct Measurement of Inter-Virus Recombination Using Landmarked Genomes

*First Author: David LV Bauer, The Francis Crick Institute**Co-Author(s): Joe Hemsley-Taylor, Nuno Santos, Catarina Nunes, Ciarán Gilbride*

BACKGROUND OBJECTIVES:

While coronaviruses can evolve through the gradual accumulation of mutations, it is their ability to undergo recombination that drives their extraordinary diversity. With the advent of viral genome sequencing and environmental and animal sampling, it became clear that recombination is widespread — consistent with classic experiments in MHV suggesting that the rate of recombination is very high. Indeed, our experience of SARS-CoV-2 evolution has been marked repeated emergence of recombinant lineages such as XBB and XEC.

On a molecular level, RNA recombination is thought to proceed via an inter-molecular copy-choice mechanism of the RNA-dependent RNA polymerase — just as subgenomic RNAs and Defective Viral Genomes (DVGs/DIs) are generated via intra-molecular recombination. Recent work has suggested that on a per-cell level, recombination may be rarer, with effects such as superinfection exclusion limiting the opportunity for two viruses to ‘meet’ during coinfection.

Direct measurement of the rate of recombination between different viruses would be essential to begin to understand the origin of coronavirus diversity and evaluate the potential for future recombination and viral evolution. Practically, however, such measurements are limited by different fitness levels, as well as biosafety concerns over experimental coinfection; we therefore aimed to measure the baseline rate of recombination in a controlled and biosafe manner.

METHODS:

We designed identical HCoV-OC43 genomes with distinct fluorescent “landmarks” and rescued viruses using our recently-described BAC-free reverse genetics system: one virus with mScarlet upstream of HE and Spike, and a second virus with mNeonGreen downstream of Spike. We measured recombination using two

approaches: (1) counting the recombinant infectious virions generated during co-infection of cells in culture by flow-sorting doubly-infected cells, seeding onto fresh monolayers, and monitoring outgrowth of fluorescent foci to distinguish co-infection events from true recombinants; (2) using direct RNA sequencing using ONT nanopore and a custom bioinformatics pipeline to count the number of true recombinant RNA molecules within doubly-infected cells.

RESULTS:

We successfully rescued two “landmarked” HCoV-OC43 viruses, which replicated with similar kinetics. Analysis of fluorescent foci from doubly-infected cells showed a reliable pattern of (i.) distinct green and red outgrowth from doubly-infected (but not recombinant) cells, and (ii.) uniform dual-colour from true recombinant viruses. The observed rate of recombination was well below the 1% limit of detection in this assay. Turning to direct RNA-seq experiments, we typically obtained >4M reads spanning the two landmarked locations, with an average recombination rate of 0.02%.

CONCLUSIONS:

Our results suggest that on a per-cell level, recombination is a rare event. However, on a whole-organism level, with millions of cells infected, it is reasonable to assume that recombination may proceed relatively frequently — and could readily overtake the parental populations if the recombinant had a fitness advantage. Future work to delineate the effect of host and viral factors that promote recombination will benefit from our controlled system, and enable precise modelling of the recombination potential between circulating viruses.



Abstract No.: 326

24 June 2026 (Wednesday) 1055-1105

Deciphering and forecasting viral evolution via AI-augmented molecular evolution

First Author: Jian Lu, Peking University

BACKGROUND OBJECTIVES:

Human history has been profoundly shaped by continual interactions with pathogenic microorganisms. Understanding how pathogens emerge, spread, and adapt requires an evolutionary framework that connects genomic change to phenotypic consequences and population-level fitness. This challenge is especially acute for rapidly evolving viruses, whose trajectories are influenced not only by mutation and transmission stochasticity but also by immune-driven antigenic change in a constantly shifting host immune landscape. To address this problem, we combine molecular evolutionary analysis with artificial intelligence to improve prediction of pathogen evolution and outbreak dynamics, with a particular focus on SARS-CoV-2.

METHODS:

We developed vTRACE, an AI-augmented evolutionary framework that links viral genotype, phenotype, and fitness to forecast evolution in antigenic space. Using SARS-CoV-2 receptor-binding domain sequences, vTRACE applies deep-learning-based genotype-to-phenotype inference to predict key functional traits, including antigenic distance and ACE2-binding affinity. These inferred phenotypes are then integrated with genomic surveillance data and population immune history to reconstruct time-resolved fitness landscapes. To capture the predictable component of viral evolution at the population level, we introduce antigenic velocity, a metric that quantifies both the speed and direction of viral movement in antigenic space, thereby distinguishing directional adaptation from stochastic drift.

RESULTS:

We developed vTRACE, an AI-augmented evolutionary framework that links viral genotype, phenotype, and fitness to forecast evolution in antigenic space. Using SARS-CoV-2 receptor-binding domain sequences, vTRACE applies deep-learning-based genotype-to-phenotype inference to predict key functional traits, including antigenic distance and ACE2-binding affinity. These inferred phenotypes are then integrated with genomic surveillance data and population immune history to reconstruct time-resolved fitness landscapes. To capture the predictable component of viral evolution at the population level, we introduce antigenic

velocity, a metric that quantifies both the speed and direction of viral movement in antigenic space, thereby distinguishing directional adaptation from stochastic drift.

CONCLUSIONS:

Our results show that while the fate of individual viral sequences remains strongly influenced by stochasticity, population-level phenotypic dynamics are substantially more predictable. Viral antigenic evolution appears pulsed in speed yet constrained in direction during adaptive episodes, making it possible to anticipate both the timing of antigenic shifts and the variants most aligned with emerging evolutionary trajectories. More broadly, vTRACE provides an AI-based encode-decode framework for studying evolution in complex adaptive landscapes by mapping high-dimensional sequence variation into biologically meaningful phenotypic space and decoding population dynamics using explicit evolutionary principles. This approach offers a powerful strategy for early identification of adaptive changes, more accurate outbreak forecasting, and improved public health preparedness against emerging infectious threats.



Abstract No.: 405

24 June 2026 (Wednesday) 1150-1205

Co-circulation and Preferential Infection of Children by the Emerging Saltation Variant BA.3.2 Suggest a Transition Towards Endemicity

First Author: Stefan Pöhlmann, German Primate Center - Leibniz Institute for Primate Research

Co-Author(s): Lu Zhang, Inga Nehlmeier, Amy Eichmann, Nianzhen Chen, Metodi V. Stankov, Christine Happle, Alexandra Dopfer-Jablonka, Georg M N Behrens, Markus Hoffmann

BACKGROUND OBJECTIVES:

The COVID-19 pandemic exerted a profound impact on global public health and caused substantial socioeconomic disruption worldwide. The World Health Organization declared the end of the global health emergency phase in 2023. However, viral circulation persisted, accompanied by the continuous emergence of SARS-CoV-2 variants harboring mutations in the spike glycoprotein that reduced susceptibility to antibody-mediated neutralization while largely preserving ACE2 engagement. These features facilitated efficient transmission and drove successive waves of infection on a global scale. In November 2024, a highly mutated saltation variant, BA.3.2, was identified in South Africa, carrying more than 50 mutations in the viral spike protein. The variant started to spread efficiently in parts of Europe and Australia in 2025, suggesting a competitive advantage and the potential for rapid dominance. Here, we aimed to characterize the epidemiological dynamics of BA.3.2 and to assess whether the determinants of host cell entry differed from those of previously circulating variants.

METHODS:

We analyzed SARS-CoV-2 sequences deposited in the Global Initiative on Sharing All Influenza Data (GISAID) database. A Vesicular stomatitis virus (VSV)-based pseudotyping system was employed to investigate viral entry into host cells and its inhibition by antibodies.

RESULTS:

We found that BA.3.2 entered multiple cell lines with high efficiency. However, entry into Calu-3 lung cells was reduced, consistent with diminished ACE2 binding. BA.3.2 exhibited pronounced evasion of antibodies elicited by infection and/or vaccination. Moreover, the current LP.8.1-adapted vaccine boosted neutralizing antibody responses against the prevalent variants NB.1.8.1 and XFG with markedly greater efficiency than against BA.3.2. Analysis of sequences deposited in the

GISAID database indicated that BA.3.2 became dominant in several European and Australian regions, but not uniformly across all settings. Its expansion occurred at the expense of XFG, whereas the spread of NB.1.8.1 was not reduced. Furthermore, comparative analyses of relative infection efficiency showed that BA.3.2 preferentially targets children, for reasons that remain unclear. Finally, characterization of viral entry demonstrated that BA.3.2 differs from SARS-CoV-2 Wuhan-1 in the determinants governing spike protein cleavage, indicating that evolutionary changes in BA.3.2 have altered the regulation of spike processing.

CONCLUSIONS:

Collectively, our findings identify BA.3.2 as an antigenically distinct SARS-CoV-2 variant characterized by pronounced antibody evasion and altered entry properties. Despite efficient spread in selected regions, its sustained co-circulation with other lineages - particularly the lack of suppression of NB.1.8.1 - suggests that current population immunity is not readily overcome and may indicate a transition toward endemicity. Finally, the apparent preferential infection of children underscores the need to consider targeted protective measures for younger age groups under defined conditions and to elucidate the biological mechanisms underlying this tropism.



Abstract No.: 116

24 June 2026 (Wednesday) 1205-1220

Neutralizing antibody profiles in different age cohorts reveal evidence of antigenic drift in human coronavirus 229E that is not seen with OC43 and NL63

First Author: Kanta Subbarao, Université Laval

Co-Author(s): Mingyang Wang, Francesca Mordant, Jessica Neil, Matthew Gartner, Kevin Selva, Amy Chung, Eileen Roulis, Siddhartha Mahanty

BACKGROUND OBJECTIVES:

Human seasonal coronaviruses (sCoV) NL63, OC43, and 229E circulate widely in the population. However, population-level binding and neutralizing antibody profiles and their relationship to antigenic evolution have not been systematically compared.

METHODS:

In this study, we analyzed 400 serum samples collected in 2020 from healthy individuals in Australia, grouped into five age decades from their 20s, 30s, 40s, 50s and 60s (n=80 from each decade) for antibodies against sCoVs. In a subset of 300 people we measured IgG antibody levels against recombinant S protein trimers from reference strains of four seasonal CoVs (NL63, OC43, HKU1 and 229E), and representative control proteins (SARS-CoV2 S trimer, Influenza HA and SIV gp120) coupled to magnetic carboxylated beads (Bio-Rad) in a customised Luminex multiplex assay. We established neutralizing antibody assays against laboratory reference strains of NL63, OC43, and 229E using a cytopathic effect-based assay in LLC-MK2 or MRC-5 cells for the full sample set and for subsequent 229E strains, we established an immunospot assay in Huh7-TMPRSS2 cells.

RESULTS:

We observed a substantial overlap in IgG levels to the four sCoV among the five age cohorts without consistent patterns or differences in rates or titers of antibodies or age-associated patterns of binding IgG among the viruses. Binding antibodies against OC43, NL63 and HKU1 correlated with each other but not with 229E. Binding and neutralizing antibodies correlated well for OC43 and NL63 but not for 229E. While neutralizing antibodies against NL63 and OC43 were present at all

ages, they were only detected against 229E in people in their 50s and 60s, though binding antibodies to 229E were detected in all age groups. To determine whether this observation was the result of antigenic drift, we isolated 229E strains from 1988 and 2010 and, across all age groups, we found higher neutralizing antibody positivity rates and titers against the 1988 and 2010 viruses than the reference strain from 1965.

CONCLUSIONS:

Our findings are consistent with antigenic evolution in 229E that was not observed in OC43 and NL63. Notably, only a small number of serum samples showed moderate to high neutralizing activity against all three seasonal coronaviruses. Overall, this study highlights the importance of selecting a relevant virus for seroprevalence studies.



Abstract No.: 57

24 June 2026 (Wednesday) 1220-1235

Optimizing Global Genomic Surveillance for Early Detection of Emerging SARS-CoV-2 Variants

*First Author: Haogao Gu**Co-Author(s): Wanying Sun, Leo Poon*

BACKGROUND OBJECTIVES:

Timely genomic surveillance is critical for detecting emerging viral variants and informing public health responses. However, global surveillance is constrained by high costs and uneven sequencing capacity. This study aims to evaluate whether targeted surveillance of international travelers can enhance early detection of emerging SARS-CoV-2 variants under resource limitations.

METHODS:

We developed a multi-strain metapopulation model integrating epidemiological, phylogenetic, and global air travel data to simulate the spread and detection of SARS-CoV-2 variants. The model was calibrated using data from the Omicron BA.1 and BA.2 waves and extended to evaluate alternative surveillance strategies, including targeted traveler-focused approaches at high-connectivity travel hubs.

RESULTS:

Simulation results indicate that targeted surveillance of travelers at key international hubs can reduce global detection delays compared with baseline strategies. Practical approaches focusing on a small number of highly connected hubs consistently improved detection timeliness without requiring additional global resources. Under constrained diagnostic and sequencing capacities, these strategies remained effective and demonstrated robustness across varying levels of transmissibility and vaccine effectiveness.

CONCLUSIONS:

Targeted genomic surveillance at major travel hubs provides a scalable and resource-efficient strategy to complement local surveillance systems. This approach enhances early detection of emerging variants and supports improved global pandemic preparedness.

Abstract No.: 261

24 June 2026 (Wednesday) 1235-1250

PhyloRt: scalable deep-learning-based inference of time-varying transmission dynamics from outbreak phylogenies

*First Author: Ruopeng Xie, University of Oxford**Co-Author(s): Anna Zhukova, Moritz U. G. Kraemer, Oliver G. Pybus, Olivier Gascuel*

BACKGROUND OBJECTIVES:

Reconstructing epidemic dynamics in real-time has become crucial for effective disease management, as demonstrated by the COVID-19 pandemic. Most methods rely on epidemiological time series data (e.g., reported cases), which can be biased or incomplete due to variable testing policies, particularly in resource-limited settings. Phylodynamics, which leverages viral phylogenies, offers an independent, alternative data source but can be computationally intensive and suited mainly for retrospective analysis. Recent deep-learning approaches (e.g., PhyloDeep, PhyloCNN) have improved scalability, yet challenges remain due to poorly-resolved phylogenies caused by limited genetic divergence during outbreaks. We present a deep-learning framework that integrates pathogen-specific evolutionary and epidemiological signals to enable real-time phylodynamic inference from imperfect genomic data.

METHODS:

To assess the impact of realistic genomic conditions, we simulated transmission trees using multi-type birth-death models for major respiratory viruses (SARS-CoV-2, influenza, and RSV), and transformed them into poorly resolved phylogenies with virus-specific levels of molecular resolution. PhyloCNN models were retrained and performance was evaluated. To account for temporal variation in transmission rates (e.g. driven by public health interventions, population immunity, or the emergence of variants with increased transmissibility), we introduce PhyloRt, a scalable framework for estimating the time-varying reproductive number (R_t)

from large outbreak phylogenies.

RESULTS:

Significant discrepancies emerged when models trained on one type of phylogeny were applied to another, underscoring the importance of jointly aligning the birth-death model and phylogenetic resolution with the characteristics of each pathogen. PhyloRt decomposes large trees into overlapping subtrees and applies a hierarchical deep-learning-based inference strategy to classify subtrees as exhibiting constant or time-varying reproduction numbers, enabling identifiable and computationally efficient estimation of R_t as a piecewise-constant trajectory through time. Applications to SARS-CoV-2 and influenza outbreaks show that PhyloRt recovers transmission dynamics consistent with estimates derived from mathematical epidemiological and Bayesian phylodynamic analyses.

CONCLUSIONS:

Our work enables scalable and rapid estimation of time-varying transmission dynamics from very large-scale outbreak genomic data sets, supporting real-time genomic epidemiology of emerging pathogens.



Abstract No.: 430

24 June 2026 (Wednesday) 1250-1300

Surveillance, Characterisation and Organ Tropism of Coronaviruses in UK Wildlife

First Author: Ternenge Apaa, Animal & Plant Health Agency

Co-Author(s): Ternenge Apaa, Ternenge Apaa, Thosmas, S.S, Maxwell, C, Goharriz, H., Lavery, J., Bagley, M, Shukla, S., Gould, V., Shipley, R., Wu, G, Delahay, R, Cox, R, McElhinney, L.M, Seekings, A.H

BACKGROUND OBJECTIVES:

Coronaviruses circulate widely across animals species and continue to pose risk of emergence through viral recombination and spillover. National-scale surveillance and detailed characterisation in wildlife are therefore critical to understand virus distribution, host range and organ tropism. This work draws on outputs from a coordinated coronavirus surveillance and pathogen investigation programme in animals, combining optimised diagnostics and characterisation tools to investigate coronavirus circulation in UK wildlife and assessed potential implications for animal and human health.

METHODS:

Optimised molecular diagnostics, including pan-coronavirus and SARS-CoV-2 specific PCR assays and sequencing, were applied to archived and newly collected wildlife samples. Surveillance targeted bats and other UK wildlife species, including deer, badgers, foxes, squirrels and additional mammals. Sample types comprised swabs, tissues, sera and whole carcasses obtained through established passive surveillance and wildlife management activities. Organ-level analyses were undertaken in selected bat carcasses to investigate tissue distribution of coronavirus RNA. Virus isolation was attempted from PCR-positive bat samples using cell culture systems. Serological screening of wildlife sera, including samples from deer, badgers, foxes and other species was performed using a SARS-CoV-2 nucleocapsid (N) gene ELISA. Reactive samples were further assessed using SARS-CoV-2 virus neutralisation test to evaluate the presence of neutralising antibodies.

RESULTS:

From 2021 to 2025, coronaviruses were detected in 111 of 1,978 bat tested (5.6%), spanning multiple species. Both alpha and beta coronaviruses were identified, including pedacovirus, merbecovirus and sarbecoviruses. In 2025 alone, 28 of 403 bat swab samples were positive, with rectal swabs showing higher detection rates than oral swabs. No bat samples were positive for SARS-CoV-2. Organ tropism investigations demonstrated predominant detection of coronavirus RNA in intestinal tissues, with occasional detection in respiratory organs and kidneys. Across more than 2,500 non-bat samples assessed, viral RNA for SARS-CoV-2 was not detected; however, serological reactivity was identified in several species, suggesting exposure to SAR-CoV-2 or related coronaviruses. Whole genome sequencing and bioinformatic analysis provided high resolution genomic characterisation of Merbecovirus in brown-long eared bat, highlighting receptor binding domain characteristics of the MERS-like coronavirus.

CONCLUSIONS:

This study demonstrates active circulation of genetically diverse coronaviruses in UK bats, with clear gastrointestinal tropism and limited extra-intestinal detection. Absence of SARS-CoV-2 RNA in surveyed wildlife suggests no established animal reservoir, while serological findings highlights the need for cautious interpretation. Continued integrated surveillance and advanced characterisation remain essential to monitor coronavirus diversity, host range and emergence risk at the wildlife-human interface



Abstract No.: 51

24 June 2026 (Wednesday) 1425-1440

Structural basis of mismatch recognition by a SARS-CoV-2 proofreading enzyme

First Author: Chang Liu, The Johns Hopkins University School of Medicine

Co-Author(s): Bin Liu, Yang Yang

BACKGROUND OBJECTIVES:

The coronavirus 3'-to-5' exoribonuclease (ExoN) within the nsp10-nsp14 complex is essential for viral replication, enhancing fidelity by proofreading nascent RNA. ExoN also removes incorporated nucleotide analogs, reducing the efficacy of many RdRp-targeting antivirals. Despite its central roles in genome maintenance and drug resistance, the molecular basis of ExoN substrate specificity and mismatch correction remains incompletely defined, limiting rational therapeutic design that can withstand ExoN-mediated excision. To address this gap, we determined high-resolution cryo-electron microscopy structures of wild-type and mutant SARS-CoV-2 nsp10-nsp14 complexes bound to RNA bearing a 3'-end mismatch. These structures will define the determinants of ExoN substrate recognition and elucidate the mechanisms of mismatch correction during coronavirus RNA synthesis.

METHODS:

We engineered a hairpin RNA (T35P31) containing a portion of 3' terminus of the SARS-CoV-2 genome and a 3' C-U mismatch. Recombinant nsp10-nsp14 was reconstituted with T35P31 and assayed for exoribonuclease activity in Mg²⁺. For structural trapping, we used Ca²⁺ or the nsp14 E191A mutant to preserve RNA binding while abolishing cleavage. Complexes were purified by SEC and analyzed by single-particle cryo-EM, yielding 3.9 Å (WT) and 3.4 Å (E191A) maps.

RESULTS:

The nsp14 ExoN active site, supported by nsp10, engages the RNA 3' end and separates it from the 5' overhang, leaving much of the duplex solvent exposed. A tetrameric nsp10-nsp14-RNA assembly (E191A) yielded a 2.5 Å map used for active-site analysis. RNA binding narrows the pocket via '4-'5 and '2-'3 loop shifts,

assembles a two-metal center, and moves H268 ~ 2.6 Å toward the scissile phosphate. RNA-contacting residues in nsp14 are highly conserved across coronavirus genera, indicating a shared recognition mechanism. ExoN requires the 2'- and 3'-OH of the 3'-terminal nucleotide, removing either reduces degradation, and loss of the 3'-OH nearly abolishes activity.

CONCLUSIONS:

Our study elucidates the mechanism of mismatch correction during SARS-CoV-2 RNA synthesis and defines substrate features critical for ExoN recognition and catalysis, enabling structure-guided design of potent, specific ExoN inhibitors. Such inhibitors, coadministered with nucleotide analog RdRp antivirals, could enhance COVID-19 therapy. We also inform the development of ExoN-resistant nucleotide analogs: a free 3'-OH is required for ExoN excision, suggesting that 3'-deoxy nucleotide analogs, known to be incorporated and act as chain terminators in other positive-strand RNA viruses, may both terminate coronavirus RNA synthesis and resist ExoN removal.



Abstract No.: 151

24 June 2026 (Wednesday) 1440-1455

Capturing the first translation and replication events: single-molecule imaging of early coronavirus infection.

First Author: Martin Gonzalez Burgos, Institute of Virology and Immunology

Co-Author(s): Bettina Trueeb, Annika Kratzel, Agata Misiaszek, Tobias Hochstoeger, Jeffrey Chao, Volker Thiel

BACKGROUND OBJECTIVES:

Coronaviruses are a family of enveloped, positive-sense single-stranded RNA viruses that infect mammals and birds, causing diseases ranging from mild to lethal. Their importance has been highlighted globally following the COVID-19 pandemic. Mouse Hepatitis Virus (MHV), a member of the Betacoronavirus genus like SARS-CoV-2, serves as a model to study coronavirus tropism, pathogenicity, and replication. This project aims to elucidate the early stages of coronavirus infection, focusing on events occurring immediately after viral binding and cellular entry. Understanding how infection is initiated and how viral replication complexes are established is essential to uncover the molecular mechanisms governing coronavirus infection and virus–host interactions.

METHODS:

To investigate these early infection events, we implemented a live-cell imaging approach based on the SunTag technology, enabling real-time visualization of single viral translation events. In this system, SunTag peptide epitopes were fused to the viral nonstructural protein nsp2 of MHV, associated with the viral Replicase-Transcriptase Complex.

RESULTS:

The SunTag based system enabled detection of translation from the first incoming viral genome immediately after cell entry, followed by translation from newly synthesized positive strand RNAs. Allowing also visualization of nsp2 accumulation. Using this approach, we identified distinct infection associated phenotypes in mouse fibroblasts, revealing substantial heterogeneity between cells in the establishment of infection and the kinetics of viral replication.

CONCLUSIONS:

This work establishes a framework for visualizing the early stages of coronavirus infection at single-molecule resolution. The approach provides direct insight into viral translation dynamics immediately following cell entry and reveals cell-to-cell heterogeneity in infection outcomes. Further refinement and expansion of this system will enable systematic mapping of the molecular processes governing early coronavirus infection.

Abstract No.: 418

24 June 2026 (Wednesday) 1455-1510

The first foothold: the initiating steps in coronavirus replication visualized in real time and by 4i/smFISH analysis

First Author: Sypke van Terwisga, Utrecht University

Co-Author(s): Chiara Aloise, Arno L.W. Van Vliet, Richard Wubbolts, Sereina O. Sutter, Cornelis A. M. de Haan, Frank J. M. van Kuppeveld, Raoul J. de Groot

BACKGROUND OBJECTIVES:

Coronavirus (CoV) RNA synthesis occurs within the confines of cytosolic virus-induced double membrane vesicles. Newly made genomic (gRNA) and subgenomic (sg) mRNAs are exported from these replication organelles (ROs) via specialized pores. Although the basic principles of CoV replication are understood in considerable detail, critical gaps remain, particularly regarding the early molecular and cellular events that shape the course and outcome of infection. Here, we investigated the earliest stages of replication at the level of the individual cell using mouse hepatitis virus as a model system.

METHODS:

We used live cell imaging and quantitative iterative indirect immunofluorescence imaging combined with differential single molecule FISH (q4i-smFISH) to capture the initiating steps of the CoV replication cycle after single infection events. To allow high sensitivity/high specificity detection, we constructed recombinant viruses encoding genetically-tagged key proteins of the replication complex and ROs.

RESULTS:

Visualization of early infection in real time revealed considerable cell-to-cell heterogeneity. Initial translation of the viral genome, evidenced by focal accumulation of RO constituents at single sites within each infected cell, was detected from 2h pi onwards, followed by incremental accumulation of satellite sites in successive wave-like fashion as the infection progressed. As found by q4i-smFISH, the primary foci develop into sites of replication as evidenced by the detection of (-)strand RNAs, which in turn served as templates for the synthesis of

gRNA and sgRNAs. These (+)RNAs were found to accumulate in situ initially, apparently within a membrane-protected compartment consistent with a primary RO, prior to their dispersal in the cytoplasm. Upon translation, newly synthesized N protein was recruited at the primary RO, coinciding with the appearance of encapsidated gRNA in the cytoplasm and subsequent emergence of satellite ROs. gRNA encapsidation seems key to efficient RO formation. Substitutions in N affecting RNA binding were predictably lethal, but recombinant viruses harboring such mutations could be salvaged in a supporting cell line. Infection with these viruses typically was abortive in non-supporting cells, with either no evidence of RNA synthesis at all or arrest at the primary RO stage.

CONCLUSIONS:

Our findings provide a comprehensive, stepwise view of the earliest stages of CoV infection, shedding light on the biogenesis and maturation of primary ROs and on the importance of early N protein expression. These insights may clarify the role of N in CoV replication, which has been a topic of longstanding debate.



Abstract No.: 423

24 June 2026 (Wednesday) 1510-1525

A new model of coronavirus replication and transcription

First Author: Nuno Santos, The Francis Crick Institute

Co-Author(s): Joe Hemsley-Taylor, Ciaran Gilbride, Yew Mun Yip, Anob Chakrabarti, David LV Bauer,

BACKGROUND OBJECTIVES:

The RNA-dependent RNA polymerase (RdRp) of nidovirales is a molecular acrobat, coordinating both genome replication and transcription of subgenomic RNA. Together with its replicase complex, it carries out this task in highly structured genomes, making initiation an intricate challenge. In coronavirus, the overall organisation of the 3'-UTR is conserved. Specifically, in betacoronavirus, it includes the Bulged Stem Loop (BSL) and the Stem Loop 2 (SL2), whose top can form a pseudoknot (PK) with the 3' side of the BSL stem. Previous models suggest that PK formation, after the unpairing of the polyA-proximal region (pAprox), brings the RdRp closer to the extension site, thus being the molecular switch governing initiation. However, these rely on initial primase activity, do not address where the potential promoter region is, and how is the RdRp translocated to the active site.

METHODS:

Here, we report on RdRp dynamics during SARS-CoV-2 infection in cultured cells using improved individual-nucleotide resolution crosslinking and immunoprecipitation (iiCLIP) and RNA hybrid and individual-nucleotide resolution ultraviolet crosslinking and immunoprecipitation (HiCLIP), which generate a 2D snapshot of nsp12 and nsp8, components of the SARS-CoV-2 RdRp, along the viral genome. We then modelled the 3D structure by running molecular dynamics (MD) simulations, and validated it by engineering a comprehensive panel of mutations.

RESULTS:

We observed consistent patterns of dynamic RdRp association with the BSL (but not with SL2) as well as that the PK is not the predominant conformation upon

infection. In agreement, MD modelling showed that the RdRp docking into the BSL is sterically permissible. Importantly, it also showed that the formation of the PK does not require unpairing of the pAprox region. Our panel of mutants revealed that length and structural integrity of the BSL stem are essential, while the top loop is dispensable. Moreover, mutations increasing PK probability were tolerated, opposing to mutations preventing it, which proven lethal. Based on our new insights, we propose an updated model of betacoronavirus transcription and replication initiation, where nsp12 acts to initiate transcription without any independent primase functions of other nsps.

CONCLUSIONS:

Our data revealed striking patterns of RdRp association with the viral genome, allowing us to identify the putative promoter for genome replication and transcription in SARS-CoV-2 in the 3'UTR, as well as the conformation of key RNA structures to initiate transcription and replication. Ultimately, the generated knowledge will advance the understanding of fundamental and conserved mechanisms regulating viral replication and driving evolution.



Abstract No.: 358

24 June 2026 (Wednesday) 1525-1535

Perturbing MHV:nsp5 to unveil the role of proteolytic processing on viral RNA synthesis

First Author: Maximilian Erdmann, University of Liverpool

Co-Author(s): Rebekah Penrice-Randal, Leandro X Neves, I'ah Donovan-Banfield, Frazer Buchanan, Andrew Davidson, Edward Emmott

BACKGROUND OBJECTIVES:

Coronaviruses express the components of their replicase-transcriptase complex (RTC), the non-structural proteins (nsps) as part of a large viral polyprotein. The polyprotein is autoproteolytically processed via the two encoded viral proteases nsp3 (nsp1-3) and nsp5 (nsp4-16). In alphaviruses this process is highly regulated; larger precursors control the synthesis of anti-sense RNA whereas, smaller, more cleaved precursors/nsps control positive-sense RNA replication. This is less defined in the coronavirus family. For murine hepatitis virus (MHV), previous work demonstrated certain cleavage sites as essential for viability, however, the role of specific precursors remains largely unknown.

METHODS:

To continue from this previous work to explore the role of polyprotein cleavage and its individual precursors we employed an emerging proteomics methodology (N-terminomics) capable of identification and quantitation of proteolytic cleavage events. Additionally, we utilised strand-specific RT-qPCR to assess viral RNA synthesis (genome and antigenome) to determine the relationship between polyprotein processing and regulation of viral RNA replication. We generated recombinant wild-type (r)MHV and a previously described temperature-sensitive mutant by introducing a F219L mutation in nsp5 (rMHV-ts) using yeast-based reverse genetics. rMHV infected cultures were subjected to perturbation using temperature shifts and nsp5 inhibitor nirmatrelvir. Samples were assessed for viral cleavage and genome/antigenome replication kinetics using N-terminomics and strand-specific RT-qPCR respectively.

RESULTS:

rMHV generated were free of undesired mutations and replicated comparably at 33 °C; while rMHV-ts was lethally attenuated at 39 °C. In cultures maintained at 33 °C, we identified cleavage between nsp1-2 (YRG247VKP), nsp4-5 (FLQ3333SGI), nsp7-8 (ALQ4015SEF), nsp8-9 (VLQ4209NNE), nsp9-10 (RLQ4319AGT) and nsp12-13 (VLQ5382SVG). When infected cultures were shifted to 39 °C early during

replication; viral titres were reduced in rMHV-ts but not rMHV. For rMHV-ts this was accompanied by reduced cleavage at these sites, as well as a failure to synthesise antigenome. Temperature-shift during late replication did not result in defects at virus release and RNA synthesis; however, viral cleavage was still reduced compared to rMHV.

CONCLUSIONS:

Here we provide experimental evidence of MHV polyprotein processing, confirming a nsp5-mediated proteolytic regulation of the coronavirus RNA synthesis. We provide and confirm experimental evidence for the occurrence of cleavages in the RTC resulting in 'conversion', directing the RTC from antisense to sense RNA synthesis.



Abstract No.: 69

24 June 2026 (Wednesday) 1620-1635

Intermediate hosts prime merbecovirus spike for human compatibility

First Author: Michael Letko, Washington State University

Co-Author(s): Victoria Jefferson, Jarel Elgin Tolentino, Nick Catanzaro, Spyros Lytras, Ralph Baric, Kei Sato

BACKGROUND OBJECTIVES:

Most human coronaviruses likely originated in wildlife before spilling over into humans. Although the exact source species is often unclear, bats, rodents, and birds are suspected reservoirs. Bat-human interactions are not always obvious, making origins difficult to trace. Intermediate hosts may bridge transmission by amplifying the virus and enabling mutations that improve adaptation to new hosts. Merbecoviruses, a coronavirus subgenus that includes Middle East Respiratory Syndrome Coronavirus (MERS-CoV), occur globally in bats, hedgehogs, pangolins, and camels. In the past five years, two bat merbecoviruses have spilled over into new hosts: HKU4-CoV, originally found in *Tylonecteris* bats, has been detected in Malaysian pangolins, and HKU5-CoV, previously unique to *Pipistrelle* bats, has been identified in farmed mink in China. These host jumps raise the question of whether viral species tropism has shifted compared with earlier HKU4 and HKU5 strains.

METHODS:

Because host receptor compatibility is a key determinant of coronavirus cross-species transmission, we tested the host receptor interactions for the spike proteins from both HKU4-CoV and HKU5-CoV viruses. We generated replication-defective pseudotypes and measured viral entry in various cell lines expressing coronavirus receptor orthologs from different species.

RESULTS:

Our results show that the spike proteins from the Malaysian pangolin strain of HKU4-CoV and farmed mink strain of HKU5-CoV have broadened species compatibility as compared to other HKU4-CoV and HKU5-CoV viruses. The spike protein from mink HKU5-CoV is recombinant from least two other viruses, endowing the mink virus with a unique species adaptability for the other orthologs of its receptor, including from humans. We track receptor use through the parental strains to show how the resulting HKU5 that emerged in mink with an intrinsic ability expand to new hosts.

CONCLUSIONS:

These results provide molecular support for the concept of intermediate hosts playing an essential role in the cross-species transmission of coronaviruses from wildlife to humans.



Abstract No.: 162

24 June 2026 (Wednesday) 1635-1650

CEACAM6 is a newly discovered receptor for different alphacoronaviruses

First Author: Giulia Gallo, The Pirbright Institute

Co-Author(s): Antonello Di Nardo, Adam Roberts, Aghnianditya Kresno Dewantari, Moses Okombo, Bernadette Ataku Kutima, Doreen Lugano, Florence M. M. Buckley, Gavin J. Wright, James Nyagwange, Bernard Agwanda, Stephen C. Graham, Dalan Bailey

BACKGROUND OBJECTIVES:

Cross-species transmission of viruses is initiated by successful host cell entry, where viral attachment proteins interact with cellular receptor(s). Defining receptor usage is therefore central to understanding host range and zoonotic potential. Alphacoronaviruses (alphaCoVs) represent a diverse and widespread genus with established capacity for cross-species transmission, yet their receptor usage and host tropism remain poorly characterised at the genus level. To date, only two entry receptors have been described for alphacoronaviruses, APN and ACE2, suggesting that additional receptors likely exist. Here, we aimed to systematically explore receptor usage across the alphacoronavirus genus by combining computational sequence selection with experimental screening approaches, with the goal of characterising known receptors, identifying novel receptors and improving our understanding of alphacoronavirus host range and zoonotic risk.

METHODS:

We used pseudotyped alphaCoV Spikes (S) to assess their dependency on APN and ACE2 from different mammalian species, and to evaluate potential for human zoonosis by transduction of human cell lines. Using a receptor discovery platform, we assessed if any of the S could interact with potential human receptor(s). Using cellular and molecular approaches, including X-ray diffraction, we validated the identified hits. We performed immunological studies, such as ELISA, to detect potential evidence of spillover in the human population of these alphaCoVs. Finally, through bioinformatic analysis, we expand our understanding of receptor usage among alphaCoVs.

RESULTS:

Using a greedy algorithm, we assembled a representative library of alphacoronavirus spike proteins to assess their dependence on APN and ACE2 for viral entry. Most viruses were unable to utilize any of the tested receptors, suggesting that additional, as yet unidentified, proteinaceous receptors remain

to be discovered. Entry assays in human cell lines revealed that CcCoV-KY43 can use a previously uncharacterized human receptor, which we identified as CEACAM6. We determined the structure of CEACAM6 in complex with the receptor-binding domain of CcCoV-KY43 by X-ray crystallography, providing molecular insight into the interaction interface. Although CcCoV-KY43 was originally identified in Kenya, sero-surveillance analyses showed no evidence of spillover into the human population. We further identified KY43-related viruses in Asia and Europe that also utilize CEACAM6 for entry, albeit not the human orthologue.

CONCLUSIONS:

By integrating computational sequence selection with high-throughput screening, receptor identification, structural characterisation, and sero-surveillance, we identify CEACAM6 as a previously unrecognised receptor for alphacoronaviruses circulating across Africa, Europe, and East Asia. These findings reveal a new pathway for viral entry, highlight a potential risk for zoonotic emergence, and provide a mechanistic framework to inform future surveillance and pandemic preparedness efforts.



Abstract No.: 113

24 June 2026 (Wednesday) 1650-1705

Sialoglycan Binding in HKU1 Cell Entry: Not Such an Open-and-Shut Case

First Author: Daniel Hurdiss, Utrecht University

Co-Author(s): Robert Creutzmacher, Louisa Wallace, Oliver Debski-Antoniak, Ieva Drulyte, Ruben J.G. Hulswit, Jeffrey Beekman, Frank J.M. van Kuppeveld, Berend-Jan Bosch, Xander de Haan, Martin Frank, Raoul J. de Groot

BACKGROUND OBJECTIVES:

Human coronavirus HKU1 initiates infection by binding 9-O-acetylated sialoglycans via the spike S1A domain, a step previously shown to promote spike opening and enable engagement of the secondary receptor TMPRSS2. However, HKU1 comprises two serotypes with divergent spike proteins, raising the question of whether receptor binding and activation mechanisms are conserved. Recent structural studies have further suggested that HKU1-B spikes may undergo spontaneous, ligand-independent opening, challenging a strictly receptor-triggered activation model. Here, we aimed to define the ligand specificity and structural basis of sialoglycan recognition across HKU1 serotypes, and to determine how receptor engagement contributes to spike activation and viral entry.

METHODS:

We combined biochemical, structural, and cell-based approaches to investigate HKU1 spike-receptor interactions. Glycan binding specificity and kinetics of HKU1-A and -B spike proteins were analyzed by biolayer interferometry using defined O-acetylated sialoglycans. High-resolution cryo-electron microscopy structures of the HKU1-B spike in apo and ligand-bound states were determined and complemented by molecular dynamics simulations and structure-guided mutagenesis. The role of N-linked glycans in modulating binding affinity and specificity was assessed using engineered glycoforms. Finally, spike binding and viral infection were examined in differentiated human nasal epithelial air-liquid interface cultures.

RESULTS:

HKU1-A and -B spike proteins exhibited a shared and highly selective preference for α 2,8-linked 9-O-acetylated disialosides, mediated by a conserved extended binding site accommodating both the terminal (Sia2) and penultimate (Sia1) sialic acid residues. Structure-guided mutagenesis revealed a critical contribution of Sia1 to binding affinity. In addition, conserved N-linked glycans modulated both binding strength and ligand selectivity, indicating that receptor recognition

is governed not only by protein–glycan interactions but also by glycan shielding. Strikingly, cryo-EM analysis revealed no detectable open spike conformations in either apo or ligand-bound HKU1-B samples under near-native conditions. Despite extensive ligand occupancy and adoption of the activated S1A state, all spike trimers remained in a closed conformation, highlighting a lack of spontaneous or receptor-induced opening. In human nasal epithelial cultures, infection was restricted to ciliated cells and strictly dependent on 9-O-acetylated sialoglycans, likely presented on gangliosides such as GT3.

CONCLUSIONS:

HKU1 spike proteins from both serotypes share a conserved, glycan-regulated specificity for 9-O-acetylated disialoside receptors that are functionally relevant in the human airway epithelium. However, receptor binding alone does not universally trigger spike opening, revealing serotype-dependent differences in activation mechanisms. Notably, our data challenge recent reports of spontaneous, ligand-independent opening of HKU1-B spikes and instead indicate that this serotype is less predisposed to receptor-induced conformational transitions than its HKU1-A counterpart.



Abstract No.: 42

24 June 2026 (Wednesday) 1705-1715

The Omicron tropic shift from SARS-CoV-2 Arises from Variant Specific Exploitation of Distinct ACE2 Compositions

First Author: Timothy Ison, Kirby Institute, UNSW

Co-Author(s): Anupriya Aggarwal, Samantha Ognenovska, Stuart G Turville, Christina Fichter, Vanessa Milogiannakis, Madeeha Afzal, Stefan Pöhlmann, Markus Hoffmann, Matt D Johansen, Melissa Churchill, Thomas Angelovich, Anthony D Kelleher

BACKGROUND OBJECTIVES:

SARS CoV 2 entry and tropism are shaped by Spike-ACE2 engagement and protease activation, yet the basis for Omicron's airway biased replication and altered TMPRSS2 use remains unresolved. ACE2 receptor exists in various protein complexes in vivo based upon organ type. In the gastrointestinal system, ACE2 is in complex with a solute carrier protein, SLC6A19. The role that this ACE2-SLC6A19 complex plays with SARS-CoV-2 entry remains undefined. Here we resolve the mechanisms that reshape Omicron replication dynamics.

METHODS:

Using clinical isolates spanning the pandemic we resolved the phenotypic outcomes when viral variants were exposed to different ACE2 complexes in the presence of TMPRSS2. To achieve this, we systematically generated clonal cell lines to incorporate ACE2 and TMPRSS2 (RAS-functional ACE2 pool) or ACE2 + SLC6A19 and TMPRSS2 (ACE2 chaperone function) and then directly compared them with outcomes in animal and primary tissue models.

RESULTS:

We observe Omicron replication to be dominated through cell-to-cell spread which can only be sustained in a chaperone ACE2 pool (ACE2-SLC6A19/20). In contrast when ACE2-TMPRSS2 complexes form, Omicron lineages are attenuated. Mechanistically, this reflects extracellular, membrane-bound Spike undergoing S1/S2 cleavage at the canonical furin site following only chaperone ACE2 engagement on adjacent cells, licensing subsequent TMPRSS2 activity during cell-to-cell transmission. In the presence of ACE2-TMPRSS2 complexes Spike S1/S2 cleavage and TMPRSS2 use is restricted to early circulating pre-Omicron lineages and not subsequent Omicron lineages. Observations in vivo and with primary cells support this later mechanism to contribute towards attenuation in the lung, whilst in nasal epithelia this results in augmentation and greater viral fitness.

CONCLUSIONS:

Omicron is preferentially and rapidly amplified by chaperone ACE2 composition (ACE2-SLC6A19), providing a mechanistic explanation for its hyper-transmissible spread phenotype.



Abstract No.: 63

24 June 2026 (Wednesday) 1715-1725

The SLC39A9-C1GALT1 Axis Promotes Coronavirus Infection by Coupling Viral Entry with Innate Immune Suppression

First Author: Wentao Li, Huazhong Agricultural University

BACKGROUND OBJECTIVES:

Porcine epidemic diarrhea virus (PEDV) has caused devastating economic losses to the global swine industry; however, the key host factors regulating its infection cycle and the underlying molecular mechanisms remain poorly understood. The objective of this study was to identify these essential host factors and elucidate their mechanisms of action during PEDV infection.

METHODS:

The research utilized a genome-wide CRISPR/Cas9-knockout (KO) screen to identify candidate host factors. To understand the underlying mechanisms, the study employed SLC39A9-KO models to investigate protein stability, evaluate cell-surface sialic acid abundance during the viral attachment phase, and analyze immune regulation pathways. Furthermore, the effects of SLC39A9-KO were tested against multiple other coronaviruses to assess broad-spectrum antiviral potential.

RESULTS:

We demonstrated that the stability of core 1 β 1,3-galactosyltransferase (C1GALT1) is highly dependent on SLC39A9 and its critical zinc-transporting site (H155), and that SLC39A9-KO resulted in the degradation of C1GALT1. Further mechanistic investigations revealed that the SLC39A9-C1GALT1 axis promoted viral infection via a dual mechanism: during the viral attachment phase, SLC39A9-KO and the subsequent C1GALT1 degradation led to a marked reduction in the overall abundance of cell-surface sialic acids—specifically β -2,3- and β -2,6-linked sialic acids—thereby significantly impairing effective viral attachment. Regarding immune regulation, we found that C1GALT1 exerted an enzyme-independent immunomodulatory function; specifically, its N-terminal domain suppressed the transcription of IRF3, thereby blocking the activation of the type I interferon signaling pathway. Furthermore, SLC39A9-KO markedly inhibited infection by multiple coronaviruses, including porcine enteric coronaviruses: porcine deltacoronavirus (PDCoV), transmissible gastroenteritis virus (TGEV), and human coronavirus SARS-CoV-2.

CONCLUSIONS:

These results indicate that the host factor SLC39A9 acts as a dual pivotal hub in regulating both cell-surface sialic acid abundance and the innate immune response. Consequently, SLC39A9 provides a novel potential target for the development of broad-spectrum antiviral strategies against multiple coronaviruses.



Abstract No.: 118

24 June 2026 (Wednesday) 1725-1735

Dynamic binding to proteoglycans in airway epithelium drives SARS-CoV-2 evolution

First Author: Bo Meng, University of Cambridge

Co-Author(s): Pan Liu, Mazharul Altaf, Wenrui Guo, Mark Tsz Kin Cheng, Sathuwarman Raveenthiraraj, Alice Chernaik, Tomokazu Tamura, Takasuke Fukuhara, Frank McCaughan, Xiangxi Wang

BACKGROUND OBJECTIVES:

Diverse viruses have explored ubiquitously expressed proteoglycans as attachment factors for cell entry, leading to tropism expansion. SARS-CoV-2 has evolved multiple positively charged residues over the course of the pandemic, some of which coincide with the heparan sulfate (HS) binding site on spike, including a pocket at N354 that is modified by the K356T mutation via N354-linked glycosylation. Convergent evolution at position K356T has occurred across the global SARS-CoV-2 phylogeny.

METHODS:

Here, we investigated the influence of proteoglycan on SARS-CoV-2 infection by using comparative viruses during the virus evolution, both clinical isolates and recombinant viruses, in a multidonor-based physiologically relevant air-liquid interface (ALI) system.

RESULTS:

We found that despite a more rapid and pronounced replication than early VOCs, Omicron BA.2.86 has become susceptible to trapping by surface proteoglycans due to higher spike-HS binding affinity, thereby limiting infection. This tight binding augments virus entry in low-ACE2 expressing cells by synergising spike-ACE2 interaction. However, when ACE2 is abundant, HS becomes inhibitory and can be mitigated by rapid TMPRSS2-dependent fusion.

CONCLUSIONS:

Our study reveals context-dependent roles of HS in orchestrating the intricate interplay between viral glycoprotein and proteinaceous receptors that together dictate entry efficiency and potentially human-to-human transmission.

Abstract No.: 53

24 June 2026 (Wednesday) 1735-1745

A pH-driven receptor switch from ACE2 to NPC1 enables endosomal entry of SARS-CoV-2

*First Author: Yonghui Zheng, University of Illinois Chicago**Co-Author(s): Zhongshun Liu, Yu Pang, Shuvankar Dey, Yixiang Hu*

BACKGROUND OBJECTIVES:

SARS-CoV-2 enters host cells either through TMPRSS2-mediated membrane fusion at the plasma membrane or via a cathepsin-dependent pathway following endocytosis. While ACE2 is the primary cell-surface receptor, how the viral spike protein remains fusion-competent during endosomal trafficking and mediates fusion in acidic compartments has remained unclear. Here, we investigated whether Niemann–Pick C1 (NPC1), a late endosomal/lysosomal membrane protein previously identified in CRISPR screens for SARS-CoV-2 host factors, functions as an intracellular receptor enabling endosomal entry.

METHODS:

NPC1 function was evaluated using CRISPR–Cas9 knockout cell lines, pharmacological inhibition, and rescue experiments across multiple cell types. Viral entry was assessed using SARS-CoV-2 spike-pseudotyped particles and authentic SARS-CoV-2 variants. Spike–NPC1 interactions were examined using bimolecular fluorescence complementation, bilayer interferometry, and competition assays with neutralizing antibodies. Fusion activity was analyzed using cell–cell fusion assays and virus-like particle fusion systems under neutral and acidic conditions

RESULTS:

Loss of NPC1 strongly inhibited SARS-CoV-2 infection in cell types that rely on endosomal entry, while having minimal effects when TMPRSS2-mediated surface fusion was available. NPC1 knockout caused virions to accumulate within endosomal vesicles, indicating a block at a post-endocytic entry step. Spike–NPC1 complexes localized to Rab7-positive late endosomes and LAMP1-positive lysosomes. Biochemical analyses showed that NPC1 loop C binds the spike receptor-binding domain preferentially at acidic pH through a surface distinct from the ACE2 interface. Engineered receptor assays further demonstrated that NPC1 loop C alone can promote spike-mediated fusion under low-pH conditions. Consistent with its reduced fusogenicity at the cell surface, the Omicron variant

displayed heightened dependence on NPC1.

CONCLUSIONS:

These findings identify NPC1 as an intracellular receptor that enables SARS-CoV-2 fusion within acidic endolysosomal compartments. We propose a pH-driven receptor switch in which the virus initially engages ACE2 at the cell surface and subsequently binds NPC1 during endosomal maturation, thereby sustaining fusion competence. This work expands the receptor logic of coronavirus entry and highlights an intracellular receptor step that may be targeted for antiviral intervention.



Abstract No.: 84

24 June 2026 (Wednesday) 1745-1755

Structural basis and regulation of sialic acid receptor recognition by infectious bronchitis virus

*First Author: Harry Libbey, Utrecht University**Co-Author(s): Ruben Hulswit, Daniel Hurdiss, Berend-Jan Bosch*

BACKGROUND OBJECTIVES:

Infectious bronchitis virus (IBV) is a globally endemic avian gammacoronavirus that causes major economic losses in poultry. IBV engages $\alpha 2,3$ -linked sialic acids via its spike (S) glycoprotein, yet unlike other sialic acid-binding viruses, it lacks a receptor-destroying enzyme. The structural basis of receptor recognition and regulation of engagement therefore remain poorly understood. We aimed to define the molecular basis of sialic acid binding by IBV spike and to determine how receptor interactions are regulated.

METHODS:

High-resolution cryo-electron microscopy (cryo-EM) was used to determine structures of spike ectodomains from two genetically and geographically distinct IBV strains, Ma5 and D274. Spikes were expressed in both sialylation-competent and sialic acid-deficient cells to assess glycan-dependent effects. Biochemical binding assays and hemagglutination assays were performed to evaluate sialic acid interaction. Structure-guided mutagenesis was used to identify key receptor-binding residues, and sequence analysis assessed conservation across IBV genotypes.

RESULTS:

Cryo-EM reconstructions revealed density consistent with a sialic acid bound within a conserved pocket in the S1A domain. This density was present in spikes produced in sialylation-competent cells but absent in spikes from sialic acid-deficient cells. Spikes lacking associated sialoglycans exhibited markedly enhanced sialic acid-binding activity in biochemical and hemagglutination assays, indicating that spike-associated glycans regulate receptor engagement. Structure-guided mutagenesis identified key residues within the receptor-binding site, and sequence analysis demonstrated functional conservation across IBV

genotypes.

CONCLUSIONS:

These findings provide the first structural definition of the IBV sialic acid-binding site and reveal a regulatory mechanism in which spike-associated sialoglycans modulate receptor interactions. This advances understanding of IBV attachment and highlights conserved functional features that may inform future antiviral and vaccine design strategies.



Abstract No.: 29

25 June 2026 (Thursday) 0955-1010

Betacoronavirus internal protein: role in immune evasion and viral pathogenesis

First Author: Roy Wong, Rutgers Health

Co-Author(s): Chaminda D. Gunawardene, Isha Pandey, Shruti Chatterjee, Yoatzin Peñaflor-Téllez, Abby Odle, Abbey N. Warren, Adriana Messias, Ricardo Rajsbaum, Alan Sariol

BACKGROUND OBJECTIVES:

Coronaviruses express a repertoire of accessory proteins for evading host immune responses. Notably, a small internal (I) accessory gene that overlaps in an alternative reading frame with the nucleocapsid (N) gene is encoded by the genus Betacoronavirus. The earliest report on the internal protein of mouse hepatitis virus suggested that the internal protein is a structural protein without significant functions in virus replication and virulence. However, the internal protein of human betacoronaviruses has been relatively understudied. While the internal proteins of SARS-CoV, MERS-CoV, and SARS-CoV-2 have been shown to evade immune responses, their roles in mediating pathogenesis have not been extensively characterized. In addition, common cold coronaviruses such as HCoV-OC43 and HCoV-HKU1 similarly encode an internal protein of previously unknown functions. We aim to dissect the functions of these internal proteins of different human betacoronaviruses in relation to pathogenesis.

METHODS:

Using established reverse genetic systems, we abrogated the expression of the internal proteins in MERS-CoV (protein 8b) and SARS-CoV-2 (protein 9b) by introducing premature stop codons to the I proteins without causing changes in the N protein sequences. Using mouse models of MERS-CoV and SARS-CoV-2 infection, MERS-CoV lacking protein 8b expression and SARS-CoV-2 lacking protein 9b expression were evaluated for changes in pathogenicity with the corresponding wild-type virus as a control. Similarly, the role of the I proteins of HCoV-OC43 (protein 8b) and HCoV-HKU1 (protein 7b) in mediating pathogenesis were investigated using an animal model. We examined how the heterologous expression of the HCoV-HKU1 and HCoV-OC43 I proteins impact pathogenesis in a mouse model of infection using a prototypic betacoronavirus due to the lack of robust reverse genetic systems, tissue culture and animal models for HCoV-

HKU1 and HCoV-OC43. We inserted the I genes of HCoV-HKU1 (ORF 7b) and HCoV-OC43 (ORF 8b) and the corresponding control sequences (with premature stop codons) independently into the genome of a neurotropic strain of mouse hepatitis virus (J2.2). The pathogenicity of these viruses expressing or lacking the I proteins of different human betacoronaviruses was evaluated to determine the role of these proteins in pathogenesis.

RESULTS:

We found that MERS-CoV lacking protein 8b expression was more virulent, while SARS-CoV-2 lacking protein 9b expression was attenuated compared with the respective wild-type viruses. Upon further analysis, we detected increased levels of type I interferon and enhanced infiltration of immune cells to the lungs of mice infected with MERS-CoV lacking protein 8b expression. These data suggest that the I protein of MERS-CoV plays a role in limiting pathogenesis while that of SARS-CoV-2 enhances disease severity. Furthermore, we showed that ORF 7b of HCoV-HKU1, but not ORF 8b of HCoV-OC43, ameliorated MHV-J2.2 pathogenesis. The presence of HCoV-HKU1 ORF 7b decreased virus titers and cytokine expression in vivo. Moreover, proteins expressed by ORF 7b of HCoV-HKU1 and ORF 8b of HCoV-OC43 showed different patterns of subcellular localization. Overall, our findings suggest that the I genes of different betacoronaviruses play unique roles in pathogenesis.

CONCLUSIONS:

Our data indicated that despite the similar genomic location and expression strategy of these internal proteins, their effects on virulence are vastly different and virus-specific, highlighting the complexity between host-virus interaction and disease outcome.



Abstract No.: 354

25 June 2026 (Thursday) 1010-1025

Why Did SARS-CoV Not Cause a Pandemic: Comparative Pathogenesis and Transmission Studies in Hamsters

First Author: Melanie Rissmann, Erasmus Medical Center

Co-Author(s): Debby van Eck - Schipper, Laura van Dijk, Mark Power, Edwin J.B. Veldhuis Kroeze, Anna Mykityn, Merel R. te Marvelde, Daryl Geers, Peter van Run, Bart L. Haagmans

BACKGROUND OBJECTIVES:

Over the last two decades, two zoonotic coronaviruses of the genus Sarbecovirus, severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2, caused significant global outbreaks in the human population. Although both coronaviruses are genetically relatively closely related and use the same cellular receptor for entry (ACE-2), the global impact of these outbreaks differed significantly with respect to hospitalizations and fatalities. We used hamsters to comparatively study transmission and pathogenesis of SARS-CoV and SARS-CoV-2.

METHODS:

We performed a direct comparative analysis of the replicative potential in the respiratory tract, transmission efficiency and pathogenesis of SARS-CoV (HKU-39849) and SARS-CoV-2 D614G in the Syrian golden hamster model. To define if observed phenotypes can be translated to the situation in humans, we furthermore compared our *in vivo* findings to replication kinetics of those viruses in multi-donor human nasal epithelial air-liquid interface cultures (hNEC-ALI).

RESULTS:

Surprisingly, SARS-CoV replicated to very high titers in the upper respiratory tract of hamsters, comparable to that of SARS-CoV-2. Also, in hNEC-ALI cultures, both viruses replicated to high titers. In addition, direct contact transmission in hamsters was efficient for both viruses in male and female animals. However, clear differences in the pathogenicity of the two viruses were observed. Experimental infection with SARS-CoV caused more significant clinical symptoms (body weight loss, respiratory symptoms), associated with a higher viral replication and inflammation in the lower respiratory tract in SARS-CoV infected animals in comparison to SARS-CoV-2 D614G infected hamsters.

CONCLUSIONS:

It has been postulated that SARS-CoV did not cause a pandemic because it showed limited replication in the upper respiratory tract and did not spread efficiently between humans. Our data in hamsters reveal no differences between tropism and contact transmission potential between SARS-CoV and SARS-CoV-2. However, we observed that SARS-CoV is more pathogenic in comparison to SARS-CoV-2. Therefore, effective isolation or containment measures due to severe disease were potentially the main reason that prevented SARS-CoV from a global spread as observed for SARS-CoV-2.



Abstract No.: 379

25 June 2026 (Thursday) 1025-1040

An adult stem cell-based organoid platform to assess virus tropism in the human upper and lower respiratory tract

First Author: Trena WX Lee, Duke NUS

Co-Author(s): Sophie W van Leur, Nicholas KW Yeo, Alan CY Hsu, Gavin JD Smith, Daniel SW Tan, Mart M Lamers

BACKGROUND OBJECTIVES:

Realistic models of the human respiratory tract are critical for studying respiratory viruses, enabling dissection of disease mechanisms and transmission, testing medical countermeasures, and supporting risk assessment of emerging viruses. Insights into upper (URT) and lower respiratory tract (LRT) tropism can help predict pandemic potential based on the premise that URT preference correlates with greater transmissibility, whereas LRT tropism is linked to severe disease. However, our predictive capacity is currently limited by the lack of well-validated physiologically relevant respiratory in vitro models. Developing such models is hindered by the limited proliferative capacity of key cell types, phenotypic plasticity in culture, donor-to-donor variability, and the scarcity of robust differentiation protocols. Here, we aimed to establish and validate an adult stem cell-derived organoid platform that accurately represents the human airway, serving as a risk assessment tool for emerging viruses.

METHODS:

We established human organoid models of the nasal and alveolar epithelium. Nasal basal cells obtained by nasal brushing of healthy donors were expanded and differentiated at air-liquid interface (ALI). Distal lung tissues derived from non-tumor regions adjacent to primary tumors were digested and sorted to isolate alveolar type 2 (AT2) cells, which were cultured as three-dimensional organoids. Organoid models were characterized by immunofluorescence and transcriptomics to investigate cell diversity and receptor expression. To further validate our organoids as infection models, they were infected with Human Coronavirus NL63 (HCoV-NL63), ancestral SARS-CoV-2, and SARS-CoV-2 Omicron.

RESULTS:

We confirmed that nasal ALI cultures recapitulated key epithelial cell types

(ciliated, goblet, club, and basal), while AT2 organoids expressed hallmark AT2 markers (HTII-280 and surfactant protein C). Both models express ACE2, the SARS-CoV-2 and HCoV-NL63 receptor, and TMPRSS2, a protease essential for SARS-CoV-2 entry. Using these organoid platforms, we found that the endemic HCoV-NL63 robustly infects nasal ALI cultures but is unable to infect AT2 organoids, consistent with a primarily upper airway tropism. The ancestral SARS-CoV-2 strain efficiently infects both the nasal ALI and AT2 organoids, whereas the Omicron strain is specifically attenuated in AT2 cells.

CONCLUSIONS:

Here, we established a physiologically relevant human organoid platform for dissecting virus tropism across the human airway. We validated the platform by accurately recapitulating coronavirus tropism, with tropism preferences correlating with reported clinical severity. The contrasting tropism of HCoV-NL63 and SARS-CoV-2 highlights that cell preference is governed by factors beyond receptor expression. Our platform can be used for characterization of emerging viruses to support risk assessment.



Abstract No.: 209

25 June 2026 (Thursday) 1040-1050

A nucleocapsid R204P mutation in SARS-CoV-2 Omicron XEC enhances inflammation and pathogenicity

First Author: Shuhei Tsujino, Kyushu University

Co-Author(s): Masumi Tsuda, Sayaka Deguchi, Jumpei Ito, Taha Y Taha, Hesham Nasser, Lei Wang, Julia Rosecrans, Rigel Suzuki, Saori Suzuki, Kumiko Yoshimatsu, Melanie Ott, Terumasa Ikeda, Kei Sato, Kazuo Takayama, Shinya Tanaka, Tomokazu Tamura, Takasuke Fukuhara,

BACKGROUND OBJECTIVES:

SARS-CoV-2 continues to circulate in human populations, and recombination between co-circulating variants contributes to its genetic diversity. The Omicron XEC variant, which became globally predominant in early 2025, emerged through recombination between two JN.1 descendants, KS.1.1 and KP.3.3. Although spike (S) mutations have been extensively studied, accumulating evidence suggests that non-spike mutations also contribute to viral fitness and pathogenicity. In particular, mutations in the nucleocapsid (N) protein at residues 203–204 have been implicated in viral replication and pathogenicity. Here, we characterize the virological features of XEC and identify mutations underlying its phenotype, with a focus on their functional impacts.

METHODS:

Mathematical modeling was used to estimate the contribution of mutations to the effective reproduction number. Spike-mediated membrane fusion assays and antiviral susceptibility tests were performed. Clinical isolates of JN.1 and XEC were analyzed in VeroE6/TMPRSS2 cells and in 4-week-old male Syrian hamsters. To assess the role of non-spike mutations, recombinant viruses were generated using the CPER method, in which the N:R204P mutation was reverted. Viral replication, body weight changes, viral RNA levels, lung pathology, and inflammatory responses were evaluated.

RESULTS:

Mathematical modeling indicated that XEC has a higher effective reproduction number than its parental lineages, with S:T22N and S:F59S contributing to increased transmissibility. In vitro, XEC exhibited comparable fusogenicity, antiviral susceptibility, and replication efficiency to JN.1. In contrast, XEC infection resulted in transient body weight loss, accompanied by increased lung pathology and

inflammatory responses, without increased viral replication in vivo. These findings indicate that enhanced pathogenicity is not attributable to increased viral replication. Recombinant virus analysis identified N:R204P as a key determinant. We found that this mutation impairs nucleocapsid-mediated suppression of NF- κ B signaling, thereby enhancing inflammatory responses.

CONCLUSIONS:

These results show that a single non-spike mutation can enhance pathogenicity by modulating host immune responses rather than viral replication. The R204P mutation promotes NF- κ B-mediated inflammation via disruption of nucleocapsid function. Collectively, these findings highlight a critical role for non-spike mutations in shaping SARS-CoV-2 pathogenicity through modulation of innate immune responses and provide mechanistic insight into host-virus interactions. Recombination among co-circulating lineages may facilitate the re-emergence of mutations with pathogenic potential, underscoring the importance of continued genomic surveillance and functional characterization of non-spike regions.



Abstract No.: 182

25 June 2026 (Thursday) 1050-1100

Strain-dependent Infection Outcomes of Feline Infectious Peritonitis Virus in Conventional Mice

First Author: Keisuke Nakagawa, Gifu University

Co-Author(s): Saya SUZUKI, Wataru KAMITANI, Taizo SAITO, Yasuhiro TAKASHIMA

BACKGROUND OBJECTIVES:

Feline infectious peritonitis virus (FIPV) is generally considered unable to infect mice. Thus, no robust small-animal model has been established for studying FIPV pathogenesis and transmission. In this study, we investigated the susceptibility of conventional mice to FIPV and whether different viral strains produce distinct disease phenotypes, with particular focus on neonatal infection, lethality, and transmission. Although the pathogenic differences between these two strains in cats have not been directly compared in this study, we used them to evaluate strain-dependent disease phenotypes in mice.

METHODS:

Type I FIPV C3663 (C3663) and type II FIPV WSU (WSU) strains were examined in conventional ddY and ICR mice. Adult mice were inoculated intracranially, orally, or intraperitoneally, and 2-day-old suckling mice were inoculated intracranially, orally, or intraperitoneally. For both virus strains, inoculations were performed using undiluted virus stocks at the highest available dose (C3663: 4.0×10^5 pfu/ml; WSU: 1.5×10^7 pfu/ml). Clinical signs, body weight, and survival were monitored. To evaluate transmission, co-housing experiments and dam-swapping experiments were performed in the oral WSU model. Viral RNA detection by RT-PCR was conducted using fecal samples from dams.

RESULTS:

Adult mice receiving intracranial, oral, or intraperitoneal inoculation did not show changes in body weight or lethal outcomes. In the neonatal mice, intracranial inoculation showed strain-dependent differences: C3663 was associated with severe neurologic signs and mortality, whereas WSU was associated with neither neurologic signs nor mortality. On the other hand, oral inoculation of WSU into suckling mice was associated with reduced weight gain and mortality, whereas

oral inoculation of C3663 did not show changes in body weight or lethal outcomes. Similar growth impairment was observed in co-housed, non-inoculated littermates, suggesting horizontal transmission. In the dam-swapping experiments, previously uninfected pups nursed by dams exposed to infected litters also showed reduced weight gain, indicating transmission via the dam. Viral RNA was detected in fecal samples of dams, suggesting fecal-oral and/or mother-mediated transmission. Intraperitoneal inoculation of either C3663 or WSU into suckling mice did not show changes in body weight or lethal outcomes.

CONCLUSIONS:

FIPV exhibited strain-dependent phenotypes in conventional mice under neonatal conditions. C3663 showed intracranial lethality in suckling mice, whereas WSU demonstrated oral infectivity and dam-mediated transmission. Although preliminary, this study suggests that FIPV can establish both infection and transmission cycles in mice, supporting the feasibility of developing a novel small-animal model for FIPV pathogenesis and transmission.



Abstract No.: 39

25 June 2026 (Thursday) 1100-1110

Mechanism of palmitoylation of MERS-CoV S protein

First Author: Zhaohui Qian, Chinese Academy of Medical Sciences & Peking Union Medical College

BACKGROUND OBJECTIVES:

Palmitoylation is a crucial post-translational modification that regulates protein function by attaching palmitic acid to target proteins. The spike (S) protein of coronaviruses (CoVs) undergoes palmitoylation, which regulates its membrane localization and facilitates viral fusion with the host cell membrane. However, the precise mechanism of S protein palmitoylation remains elusive.

METHODS:

cell fusion assays. In addition, the Acyl-Biotin Exchange (ABE) assay was employed to detect S protein palmitoylation.

RESULTS:

We found that the host acyltransferases ZDHHC18 and ZDHHC20 are likely key enzymes responsible for catalyzing palmitoylation of the MERS-CoV S protein. Notably, knockout of either ZDHHC18 or ZDHHC20 markedly reduced MERS-CoV infectivity and replication. This defect could be rescued only by reconstitution with the corresponding enzyme, suggesting that they function as heterodimeric or hetero-oligomeric complexes. Furthermore, co-immunoprecipitation (co-IP) and immunofluorescence assays (IFA) confirmed that ZDHHC18 and ZDHHC20 form hetero-oligomers.

CONCLUSIONS:

The MERS-CoV S protein is palmitoylated by ZDHHC18 and ZDHHC20, which act as heterodimeric or hetero-oligomeric complexes. The S protein interacts with ZDHHC18/20 through its transmembrane domain.



Abstract No.: 248

25 June 2026 (Thursday) 1150-1205

Dynamics of coronavirus-host cell protein interactions in assembly and egress

First Author: Brenda Hogue, Arizona State University

Co-Author(s): Bereket Stefanos, Noe Green, Isha Nadig, Ian Hogue, Honor Glenn

BACKGROUND OBJECTIVES:

Coronaviruses assemble at intracellular endoplasmic reticulum Golgi intermediate compartment (ERGIC) membranes and exit cells in cargo vesicles. Virus-host protein interplay is not fully defined. There is no consensus regarding multiple proposed pathway(s) taken for egress. Our objective is to gain complete mechanistic understanding of virus-host protein interactions and the pathway(s) taken during assembly and egress. We recently described a fluorescent tagged virus that is a significant new tool for assembly, trafficking and egress studies in live cells (doi:10.64898/2025.12.29.696688; J. Virol. revision submitted).

METHODS:

Two betacoronaviruses, mouse hepatitis virus (MHV) and SARS-CoV-2, are used as models for our studies. Reverse genetics was used to produce fluorescently labeled virus particles to help further understanding of coronavirus assembly and egress and the roles of virus-host proteins involved in the processes. Mouse hepatitis coronavirus (MHV-A59) was genetically engineered to express the M protein fused to green fluorescent protein (M-GFP). The chimeric gene was cloned in place of the open reading frame (ORF) 4 coding region in the RNA genome. The wild-type M protein (M WT) gene was left in its native ORF in the genome. Standard methods for studying viruses during infection were used to characterize the recovered M-GFP virus in comparison with the WT virus and understand assembly of the chimeric M-GFP protein into virus particles. Cryo-EM was used to image virus particles. Confocal microscopy was used to image protein expression and localization in fixed and live infected cells. Total Internal Reflection Fluorescence (TIRF) microscopy imaging was used for virus particle tracking in live cells. Analysis pipelines were developed using Nikon Elements software for image analyses. M-GFP virus was also used to identify and track host protein interacting partners. Cells were transduced with adenoviral vectors expressing fluorescently labeled Rab proteins before infection with M-GFP virus and analyzed

using standard molecular approaches and both fixed and live cell imaging.

RESULTS:

A coronavirus expressing a functional, GFP-tagged M protein was recovered through reverse genetic engineering of MHV to express and assemble the fluorescently tagged protein into virus particles for tracking in infected cells in real time in live cells. Purified extracellular green virus particles were recovered that exhibit strong fluorescence in tightly packed puncta when viewed by confocal microscopy. Particles imaged by cryo-EM display a diameter of 80-120 nm with spike trimer projections typical of coronavirus particles. Analysis of particles showed that WT M and M-GFP are co-assembled into virion particles through interaction of the two proteins. The M-GFP virus growth properties are like WT virus and both M and M-GFP proteins co-localize and co-traffic in virus particles to the cell periphery in infected cells. Using TIRF imaging of M-GFP virus trafficking in live cells shows thousands of GFP-positive particles moving at the cell surface and what appears to be exocytosis events. Our previous investigations using standard immunofluorescence showed that M protein colocalizes with cell Rab GTPase adaptor proteins in MHV and SARS-CoV-2 infected cells. The new M-GFP virus is being used to identify with confocal time course imaging and high-speed live cell TIRF imaging of Rab proteins associated with M-GFP positive particles and trafficking at the cell surface. M-GFP and Rab6 exhibit high colocalization in the Golgi early in infection, but M-GFP positive virus particles track away from the Rab6 signal, suggesting that Rab6 is involved in assembly, but not playing a direct role in egress. Very high colocalization of actively moving green virus particles with specific Rabs near the cell surface point to multiple pathways involved in egress that appear to be cell specific.

CONCLUSIONS:

We have demonstrated, to our knowledge for the first time, a coronavirus expressing a functional, fluorescent-tagged M protein that is assembled into virus particles and allows tracking in live cells. Assembly of M-GFP into particles serves as a selective probe for details of post-assembly trafficking and egress. The association of M-GFP with specific Rab proteins during infection suggests a model where multiple secretory pathways may function in parallel or may represent functional redundancy for coronavirus egress. This new tool opens significant, exciting opportunities for detailed functional studies using state of the art microscopy to study virus assembly, trafficking and egress in live cells to facilitate deeper understanding of the molecular and cell biology of these processes. The ability to visualize particles also opens opportunities to help further understanding of steps in viral replication and pathogenesis for potential therapeutic targets.

Abstract No.: 93

25 June 2026 (Thursday) 1205-1220

Host-mediated ADP-ribosylation targets multiple stages of the coronavirus replication cycle

First Author: Anthony Fehr, University of Kansas

Co-Author(s): Joseph O'Connor, Anuradha Roy, Reem Khattabi, Yousef Alhammad, Catherine Kerr,

BACKGROUND OBJECTIVES:

All coronaviruses (CoVs) encode a macrodomain, termed Mac1, that counters antiviral ADP-ribosyltransferase (PARP) activity. It has both ADP-ribose binding (reader) and ADP-ribosylhydrolase (eraser) activity. Recombinant MHV strain JHM (JHMOV) with a mutation in the Mac1 adenine binding site, JHMOV-D1329A, was extremely attenuated in all tested cell types independent of interferon (IFN). In contrast, a Mac1 mutant virus with a catalytic mutation (JHMOV-N1347A) only replicates poorly in IFN-competent cells. We hypothesized that these mutations separately affect Mac1 reader and eraser activity and that Mac1 uses both activities to counter host-mediated ADP-ribosylation to promote multiple stages of the coronavirus lifecycle.

METHODS:

We generated recombinant Mac1 protein and recombinant MHV with mutations predicted to that ablate Mac1 ADP-ribose binding (D-A) and ADP-ribosylhydrolase activity (N-A). We tested the ability of the recombinant proteins to bind ADP-ribose or ADP-ribosylated peptides using an alphascreen assay, differential scanning fluorimetry, and isothermal calorimetry. We also tested their ability to remove ADP-ribose from protein using a gel-based deMARylation assay. We tested the ability of recombinant viruses to replicate in several cell types and tested the impact of these mutations at several stages of the viral lifecycle using quantitative PCR, western blotting, and confocal microscopy.

RESULTS:

To clarify how these mutations affected the biochemical activities of Mac1, we generated Mac1 proteins encoding the same mutations. As expected, the D-A mutation was extremely defective in ADP-ribose binding but maintained enzyme activity. In contrast, we found that the N-A mutation had WT levels of ADP-ribose binding but low enzyme activity, confirming that these mutations differentially

effect the biochemical functions of Mac1. Both viruses replicated poorly in BMDMs, and mutant virus replication can be enhanced by PARP inhibition and further reduced by PARP activation. D1329A displayed a large defect in the accumulation of viral RNA compared to WT or N1347A in all cells tested. Alternatively, N1347A infection produced normal levels of viral RNA but produced reduced levels of viral protein in interferon competent bone-marrow derived macrophages (BMDMs).

CONCLUSIONS:

We conclude that Mac1 ADP-ribose reader and eraser activities counter PARP-mediated ADP-ribosylation that aims to restrict coronavirus replication at different stages of the viral lifecycle, highlighting the critical importance of Mac1 for JHMV replication.



Abstract No.: 49

25 June 2026 (Thursday) 1220-1230

From Bat to Human and Back: A Single Nucleocapsid Mutation Enables SARS-CoV-2 Replication in Greater Horseshoe Bat Cells

First Author: Magdalena Rutkowska, Icahn School of Medicine at Mount Sinai

Co-Author(s): Jyoti Batra, Anastasija Cupic, Thomas Kehrer, Fernando Villalon-Latelier, Zain Khalil, Chengjin Ye, Ignacio Mena, Marta Mayoral Cifuentes, Shivali Malpotra, Rithika Adavikolanu, Daniel Fuentes, Carles Martinez-Romero, Jack M Moen, Harm van Bakel, Sumit Chanda, Luis Martinez-Sobrido, Nevan Krogan, Lisa Miorin, Adolfo García-Sastre

BACKGROUND OBJECTIVES:

Horseshoe bats (genus *Rhinolophus*) are natural reservoirs of coronaviruses, including the ancestors of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), yet the molecular barriers that constrain virus replication in bat cells remain poorly defined. Notably, highly pathogenic human coronaviruses can replicate in bats without apparent disease, suggesting fundamental differences in host-virus interactions. Here, we identified an early replication block restricting SARS-CoV-2 replication in greater horseshoe bat cells and demonstrated that a single amino acid substitution in the viral nucleocapsid enables the virus to overcome this barrier.

METHODS:

We established a lung fibroblast cell line from the greater horseshoe bat. To enable viral entry, cells were transduced with human angiotensin-converting enzyme 2 (hACE2) and transmembrane protease serine 2 (TMPRSS2). SARS-CoV-2 infection and serial passaging were performed to adapt the virus to bat cells, followed by whole genome viral RNA sequencing to identify adaptive mutations. Recombinant viruses encoding these mutations were generated to test their impact on replication. We mapped the replication block using single-molecule RNA fluorescence in situ hybridization (smRNA FISH) against SARS-CoV-2 genomic RNA. Bat-human heterokaryon assay together with a targeted siRNA screen of host nucleocapsid interactors were then used to identify host dependency factors.

RESULTS:

Despite efficient viral entry, SARS-CoV-2 WA1 failed to replicate in greater horseshoe bat cells, revealing a post-entry replication block. Serial passaging of the virus in bat cells rapidly selected missense mutations in the viral genome. Functional analyses with recombinant viruses carrying these mutations showed that a single amino acid substitution in nucleocapsid was sufficient to restore robust viral replication in bat cells. Virus replication evaluation by smRNA FISH revealed that initial viral RNA amplification occurs but is subsequently aborted in bat cells infected with WT virus, indicating an early replication block, which can be overcome by the bat-adapted virus. Rescue of infection in bat-human heterokaryon suggested that the restriction arises from suboptimal interactions between wild-type nucleocapsid and bat host dependency factors and can be overcome by adaptive mutation. Consistent with this model, a targeted siRNA screen of host factors showing enhanced interaction with bat-adapted nucleocapsid identified proteins that modulate viral growth in bat cells.

CONCLUSIONS:

These findings identify an early replication barrier to SARS-CoV-2 infection in bat cells that can be overcome by minimal viral genetic adaptation. Our work reveals how subtle changes in the viral nucleocapsid can rewire host dependency networks, providing mechanistic insight into sarbecovirus host adaptation and potential bat-to-human transmission pathways. The bat cell model described here offers a powerful platform to dissect bat-specific antiviral responses and coronavirus zoonotic potential.



Abstract No.: 31

25 June 2026 (Thursday) 1230-1240

Comparative analysis in human respiratory epithelial cultures reveals patterns that distinguish pathogenic from seasonal human coronaviruses

First Author: Matthew Gartner, University of Melbourne

Co-Author(s): Jessica Neil, Arada Hirankitti, Stephany Sanchez, Randy Suryadinata, Joseph Chen, Guizhi Sun, Monika Mohenska, Annette Fox, Philip Robinson, Jose Polo, Kanta Subbarao

BACKGROUND OBJECTIVES:

Human coronaviruses (CoVs) range from minimally symptomatic seasonal strains to highly pathogenic viruses capable of causing severe pneumonia. The biological features that distinguish pathogenic coronaviruses (SARS-CoV, MERS-CoV, SARS-CoV-2) from seasonal strains (HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1) remain poorly defined. To identify determinants of pathogenicity, we systematically compared replication dynamics, cellular tropism, innate immune responses, and cytopathogenicity of five human CoVs (MERS-CoV, SARS-CoV-2, HCoV-229E, HCoV-NL63 and HCoV-OC43) in a panel of physiologically relevant airway and alveolar models.

METHODS:

We established four human respiratory epithelial systems spanning the upper and lower respiratory tract: primary nasal epithelial cells (HNECs), BCI-NS1.1 (BCi) and hSABCI-NS1.1 (SABCI) air-liquid interface cultures, and alveolar type II (AT2) cells differentiated from embryonic stem cells. Cultures were infected with seasonal (HCoV-229E, HCoV-NL63, HCoV-OC43) or pathogenic (SARS-CoV-2, MERS-CoV) viruses. Viral growth was quantified by infectivity assays, innate responses by cytokine bead arrays (CBA), cell death by lactate dehydrogenase (LDH) release, infected cell types by immunohistochemistry (IHC) and transcriptional responses in HNECs by single-cell RNA sequencing (scRNA-seq).

RESULTS:

SARS-CoV-2 showed the highest peak viral titres across all four models. MERS-CoV demonstrated robust replication in AT2 cells, similar replication to seasonal CoVs in HNECs and SABCI cultures, and limited replication in BCi cultures. Seasonal coronaviruses showed modest replication throughout all epithelial compartments.

All five viruses preferentially infected ciliated cells, with HCoV-229E, MERS-CoV and SARS-CoV-2 also infecting low proportions of secretory and basal cells. ScRNA-seq and CBA showed SARS-CoV-2 induced the strongest type I interferon (IFN) response in HNECs, followed by HCoV-229E and MERS-CoV. CBA showed a gradient in CXCL10, IFN- λ , IFN- λ 1 and IFN- λ 2 secretion across the different respiratory models for each virus, with the highest responses seen in HNECs and the lowest in AT2 cells. Principle component analysis of bulk RNAseq data from infected AT2 cells showed pathogenic CoVs clustered separately from seasonal CoVs. ScRNAseq showed MERS-CoV, SARS-CoV-2 and HCoV-229E induced strong expression of genes associated with apoptosis in HNECs. Analysis of LDH secretion showed MERS-CoV and HCoV-229E showed high cell death in AT2 cells.

CONCLUSIONS:

Replication efficiency in Lung AT2 cells differentiated pathogenic from seasonal CoVs. However, high cytotoxicity in AT2 cells by MERS-CoV and HCoV-229E indicates that viral pathogenicity in humans is not solely explained by cytopathic potential. In addition, HCoV-229E showed high cytokine expression and apoptotic signaling in HNECs despite modest viral replication, suggesting HCoV-229E may display distinct virus-host interactions compared to other seasonal CoVs.



Abstract No.: 68

25 June 2026 (Thursday) 1240-1250

Gasdermin Family Proteins as Innate Immune Effectors in Coronavirus Infection

*First Author: Pinghuang Liu, China Agricultural University**Co-Author(s): Yao Yao*

BACKGROUND OBJECTIVES:

Coronaviruses cause severe respiratory and enteric diseases in humans and animals and continue to pose major threats to public health and livestock production. Host cell death pathways and innate immune responses are critical determinants of viral replication and pathogenesis. Gasdermin (GSDM) family proteins are pore-forming effectors that mediate pyroptosis and inflammatory signaling. While pyroptosis has been implicated in viral infections, the specific roles and mechanisms of individual GSDM family members during coronavirus infection remain poorly defined. This study aimed to elucidate the antiviral functions of gasdermin proteins, particularly GSDMD and GSDMB, and to define their mechanisms in regulating coronavirus infection and host innate immune responses.

METHODS:

Using porcine enteric coronavirus models—including transmissible gastroenteritis virus (TGEV), porcine deltacoronavirus (PDCoV), and porcine epidemic diarrhea virus (PEDV)—we investigated the roles of gasdermin proteins in intestinal epithelial cells *ex vivo* and *in vitro*. Functional analyses were performed using gene overexpression and knockout systems, together with assays for viral replication, pyroptosis, and innate immune responses.

RESULTS:

Our results demonstrate that gasdermin family proteins function as important innate immune effectors against coronaviruses. GSDMD was activated during coronavirus infection and restricted viral replication by forming membrane pores that promote the unconventional secretion of interferon- β , thereby enhancing interferon-stimulated gene responses and establishing an antiviral state. In contrast, GSDMB suppressed PEDV and other swine enteric coronaviruses primarily through the induction of pyroptosis. PEDV infection triggered caspase-3/6/7-

mediated cleavage of GSDMB at residue D237, generating an active N-terminal fragment that forms membrane pores and induces pyroptotic cell death, thereby limiting viral propagation. Notably, PEDV evolved a countermeasure to evade this host defense by suppressing GSDMB expression through the viral proteins nsp1 and nsp15, revealing a novel immune evasion strategy targeting gasdermin-mediated antiviral responses.

CONCLUSIONS:

Our findings identify gasdermin family proteins as key regulators of coronavirus infection through distinct yet complementary antiviral mechanisms. GSDMD enhances antiviral interferon signaling through noncanonical cytokine secretion, whereas GSDMB directly restricts viral replication by inducing pyroptosis. In addition, coronaviruses have evolved strategies to antagonize gasdermin-mediated host defenses. These findings broaden our understanding of host-coronavirus interactions and highlight gasdermin proteins as potential targets for antiviral therapeutic strategies.



Abstract No.: 81

25 June 2026 (Thursday) 1250-1300

The Accessory Protein 3a of IBV Activates the NLRP3 Inflammasome in Renal Collecting Ducts via ER Calcium Mobilization and Mitochondrial ROS-Driven Mechanisms Contributing to Nephropathogenesis

First Author: Ye Zhao, China Agricultural University

Co-Author(s): Min Huang, Chengyin Liukang, Yingfei Li, Ruihua Yang, Rong Liang, Jing Zhao, Guozhong Zhang

BACKGROUND OBJECTIVES:

Infectious bronchitis virus (IBV) causes severe respiratory and renal inflammation and tissue damage in chickens. However, the molecular mechanisms underlying IBV-induced renal lesions remain unclear. The NLRP3 inflammasome is critical for antiviral and inflammatory responses, yet how IBV regulates its activation and the role of viral proteins in this process are unknown. This study explores the mechanisms by which IBV activates NLRP3 inflammasome to trigger renal inflammation and injury.

METHODS:

We investigated IBV-induced NLRP3 inflammasome activation and renal inflammation with a focus on accessory protein 3a. Transcriptomic analysis was performed in chicken kidneys and CEK cells. In vivo studies using SPF chickens were applied to detect histopathology, inflammatory factors, and inflammasome assembly. Co-IP and GST pull-down assays identified 3a-NLRP3 interactions. A 3a-deletion IBV strain was constructed, and intracellular K^+ , Ca^{2+} , and mitochondrial ROS were measured to verify the regulatory mechanism.

RESULTS:

Our findings demonstrated that IBV infection induces renal inflammation and activates the NLRP3 inflammasome in vivo. Pathological manifestations included renal swelling, urate crystal deposition, and tubular necrosis, accompanied by the significant upregulation of pro-inflammatory cytokines IL-1 β , IL-18, and key chemokines. Immunohistochemical analysis revealed that NLRP3 inflammasome activation is predominantly localized to AQP2-positive collecting duct cells. Both in vivo and in vitro experiments confirmed that IBV promotes NLRP3 oligomerization and enhances pro-inflammatory cytokine secretion through a Caspase-1-dependent mechanism. Notably, pharmacological inhibition of NLRP3 effectively mitigated renal inflammation and tissue damage without affecting viral

replication. Mechanistically, we found that the accessory protein 3a directly binds to specific domains of NLRP3, inducing its aggregation. Deletion or mutation of the 3a protein significantly attenuated inflammasome activation and subsequent renal injury. Further mechanistic studies revealed that IBV and its 3a protein trigger the release of Ca^{2+} from the endoplasmic reticulum (ER), leading to mitochondrial Ca^{2+} overload and subsequent ROS accumulation, which specifically activates the NLRP3 inflammasome and drives inflammation and dysfunction in renal collecting ducts.

CONCLUSIONS:

In summary, our study reveals that IBV-induced kidney injury is driven by NLRP3 inflammasome activation in renal collecting duct cells. This process is specifically mediated by the viral protein 3a via a novel pathway involving ER Ca^{2+} release and mitochondrial ROS production. These findings not only uncover a key molecular pathway underlying IBV-mediated renal pathology but also provide critical insights for the development of targeted antiviral strategies and the rational design of attenuated vaccines.



Abstract No.: 95

25 June 2026 (Thursday) 1400-1415

Comparing SARS-CoV-2 and MERS-CoV host responses to understand transmission and pathogenesis

First Author: Ziqi Zhou, Queen Mary University of London

Co-Author(s): Ann-Kathrin Reusch, Tom Dubois, Linran Wang, Clare Jolly, Malik Peiris, Greg J Towers

BACKGROUND OBJECTIVES:

Middle East respiratory syndrome coronavirus (MERS-CoV) is a zoonotic coronavirus endemic to dromedary camel populations in Africa and the Arabian Peninsula. Intriguingly, MERS-CoV zoonotic transmission has only been reported in the Arabian Peninsula. Since zoonosis requires evasion of human antiviral innate immune defences, we hypothesize that Arabian MERS-CoV may have developed better evasion of human innate immunity than African viruses. Furthermore, MERS-CoV and SARS-CoV-2 differ hugely in transmission potential and disease severity (MERS-CoV >36% mortality compared to SARS-CoV-2 <4% in unvaccinated populations). We hypothesise that the greater observed MERS-driven inflammation and disease severity contributes to poor transmission, compared to SARS-CoV-2.

METHODS:

Comparative molecular virology was used to test our hypotheses. We infected human bronchial (BEAS-2B-DPP4) and lung epithelial (Calu-3) cell lines with equivalent genome doses of MERS-CoV isolates: Arabian human EMC (clade A), Arabian camel AH13 (clade B), and African camel BF785, CAC9690, Nig1657 and CAC270 (clade C) and early-lineage SARS-CoV-2 isolate IC19. Virus replication, innate immune and inflammatory responses were measured by RT-qPCR, RNASeq and proteomics. We also measured activation of inflammatory cell death by LDH release and detection of caspase 3 and GSDME-cleavage by Western blot.

RESULTS:

Arabian human and camel viruses replicated similarly but outpaced African camel viruses in human cells. Strikingly, the Arabian human-isolated virus triggered significantly less antiviral IFN/ISG responses than camel-isolated viruses, despite similar levels of replication. RNASeq and proteomics confirmed reduced activation of innate immune and inflammatory pathways by the human MERS isolate. African

camel clade C viruses—which have not naturally infected humans and replicated more slowly than Arabian viruses—elicited the strongest and earliest activation of innate immunity, consistent with reduced zoonotic potential through activation of defensive pathways.

Concordantly, we found that MERS-CoV infection caused greater inflammation in lung epithelial Calu-3 cells as compared to SARS-CoV-2, despite similar replication. Specifically, MERS-CoV triggered substantially stronger pro-inflammatory responses, including expression of IL6 and IL1 α , and stronger activation of GSDME-mediated pyroptosis, evidenced by caspase-3 and GSDME cleavage.

CONCLUSIONS:

Collectively, these findings suggest that successful zoonotic emergence of MERS-CoV is linked to innate immune evasion, while the severe disease caused by MERS-CoV associates with activated inflammation and inflammatory cell death during epithelial infection. Further understanding of the viral genetic determinants underlying these differences will inform risk assessment of coronavirus zoonotic potential and help us understand the relationships between transmission potential and pathogenesis.



Abstract No.: 126

25 June 2026 (Thursday) 1415-1425

Functional Genomics and Transcriptomics of Porcine Alphacoronavirus Transmissible Gastroenteritis Virus Identifies Host Factors Associated with Viral Replication and the Cellular Immune Response

First Author: Andrew Hanton, University of Edinburgh

Co-Author(s): Amanda Warr, Marie O'Shea, Sarah Fletcher, Teagwen Cameron, Isabella Hoskins, Christine Tait-Burkard

BACKGROUND OBJECTIVES:

Pigs are highly susceptible to infection with porcine coronaviruses (pCoVs), causing illness and economic losses. High-throughput functional genomics technologies such as RNA interference screening can enable significant insight into host-pathogen interactions. However, many such technologies to study livestock animals are underdeveloped relative to model species. We developed a porcine short interfering RNA (siRNA) screening platform and applied this to pCoV transmissible gastroenteritis virus (TGEV), and performed additional RNAseq to identify host factors and pathways associated with viral replication and the host response.

METHODS:

A library of siRNAs was designed for 6,727 genes within the Sscrofa11.1 genome and applied to a high-throughput screen by reverse-transfection into swine testes (ST) cells before infection with TGEV Miller M6 (MOI = 0.01, 48h). RNAseq was performed on RNA from TGEV Miller M6-infected (MOI = 0.01 & 1) ST cells at 24h and 48h before Illumina sequencing. Knockout (KO) cell lines were generated using CRISPR/Cas9 gene editing.

RESULTS:

A total of 301 proviral and 479 antiviral hits were identified through siRNA screening, including several previously described host factors including COPB2, DYRK1A, HIF1A, JAK2, and IFNLR1. Pathway analysis identified proviral mechanisms including serine/threonine kinase activity and calcium regulation, while antiviral pathways included PI3K/Akt signalling and the unfolded protein response.

Using RNAseq we identified a total of 2,283 unique differentially expressed genes

(DEGs). Pathway analysis identified a significant upregulation of the innate immune and stress responses, typified by JAK-STAT signalling and IFN-stimulated gene expression. Further, downregulated DEGs were strongly involved in cellular metabolic and ion transport processes. Through a combinatorial analysis, dozens of host factors were identified as antiviral with upregulated expression, likely significant elements of the cellular immune response.

Proviral siRNA screen hits included the Ras-related proteins Rab4a and Rab4b, directors of early endosomal trafficking, in addition to Cd2-associated protein (Cd2ap), a Rab4 effector protein. We sought to validate this result by generating CRISPR/Cas9 RAB4A and RAB4B KO cell lines and explore the roles of these factors through infection assays and microscopy.

CONCLUSIONS:

Our multi-omics approach involved the first application of siRNA screening in a livestock model, and identified a wide range of host factors and pathways involved in viral replication and the host response. This expansion of the molecular toolkit available to porcine coronaviruses offers numerous avenues of follow-up, relieves gaps in research and knowledge, and offers the prospect of expansion to other commercially important porcine viruses.



Abstract No.: 161

25 June 2026 (Thursday) 1425-1435

Contribution of SARS-CoV-2 ORF7a to virion release and virus virulence

First Author: Ailén Schonvandt, National Center for Biotechnology (CNB-CSIC)

Co-Author(s): Jesus Hurtado-Tamayo, Ricardo Requena-Platak, Diego Muñoz-Santos, Jorge Ripoll-Gomez, Luis Enjuanes, Isabel Sola

BACKGROUND OBJECTIVES:

Although COVID-19 is no longer a Public Health Emergency, SARS-CoV-2 continues circulating in humans and causing deaths in vulnerable people. Understanding virus-host interactions is essential to identify new therapeutic targets. Coronavirus accessory proteins, which are not absolutely essential for virus replication or virion structure, are involved in pathogenesis and in the antagonism of antiviral responses through different mechanisms. SARS-CoV-2 genome encodes six accessory proteins (3a, 6, 7a, 7b, 8 and 9b), for which limited information is available. Our main objective is to study the contribution of ORF7a to virus-host interactions in the context of infection and to pathogenesis.

METHODS:

SARS-CoV-2 recombinant viruses in which ORF7a was deleted, either alone or in combination with other accessory genes, were engineered using a reverse genetics system. Deletion mutants were evaluated in cell lines, human bronchoalveolar organoids and mouse models to determine the contribution of ORF7a to the virus biology and virulence. The ORF7a interactome was studied during infection, using a proximity-labeling approach. Recombinant viruses including an in-frame fusion of the TurboID biotin-ligase to ORF7a were engineered. Affinity purified proteins derived from biotin-treated SARS-CoV-2-TurboID-ORF7a-infected cells were subjected to mass spectrometry analysis.

RESULTS:

Viruses without ORF7a showed lower titers than those expressing ORF7a in cell lines, human bronchoalveolar organoids and mouse bronchoalveolar lavages. Electron microscopy analysis of infected cells showed that SARS-CoV-2- Δ 7a, but not SARS-CoV-2-WT, virions were bound to the plasma membrane. Together, these results suggest that the absence of ORF7a leads to a defect in virion release. BST-2 is a cellular restriction factor that hinders the release of some enveloped viruses

from infected cells. Overexpression of human, murine or bat BST2 in infected cells had a modest effect on SARS-CoV-2-WT, while significantly reduced SARS-CoV-2- Δ 7a titers. Accordingly, BST2 silencing significantly increased SARS-CoV-2- Δ 7a titers. More than 200 host proteins were enriched in SARS-CoV-2-TurboID-ORF7a-infected cells over the negative control samples. Protein 7a-proximal factors are related to transport pathways, post-translational modification processes, and translation. Deletion of ORF7a along with ORFs 6, 7b and 8 in a mouse adapted SARS-CoV-2- Δ [6,7ab,8]-MA reduced by 60% the mortality in conventional BALB/c mice as compared to SARS-CoV-2- Δ [6,7b,8]-MA virus expressing ORF7a.

CONCLUSIONS:

SARS-CoV-2 7a accessory protein promoted the release of virions in cell lines, human bronchoalveolar organoids and in the lungs of mice. ORF7a antagonized the antiviral effect of BST-2, a host restriction factor that retains virions on the cell membrane. SARS-CoV-2 ORF7a is a virulence factor in a mouse adapted SARS-CoV-2-MA in which other accessory genes (6, 7b and 8) were also deleted.



Abstract No.: 159

26 June 2026 (Friday) 0955-1010

Investigating the role of Toll-like receptor 7 in SARS-CoV-2 infection using golden hamsters

First Author: Ziyi Guo, The University of Tokyo

Co-Author(s): Keiya Uriu, Yukun Zhu, Luo Chen, Nozomi Shiwa-Sudo, Seiya Ozono, Tadaki Suzuki, Kazuto Yoshimi, Tomoji Mashimo, Kei Sato

BACKGROUND OBJECTIVES:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection induces inflammation and antiviral interferon response in lung tissues, which is mediated by pattern-recognition receptors. Toll-like receptor 7 (TLR7) recognizes single-stranded RNA (ssRNA), the genomic material of SARS-CoV-2, thereby may induce innate immune response during SARS-CoV-2 infection. Although mice are widely used for studying host response, they are not naturally susceptible to SARS-CoV-2 infection. The mouse-adapted SARS-CoV-2 or genetically engineered mouse model may introduce non-physiological effects during infection, limiting its relevance to natural infection. In contrast, golden hamsters can be infected by SARS-CoV-2 without additional manipulation and can recapitulate major respiratory symptoms of human infection. However, the role of TLR7 in hamsters during SARS-CoV-2 infection remains unclear.

METHODS:

To study the contribution of TLR7 to SARS-CoV-2 pathogenesis in golden hamsters, we constructed Tlr7 knock-out (KO) hamsters by CRISPR-Cas9 technology. Wild-type and Tlr7 KO hamsters were intranasally infected with ancestral SARS-CoV-2 (WK-521). The body weight, lung function and viral burden in oral swab was measured to assess the disease severity. At 2 and 5 days post infection, lung tissues were harvested for histopathological analysis. Viral burden in lung tissues was examined by N protein staining. In addition, immune cell infiltration was analyzed by immunohistochemistry staining.

RESULTS:

Tlr7 KO hamsters showed lower disease severity than wild-type after SARS-CoV-2 infection, manifested as alleviated body weight loss, ameliorated lung dysfunction and reduced lung pathological changes. However, the viral burden in upper respiratory tract, including oral swab and trachea, was increased in Tlr7 KO

hamsters compared with wild-type, suggesting that TLR7 signaling may promote the viral clearance. Immunohistochemistry analysis further showed that the number of macrophages in lung tissues was decreased in Tlr7 KO hamsters at 2 and 5 days post infection, which may account for the attenuated inflammation and increased viral burden in SARS-CoV-2 infection.

CONCLUSIONS:

Our results identified that TLR7 played a dual role in SARS-CoV-2 infection by mounting inflammatory response and promoting viral clearance. The effect of TLR7 on mediating inflammation is more prominent, thus contributing to immunopathology of SARS-CoV-2 infection.



Abstract No.: 420

26 June 2026 (Friday) 1010-1025

SARS-CoV-2 Balances Antigenic Evolution and Replicative Fitness in Human Airways

First Author: Anna Mykytyn, Erasmus MC

Co-Author(s): Merel te Marvelde, Tim I. Breugem, Melanie Rissmann, Alexandre H. J. Bouffier, Petra B. Van den Doel, Debby Schipper, Sivana Baptista Varela, Jingshu Zhang, Bas B. Oude Munnink, Mart M. Lamers, Bart L. Haagmans

BACKGROUND OBJECTIVES:

SARS-CoV-2 variants continuously acquire mutations, which could alter their fitness, influencing emergence and spread. Identifying emerging variants which will dominate is crucial for public health policy and updating vaccines. Multiple factors drive the evolution of SARS-CoV-2, but the interaction between antigenicity and population immunity, combined with replicative fitness are likely key contributors to the overall fitness of dominating variants. The recently emerged Omicron BA.3.2 variant, which is increasing in prevalence in some countries, including England and Denmark, suggests properties that may have an advantage over the currently globally dominant XFG variant.

Here, we modelled the evolution of variants over time from the early pandemic D614G isolate to the most recently emerged Omicron BA.3.2, by investigating the replicative fitness of SARS-CoV-2 in the human respiratory tract combined with antigenic evolution.

METHODS:

Replication and fitness of SARS-CoV-2 variants was quantified using human airway organoids. Antigenic cartography was performed using neutralization titers from sera of experimentally infected golden Syrian hamsters.

RESULTS:

Variant replication and replicative fitness increased over time until plateauing among Omicron variants. The largest increase in replication occurred between the Omicron and pre-Omicron variants. Slight increases and dips were observed for Omicron EG.5.1.1 and JN.1, respectively. Using antigenic cartography, we observed increasing immune evasion among newer variants, with predominantly

incremental changes and occasional larger jumps. The first large antigenic jump away from earlier Omicron variants occurred with the emergence of Omicron BA.2.86.1 and JN.1. All circulating variants up to Omicron XFG remain within this cluster. However, the recently emerged, highly divergent Omicron BA.3.2 variant was poorly neutralized by all sera, including pre-Omicron, early Omicron and JN.1-derived variant sera, pointing toward further antigenic diversification among emerging variants.

CONCLUSIONS:

We did not observe a noticeable trade-off between immune evasion and replicative fitness. Omicron variants continue to replicate efficiently in the human airway, with little change in this capacity over time. At the same time, SARS-CoV-2 evolution favors continued immune evasion, with larger antigenic jumps observed every few years. The ability of variants to retain high replicative fitness despite continued antigenic change indicates that the evolutionary space of SARS-CoV-2 to accommodate phenotypic changes is remarkably large.



Abstract No.: 88

26 June 2026 (Friday) 1025-1035

Chronic Infection Drives Evolution of SARS-CoV-2 Innate Immune Antagonism

First Author: Xiaohao Xu, King's College London

Co-Author(s): Thomas Courty, Luke Blagdon Snell, Suzanne Pickering, Gaia Nebbia, Michael Malim, Stuart Neil, Rui Pedro Galao

BACKGROUND OBJECTIVES:

SARS-CoV-2 variants of concern (VOCs) have repeatedly evolved enhanced capacity to remodel host antiviral responses, yet the minimal genetic determinants sufficient to tune innate immune antagonism remain incompletely defined. Prolonged infections in immunocompromised individuals provide a setting for within-host viral evolution under sustained immune pressure. Here, we investigated whether regulatory mutations arising during persistent infection can directly modulate innate immune antagonism.

Here, we modelled the evolution of variants over time from the early pandemic D614G isolate to the most recently emerged Omicron BA.3.2, by investigating the replicative fitness of SARS-CoV-2 in the human respiratory tract combined with antigenic evolution.

METHODS:

sequencing and functional assays. Infections of airway epithelial cells with a P65 isolate, ancestral D614G, or the Alpha VOC were assessed for induction of antiviral genes (IFNB1, CXCL10) and viral protein expression. Replication phenotypes were evaluated in relevant epithelial systems, including under JAK/STAT inhibition. Candidate regulatory mutations were identified by sequence analysis, and circular polymerase extension reaction (CPEP)-based reverse genetics was used to generate isogenic viruses differing at the nucleotide of interest.

RESULTS:

Infection of Calu-3 airway epithelial cells with the long-term infection isolate P65 was associated with attenuated induction of IFNB1 and CXCL10 despite productive replication, and both P65 and Alpha exhibited elevated abundance of the innate immune antagonist ORF9b. Consistent with reduced dependence on interferon-

mediated restriction, JAK1/2 inhibition by ruxolitinib preferentially enhanced replication of an early-pandemic D614G strain relative to P65 and Alpha. Sequence analysis identified a single-nucleotide deletion at the –3 position of the nucleocapsid (N) start-codon Kozak context that was shared by P65 and Alpha, present from the first available longitudinal sample, and stably maintained throughout infection. Using isogenic viruses, we demonstrated that introduction of this –3 Kozak deletion was sufficient to increase ORF9b protein abundance in the D614G background, whereas reciprocal restoration of the –3 nucleotide in the P65 background reduced ORF9b toward ancestral levels. In primary airway HBECs, viruses carrying the deletion displayed enhanced replication relative to matched controls, consistent with enhanced antagonism of innate immune restriction.

CONCLUSIONS:

Together, these findings identify a minimal, non-coding regulatory mutation selected during persistent infection that is sufficient to elevate ORF9b expression and modulate innate immune antagonism. This work establishes a direct genotype-phenotype link between within-host evolution and translational control of viral immune evasion and highlights regulatory mutations as an underappreciated mechanism contributing to SARS-CoV-2 adaptation.



Abstract No.: 41

26 June 2026 (Friday) 1035-1045

Bat-specific adaptations in interferon signaling and GBP1 contribute to enhanced antiviral capacity

First Author: Arinjay Banerjee, University of Saskatchewan

Co-Author(s): Victoria Gonzalez, Briallen Lobb, Jacob Cote, Arkadeb Bhuinya, Akarin Asavajaru, Yan Zhou, Vikram Misra, Darryl Falzarano, Trevor Sweeney, Sophie Gobeil, Linfa Wang, Andrew C. Doxey

BACKGROUND OBJECTIVES:

Bats are one of the most abundant and geographically diverse mammalian species in the world, with over 1500 species globally distributed on all continents with the exception of Antarctica. Accumulating data suggest that bats are hosts to multiple zoonotic viruses that have caused consequential disease outbreaks in humans, including severe acute respiratory syndrome coronavirus (SARS-CoV), SARS-CoV-2, and Middle East respiratory syndrome coronavirus (MERS-CoV). Despite these viruses causing life-threatening disease in humans, naturally or experimentally infected bats do not demonstrate overt signs of disease. This suggests the evolution of enhanced antiviral capacity in reservoir bat species. In this study, we produced bat species-specific recombinant IFNs and identified their mechanisms of action. We identified novel antiviral IFN stimulated genes and discovered broadly protective antiviral genes in bat species, along with discovering new functional motifs within these proteins.

METHODS:

To elucidate the bat IFN response, recombinant IFN β from *Pteropus alecto*, *Eptesicus fuscus*, and humans was generated. *P. alecto* (PaKiT03), *E. fuscus* (EfK3B), and human (A549, RPTEC) cells were then treated with their species-matched IFN β for various lengths to assess protection against vesicular stomatitis virus (VSV), H1N1, VSV, HSV, SARS-CoV-2, and Middle East respiratory syndrome coronavirus (MERS-CoV). The IFN β signaling pathway was profiled using RNA-sequencing.

RESULTS:

By applying a transcriptomic approach, we demonstrate a largely conserved type I IFN response across both bat species. However, lower expression levels of orthologous ISG transcripts, in addition to divergent transcripts, contributed to

the overall antiviral IFN β response in bat cells relative to human cells. Guanylate-binding protein 1 (GBP1) was one of the top upregulated transcripts in bat cells, and functional assays demonstrated that bat GBP1 is broadly protective against a range of RNA and DNA viruses compared to human GBP1. The antiviral function of bat GBP1 was dependent on a previously unknown and uncharacterized AV1 motif within the N-terminal domain, leading to restriction of the bat-derived Eptesipoxvirus.

CONCLUSIONS:

To our knowledge, no study has evaluated the efficacy of human and bat GBP1 against two coronaviruses of bat origin that have caused outbreaks in humans. Overall, our study highlights the evolutionary differences in the IFN β response between humans and bats, and between bat species. By investigating the arms race between bat GBP1 and multiple RNA and DNA viruses, we have identified a motif (AV1) within GBP1, and this knowledge can one day be harnessed to better understand and control viral infections in humans.



Abstract No.: 194

26 June 2026 (Friday) 1045-1055

Infection-induced immune imprinting following SARS-CoV-2 exposure in unvaccinated populations

First Author: Adam Abdullahi, University of Cambridge

BACKGROUND OBJECTIVES:

In regions with delayed vaccine rollout, SARS-CoV-2 exposure was largely infection-first, providing a unique setting to evaluate immune imprinting. While imprinting has been described in vaccine-primed populations, its emergence following natural infection remains less well defined. We aimed to characterize neutralizing antibody responses and assess whether early infection shapes subsequent responses to antigenically distinct variants in unvaccinated populations.

METHODS:

sampled in early 2023. Omicron exposure was determined using a BA.1 receptor-binding domain (RBD)-based serological assay. Neutralization against ancestral (Wu-1) and Omicron variants (BA.1, BA.2, BA.4) was assessed using pseudotyped virus assays. Magnitude-breadth analysis quantified antibody responses. Wu-1 spike antibody depletion experiments were performed to evaluate contribution of cross-reactive antibodies.

RESULTS:

Widespread pre-Omicron exposure was observed, with partial Omicron exposure across cohorts. Despite recent Omicron infection, neutralizing antibody titres against Omicron variants were consistently equal to or lower than those against ancestral Wu-1. Magnitude-breadth analysis demonstrated preferential responses directed toward ancestral epitopes. Depletion of Wu-1 spike-binding antibodies abrogated neutralization of both Wu-1 and Omicron variants in most participants, confirming dominance of cross-reactive ancestral responses. Similar patterns were observed in an independent cohort with higher Omicron exposure.

CONCLUSIONS:

Infection-first exposure to SARS-CoV-2 induces durable immune imprinting characterized by preferential recall of ancestral antibody responses. This pattern persists despite subsequent exposure to antigenically distinct variants. These findings highlight the importance of exposure sequence in shaping population-level immunity and have implications for vaccine strategy in settings with heterogeneous exposure histories.



Abstract No.: 117

26 June 2026 (Friday) 1056-1105

Cross-species quantification of antiviral immune responses from large-scale RNA-seq data using ISG Profiler

First Author: Luca Nishimura, The University of Tokyo

Co-Author(s): Hiroaki Unno, Kyoko Kurihara, Mai Suganami, Junna Kawasaki, Spyros Lytras, Kaho Okumura, Edward C Holmes, Jumpei Ito, Kei Sato

BACKGROUND OBJECTIVES:

Many emerging infectious diseases originate from zoonotic viruses, highlighting the need for comprehensive viral surveillance across diverse animal species. While homology-based approaches are widely used for virus detection, they are computationally demanding and may fail to detect viruses with low sequence similarity. To address these challenges, our group is developing an integrated framework for large-scale viral surveillance from RNA-seq data, consisting of three steps: (1) quantification of host immune responses, (2) prediction of viral infection status based on host transcriptomic patterns, and (3) downstream virome analysis for viral discovery. In this presentation, we focus on the first step of this framework which enables rapid and cross-species quantification of interferon-stimulated gene (ISG) expression.

METHODS:

We developed ISG Profiler, a computational tool that rapidly quantifies the expression levels of evolutionally conserved core ISGs from animal RNA-seq data. We constructed a database including 59 core ISGs and 100 internal control genes (ICGs) derived from 398 amniota species. RNA-seq reads were mapped to this database using Salmon, and normalized ISG expression levels were calculated with ICGs. It enabled cross-species comparison without requiring reference genomes.

RESULTS:

ISG Profiler was validated with experimentally interferon induced datasets from ten animal species. The results showed strong correlation with conventional RNA-seq analysis and consistently higher ISG expression in interferon positive samples. Similar ISG upregulation patterns were observed in both experimentally and

naturally viral infected samples. Analysis of 170,000 RNA-seq data revealed that ISG expression patterns vary substantially depending on host animal taxonomic order and infecting viral families. For example, while RNA viruses generally induced ISG expression, notable exceptions were observed, and certain DNA viruses also triggered strong responses. We also observed that basal ISG expression levels varied substantially across host species, with relatively higher expression observed in Primates, Carnivora, and Artiodactyla, indicating inherent differences in antiviral immune states.

CONCLUSIONS:

ISG Profiler enables rapid and scalable quantification of antiviral host responses across diverse animal species. This approach provides a practical foundation for large-scale comparative analysis of immune responses and supports future efforts in infection detection and viral discovery.



Abstract No.: 201

26 June 2026 (Friday) 1056-1105

Cellular innate immune determinants of SARS-CoV-2 clearance and disease severity

First Author: Margarida Bonifacio, The Francis Crick Institute

Co-Author(s): Javier Alvarez de Miranda, Jamie Barrett-Rodger, Katja Finsterbusch, Andreas Wack, David LV Bauer

BACKGROUND OBJECTIVES:

SARS-CoV-2 (SCV2) infection results in a range of clinical outcomes. Human and animal studies show that a rapid innate immune response is critical for early viral control. Conversely, excessive recruitment of inflammatory monocytes and neutrophils, particularly at later stages, is associated with severe infection and lung injury. While the effects of adaptive immune cells have been well defined in SCV2 infection, the roles of innate immune cells, such as dendritic cells and monocytes, and how they limit lung injury are less understood. Here, we aimed to dissect the contributions of DCs and monocytes in the response to SCV2 infection.

METHODS:

Mice lacking conventional dendritic cells via deletion of the Fms-related tyrosine kinase 3 ligand gene (Flt3l^{-/-} mice) or with a severe defect in recruitment of inflammatory monocytes and in monocyte-derived macrophages via deletion of C-C chemokine receptor type 2 gene (Ccr2^{-/-} mice) were infected with mouse-adapted SCV2 (MA20). Lung, nasal tissue, and lung-draining mediastinal lymph nodes were collected at 3, 7, and 14 days post-infection. Infection was monitored by plaque assay, RT-qPCR, and immunohistochemistry. Immune responses were characterised by flow cytometry.

RESULTS:

MA20 infection in wild-type mice is cleared by day 7 post-infection. We observed an increase in migratory dendritic cell numbers in the mediastinal lymph nodes during early infection and an expansion of lymph node-resident dendritic cells, germinal centre B cells and T cell populations, including central memory and effector memory CD8⁺ T cells, and CD4⁺ T follicular helper (T_{fh}) cells, within the first week of infection. In contrast, Flt3l^{-/-} mice experienced more severe disease,

delayed clearance, and reduced adaptive immune cell populations (including Spike-specific CD8⁺T cells, germinal centre B cells, and Tfh cells) in the mediastinal lymph nodes. Similarly, Ccr2^{-/-} mice lost more weight during acute infection and had higher viral loads in the lung and nasal compartment.

CONCLUSIONS:

inflammatory monocytes is critical for early viral control. It is likely that inflammatory monocytes act as a two-edged sword, with their association with severity depending on the magnitude and temporal dynamics of their recruitment into the lung. Our results highlight the importance of a more detailed understanding of the role of innate cell subsets in infection and have motivated current efforts to map the roles of other cells, such as natural killer cells. Overall, this work provides an important basis for understanding immune cell interplay in SCV2 infection and mechanisms underlying the development of pathology following infection.



Abstract No.: 356

26 June 2026 (Friday) 1150-1205

Population-wide SARS-CoV-2 antibody repertoire heterogeneity driven by immune imprinting

First Author: Xiao Niu, Peking University

Co-Author(s): fanchong jian, Yutong Li, Siyu Lei, Ke Li, Ruoxi Kong, Xingan Cai, Weiliang Song, Yunlong Cao

BACKGROUND OBJECTIVES:

After extensive global circulation, SARS-CoV-2 continues to co-evolve with human humoral immunity. Recently, a divergence in SARS-CoV-2 viral circulation has emerged between East and West. In Eastern countries (primarily China), the NB.1.8.1 lineage predominates while XFG remains virtually absent. Conversely, XFG has rapidly risen to dominance in Western regions, leaving NB.1.8.1 as a minor lineage. Therefore, elucidating the differences in humoral immune landscapes across populations with distinct SARS-CoV-2 exposure histories—and the mechanisms driving this divergence—is urgently needed to forecast viral evolution and guide next-generation vaccine design.

METHODS:

We compared serological profiles and single-B-cell V(D)J repertoires across a targeted vaccine-comparison cohort (n=20) and a randomly sampled cohort of over 1,000 individuals. Furthermore, we isolated over 2,000 monoclonal antibodies from China convalescents with diverse vaccination and infection histories and performed high-throughput yeast-display deep mutational scanning (DMS) for precise epitope mapping.

RESULTS:

Inactivated vaccine recipients readily develop Omicron-specific antibodies through repeated breakthrough infections, whereas mRNA-vaccinated individuals exhibit severe ancestral-strain imprinting that suppresses de novo Omicron-specific responses. DMS epitope mapping reveals that Class 1/4 antibodies

utilizing diverse V genes including IGHV2-5 and IGHV5-51 characterize the Omicron-specific repertoires, whereas Class 1 antibodies encoded by IGHV3-53/66 dominate the repertoires of WT/Omicron cross-reactive antibodies. Concordantly, IGHV3-53/66 usage is enriched both in mRNA vaccinees relative to inactivated-vaccine recipients, and in highly imprinted individuals compared to lowly imprinted ones within a large-scale, randomly sampled Chinese cohort. These repertoires with differential V(D)J usage inherently dictate distinct serological neutralization profiles against emerging variants across populations. Specifically, highly imprinted individuals retain superior neutralizing titers against ancestral and early Omicron strains compared to lowly imprinted cohorts, but display compromised responses against variants emerging post-XBB.1.5/JN.1. Interestingly, XFG exhibits greater immune evasion in highly imprinted individuals compared to NB.1.8.1, potentially driving regional disparities in variant predominance. Conversely, lowly imprinted populations exhibit unexpectedly lower neutralizing titers against BA.3.2.2 compared to highly imprinted individuals. This enhanced immune evasion may explain the localized prevalence of BA.3.2.2 among specific demographics in Europe and North America.

CONCLUSIONS:

Distinct vaccination and infection histories across populations dictate varying degrees of immune imprinting, which subsequently drive divergent antibody repertoires with different V(D)J lineage usage and distinct serological neutralization profiles. This underscores the necessity of population-specific vaccination strategies—especially for the COVID-19-naïve next generation with distinct immune baselines. Concurrently, developing vaccine platforms capable of circumventing pre-existing immune imprinting will be another paramount strategy.



Abstract No.: 385

26 June 2026 (Friday) 1205-1220

Breadth and kinetics of antibody-mediated immunity to SARS-CoV-2 variants in highly-vaccinated individuals receiving monovalent KP.2 mRNA COVID-19 booster vaccines

First Author: Daniel Egan, University of Cambridge

Co-Author(s): Rebecca Morse, Mark Tsz Kin Cheng, Mazharul Altaf, Kimia Kamelian, Rainer Doffinger, Tan Chee Wah, Ravindra Gupta

BACKGROUND OBJECTIVES:

Ongoing viral evolution of SARS-CoV-2 has led to the spread of increasingly immune-evasive variants which bypass neutralising antibodies, with steady antigenic drift between successive variants and the recent emergence of the BA.3.2 saltation variant. Seasonal 'booster' vaccines have been deployed in high-risk groups to improve titres of variant-specific neutralising antibodies. Contemporary data on the effect of seasonally-updated vaccine boosters in highly-immunised elderly individuals are limited and the benefits of such vaccines in this group of individuals at highest risk of severe disease are not fully understood. This study aimed to characterise the humoral immune landscape of a cohort of elderly individuals to contemporary viral variants following monovalent KP.2 variant mRNA booster vaccination as part of an ongoing series of bi-annual COVID-19 vaccinations.

METHODS:

We studied post-vaccine humoral responses in a cohort of 43 adults (median age: 85 years, IQR 75-88, 40% female) who received two COVID-19 booster vaccines 6 months apart in 2025. Neutralising antibody responses to the SARS-CoV-2 spike protein of 7 contemporary variants were measured by pseudovirus neutralisation assay. A 21-plex Luminex-based surrogate virus neutralisation assay was used to determine the breadth of neutralisation to the receptor-binding domain of the spike proteins of a broad range of viral variants. We used antigenic cartography to map immune landscapes across study timepoints and to evaluate breadth of immunity before and after vaccination.

RESULTS:

Neutralising antibody titres waned modestly in the 6 months between vaccines and were boosted to similar levels following KP.2 vaccination. Neutralisation of non-BA.3.2 variants remained robust following booster dose with highest responses to Wu-1 (geometric mean titre ID50 (GMT): 6765) and KP.2 (GMT: 5382), as well as a boosting for LP.8.1 (fold-change (FC): 1.3x), XEC (FC: 1.1x) and NB.1.8.1 (FC: 1.1x). Neutralisation of the BA.3.2.2 variant was poor (GMT: 900) and showed no significant boosting effect from the KP.2 vaccine. Wu-1 responses were poorly boosted by vaccination (FC: 0.8x), suggesting possible overcoming of immune imprinting. Antigenic maps showed greater breadth of neutralisation following booster vaccination, while BA.3.2.2 remained antigenically distant to all other viruses across all study timepoints, indicating its structurally distinct spike which is poorly neutralised despite repeated boosters.

CONCLUSIONS:

Seasonal monovalent mRNA booster vaccines remain effective at bolstering neutralising antibody titres against most circulating SARS-CoV-2 variants in a healthy, highly vaccinated elderly cohort. The BA.3.2.2 variant exhibits features of immune escape to established humoral immunity and may pose an increased risk to this vulnerable population.



Abstract No.: 272

26 June 2026 (Friday) 1220-1235

SARS-CoV-2 Variants Employ Diverse Strategies to Sustain Efficient Airborne Transmissibility

*First Author: Jie Zhou, Imperial College London**Co-Author(s): Anika Singanayagam, Ksenia Sukhova, Helen Wagstaffe, Helen Mcshane, Christopher Chiu, Wendy Barclay*

BACKGROUND OBJECTIVES:

Efficient airborne transmission was a key determinant of the global spread of SARS-CoV-2 since 2019. As SARS-CoV-2 has transitioned into an endemic respiratory virus, continued circulation requires ongoing viral evolution to overcome population immunity while preserving efficient transmission. This can be achieved through enhanced viral emission from infected hosts, improved environmental stability, and maintenance of a low infectious dose. This study aimed to define how successive SARS-CoV-2 variants sustain airborne transmissibility and found they have adopted distinct strategies to do so.

METHODS:

SARS-CoV-2 transmission was evaluated using both hamster models and controlled human challenge studies. Subjects were inoculated with standardised dose and monitored from the incubation period to the end of infection under controlled conditions. Viral replication in the upper respiratory tract (URT) was quantified using nasal washes, nasal swabs, and throat swabs. Environmental and airborne virus emissions were measured via a custom-made Infectious Virus Transmission Tunnel (IVTT), facemask sampling (FMS), and Coriolis air samplers. Additionally, surface and hand swabs were collected to evaluate contact contamination.

RESULTS:

In the hamster model, pre-Omicron variants (Wildtype, Alpha, and Delta) replicated efficiently and achieved 100% airborne transmission from donor to sentinel animals. Hamsters inoculated with the Alpha variant emitted fewer viral

particles than the Wildtype but transmissibility was compensated with a lower 50% infectious dose (ID50). In contrast, the Delta variant exhibited higher airborne emission but required a 14.6-fold higher ID50 (0.30 PFU). Omicron subvariants (BA.1, BA.4, BA.5, EG.5.1, BA.2.86, JN.1) displayed varying levels of airborne transmissibility, which were correlated with their airborne emission capacity. In human challenge studies using first wave SARS-CoV2, naive volunteers were inoculated with 10TCID50. Infected volunteers developed robust upper respiratory replication, with high viral loads detected in ambient air, FMS samples, and on hands and surfaces. In later studies, participants were no longer immunologically naïve, as they had either prior vaccination, infection or hybrid immunity. Even though they were inoculated with high doses (10^5 – 10^6 TCID50) of Delta or BA.5 variants, they exhibited markedly reduced URT viral replication and a lower level of detectable virus emission into the air and onto surfaces. Despite lower URT viral load, the total emitted viral load detected in FMS samples from the Delta challenge study was comparable to that from the Wildtype study, suggesting that Delta was efficiently aerosolised from human participants, in line with findings from the hamster model. The area under the curve (AUC) for Delta FMS samples represented 42% of nasal wash AUC, compared with 18% in the Wildtype study and only 1% in the BA.5 study, indicating variant-specific emission efficiency.

CONCLUSIONS:

SARS-CoV-2 variants have distinct viral strategies that maintain airborne transmission. Controlled animal and human challenge models are valuable tools for characterizing the airborne transmissibility of emerging variants, which can provide evidence for formulating corresponding public health intervention policies.



Abstract No.: 176

26 June 2026 (Friday) 1236-1240

COVID-19 vaccination induces cross-neutralisation of sarbecoviruses related to SARS-CoV-2

First Author: Grace West, University of Cambridge

Co-Author(s): Rebecca Morse, Benjamin Sievers, Adam Abdullahi, Kimia Kamelian, Mark Tsz Kin Cheng, Douglas Stewart, Divya Diamond, Mazharul Altaf, Lourdes Ceron-Gutierrez, Olga Sokolova, David Robertson, Spyros Lytras, Rainer Döffinger, Sam Wilson, Suzannah Rihn, Ravindra Gupta

BACKGROUND OBJECTIVES:

The combined threats of future sarbecovirus zoonosis and continually emerging SARS-CoV-2 variants of concern (VOCs) highlight the need to assess the breadth of existing SARS-CoV-2 vaccine-mediated protection. In particular, it remains unclear to what extent current vaccination strategies elicit cross-neutralising antibody responses against divergent sarbecoviruses.

METHODS:

Here we investigate a cohort of older individuals, who received four COVID-19 vaccine doses, for potential cross-neutralisation. Serum samples were assessed using lentiviral pseudoparticles bearing spike proteins from either Omicron VOCs or other sarbecoviruses, including SARS-CoV-1 and related animal coronaviruses, to evaluate neutralising antibody responses.

RESULTS:

Despite recent fourth bivalent mRNA vaccine doses (encoding SARS-CoV-2 Wu-1 and Omicron BA.1 spikes), neutralisation of Omicron lineage VOCs was reduced compared to Wu-1, consistent with an imprinted immune response. Similarly, particles bearing either SARS-CoV-1 or a SARS-CoV-1-related bat sarbecovirus spike were neutralised less efficiently than Wu-1. Unexpectedly, however, particles bearing spikes from two animal SARS-CoV-2-related viruses, BANAL-20-52 and a pangolin CoV, were more sensitive to serum neutralising antibodies, with NT50 values approximately 7.4-fold and 13.3-fold higher, respectively, than SARS-CoV-2 Wu-1.

CONCLUSIONS:

These surprising findings suggest that vaccine-mediated adaptive immunity may provide efficient cross-neutralisation, and potential protection, against certain animal sarbecoviruses.

Abstract No.: 189

26 June 2026 (Friday) 1425-1440

Mouse V(D)J Humanization Recapitulates human-like Severe SARS-CoV-2 Immune Imprinting

First Author: Xiao Niu, Peking University

Co-Author(s): Fanchong Jian, Yutong Li, Siyu Lei, Ke Li, Ruoxi Kong, Xingan Cai, Weiliang Song, Yifei Huang, Sai Luo, Yunlong Cao

BACKGROUND OBJECTIVES:

The underlying causes of population-wide heterogeneity in SARS-CoV-2 immune imprinting levels remain poorly understood. Importantly, conventional wild type mouse models fail to recapitulate strong human SARS-CoV-2 imprinting, hindering imprinting-related mechanistic investigation and vaccine evaluation. In this study, we aim to dissect the underlying mechanisms and establish a mouse model that faithfully recapitulates human imprinting to confirm the underlying mechanisms and provide an essential tool to guide future vaccine design.

METHODS:

To dissect the underlying mechanisms of immune imprinting, we systematically compared the imprinting phenotypes between V(D)J-humanized and wild-type mice. We isolated 583 monoclonal antibodies from these models and performed comprehensive high-throughput yeast-display deep mutational scanning (DMS) for precise epitope mapping. The identified mechanisms were validated both in vitro via competitive surface plasmon resonance (SPR) and in vivo via passive antibody transfer. Subsequently, we engineered a novel human IGHV3-53 knock-in (KI) mouse model using CRISPR/Cas9 to simulate real-world human sequential exposure histories.

RESULTS:

SARS-CoV-2 immune imprinting phenotypes. Comprehensive antibody repertoire and epitope mapping of 583 monoclonal antibodies from these models revealed that the abundance of pre-existing human IGHV3-53/66-encoded SARS-CoV-2 antibody responses determine imprinting severity following Omicron exposure

through antibody-mediated masking of Omicron-specific epitopes. Both passive transfer of IGHV3-53/66 antibodies and knock-in of the human IGHV3-53 gene were sufficient to induce severe SARS-CoV-2 imprinting in wild-type mice. Finally, we engineered an IGHV3-53 knock-in mouse model that successfully recapitulated the severe imprinting phenotypes in human mRNA vaccine recipients. Following real-world sequential vaccination regimens, these mice accurately mirrored the human-like compromised neutralization breadth against newly emerged variants (e.g., NB.1.8.1 and XFG).

CONCLUSIONS:

Together, these findings demonstrate that the V(D)J germline repertoire—even a single germline-encoded antibody response—can profoundly shape humoral imprinting severity. Mouse V(D)J humanization successfully overcomes the inherent immunological limitations of wild-type backgrounds, establishing a robust and biologically relevant in vivo platform. This model is essential for investigating human-specific antibody dynamics and provides a superior tool for the preclinical evaluation of next-generation broad-spectrum vaccines.



Abstract No.: 164

26 June 2026 (Friday) 1440-1455

mRNA-based COVID-19 JN.1 Vaccination Induces Partial Protection Against SARS-CoV

First Author: Merel te Marvelde, Erasmus Medical Center

Co-Author(s): Maarten F. Wilbrink, Daryl Geers, Anna Z. Mykytyn, Debby van Eck - Schipper, Kim Handrejk, Rory D. de Vries, Melanie Rissmann, Bart L. Haagmans

BACKGROUND OBJECTIVES:

Since the emergence of the SARS-CoV-2 Omicron variant, COVID-19 vaccines are updated annually to protect against antigenically distinct emerging variants. However, it remains unclear whether these vaccines protect against more divergent sarbecoviruses. Addressing this is critical for pandemic preparedness against related coronaviruses. Here, we evaluated whether JN.1 vaccination confers protection against homologous challenge, challenge with the recent SARS-CoV-2 variant XFG, or challenge with a more distinct sarbecovirus, SARS-CoV.

METHODS:

Golden Syrian hamsters were intramuscularly vaccinated with an mRNA-based JN.1 vaccine on day 0 and day 21. On day 42, hamsters were challenged with JN.1, XFG or SARS-CoV. Animals were sacrificed at 4 and 14 days post inoculation, and viral loads, histological changes, and T cell responses were assessed. Sera and nasal washes were collected to measure antibody responses, and sera of mock-vaccinated, challenged hamsters was used to update our previously generated antigenic map. To assess the induction of SARS-CoV cross-reactive immune responses after mRNA-based JN.1 vaccination in the context of a more complex immune background, human sera pre- and post- JN.1 mRNA vaccination were tested for neutralization of JN.1, XFG and SARS-CoV.

RESULTS:

Antigenic cartography based on hamster sera demonstrated that SARS-CoV is antigenically distant from SARS-CoV-2 variants JN.1 and XFG, which cluster closely

together. As expected, JN.1 vaccination induced JN.1 and XFG neutralizing antibodies, and lower viral loads were detected in the nose and lungs of vaccinated animals after challenge with JN.1 or XFG. Despite the presence of SARS-CoV binding antibodies post vaccination, SARS-CoV neutralizing antibodies were not detected before challenge. However, JN.1 vaccination did confer partial protection against SARS-CoV challenge in hamsters: vaccinated animals showed less bodyweight loss and had lower viral loads in the lungs, which was confirmed by histological findings. After challenge, local and systemic T cell responses to SARS-CoV-2 variants and SARS-CoV were detected. Surprisingly, in sera from humans with a more extensive SARS-CoV-2 vaccination history, relatively high levels of SARS-CoV neutralizing antibodies were detected before JN.1 vaccination that were boosted upon vaccination.

CONCLUSIONS:

JN.1 vaccination partially protected hamsters from severe disease following SARS-CoV challenge, in the absence of virus specific neutralizing antibodies. Interestingly, humans with a more complex immune background, had robust SARS-CoV neutralizing antibody responses before JN.1 vaccination, which were boosted further after vaccination. Together, these findings indicate that mRNA-based COVID-19 vaccines can offer immunity against newly emerging SARS-CoV-2 variants, as well as more divergent viruses like SARS-CoV.



Abstract No.: 421

26 June 2026 (Friday) 1455-1505

Application of Organoid-Derived Human Bronchiole Cultures for Manufacture of Human Coronaviruses Used in Controlled Human Infection Models

First Author: Jingshu Zhang, Erasmus MC

Co-Author(s): Anna Z. Mykytyn, Pim Schipper, Sofieke de Wilde, Sem Koornneef, Robbert Rottier, Pauline Meij, Marjolein Kikkert, Bart L. Haagmans

BACKGROUND OBJECTIVES:

Controlled Human Infection Models (CHIMs) for coronaviruses provide opportunities to study mucosal immunity and efficiently evaluate intranasal vaccine candidates. A critical component of CHIMs is the availability of high-quality challenge viruses that generate clinically relevant results and yet are safe to use in humans. However, the limited replication of certain coronaviruses in traditional cell lines has restricted their availability as challenge viruses, hampering the establishment of coronavirus CHIMs.

METHODS:

In this study, organoid-derived human bronchiolar cultures at air-liquid interface were set up to generate SARS-CoV-2 and common cold coronavirus OC43 as challenge viruses for two CHIM studies.

RESULTS:

Viruses propagated in these cultures resulted in rapid production of virus stocks with high infectious titres and minimal culture-related adaptations. Building on these findings, a laboratory-scale production process was transitioned into a Good Manufacturing Practice (GMP)-compliant pipeline using clinical-grade reagents. The resulting SARS-CoV-2 and OC43 batches will meet stringent regulatory standards for quality, purity, and safety, ensuring the suitability for CHIM applications.

CONCLUSIONS:

The development of coronavirus CHIM will not only provide valuable insights into human coronavirus pathogenesis but also serve as a powerful tool for early evaluation of vaccines. Additionally, this innovative use of human organoid-derived cultures for GMP manufacture of challenge viruses establishes a platform that can be adapted for CHIMs targeting other respiratory viruses, advancing the field of respiratory infection research and vaccine development.

Abstract No.: 410

26 June 2026 (Friday) 1505-1515

Broad Sarbecovirus Neutralization Elicited by a Synthetic SARS-CoV-2-Based Variant Immunogen Cocktail

First Author: Viren Baharani, The Rockefeller University

Co-Author(s): Trinity Zang, Miranda Aldis, Marie Canis, Rachel Patejak, Edmund Ossei Kuffour, Hans-Heinrich Hoffmann, Harm van Bakel, Emilia M. Sordillo, Viviana Simon, Margaret Macdonald, Charles M. Rice, Michel C. Nussenzweig, Theodora Hatzioannou, Paul D. Bieniasz

BACKGROUND OBJECTIVES:

The SARS-CoV-2 receptor-binding domain (RBD) has accumulated amino acid substitutions over time, conferring escape from neutralizing antibodies elicited by prior infection and/or vaccination. Nevertheless, most of the serum neutralization breadth following multiple exposures to SARS-CoV-2 antigens is attributable to antibodies targeting the intrinsically variable RBD. Vaccination design strategy is thus key to guide the B cell response to neutralization breadth. RBD heterodimer immunogens should selectively stimulate B cells whose antigen receptors cross-link both monomers, thereby maximizing antibody cross-reactivity to include diverse RBDs. In 2023, we constructed a cocktail of five overlapping SARS-CoV-2-based RBD heterodimers encompassing the sequence divergence present in ancestral and contemporaneous (Wuhan-hu-1 and XBB) SARS-CoV-2 strains (WX5). Each dimer was designed to limit intra-and inter-dimer divergence to maximize antibody cross-reactivity. We asked whether WX5, administered as a single vaccine, could confer neutralization and protection against future SARS-CoV-2 variants and other sarbecoviruses.

METHODS:

We immunized K18-hACE2 mice with the WX5 immunogen and collected polyclonal immune sera at different timepoints to test for neutralizing activity against a diverse panel of pseudoviruses. These included (i) KP.2/3, representing the globally dominant SARS-CoV-2 variant one year after design of the WX5 immunogen, (ii) N.J., a highly divergent cryptic wastewater SARS-CoV-2 variant, and (iii) the distantly related sarbecovirus, SARS-CoV. One year after WX5 immunization, we challenged these mice with the 'future' circulating SARS-CoV-2 variant, KP.3.1. In parallel, we immunized K18-IFNAR(-/-) mice with WX5 to assess whether the elicited neutralizing antibodies would protect against the even more divergent rVSV/SARS-CoV-1/GFP challenge virus.

RESULTS:

We found that the SARS-CoV-2-based WX5 vaccine elicited neutralizing antibodies in mice against KP.2/3, the N.J. wastewater variant, as well as SARS-CoV. These neutralizing antibody titers against divergent sarbecoviruses were universally higher, relative to those elicited by RBD homodimers, RBD heterodimers with highly divergent components, and the Wuhan and XBB.1.5 spike mRNA-LNP vaccines. In addition, WX5-immunized mice had reduced lung viral loads, upon challenge with authentic KP.3.1 virus one year after immunization. Finally, WX5 immunization conferred protection against the divergent rVSV/SARS-CoV-1/GFP.

CONCLUSIONS:

SARS-CoV-2 RBD heterodimers, when administered as a cocktail of closely-related RBDs, are able to generate neutralizing antibody breadth and protection against distantly related viruses not represented in the immunogen, such as cryptic SARS-CoV-2 lineages and SARS-CoV. Our results underscore the promise of RBD heterodimers as a tool to stimulate “broad” antibodies that inhibit both current and future sarbecoviruses with pandemic potential.



Abstract No.: 52

27 June 2026 (Saturday) 0955-1010

Allosteric Control of SARS-CoV-2 Exoribonuclease and Resistance to Nucleotide Analog-Based Antivirals

First Author: Chang Liu, The Johns Hopkins University School of Medicine

Co-Author(s): Yu Li, Xiaocong Cao, Bin Liu, Yang Yang

BACKGROUND OBJECTIVES:

The COVID-19 pandemic underscores the urgent need to understand coronavirus genome replication and transcription to support development of broad-spectrum antivirals. Coronaviruses are enveloped, positive-sense RNA viruses with large genomes (~30 kb), replicated and transcribed by a multi-subunit replication-transcription complex (RTC). A critical RTC component, the exoribonuclease ExoN, proofreads nascent RNA, correcting errors by the RNA-dependent RNA polymerase (RdRp) and thus ensuring high replication fidelity. However, this proofreading creates a barrier for nucleotide analog (NA)-based antivirals, such as remdesivir, bemnifosbuvir, and sofosbuvir. After being incorporated by RdRp, these analogs are excised by ExoN, undermining drug efficacy. The molecular mechanisms of ExoN-mediated resistance remain unclear. This study aims to elucidate how ExoN recognizes and removes NA-based antivirals, to inform strategies for overcoming resistance and advancing pan-coronavirus therapies.

METHODS:

To achieve these objectives, we employed a combination of state-of-the-art cryo-electron microscopy (cryo-EM) and advanced protein-RNA biochemistry techniques. We structurally characterized the interactions between coronavirus ExoN and RNA substrates containing remdesivir, bemnifosbuvir, or sofosbuvir using high-resolution cryo-EM. Complementary biochemical assays were performed to examine the efficiency and specificity of ExoN-mediated excision of each nucleotide analog from viral RNA. These integrated approaches allows us to dissect the molecular details of ExoN recognition and removal of NA-based antivirals, providing fundamental insights into mechanisms of coronavirus drug resistance.

RESULTS:

Our findings unveil NA incorporation alters RNA-binding dynamics, significantly increasing the affinity of RNA to ExoN while weakening its interaction with RdRp.

This shift likely facilitates RNA dissociation from RdRp, subsequent recognition by ExoN, and excision of NAs, even when they are embedded within an RNA substrate. Furthermore, we identify an allosteric regulatory loop of ExoN that promotes the full activation of ExoN but is displaced by the binding of NAs exhibiting resilience to ExoN excision.

CONCLUSIONS:

These discoveries provide a molecular framework for understanding SARS-CoV-2 resistance to NA-based antivirals and highlight mechanisms that could be exploited to improve anti-coronavirus drug design.



Abstract No.: 301

27 June 2026 (Saturday) 1010-1025

An ultrapotent bispecific nanobody delivered intranasally blocks contact transmission of SARS-CoV-1/2 and therapeutically clears MERS-CoV

First Author: Xiaoyu Zhao, Fudan University

Co-Author(s): Tianrenzheng Zhu, Xiyuan Pan, Jiayan Li, Jialu Shi, Wenhong Zhang, Lei Sun, Hin Chu, Pengfei Wang

BACKGROUND OBJECTIVES:

The recurrent spillover of SARS-CoV-1, MERS-CoV, and SARS-CoV-2 underscores the urgent need for countermeasures that combine exceptional potency, cross-lineage breadth, and practical delivery. However, marked differences in receptor usage and receptor-binding domain (RBD) antigenic architecture between MERS-CoV and sarbecoviruses make it difficult for a single nanobody to potently neutralize all three highly pathogenic human betacoronaviruses. We therefore sought to develop an ultrapotent bispecific nanobody with complementary anti-MERS-CoV and anti-sarbecovirus activity and to determine whether it could function both as an effective mucosal barrier against viral transmission and as a systemically active therapeutic.

METHODS:

Alpacas were sequentially immunized with purified MERS-CoV and Omicron XBB.1 spike (S) trimers. Two weeks after the final immunization, serum neutralizing activity was evaluated against MERS-CoV, SARS-CoV-1, wild-type SARS-CoV-2 (WT), and Omicron XBB.1 pseudoviruses, and S-specific B cells were isolated for nanobody recovery. Candidate nanobodies were identified by recombinant expression and functional screening.

Binding specificity and receptor competition were assessed by ELISA using spike trimers, RBDs, NTDs, and soluble ACE2 or DPP4. Inhibition of spike-mediated membrane fusion was examined using a GFP-based cell-cell fusion assay. To optimize antiviral activity, monovalent, multivalent, and Fc-fused formats of the selected nanobodies were generated and evaluated by SDS-PAGE, pseudovirus neutralization, and surface plasmon resonance (SPR).

Bispecific antibodies combining the two nanobodies were then constructed in both nanobody and Fc-fused formats and tested against the three highly pathogenic human coronaviruses, multiple recent Omicron subvariants, and representative SARS-CoV-1- and SARS-CoV-2-related sarbecoviruses. Cryo-electron

microscopy (cryo-EM) was used to define the structural basis of neutralization. In vivo efficacy was evaluated by intranasal prophylaxis in hamster contact-transmission models of SARS-CoV-1 and SARS-CoV-2 and by systemic treatment in hDPP4 knock-in mice infected with MERS-CoV. Potential escape sites were further assessed by structure-guided mutagenesis and epitope conservation analysis.

RESULTS:

Sequential immunization elicited broad humoral responses in alpacas and enabled the isolation of two RBD-directed nanobodies with distinct yet complementary activities. Z1 potently neutralized MERS-CoV, whereas L1 broadly neutralized the sarbecoviruses tested, including SARS-CoV-1, SARS-CoV-2 WT, multiple recent Omicron subvariants, and several related animal-derived sarbecoviruses. Both nanobodies blocked receptor engagement, with Z1 inhibiting DPP4 binding and L1 inhibiting ACE2 binding, and both also potently suppressed spike-mediated cell-cell fusion.

Multivalent engineering markedly enhanced antiviral potency. Relative to the monovalent format, trivalent Z1 showed a >1,000-fold increase in potency against MERS-CoV, whereas trivalent L1 showed an approximately 100-fold increase against SARS-CoV-1 and the recently circulating Omicron subvariant KP.3.1.1, together with a more modest improvement against SARS-CoV-2 WT. Fc fusion further enhanced activity, and SPR analysis showed that these affinity gains were driven mainly by reduced dissociation rates, indicating more stable antibody-spike engagement.

On the basis of these complementary properties, we developed a panel of bispecific antibodies and identified L1-Z1×2 as the optimal design. This bispecific antibody exhibited broad and ultrapotent neutralization against pseudoviruses representing MERS-CoV, SARS-CoV-1, SARS-CoV-2 WT, multiple currently relevant Omicron subvariants, and representative zoonotic sarbecoviruses. Among the constructs tested, L1-Z1×2-Fc consistently showed the greatest activity and also potently neutralized authentic MERS-CoV, SARS-CoV-1, SARS-CoV-2 WT, and representative currently circulating Omicron subvariants.

Cryo-EM analysis revealed distinct but complementary mechanisms of action. Z1 recognized an epitope overlapping the MERS-CoV receptor-binding motif and directly sterically blocked DPP4 engagement. By contrast, L1 bound a lateral cryptic epitope on the sarbecovirus RBD, sterically interfered with ACE2 binding, and, in SARS-CoV-2, additionally cross-linked adjacent spike trimers and constrained their conformational dynamics. These structural findings support a

dual mechanism of L1 involving receptor blockade and spike cross-linking. In vivo, intranasal administration of L1-Z1×2-Nb effectively blocked contact transmission of both SARS-CoV-1 and SARS-CoV-2 in stringent hamster co-housing models, markedly reducing viral RNA, infectious titers, viral antigen burden, and lung pathology in contact animals. In parallel, systemic administration of L1-Z1×2-Fc showed potent therapeutic efficacy in hDPP4 knock-in mice infected with MERS-CoV, reducing pulmonary viral replication by approximately 4 log₁₀ and substantially alleviating lung injury.

Structure-guided epitope conservation analysis further showed that Z1 relies on a compact but highly conserved epitope, whereas L1 recognizes a broader conserved surface with the strongest conservation among clade 1a and 1b sarbecoviruses. Consistent with these findings, mutagenesis identified D539 and Y541 as the principal escape-sensitive residues for Z1 and K378 as a major escape-sensitive residue for L1, supporting a relatively high barrier to viral escape.

CONCLUSIONS:

These findings establish heterologous sequential immunization as an effective strategy for generating nanobodies with complementary cross-lineage specificities and show that multivalent and bispecific engineering can integrate these activities into a single broadly active molecule. By combining a MERS-CoV-targeting module with a broadly sarbecovirus-neutralizing module, L1-Z1×2 achieved broad and ultrapotent neutralization against all three highly pathogenic human betacoronaviruses, multiple currently circulating SARS-CoV-2 variants, and several SARS-CoV-1- and SARS-CoV-2-related sarbecoviruses.

Mechanistically, cryo-EM analysis showed that Z1 directly blocks DPP4 engagement by targeting an epitope overlapping the receptor-binding motif, whereas L1 not only interferes with ACE2 binding but, in SARS-CoV-2, also cross-links adjacent spike trimers and constrains their conformational dynamics. Importantly, L1-Z1×2 showed robust in vivo efficacy, conferring intranasal prophylactic protection against SARS-CoV-1 and SARS-CoV-2 transmission in hamsters and systemic therapeutic efficacy against MERS-CoV infection in hDPP4 knock-in mice.

More broadly, this study offers a practical solution to the long-standing trade-off between breadth and potency in broad-spectrum coronavirus antibody development. Rather than relying on a single naturally occurring antibody to bridge highly divergent coronavirus lineages, we combined two potent RBD-directed nanobodies with complementary lineage coverage into one engineered molecule. Together with the unusual L1-mediated spike cross-linking mechanism and the distinct translational advantages of the nanobody and Fc-fused formats, these findings identify L1-Z1×2 as an ultrapotent broad betacoronavirus antibody candidate and provide a practical blueprint for modular, inhalation-ready, format-flexible antibody platforms against current and future spillover threats.

Abstract No.: 30

27 June 2026 (Saturday) 1025-1040

A SARS-CoV-2 entry inhibitor trimerizes to lock the spike protein in a closed conformation

First Author: Huihui Mou, Boston Children's Hospital, Harvard Medical School

Co-Author(s): Bo Gao, Gang Ye, Lizhou Zhang, Divyasha Saxena, Fan Bu, Jyoti Vishwakarma, Shuo Zhou, Claudia Ruiz Bayona, Li Lin, Yulia Getmanenko, Janarjan Bhandari, Charles C. Bailey, Gogce C. Crynen, Claire E. Kitzmiller, Hao Li, Yuka Otsuka, Chao Wang, Lalit Batra, Stuart Weston, Matthew B. Frieman, David K. Meyerholz, Louis Scampavia, Timothy P. Spicer, Reuben S. Harris, Michael D. Cameron, Thomas D. Bannister, Jian Zheng, Michael Farzan, Fang Li, Hyeryun Choe

BACKGROUND OBJECTIVES:

The SARS-CoV-2 spike protein binds its receptor ACE2 to initiate target-cell infection. To engage ACE2, at least one of the three receptor-binding domains (RBDs) of the spike must adopt the “up” orientation. We describe here, S22, a SARS-CoV-2-specific entry inhibitor, which we identified through a comprehensive screening of a 665,000-compound drug-like small-molecule library. Antiviral profiling revealed that S22 neutralized BA.2 and all subsequent Omicron variants. In this study, we sought to define the mechanisms of action of S22-mediated inhibition of viral entry.

METHODS:

We first performed time-of-addition experiments to define the stage of viral entry targeted by S22. Direct interactions between S22 and SARS-CoV-2 spikes were assessed using surface plasmon resonance (SPR) and nano-differential scanning fluorimetry (nanoDSF) binding assays. Flow cytometry-based binding assays were used to evaluate inhibition of spike-ACE2 engagement and explore the binding kinetics of S22 with SARS-CoV-2 spikes. To further solidify S22-spike interactions and its mechanisms of action, we determined the cryo-EM structure of the BA.5 spike in complex with S22. Finally, given its favorable pharmacokinetic properties, we evaluated the in vivo antiviral efficacy of S22 in a mouse model infected with the SARS-CoV-2 XBB.1.5 variant.

RESULTS:

Time-of-addition and binding studies demonstrated that S22 inhibits SARS-CoV-2 entry by engaging the spike glycoprotein and directly blocking spike-ACE2

interactions. Cryo-EM analyses revealed that S22 assembles as a trimer in a previously uncharacterized pocket of the spike apex, stabilizing the trimer in a fully closed conformation with all three RBDs in the down orientation, thereby preventing ACE2 association. Binding studies, especially those using mixed S22-sensitive and -resistant spikes, imply a cooperative assembly of three S22 molecules with three RBDs, resulting in an unusually slow S22 off-rate. Consistent with its slow dissociation and favorable pharmacokinetics, S22 suppressed viral replication by 100-fold in the lungs of XBB.1.5-infected mice without inducing detectable escape mutations.

CONCLUSIONS:

Thus, S22 potentially inhibits Omicron entry through a distinct mechanism of action whereby a small compound assembles cooperatively as a trimer to stabilize spike in an inactive conformation. This symmetry-matched, self-assembling mode of inhibition suggests a broader strategy for targeting multimeric viral and cellular proteins.



Abstract No.: 325

27 June 2026 (Saturday) 1041-1048

A Novel Host-Targeted Antiviral Inhibiting Glycerophospholipid Metabolism in Feline Infectious Peritonitis Virus Infection

First Author: Guoqian GU, City University of Hong Kong

Co-Author(s): Weiyi CHEN, Ákos KENÉZ, Yun Young GO

BACKGROUND OBJECTIVES:

Emerging highly pathogenic coronaviruses pose severe threats to global public health, highlighting an urgent need for novel antivirals. Coronaviruses extensively hijack host lipid metabolism to remodel intracellular membranes and establish viral replication organelles (ROs). Targeting host lipid pathways thus represents a promising antiviral strategy. This study investigated the antiviral efficacy and mechanism of action of a novel compound, KR-206238, using feline infectious peritonitis virus (FIPV) as a BSL-2 model for pathogenic coronaviruses.

METHODS:

Antiviral activity was evaluated via dose-response assays, virus growth kinetics, and time-of-addition experiments in Crandell-Rees feline kidney (CRFK) cells. Viral entry and genome replication were assessed using spike-mediated pseudovirus and replicon systems, respectively. Untargeted liquid chromatography-mass spectrometry (LC-MS) metabolomics characterized metabolic reprogramming induced by infection and treatment. Mechanistic validation included exogenous lipid supplementation and small interfering RNA (siRNA)-mediated knockdown of choline/ethanolamine phosphotransferase 1 (CEPT1). Molecular docking based on cryo-EM structures predicted ligand-target binding, while transmission electron microscopy (TEM) and immunofluorescence visualized impacts on double-membrane vesicles (DMVs) and replication centers. Synergistic effects with direct-acting antivirals and activity against multiple positive-strand RNA viruses were also tested.

RESULTS:

KR-206238 showed potent anti-FIPV activity with a 50% effective concentration (EC_{50}) of 0.34 μ M and low cytotoxicity ($CC_{50} > 100 \mu$ M). It inhibited viral replication

at an early post-entry stage without directly affecting viral attachment or RNA synthesis. Metabolomics revealed that KR-206238 reversed infection-induced accumulation of phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Exogenous PC/PE supplementation antagonized its antiviral effect, and CEPT1 knockdown phenocopied the compound's activity. Docking indicated KR-206238 acts as a competitive inhibitor of diacylglycerol (DAG) in the CEPT1 hydrophobic chamber. TEM confirmed disrupted DMVs and replication centers. KR-206238 synergized with GS-441524 and GC-376, and exerted antiviral activity against multiple positive-strand RNA viruses from three families.

CONCLUSIONS:

These findings demonstrate that CEPT1-mediated glycerophospholipid synthesis is a critical host dependency factor for coronaviruses. KR-206238 efficiently blocks viral replication by impairing the biogenesis of replication organelles, making it a promising lead compound for developing antivirals targeting positive-strand RNA viruses.



Abstract No.: 257

27 June 2026 (Saturday) 1049-1056

Intranasal administration of a broadly neutralising macrocyclic peptide inhibitor protects against SARS-CoV-2 infection

First Author: Daniel Hurdiss, Utrecht University

Co-Author(s): Antonius J.P. Hopstaken, Aneta Zegar, Oliver J. Debski-Antoniak, Preeti Hooda, Melissa van Dortmond, Joline van der Lee, Minglong Liu, Aleksandra Milewska, Krzysztof Pyrc, Berend-Jan Bosch, Frank J.M. van Kuppeveld, Seino A.K. Jongkees

BACKGROUND OBJECTIVES:

The emergence of SARS-CoV-2 variants with mutations in the spike protein has diminished the efficacy of antibody-based therapeutics, underscoring the need for antivirals targeting conserved and less accessible regions of the spike glycoprotein. We previously identified a macrocyclic peptide (S1b3inL1) that inhibits viral entry by binding a conserved, cryptic site within the receptor-binding domain. However, its limited potency and solubility hindered further development.

Here, we sought to optimize this peptide to enhance antiviral potency, solubility, and breadth of activity, and to assess whether optimized variants can confer protection against SARS-CoV-2 infection in relevant *in vitro* and *in vivo* models.

METHODS:

We performed deep mutational scanning of the parental peptide using mRNA display libraries with site-saturation mutagenesis to identify sequence variants compatible with spike binding. Selected variants were synthesized and screened for inhibition of SARS-CoV-2 pseudovirus entry and assessed for solubility using nephelometry. Lead candidates were evaluated against authentic SARS-CoV-2 variants in cell lines and primary human airway epithelial air-liquid interface cultures. Multimeric peptide constructs were designed to enhance potency and tested for antiviral activity. Finally, *in vivo* efficacy was assessed in a Syrian golden hamster model following intranasal prophylactic administration.

RESULTS:

Deep mutational scanning identified positions tolerant to mutation and enabled rational optimization of the macrocyclic peptide. The lead variant, H4v1, exhibited up to ~50-fold improved antiviral potency and ~20-fold increased solubility compared to the parental compound.

Multimerization further enhanced antiviral activity, with trimeric and tetrameric constructs achieving an additional order-of-magnitude improvement in potency. The tetrameric peptide inhibited replication of SARS-CoV-2 Omicron BA.2.86 in human airway epithelial cultures with low nanomolar potency.

Optimized peptides retained activity against multiple sarbecoviruses, including SARS-CoV-1 and related bat coronaviruses. In a hamster model, intranasal administration of multimeric peptides significantly reduced viral load in both lung and upper respiratory tract tissues following prophylactic dosing, demonstrating in vivo efficacy.

CONCLUSIONS:

Optimization of a spike-targeting macrocyclic peptide through deep mutational scanning and multimerization yielded highly potent inhibitors that engage a conserved and immunologically inaccessible site on the spike protein. Intranasal delivery of these compounds significantly reduced viral burden in vivo, supporting their potential as prophylactic agents against SARS-CoV-2 variants and potentially emerging sarbecoviruses.

These findings highlight the promise of macrocyclic peptide-based antivirals and demonstrate the utility of combining deep mutational scanning with multivalent design to enhance therapeutic performance against respiratory viruses.



Abstract No.: 33

27 June 2026 (Saturday) 1056-1105

Pan-coronavirus antiviral drug discovery driven by proteomic and gene expression analysis in alveolar type II cells

First Author: Jessica Neil, The University of Melbourne

Co-Author(s): Hannah Huckstep, Monika Mohenska, Matthew J Gartner, Monique Smith, Francesca Mordant, Guizhi Sun, Sarah Londrigan, Jose M Polo, Sean Humphrey, Kanta Subbarao

BACKGROUND OBJECTIVES:

Despite significant success in the development of vaccines there are few therapeutics options for the treatment of human coronavirus infections. Host-targeting antivirals offer the ability to provide increased durability, breadth, and resilience compared to direct-acting antivirals by targeting important shared pathways associated with coronavirus replication. Here, we aimed to use phosphoproteomic and gene expression analysis in infected lung epithelial cells to identify common host pathways modulated by coronavirus infection as possible targets for broad spectrum pan-coronavirus therapeutics.

METHODS:

Stem-cell derived alveolar type II (AT2) cells were infected with SARS-CoV-2, MERS-CoV, NL63, 229E and OC43. Virus replication was assessed by RT-qPCR and infectivity assay. Cellular protein was collected at 0 (1h post adsorption), 18 and 24 hours post infection (hpi) and the phosphoproteome analysed by mass spectrometry. Cellular RNA was collected at 24 and 72 hpi for gene expression analysis by RNASeq.

RESULTS:

All coronaviruses replicated in AT2 cells with MERS-CoV replicating to the highest levels. NL63 and OC43 displayed temperature sensitivity with replication detectable at 35°C but not 37°C within the first 24h. Only 229E and MERS-CoV induced visible cytopathic effects. Gene expression analysis showed that the

responses to pathogenic coronaviruses were distinct from seasonal coronaviruses. Beta coronaviruses reduced lung cell identity and signalling signatures while MERS-CoV also induced fibrotic signatures. Infection with all coronaviruses was associated with reduced activity of the HNF4a regulated transcriptional network. Proteomics analysis suggested that changes in phosphorylation varied over time with the most significantly regulated phosphosites observed following MERS-CoV infection. Interestingly, only MERS-CoV infection led to increased phosphorylation of proteins associated with translation initiation. Additionally, we identified an increase in EpHA2 kinase activity following infection with all coronaviruses.

CONCLUSIONS:

Overall, our data suggest that while each human coronavirus can induce distinct phosphorylation and gene expression pathways, commonalities do exist across all five viruses that could be exploited for pan-coronavirus therapies.



Abstract No.: 357

27 June 2026 (Saturday) 1150-1205

Species-specific expression of ACE2 in multiciliated airway cells determines SARS-CoV-2 susceptibility

First Author: Tim Breugem, Erasmus MC

Co-Author(s): Anna Mykityn, Alexandre Bouffier, Debby Schipper, Sivana Baptista Varela, Mart Lamers, Bart Haagmans

BACKGROUND OBJECTIVES:

SARS-CoV-2 is a zoonotic virus that infects a diverse array of animal species. This host range is usually determined by binding of the viral Spike protein to the angiotensin converting enzyme 2 (ACE2) receptor. However, some species such as pigs are remarkably resistant to SARS-CoV-2 infection, despite the ability of Spike to bind porcine ACE2 and the ability of the virus to replicate in porcine cell lines, suggesting that additional barriers limit cross species transmission. Therefore, we aimed to investigate the resistance of pigs to SARS-CoV-2 infection.

METHODS:

We investigated the resistance of pigs to SARS-CoV-2 infection using cell lines and well-differentiated porcine and human airway organoid-derived epithelial cultures. We used MERS-CoV as control with validated tropism for pigs. We performed immunohistochemistry and immunofluorescent staining on airway cultures and tissues to assess the expression of coronavirus receptors in the airway cells of both species.

RESULTS:

In contrast to porcine cell lines and human airway cultures, porcine airway cultures were resistant to SARS-CoV-2 infection. Whereas human airway cultures and tissues expressed ACE2 in multiciliated cells at the base of the motile cilia, porcine airway cultures and tissues expressed ACE2 in the cytoplasm of multiciliated airway cells. Ectopic expression of human ACE2 led to the apical localization in porcine ciliated cells and conferred permissibility to SARS-CoV-2 infection. In contrast, DPP4 expression was efficiently expressed on the surface of

both porcine and human airway cells, correlating with the tropism of MERS-CoV in these cells of both species

CONCLUSIONS:

These data indicate that the lack of susceptibility of pigs to SARS-CoV-2 is related to the expression patterns of the ACE2 receptor, and suggest that pigs are no viable reservoir host for ACE2-utilizing coronaviruses but are for DPP4-utilizing coronaviruses. Overall, our studies indicate that receptor localization must be considered carefully for evaluating species susceptibility and risk posed by zoonotic coronaviruses.



Abstract No.: 166

27 June 2026 (Saturday) 1205-1220

Animal Organoids to Study Zoonotic Coronaviruses

*First Author: Alexandre Bouffier, Erasmus Medical Center**Co-Author(s): A.Z. Mykytyn, I. I. Tosheva, M.R. te Marvelde, M. Rissmann, B.L. Haagmans*

BACKGROUND OBJECTIVES:

Several animal hosts, including bats, rodents, and mustelids, are known reservoirs of coronaviruses with zoonotic potential, as these can spillover into humans and cause significant outbreaks. A notable example highlighting the importance of animal hosts is the outbreak of SARS-CoV-2 in farmed mink. Mink were shown to be highly susceptible to infection and sustained rapid viral transmission within dense farming environments. More importantly, evidence demonstrated mink-to-human transmission, raising concerns about the establishment of animal reservoirs and the potential for viral evolution outside the human population. On the other hand, related species like ferrets did not develop disease upon SARS-CoV-2 infection. This underscores the need for improved models and surveillance strategies to better understand coronavirus ecology and prevent future spillover events.

METHODS:

Therefore, we established animal organoids to bridge the gap between virus receptor overexpression systems and in vivo studies, and to deliver an ethically acceptable and reproducible system to more accurately predict coronavirus spillover risks. Organ-specific organoids were generated from stem cells isolated from digested tissues and cultured in an extracellular matrix with growth factor-rich media. These cells differentiate into complex systems resembling native organs. Organoids were infected with different SARS-CoV-2 variants to study viral tropism.

RESULTS:

We established organoids from different animal species, such as mink, ferrets, pigs, sheep, and goats. Differentiation of airway organoids was confirmed by ciliation of cultured cells. SARS-CoV-2 productively infected both ferret and mink organoid cultures. Interestingly, we observed a clear divergence in tissue tropism

between the two mustelid hosts. In mink, bronchial organoids supported viral replication, while ferret bronchial and tracheal organoids remained resistant to SARS-CoV-2 infection, while infection was observed in nasal organoids. In line with these observations, we also showed different ACE2 receptor patterns of expression in these organoid cultures. While ACE2 was not detected in the trachea and bronchus organoids of ferrets, ACE2 can be found in organoids from the nose of ferrets and the bronchus of mink. These contrasting profiles suggest that, despite the susceptibility of both species, the SARS-CoV-2 tropism differs substantially between ferrets and minks, indicating the difference in SARS-CoV-2 pathogenesis between these two species.

CONCLUSIONS:

Thus, the established animal organoids provide a relevant tool to study the pathogenesis of coronaviruses.



Abstract No.: 278

27 June 2026 (Saturday) 1220-1235

Clade C MERS-CoV camel strains vary in protease usage during viral entry and interferon antagonism

*First Author: Helena Winstone, University of Pennsylvania**Co-Author(s): Helen Stillwell, Li Hui Tan, Noam Cohen, Ranawaka Perera, Susan Weiss*

BACKGROUND OBJECTIVES:

Middle East respiratory syndrome coronavirus (MERS-CoV) clade A and B strains have caused periodic outbreaks in the Middle East when they spillover from camels to humans. Clade C MERS-CoV strains however, are only found in camels across Africa and do not appear to cause disease in humans. Clade A/B MERS-CoV strains have been reported to utilize the TMPRSS2-mediated viral entry pathway in the human airways, and effectively shutdown interferon induction and the activation of RNase L, an antiviral endonuclease, through the activity of accessory genes NS3-NS5. Clade C virus genomes however encode substitutions across spike, and both substitutions and varying length deletions in accessory genes NS3-5. We sought to understand whether these sequence differences impact viral entry and interferon induction of clade C viruses in the human airways.

METHODS:

We utilized a panel comprising six clade C viruses (3 from West Africa and 3 from East Africa), a clade A and a clade B virus to compare viral replication, viral entry, protease usage, and interferon induction in human-derived lung cell lines and primary nasal epithelial cells.

RESULTS:

We report that all six clade C viruses were attenuated for replication in human lung cell lines relative to clade A/B viruses. Additionally, we found that East African strains, but not West African strains, were less able to utilize the TMPRSS2-mediated entry pathway for viral entry in human lung cell lines and primary nasal cultures. This was mirrored by an increased reliance on the endosomal entry pathway. We identified the D158Y substitution in the N-terminal domain of spike, a substitution that is present in all East African sequences to date, as a key determinant of this differential protease usage. We also found that West African clade C strains

induced higher interferon-stimulated gene and pro-inflammatory cytokine induction than clade A/B strains. Furthermore, West African strains with significant truncations in the NS4b protein induced marked activation of RNase L.

CONCLUSIONS:

Overall, we suggest that the reduced ability to use the TMPRSS2-mediated pathway for viral entry contributes to the attenuation of East African clade C strains in the human airways and could limit cellular tropism. Moreover, we propose that West African clade C viruses cannot antagonize interferon signaling as effectively as clade A/B viruses, which contributes to their reduced replication in the human host. These data indicate geographically distinct selection pressures on MERS-CoV evolution in camel populations which contribute to altered pandemic potential.



Abstract No.: 172

27 June 2026 (Saturday) 1235-1250

Experimental SARS-CoV-2 infection using horseshoe bats

First Author: Kaoru Usui, The University of Tokyo

Co-Author(s): Masumi Tsuda, Keiya Uriu, Shigeru Fujita, Yukie Kashima, Yutaka Suzuki, Lei Wang, Shinya Tanaka, Jumpei Ito, Kei Sato

BACKGROUND OBJECTIVES:

The majority of viruses causing disease in humans have emerged through spillover from wild animals. In particular, bats, which are the second largest group in mammals, are well known as natural reservoir hosts of diverse zoonotic viruses, including Hendra virus, SARS-CoV, MERS-CoV, and SARS-CoV-2. Sarbecoviruses, a subgenus of Betacoronavirus that includes SARS-CoV and SARS-CoV-2, are single-stranded RNA viruses that primarily cause respiratory diseases in humans. Horseshoe bats (Genus *Rhinolophus*) are considered natural hosts of sarbecoviruses, as these viruses are frequently detected in their swabs. To understand how horseshoe bats coexist with sarbecoviruses in nature, experimental infection can provide direct evidence. However, in vivo infection studies using horseshoe bats have been lacking because of the difficulty of maintaining insectivorous bats in a laboratory setting.

METHODS:

Here, we established a stable husbandry system for greater horseshoe bats (*Rhinolophus ferrumequinum*) and investigated SARS-CoV-2 infection dynamics in this natural host. Bats were captured and acclimated to laboratory conditions and trained to feed on mealworms. The kept bats were then intranasally infected with SARS-CoV-2. After infection, we longitudinally monitored body weight, viral shedding, and tissue viral loads, and performed histological and transcriptomic analyses.

RESULTS:

In contrast to Syrian hamsters which showed robust viral replication, infected horseshoe bats exhibited low-level but persistent viral replication in the lung without overt disease. Histological analyses revealed that inflammatory lesions in the bat lungs were spatially restricted and temporally delayed compared with those in hamsters. Transcriptomic analyses further showed preferential activation

of tissue repair pathways but limited inflammatory responses following infection. Notably, bats expressed several interferon-stimulated genes prior to infection.

CONCLUSIONS:

Together, these findings indicate horseshoe bats mount a distinct host response to SARS-CoV-2 infection, characterized by effective containment of viral replication without the development of systemic symptoms. We propose that this response may involve: (1) a constitutive antiviral state in the respiratory epithelium that suppresses viral replication at the earliest stage of infection; (2) preferential activation of tissue repair-associated pathways; and (3) avoidance of excessive inflammatory and adaptive immune activation. These immune features may underlie the ability of horseshoe bats to tolerate sarbecovirus infection and support their role as natural reservoir hosts.



Abstract No.: 179

27 June 2026 (Saturday) 1250-1305

Incidence, Genomic, and Phenotypic Characterization of Recent MERS-CoV Strains in East Africa

First Author: Marcel Müller, Charité - Universitätsmedizin Berlin

Co-Author(s): Brian Ogoti, Jordi Rodon, Johanna Wildemann, Diana Bösel, Johanna Bork, Victor Riitho, Julius Oyugi, Marianne W. Mureithi, Barbara Mühlemann, Sarah Metzger, Kim Heimsch, Victor M. Corman, Terry Jones, Samuel M. Thumbi, Christian Drosten

BACKGROUND OBJECTIVES:

The Middle East respiratory syndrome coronavirus (MERS-CoV) can cause severe atypical pneumonia in humans. MERS-CoV is endemic in Arabian and African dromedary camels with occasional spillovers to humans. Phylogenetic analyses suggest circulation of geographically distinct MERS-CoV clades B (Arabian Peninsula) and C (African continent). Limited surveillance has constrained our understanding of ongoing zoonotic transmissions, viral diversity, and evolution in Africa. This study aimed to characterize the incidence, diversity, evolution, and fitness of currently circulating MERS-CoV strains in East Africa.

METHODS:

Dromedaries were sampled at an abattoir hub in northern Kenya from September 2022 to September 2023 (n=2,711), and from March to April 2024 (n=386). Specimens were screened for MERS-CoV via rapid antigen tests and/or diagnostic reverse transcription quantitative polymerase chain reaction (RT-qPCR) using upE assays. Samples with high viral RNA loads ($\geq 10^6$ genome copies/ml) were sequenced using Illumina technology and subjected to in-depth genomic and phylogenetic analyses. Novel strains were isolated from field samples and subjected to phenotypic assays. Using a reverse genetics system, recombinant chimeric viruses were generated to attribute specific fitness effects to virus proteins.

RESULTS:

MERS-CoV RNA detection rates were 1.3% (36/2,711) during 2022-23, and 0.8% (3/386) in 2024. During the one-year sampling period, camels exhibited a biphasic MERS-CoV incidence, with peak viral RNA loads during the dry seasons in October 2022 and February 2023. Twelve MERS-CoV whole-genome sequences were obtained. Phylogenetic analyses revealed that all contemporary sequences belong to a monophyletic group within clade C2.2, suggesting that this lineage

is predominant in East Africa. The novel strains showed capability to infect human respiratory cells, although African viruses exhibited lower replication fitness in vitro compared to Arabian counterparts. When studied in isolation, both spike and backbone proteins of the strains contribute to the attenuated phenotype of African MERS-CoV.

CONCLUSIONS:

Our epidemiological and phylogenetic analyses suggest ongoing evolution of MERS-CoV in East Africa, with biphasic MERS-CoV incidence in camels that might facilitate public health risk assessment. Contemporary East African MERS-CoV strains exhibit an attenuated phenotype compared to their Arabian counterparts.



Abstract No.: 128

27 June 2026 (Saturday) 1305-1310

Evolution of receptor usage plasticity in merbecoviruses through a computational approach

First Author: Jarel Elgin Tolentino, The University of Tokyo

Co-Author(s): Victoria Jefferson, Nicholas J. Catanzaro, Ralph S Baric, Michael C. Letko, Spyros Lytras, Kei Sato

BACKGROUND OBJECTIVES:

Merbecoviruses within the Betacoronavirus genus exhibit remarkable plasticity in receptor usage that affects their host range and zoonotic potential. This group includes Middle East respiratory syndrome coronavirus (MERS-CoV) and several bat-associated viruses, such as HKU5-related coronaviruses (HKU5r-CoVs), some of which have recently been detected in farmed mink. Understanding how merbecoviruses evolve new receptor specificities is critical for anticipating spillover risk and guiding emergence preparedness. Two complementary studies in merbecovirus evolution is presented here. The main aim of these studies is to dissect the molecular and evolutionary events underlying receptor usage plasticity in merbecoviruses.

METHODS:

Using large-scale phylogenetic reconstruction, recombination analysis, molecular dating, and AI-guided structural modeling, the results obtained suggest that receptor adaptation can arise through both genomic recombination and localized structural plasticity within the Spike protein.

RESULTS:

In the first study, we demonstrate that MERS-CoV acquired its dipeptidyl peptidase 4 (DPP4) usage through a recombination event involving the Spike gene. Although the MERS-CoV genome is most closely related to ACE2-using bat viruses such as NeoCoV and PDF-2180, recombination analyses reveal that its receptor-binding domain (RBD) clusters with DPP4-using merbecoviruses. This finding explains the evolutionary origin of MERS-CoV and indicates that receptor switching might have occurred in bats prior to spillover into camels and humans. In the second study, we investigated the newly-detected HKU5r-CoV in mink and identified the RBD loop2 region as a key determinant of ACE2 usage and host tropism. Structural modeling and recombination analyses show that loop2 exhibits substantial

structural and sequence plasticity. Notably, a single amino acid substitution (R548S) is sufficient to enable human ACE2 usage showing the latent zoonotic potential of HKU5r-CoVs circulating at the wildlife–livestock interface.

CONCLUSIONS:

Together, our results demonstrate that receptor usage in merbecoviruses is governed by recombination and structural changes within the Spike protein, particularly in the RBD region. From the recombination-driven emergence of MERS-CoV to the loop2-mediated adaptation of HKU5r-CoVs in mink, both cases show how minor genetic events can unlock new host ranges and zoonotic potential. These findings highlight the importance of continued efforts to dissect the evolutionary drivers of merbecoviruses, as understanding these events is critical for predicting and mitigating their emergence.



Abstract No.: 47

Poster Code: A-0001

An updated dataset of early SARS-CoV-2 diversity supports a wildlife market origin

First Author: Florence Débarre, Sorbonne Université / CNRS

Co-Author(s): Zach Hensel

BACKGROUND OBJECTIVES:

The origin of SARS-CoV-2 has been intensely scrutinized, and epidemiological and genomic evidence has consistently pointed to Wuhan's Huanan Seafood Wholesale Market as the epicenter of the COVID-19 pandemic. Early cases were associated with this market, and environmental sequencing placed the common ancestor of SARS-CoV-2 genomic diversity within the market. Phylogenetic analysis also suggested separate introductions of lineages A and B into the human population, a finding that can be tested with additional data.

METHODS:

We curated an expanded dataset of early SARS-CoV-2 viral genomes, incorporating newly available sequences from mid-January 2020. We analyzed this dataset to assess support for previously proposed alternative progenitor sequences and to identify potential evolutionary intermediates between lineages A and B in the human population.

RESULTS:

evolutionary intermediates between lineages A and B in the human population. Instead, we identified SARS-CoV-2 lineages that may have spread from the market, and additional samples of a sublineage of lineage A with three mutations, including one found in closely related bat coronaviruses. Although our analysis of early pandemic genomes suggests that this mutation is unlikely to characterize the immediate SARS-CoV-2 ancestor, it is more plausible than two previously proposed ancestral genomes.

CONCLUSIONS:

These findings reinforce the proposed emergence of SARS-CoV-2 from the wildlife trade at the Huanan market, demonstrating how new data continues to both solidify and clarify our understanding of how the pandemic began.

Abstract No.: 60

Poster Code: : A-0002

A SARS-CoV-2-Informed Simulator of Time-Resolved and Nested Viral Recombinants for Benchmarking Recombination Inference

First Author: Ruixuan Wang, The University of Hong Kong

Co-Author(s): Zach Hensel, Thomas King-Fung Wong, Bui Quang Minh, Leo L.M. Poon

BACKGROUND OBJECTIVES:

Recombination is a major driver of viral evolution, generating mosaic genomes from distinct lineages and potentially altering transmissibility, antigenicity, and fitness. In SARS-CoV-2, such events can arise when different lineages co-circulate, as illustrated by XBB, which originated from BJ.1 and BM.1.1.1. Despite its importance, rigorous evaluation of recombination detection methods remains challenging. Many current simulation approaches simply concatenate empirical genomes, which may retain undocumented recombination signals and other background noise. In addition, these approaches often do not explicitly account for whether parental lineages overlapped in time or whether recombinant genomes could give rise to subsequent recombination events. To address these limitations, we developed a SARS-CoV-2-oriented simulation framework that generates time-resolved and nested recombinant genomes for biologically grounded benchmarking of recombination detection.

METHODS:

Using a dated global SARS-CoV-2 phylogeny as the temporal backbone, we defined parental lineages according to their inferred circulation windows. Recombinants were generated only from parent pairs with overlapping circulation periods, and each recombination event was assigned a date within the shared interval. The framework allows user-defined dataset sizes, configurable numbers of breakpoints, minimum spacing between breakpoints, and divergence thresholds between parent pairs. Newly generated recombinants can be returned to the parental pool and participate in later rounds of recombination, thereby enabling multi-generational recombinant histories. To minimize confounding from undocumented recombination in empirical genomes, the framework first reconstructs a recombination-free sequence background before introducing controlled mosaic genomes. Ground-truth annotations include parental identities, breakpoint architecture, recombinant dates, overlap intervals, and full multi-

generational histories.

RESULTS:

The framework generates benchmark datasets at user-defined scales, ranging from simple one- or two-breakpoint chimeras to complex genomes with multiple breakpoints and nested recombination histories. For each simulated genome, it records parental origin, breakpoint positions, event dates, and pedigree structure, enabling direct comparison between inferred and true recombination histories. By restricting recombination to temporally plausible parental pairs and introducing events on a recombination-free background, the framework reduces confounding from undocumented mosaic ancestry and supports more objective assessment of breakpoint localization and event dating.

CONCLUSIONS:

This framework provides a biologically informed platform for simulating viral recombination by incorporating temporal plausibility, event dating, and nested recombinant histories within a single benchmarking system. It enables rigorous and interpretable benchmarking of recombination detection methods and provides a practical methodological resource for improving the identification of complex recombinant genomes, particularly in high-resolution surveillance settings represented by SARS-CoV-2.



Abstract No.: 134

Poster Code: : A-0003

Relaxed Selection and Convergent Evolution Shape SARS-CoV-2 Variant Dynamics in Immunocompromised Patients

First Author: Alen Suljič, University of Ljubljana

Co-Author(s): Rok Kogoj, Tereza Rojko, Peter Adamič, Tatjana Lejko Zupanc, Miroslav Petrovec, Julian A. Hiscox, Miša Korva

BACKGROUND OBJECTIVES:

Prolonged SARS-CoV-2 infection in immunocompromised patients provides an extended window for within-host viral evolution and may facilitate adaptive change. However, most studies focus on consensus-level substitutions, while the contributions of minor variants, synonymous mutations, and host-driven mutational processes remain incompletely understood. We aimed to characterise within-host evolutionary dynamics during prolonged infection, compare immunocompromised (IC) and non-immunocompromised (nIC) hosts, and identify clinical factors associated with changes in viral evolutionary trajectories.

METHODS:

Longitudinal sequencing was performed on 677 respiratory samples from 82 patients (52 IC and 30 nIC) with prolonged SARS-CoV-2 infection. Mutations were classified as transient, persistent, convergent, emergent, or unclassified based on allele-frequency dynamics and recurrence across viral lineages. Mutation accumulation was analysed using linear regression models and expressed as substitutions per site per year. Analyses were stratified by viral lineage (early vs. Omicron), allele frequency (major vs. minor variants), and mutation type (synonymous vs. non-synonymous). Evolutionary trajectories in IC patients were further evaluated using synonymous/non-synonymous (S/N) ratios, transition/transversion (Tv/Ts) ratios, and mutational signatures.

RESULTS:

Viruses from IC patients showed fewer transient mutations than those from nIC patients (13.4% vs. 28.3%) and more convergent mutations (52.3% vs. 41.7%). Among IC infections, two distinct evolutionary trajectories were identified: accelerated and neutral. Accelerated infections reached mutation rates of 118.4×10^{-4} substitutions per site per year, whereas neutral infections showed

minimal temporal accumulation. Early SARS-CoV-2 lineages accumulated mutations faster than Omicron variants (67.1×10^{-4} vs. 20.8×10^{-4} substitutions per site per year) and were dominated by temporally structured minor variants, while Omicron infections showed higher baseline mutation counts and enrichment of major Spike mutations. Accelerated trajectories were associated with corticosteroid and antiviral exposure, lower S/N ratios, higher Tv/Ts ratios, and mutational signatures consistent with host-mediated RNA editing.

CONCLUSIONS:

Prolonged SARS-CoV-2 infection in immunocompromised hosts can follow distinct evolutionary trajectories shaped by host immunity and treatment exposure, highlighting the importance of integrating longitudinal genomics with clinical data to understand the emergence of adaptive viral variants.



Abstract No.: 141

Poster Code: : A-0004

Selection of Spike Deletions Under Sustained Antibody Pressure in Omicron Variants of Severe Acute Respiratory Syndrome Coronavirus 2

*First Author: Songhyeok Oh, Seoul National University**Co-Author(s): Yujin Kim, Sohee Kim, Jiwon Choi, Namhyuk Cho*

BACKGROUND OBJECTIVES:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has undergone continuous genetic evolution since its emergence in 2019. Among the variants, the Omicron lineage rapidly became globally dominant despite carrying more than 30 spike mutations without a clearly identified immediate ancestor. While antibody escape has been a major driver of variant emergence, the specific evolutionary patterns favored under sustained antibody pressure remain incompletely characterized. This study aimed to define the mutational trajectories of an Omicron variant under continuous neutralizing antibody selection.

METHODS:

SARS-CoV-2 D614G variant (GenBank accession OR447552) and Omicron BA.5 isolate (NCCP 43426) were propagated in Vero cells. For antibody selection, 106 focus-forming units (FFU) of virus were incubated with sera obtained from convalescent individuals or vaccine recipients prior to infection. Virus-serum mixtures were then inoculated onto Vero cell cultures and maintained until the development of cytopathic effect (CPE). For serial passaging, harvested supernatants were diluted 1:100 and re-incubated with fresh human sera prior to subsequent infection of Vero cells, thereby maintaining continuous antibody selection across passages. For downstream analysis, harvested viruses were separately expanded in T-25 flasks. Viral populations from each passage were subjected to whole-genome sequencing (WGS) to identify mutations enriched during adaptation. Spike alterations were further characterized by Western blot and PCR for each passage.

RESULTS:

During serial passaging under antibody pressure, a deletion of approximately 1,570 base pairs near the receptor-binding domain (RBD) was observed in the

BA.5 population. This deletion was consistently found in serial passages. Truncated spike proteins and corresponding shortened sequences were detected in infected cells and supernatants, supporting the emergence of deletion-containing variants at both protein and sequence levels. In contrast, no deletion events of comparable size or pattern were detected in the D614G variant across passages under the same experimental conditions. Analysis of viral sequences obtained from patient nasal swab samples further showed that deletion events occur at a significantly higher frequency in Omicron lineages compared to earlier variants.

CONCLUSIONS:

Under sustained antibody pressure, the Omicron BA.5 variant exhibits a distinct pattern of antibody escape characterized by the selective enrichment of spike deletions. This observation can provide a basis for further interpretation of Omicron evolutionary dynamics.



Abstract No.: 155

Poster Code: : A-0005

Comparative genomic analysis of feline coronaviruses with different virulence

First Author: Izumi Kida, Hokkaido University

Co-Author(s): Shusuke Kawakubo, Terza Brostoff, Petra Cerna, Michael R Lappin, Patricia A Pesavento, Kei Sato, Keita Matsuno

BACKGROUND OBJECTIVES:

Feline infectious peritonitis (FIP) is a fatal systemic disease in cats caused by genetic variants of feline coronavirus (FCoV). FCoV is classified into two distinct biotypes: feline enteric coronavirus (FECV), which typically causes mild or asymptomatic enteric infection, and feline infectious peritonitis virus (FIPV), lethal variants of FECV causing systemic infection. Despite clinical significance of FIP in veterinary field, the genetic mechanisms underlying FIP pathogenesis remain unclear, given the limited genome sequences with biotype annotation. Thus, this study aimed to obtain numerous whole-genome sequences from cats diagnosed with FIP and from non-FIP cats, that allow to analyze potential key mutations in the spike, 3c, and 7b genes as molecular determinants of FIPV, particularly in serotype 1 FCoV (FCoV-1), globally predominant serotype.

METHODS:

RNA was extracted from effusions and tissues of cats with FIP, and from feces of non-FIP cats. FIP samples were sequenced using multiplex PCR-based targeted enrichment. Fecal samples were sequenced by RNA sequencing following rRNA depletion. Sequencing was performed using Illumina sequencer, with any remaining genomic gaps filled by conventional PCR and Sanger sequencing. To identify amino acid mutations associated with FIPV, Fisher's exact test was used to screen for mutations enriched in FIPV compared to FECV, using both publicly available sequences and those newly sequenced in this study. Recombination analysis of the spike gene was then conducted using RDP5. Finally, phylogenetic analysis and ancestral sequence reconstruction were performed based on the non-recombinant regions (NRRs) containing the sites identified as potential responsible mutations in this study.

RESULTS:

A total of 41 whole-genome sequences (19 FECV and 22 FIPV) were determined in the present study and analyzed together with those in the public database, resulting 124 sequences included in the analysis. We identified 17 potential amino acid substitution sites in the spike protein significantly correlated with FIPV; including the furin cleavage site and multiple residues within the S2 domain. These mutations were repeatedly observed in independent external branches of the phylogenetic trees, suggesting convergent emergence. Notably, FCoV-1 variants harboring deletions in the 5'-terminus of the spike gene were detected in 10/74 of FIPV sequences. While no specific amino acid substitutions in the 3c or 7b genes showed a significant correlation with FIPV, internal stop codons in 3c were observed in 19/67 of FIPV sequences, whereas none were detected in FECV.

CONCLUSIONS:

Our analysis provided genetic basis potentially associated with FIPV within the spike and 3c genes.



Abstract No.: 175

Poster Code: : A-0006

Development and characterization of a mouse model for persistent SARS-CoV-2 infections

First Author: Javier Alvarez de Miranda, The Francis Crick Institute

Co-Author(s): Margarida Bonifacio, James Boot, Jamie Barrett-Rodger, Katja Finsterbusch, Daniel Schnepf, Emma Nye, Robert Goldstone, Andreas Wack, David Bauer

BACKGROUND OBJECTIVES:

SARS-CoV-2 (SCV2) evolution was expected to be a steady process of accumulating mutations. However, the evolution pattern observed during the pandemic was characterised by antigenic 'leaps': sudden evolutionary events in which multiple mutations appeared simultaneously and gave rise to new variants of concern (VOCs). A potential explanation for this accelerated evolution is that persistent infection of immunocompromised patients may have allowed for extended periods of viral evolution within single hosts, providing the perfect environment for adaptive mutations to appear. This is supported by the early finding of specific mutations in persistent infections that were later found in VOCs.

METHODS:

We have used a mouse-adapted SCV2 strain (MA20) that recapitulates infection in WT mice to infect immunocompromised mouse models (Rag1^{-/-} mice, lacking T and B cells) aiming to establish persistent infections and study them over long periods. Once the model of SCV2 persistence was validated, we performed a deep characterisation of infection over time including viral dissemination, CT scans, histopathology and host immune response. Then, we sequenced viral variants arising from long-term infections to study potential intra-host adaptations and the possibility of viral compartmentalization within single hosts.

RESULTS:

We find that SCV2 can persistently infect animals for over 120 days in our model. Contrary to prior reports, we observed increased and age-dependent pathology during acute infection of Rag1^{-/-} mice compared to WT mice. Interestingly, the persistently-infected mice showed a striking recovery after the first week, rapidly

regaining weight and showing no external signs of illness — despite bearing high viral titres in their respiratory tract and maintaining a sustained antiviral response during the long-term infection. We also observed differences in persistence between upper and lower respiratory tract. In contrast with the stable infection in lung tissue, nasal cavities showed progressive viral clearance over time, hinting at different clearance mechanisms between these compartments.

Finally, we analysed viral evolution during persistence and compared to that of acute infections in WT mice, with persistent variants showing signs of increased intra-host diversification.

CONCLUSIONS:

Persistent infections, which remain largely uncharacterised, may have had an outsized impact in driving the evolution of SCV2 and the course of the pandemic. We have developed and studied an in vivo model of persistent SCV2 infections, enabling further studies of this clinical condition. Future work will focus on understanding how different types or levels of immunocompromise impact pathogenesis and viral evolution.



Abstract No.: 177

Poster Code: : A-0007

The +1 reading frame of the coronavirus N gene is a reservoir of accessory genes

First Author: Federico De Angelis, University of Edinburgh

Co-Author(s): Hou Wei Chook, Samantha Lycett, Paul Digard,

BACKGROUND OBJECTIVES:

The Nucleocapsid (N) gene of coronaviruses (CoVs) encodes a structural protein necessary for genome packaging and viral assembly. In characterised CoVs such as SARS-CoV/CoV-2, MERS-CoV and PDCoV, N encodes an accessory gene in its second reading frame that produces an innate immune antagonist, but the extent of this dual coding capacity has not been explored at the family level, and the genetic characteristics that drive formation of overlapping ORFs in CoV genomes remain unknown. The aim of this study were to determine the extent of the dual coding capacity of the coronavirus N gene as well as understanding the genetic reasons underpinning such capacity.

METHODS:

To determine the coding capacity of the coronavirus N gene, we developed ORFan (Open Reading Frame ANalyser), a bioinformatic tool which mimics the translation initiation dynamics of a mammalian ribosome and scores and ORF according to their probability of being accessed via leaky scanning. We then performed nucleotide, codon and amino acid composition analysis, paired with phylogenetic analyses of each of the CoV structural genes to identify the causes that lead to the development of bicistronic genes.

RESULTS:

Our results suggest that the N gene of alpha-, beta - and delta-CoVs contains an elevated number of accessory gene (AcORF) candidates likely to be expressed via ribosomal leaky scanning. Structure and sequence conservation analyses showed that, at the subgenus level, AcORF candidates are conserved in length, genomic coordinates and likelihood of translation initiation, but these similarities are not due to sequence restrictions imposed by N functional domains. Sequence

composition analyses showed that the high abundance of ORFs in N frame 2 is due to codon and nucleotide biases that lead to an overrepresentation of start codons while suppressing stop codons. Comparisons between structural genes suggest that N is a better AcORF reservoir due to restrictions imposed by its function and cellular distribution. Functional analyses on a sub-group of identified AcORFs suggests that their protein products act as antagonists of the interferon induction cascade by interfering with IKK-beta phosphorylation of I κ B , impeding Nf-kB translocation in the nucleus.

CONCLUSIONS:

In conclusion, our findings suggest that the N gene of CoVs acts as a reservoir for accessory genes in its second reading frame, that AcORFs are overall conserved at the subgenus level, and that the dual coding capacity of N is driven by the combination of evolutionary pressures at the protein and genome level. These insights further enhance our understanding of the coding capacity of coronaviruses, but more broadly suggest that there might be AcORF reservoir 'candidate' genes in other viral families, based on cognate protein function and overall genome coding biases. Future research looking at other viral families is envisaged, as it could lead to the identification of novel genes and to a better understanding of their biology.



Abstract No.: 249

Poster Code: : A-0008

The evolution of an antigenically distinct genotype of the human coronavirus NL63

First Author: Keiya URIU, The Institute of Medical Science, The University of Tokyo

Co-Author(s): Kei Sato, Spyros Lytras, Jarel Elgin Tolentino, Maximilian Stanley Yo, Keno Strotjohann, Kenichi Komabayashi, Yohei Matoba, Kouji Kobiyama, Asuka Joy Tobuse, Ken J Ishii, Jumpei Ito, Katsumi Mizuta, Tsutomu Itagaki

BACKGROUND OBJECTIVES:

Human coronavirus NL63 (HCoV-NL63) is a common cold coronavirus in the genus Alphacoronavirus that circulates among humans. Since its discovery in 2004, HCoV-NL63 has been recognized as a significant cause of respiratory tract infections, particularly in children, the elderly, and immunocompromised individuals, being associated with group in some pediatric cases. Unlike other seasonal human coronaviruses (HCoV-OC43, HCoV-HKU1, and HCoV-229E) or recently emerged pathogenic coronaviruses (MERS-CoV, SARS-CoV, and SARS-CoV-2), little is known about the epidemiology and evolution of HCoV-NL63, primarily due to the virus's moderate disease and smaller impact on public health. To fill this research gap, we aimed to explore the evolutionary history of the three HCoV-NL63 genotypes circulating in humans and determine whether different genotypes have evolved distinct antigenic phenotypes.

METHODS:

We analyzed a longitudinal dataset comprising HCoV-NL63 patient virus samples and sera, retrieved between 2010 and 2022 in Yamagata Prefecture, Japan. We sequenced the S1 segment of the spike gene for all samples – containing the receptor binding domain (RBD) and most neutralizing antibody binding sites on the spike glycoprotein – and determined the genotype of each virus. We present more than 200 S1 sequences representing all three known HCoV-NL63 genotypes (A, B, and C). Our new sequences were combined with all publicly available S1s to perform recombination and molecular dating phylogenetic analyses. In addition, we performed neutralization assays with spike pseudotyped reporter virus using the collected sera to test for antigenic differences between genotypes.

RESULTS:

Consistent with previous observations, we confirmed that genotype B sequences differ substantially from those of the other two genotypes (A and C), with most mutations occurring in the N-terminal domain (NTD) of S1. We demonstrate that the NTD of genotype B S1 diverged from genotypes A and C more than 100 years ago. However, the C-terminal domain of genotype B S1, containing the RBD, is most closely related to genotype C, a result of recombination within the S1. Despite all three genotypes having very similar RBDs, genotype B Spike is neutralized less efficiently by antibodies elicited by infections with genotypes A and C.

CONCLUSIONS:

Our study describes the recent emergence of a recombinant, antigenically distinct HCoV-NL63 genotype with a divergent S1 NTD. These findings highlight how NTD, rather than RBD, mutations can modulate HCoV-NL63 antigenicity and demonstrate the importance of monitoring not only newly emerging coronaviruses, but also ones with established human circulation.



Abstract No.: 307

Poster Code: : A-0009

Within-host evolution of SARS-CoV-2 in immunocompromised patients and mapping antibody escape via experimental evolution in human airway epithelium model

First Author: Nirmegh Basu, University of Geneva

Co-Author(s): Fedor Bezrukov, Pauline Vetter, Samuel Cordey, Florian Laubscher, Yoann Sarmiento, Damien Dbeissi, Krisztina Hosszu-Fellous, Manel Essaidi-Laziosi, Isabella Anne Eckerle,

BACKGROUND OBJECTIVES:

The COVID-19 pandemic has revealed a continuing evolutionary contest between SARS-CoV-2 and host immunity at both individual and population level. The emergence of Omicron marked a turning point, showcasing extensive antibody escape and viral diversification under complex immune landscapes shaped by vaccination, infection, and hybrid immunity. Conversely, chronically infected immunocompromised (IC) patients provide a unique environment for virus evolution. Insufficient immune clearance and/or therapeutics in such patients allow viruses to rapidly diversify within a single host. Here, we integrate experimental evolution with longitudinal sequencing of virus from IC patients to identify indicators of immune escape and fitness trade-offs shaping coronavirus evolution.

METHODS:

We combined (A) controlled experimental evolution of SARS-CoV-2 (Omicron BA.1) under graded sera panels in Vero-TMPRSS2 cells and human airway epithelial (HAE) cultures to model physiological infection, with (B) longitudinal whole-genome sequencing of clinical IC cohorts with persistent SARS-CoV-2 infection, linking genomic and clinical data. Longitudinal intra-host variant dynamics, resistance-associated mutations, and structural impacts on monoclonal antibody (mAb) binding were analyzed in silico.

RESULTS:

Long-term experimental models established physiologically relevant systems for sustained viral replication under sera pressure or immune-deficient state. Across clinical IC cohort, we observed accumulation of multiple nonsynonymous mutations arising sequentially, particularly in the spike region. Both established immune-escape/fitness-associated mutations (e.g., E340K, L452R, K356T) and poorly characterized substitutions (e.g., S810A, S967N, P209L) were observed.

Therapeutic pressure drove rapid convergent resistance: sotrovimab exposure selected for P337S, E340D/K, and K356T, while cilgavimab exposure selected for K444R and G446S. In silico structural modeling showed disruption of key antibody-spike contacts, explaining predicted loss of neutralization. Notably, K356T appeared early within-host before global dominance. Cleavage-site specific mutations were also observed, notably, P681Y and R683Q, and S2' -proximal S810A-which has been recently implicated in escape from GALNT2-mediated restriction and may reflect fitness trade-offs.

CONCLUSIONS:

Together, these complementary approaches aim to better illuminate how immune landscapes and persistent infections in IC patients drive viral evolution. The clinical findings validate persistent SARS-CoV-2 infection in IC cohorts as a potent driver of rapid within-host diversification, including emergence of clinically significant resistance-associated and cleavage-site mutations. Ongoing work will complete experimental passages, functionally characterize novel spike and non-spike mutation to correlate them to phenotypes and clinical observations. Implications include the need for tailored antiviral strategies, continued genomic surveillance in IC cohorts, while illuminating how immune landscapes influence coronavirus evolution.



Abstract No.: 342

Poster Code: : A-0010

Pathogenicity, virological features, and immune evasion of SARS-CoV-2 KP.3.1.1

First Author: Jialu SHI, The University of Hong Kong

Co-Author(s): Yuanchen LIU, Huan LIU,

BACKGROUND OBJECTIVES:

The Omicron variant of SARS-CoV-2, first identified in late 2021, exhibited attenuated pathogenicity and enhanced transmissibility compared to ancestral SARS-CoV-2 and earlier variants of concern, rapidly becoming the globally dominant strain. In 2023, the emergent JN.1 lineage acquired the spike L455S mutation, which conferred further immune evasion and transmissibility alongside reduced pathogenicity, allowing it to replace prior circulating variants and become the new predominant lineage. Throughout 2024, multiple JN.1-derived sublineages successively emerged. Among these, KP.3.1.1 rapidly rose to global dominance and was classified by the World Health Organization as a variant under monitoring (VUM). Several recent studies have characterized the immune evasion capacity and altered infectivity mediated by the KP.3.1.1 spike protein. However, the comprehensive virological features of KP.3.1.1, including its intrinsic pathogenicity, replication fitness, and the functional contributions of non-spike mutations, remain incompletely understood. In this study, we systematically investigated the in vitro and in vivo virological characteristics of KP.3.1.1, encompassing its immune evasion, infectivity, replication capacity, and intrinsic pathogenicity, with parallel comparative analyses of JN.1.7, KP.2, and KP.3 as reference variants. At the genomic level, we performed detailed sequence comparisons between KP.3.1.1 and other JN.1-derived sublineages and further dissected how specific amino acid mutations contribute to the distinct virological phenotypes observed. Collectively, our findings provide deeper insights into the evolutionary adaptation of SARS-CoV-2 and coronaviruses more broadly, and establish a robust foundation for future strategies aimed at preventing coronavirus transmission and treating coronavirus-induced diseases.

METHODS:

In this study, we first analyzed viral genome sequences from public databases to confirm the widespread circulation of KP.3.1.1 as a dominant lineage in 2024 and to identify key amino acid mutations associated with this variant. Using pseudovirus-based in vitro neutralization assays with convalescent sera from infected patients or immunized hamsters, as well as monoclonal antibody escape assays, we characterized the cross-neutralization profiles among different variants and demonstrated the pronounced immune evasion capacity of KP.3.1.1. Cryo-electron microscopy (cryo-EM) was employed to elucidate how specific amino acid mutations alter the structural conformation of the viral spike protein, thereby modulating virological characteristics and conferring competitive advantages. To systematically evaluate the fundamental virological features of KP.3.1.1, we performed cell-cell fusion assays, in vitro viral replication kinetics, spike protein cleavage analysis, immunofluorescence staining, and pseudovirus infection assays. These approaches allowed us to dissect the mechanisms underlying KP.3.1.1's global dominance, particularly with respect to its enhanced infectivity and improved adaptability to the upper respiratory tract. In animal studies, we assessed the intrinsic pathogenicity of KP.3.1.1 by quantifying viral loads and analyzing histopathological damage in infected hamster tissues. Finally, using reverse genetics, we identified how unique amino acid mutations in KP.3.1.1 confer enhanced immune evasion and increased infectivity, and further revealed the underlying mechanism by which non-spike mutations counterbalance viral pathogenicity to maintain overall viral fitness.

RESULTS:

Evolution and mutation landscape of JN.1-derived subvariants Phylogenetic analysis revealed that KP.3.1.1 evolved from KP.3 and acquired a unique spike S31del mutation along with an additional ORF1a S4286C (NSP10 S33C) mutation. Global surveillance data confirmed that KP.3.1.1 rapidly became the dominant lineage by the second half of 2024, demonstrating a substantial competitive fitness advantage over other JN.1-derived subvariants including JN.1.7, KP.2, and KP.3. Enhanced immune evasion of KP.3.1.1 Pseudovirus neutralization assays using clinically authorized monoclonal antibodies demonstrated that KP.3.1.1 exhibited further reduced susceptibility to Pemivibart compared to KP.3. Using

convalescent sera from two independent breakthrough infection cohorts, we showed that KP.3.1.1 possessed the greatest immune evasion capacity among all tested JN.1-derived subvariants. The enhanced immune escape was attributed to the spike S31del mutation, which introduced a novel N30 glycosylation site that disrupted the binding of NTD-targeting neutralizing antibodies, as validated by mutagenesis studies. Structural basis of immune evasion and receptor interaction Cryo-EM structures of KP.2 spike-ACE2 and KP.3.1.1 spike complexes revealed that the F456L mutation facilitated improved ACE2 binding by allowing repositioning of ACE2 residue K31. The Q493E mutation in KP.3 and KP.3.1.1 introduced additional salt bridges with ACE2 H34, further enhancing receptor affinity. Importantly, the S31del mutation induced a conformational rearrangement of residue F32 and created a novel glycosylation site at N30, which sterically hindered the binding of NTD-directed antibodies, providing a structural explanation for the increased immune evasion of KP.3.1.1. Increased infectivity conferred by spike S31del Pseudovirus entry assays across multiple cell lines, including human primary nasal epithelial cells, consistently demonstrated that KP.3.1.1 exhibited markedly enhanced entry efficiency compared to KP.3. This enhancement was directly attributable to the S31del mutation, as introduction of S31del into heterologous spike backgrounds (BA.2, XBB.1, JN.1) similarly increased pseudovirus entry. Mechanistically, S31del increased both total and cell surface expression levels of the spike protein, thereby promoting viral infectivity. Attenuated replication and pathogenicity of KP.3.1.1 in vitro and in vivo Despite the enhanced infectivity conferred by S31del, authentic virus replication assays in Calu-3 cells and differentiated hNECs revealed that KP.3.1.1 did not replicate more efficiently than KP.3. In a golden hamster model, KP.3.1.1 exhibited similar viral loads, antigen expression, and histopathological changes in lung tissues compared to JN.1 and KP.3, with no evidence of increased pathogenicity. Notably, KP.2 showed the most attenuated replication and pathogenicity among all tested sublineages. Counterbalancing role of NSP10 S33C mutation Sequence alignment identified a unique NSP10 S33C mutation in KP.3.1.1 that was absent in other JN.1-derived variants. Using reverse genetics in a recombinant BA.1 background, we demonstrated that the NSP10 S33C mutation significantly attenuated virus replication in vitro and in vivo. Structural modeling suggested that the S33C substitution disrupts the native hydrogen bonding network between NSP10 and NSP14, impairing the exoribonuclease activity essential for viral replication. Importantly, introducing the spike S31del mutation into the NSP10 S33C

background rescued the attenuated replication phenotype. These findings reveal that the increased infectivity conferred by spike S31del is counterbalanced by the attenuating effect of NSP10 S33C, resulting in overall pathogenicity of KP.3.1.1 that remains comparable to its precursors.

CONCLUSIONS:

In this study, we comprehensively characterized the virological features of the globally dominant SARS-CoV-2 Omicron subvariant KP.3.1.1. We identified the spike S31del mutation as a key determinant that confers dual advantages: enhanced immune evasion through the introduction of a novel N30 glycosylation site that disrupts NTD-directed neutralizing antibody binding, and increased viral infectivity by promoting spike protein expression and entry efficiency, particularly in human primary nasal epithelial cells. Despite these fitness gains, KP.3.1.1 does not exhibit increased intrinsic pathogenicity compared to JN.1 or KP.3, as demonstrated by in vitro replication kinetics and in vivo hamster model analyses. Strikingly, we uncovered that the unique non-spike NSP10 S33C mutation in KP.3.1.1 attenuates viral replication, effectively counterbalancing the enhanced infectivity conferred by S31del. This epistatic interplay between spike and non-spike mutations reveals a sophisticated evolutionary strategy that allows KP.3.1.1 to achieve immune escape and transmissibility advantages while maintaining overall pathogenicity at a level comparable to its predecessors. Our findings provide critical insights into the ongoing adaptive evolution of SARS-CoV-2 and highlight the high plasticity of coronaviruses in navigating the complex trade-offs between immune evasion, infectivity, and replication fitness. The discovery that a single spike mutation can simultaneously enhance both immune escape and viral infectivity, coupled with the identification of compensatory non-spike mutations that temper pathogenicity, underscores the importance of moving beyond spike-centric surveillance. Comprehensive genomic and functional characterization of emerging variants, including both spike and non-spike mutations, is essential for accurately predicting viral evolution trajectories and for informing the development of next-generation vaccines and therapeutics. This study establishes a robust framework for understanding how coronaviruses evolve to persist in human populations and provides a foundation for future pandemic preparedness strategies.



Abstract No.: 403

Poster Code: : A-0011

Accelerated adaptive mutations in persistent SARS-CoV-2 infections predict future virulent mutations

First Author: Tsz Kin Mark Cheng, University of Cambridge

Co-Author(s): Luis Daniel González-Vázquez, Theo Bell, Boyan Wang, Luke Tillman, Ibrahim Ahmed Khan, Oscar Tam, Angelette Mendonca, David J. Pascall, Kimia Kamelian, Darren P Martin, Ravindra K Gupta,

BACKGROUND OBJECTIVES:

Prolonged SARS-CoV-2 infection in immunocompromised individuals has been proposed as a driver of the accelerated viral evolution observed in variants of concern (VOCs), particularly saltation variants characterised by long phylogenetic branch lengths such as Alpha, Omicron BA.1, Omicron BA.2.86, and most recently Omicron BA.3.2. Prior large-scale studies have been limited either by a pre-VOC sampling frame or by reliance on inferred timelines in the absence of linked clinical metadata. Here we present a focused analysis designed to address these limitations.

METHODS:

We systematically searched the literature for case reports and case series for persistent SARS-CoV-2 infections, defined by infections lasting longer than 14 days, confirmed by positive polymerase chain reactions (PCRs). We classified patients by degree of immune suppression and comorbidities. We used the four-gamete test for read-level detection of intra-host recombination, TreeTime to measure rates of evolution, and Periscope to estimate subgenomic RNA (sgRNA) production.

RESULTS:

Our search yielded 609 patients. We observed accelerated substitution rates correlates with the degree of immunocompromise. Notably, patients with complete B and T deficiency have a significantly accelerated rate of 7.0×10^{-4} (95% CI= 5.2×10^{-4} - 9.4×10^{-4}) substitutions per site per year, whilst healthy patients have a rate of 8.5×10^{-5} (95% CI= 2.8×10^{-5} - 2.5×10^{-4}), lower than global estimates of 8.9×10^{-4} to 1.0×10^{-3} . Mutations associated with viral load rebounds are predictive of future dominant mutations. These include S: D950N, which we first detect in 12/2020 and later emerged to global dominance in Delta variants in

06/2021; S: D796Y, which we first find in 02/2021 and later defined Omicron variants from 11/2021; and S:A704L was first found in our dataset as early as 05/2022 before BA.3.2 emergence in 11/2024. We note increased structural (S, E, and M) sgRNA expression in patients with higher degree of immunodeficiency. Amongst all gene segments, we note the highest non-synonymous-to-synonymous mutation (dN/dS) ratio in the Spike gene and ORF1b:nsp12 (RdRp). We also identified multiple recombination hotspots across the S gene that coincided with breakpoint regions reported in population-level recombinant lineages.

CONCLUSIONS:

These results strengthen the hypothesis that persistent infections are sources of saltation VOCs. The analysis further defines the importance of the spike gene, likely due to strong selection pressure secondary to its immunodominance in serological and cellular responses and its key role in infectivity and persistence. This work highlights the value of monitoring persistent infection for mutations, as these mutations can herald future mutations in pandemic variants.



Abstract No.: 404

Poster Code: : A-0012

Signatures of omicron-like adaptation in early SARS-CoV-2 variants and chronic infection

First Author: Tsz Kin Mark Cheng, University of Cambridge

Co-Author(s): Mazharul Altaf, Jesu Castin, Ann-Kathrin Reuschl, Benjamin L. Sievers, Kimia Kamelian, Dejan Mesner, Rebecca B. Morse, Adam Abdullahi, Bo Meng, Kata Csiba, Steven A. Kemp, Darren P. Martin, Clare Jolly, Christopher Ruis, Lipi Thukral, Ravindra K. Gupta,

BACKGROUND OBJECTIVES:

Persistent SARS-CoV-2 infections, particularly in immunocompromised or chronically infected individuals, exhibit enhanced viral diversity due to extended infection clearance or unchecked viral proliferation. We treated a septuagenarian, chronically-infected, immunocompromised male with extended SARS-CoV-2 shedding. Using whole genome sequencing, we identified the P812S mutation within the Spike fusion peptide (FP).

METHODS:

Throughout his 101-day hospital admission, we systematically collected nasopharyngeal swabs and endotracheal aspirates and performed whole genome sequencing using the Illumina platform. Genomes were analysed for mutations and trajectories of minor variants were tracked over 101 days. We queried the GISAID database to understand the global context. We used a lentiviral pseudovirus neutralisation assay to scrutinise the neutralising efficacy of polyclonal plasma antibodies from individuals administered with two doses of AZD1222 and fusion-peptide specific monoclonal antibodies. We also assessed spike entry efficiency in cell lines bearing different levels of ACE2 and TMPRSS2, and spike cleavage efficiency using a western blot. Finally, we assessed the structural significance of P812S using molecular dynamics simulations.

RESULTS:

After remdesivir and convalescent plasma treatment, Spike $\Delta 69/70$ + D796H emerged, and P812S mutation was only discernible from nasopharyngeal swab (99.1% prevalence), yet absent in endotracheal aspirate. Globally, P812S have been detected predominantly in Delta lineages. In the context of a Wuhan-Hu-1 + D614G (WT) genetic background, P812S induced a modest 1.1-fold diminution in geometric mean titre (GMT). Contrastingly, within the WT+ $\Delta 69/70$ + D796H

(previously characterised by a 2-fold GMT decrement relative to WT) and Delta framework, P812S caused significant immune evasion, with a 1.4-fold and 1.3-fold reduction in GMT, respectively. P812S provides marked immune evasion to fusion-peptide-specific monoclonal antibodies in both WT+ $\Delta 69/70$ + D796H (x5.6 decrease in IC₅₀) and Delta framework (x3.0). P812S also causes infectivity defect across all three backgrounds in both ACE2-High TMPRSS2-low and ACE2-low TMPRSS2-low cell lines, likely explained by a cleavage deficit secondary to localised instability in the S1/S2 region induced by P812S.

CONCLUSIONS:

This data underscores the epistatic interactions of Spike mutations, as opposed to a mere additive effect on immune evasion. The compartmentalised emergence of P812S in the upper respiratory tract suggests a potential influence of differential IgA and IgG abundance and specificity between upper and lower respiratory domains. We stress the importance of refining neutralisation assays using mucosal samples, which will better capture the immune response in the upper respiratory tract, essential for understanding protective mechanisms against infection.



Abstract No.: 447

Poster Code: : A-0117

Local and introduced lineages drive MERS-CoV recombination in Egyptian dromedary camels

*First Author: Kimberly Edwards**Co-Author(s): Ruixuan Wang, Vijaykrishna Dhanasekaran, Ghazi Kayali, Rabeh El-Shesheny*

BACKGROUND OBJECTIVES:

Middle East respiratory syndrome coronavirus (MERS-CoV) strains that belong to clade B circulate in the Arabian Peninsula and frequently cause human infections, whereas clade C strains in African camels do not infect humans. Here we describe the first detection of clade B in North Africa and recombination between clades.

METHODS:

MERS-CoV genomes from dromedary camel nasal swabs were assessed for recombination using RDP5 tools: RDP, GENECOV (for deep divergence), MaxChi (for small breakpoints), and 3SEQ (for recent events), with Bonferroni correction to reduce false positives. Potential breakpoints were further assessed with GARD in HyPhy. Topology discordance was confirmed with IQ-TREE and visualized with Bootscan.

RESULTS:

Genomic analysis confirmed the introduction of clade B and subsequent recombination with clade C viruses. Clade C viruses acquired portions of ORF1ab, Spike, and Nucleocapsid proteins from the newly introduced clade B strains.

CONCLUSIONS:

The recent introduction of a zoonotic clade B virus from the Arabian Peninsula and its recombination with local African clade C viruses could lead to the emergence of novel variants with unknown zoonotic potential. These findings challenge assumptions of limited cross-regional virus exchange and highlight the role of international trade in driving viral evolution. Enhanced surveillance, biosecurity, and a One Health approach are crucial to mitigate the potential threat of recombinant MERS-CoV strains.

Abstract No.: 133

Poster Code: : B-0013

Genomic surveillance reveals the evolutionary diversity of HKU2-related bat coronaviruses in Vietnam.

First Author: Shusuke Kawakubo, The University of Tokyo

Co-Author(s): Sakurako Seki, Hai The Le, Quynh Diem Nguyen, Hien Thi Tran, Mai Phuong Thi Dang, Murasaki Amano, Haruka Abe, Futoshi Hasebe, Vu Dinh Thong, Kei Sato

BACKGROUND OBJECTIVES:

Coronaviruses (CoVs) exhibit a high zoonotic potential due to their ability to infect a wide range of host species and to rapidly adapt to new hosts following cross-species transmission. Among these hosts, bats are recognized as important natural reservoirs. Therefore, genomic surveillance of bat CoVs is essential for understanding their evolutionary dynamics and assessing the risk of future emergence. Swine acute diarrhea syndrome coronavirus (SADS-CoV), a causative agent of SADS in piglets, emerged in pig farms in China in 2016 and is considered to have originated from bat HKU2-related CoVs. Previous studies have shown that SADS-CoV can infect human airway epithelial cells, suggesting its high potential risk for human as well. However, genomic information on HKU2-related CoVs remains limited, hence the evolutionary history and diversity of HKU2-related CoVs remain poorly understood. In this study, we aimed to characterize the genetic diversity of HKU2-related bat CoVs in Vietnam.

METHODS:

We collected 399 rectal swab samples from *Rhinolophus* bats across three provinces in Vietnam (Lao Cai, Tam Dao, and Dong Nai) between May and October 2025. After extracting RNA, samples were screened using Watanabe method pan-CoV PCR assay targeting the partial RdRp region, and PCR-positive products were sequenced by Sanger sequencing. A total of 44 samples were identified as HKU2-related CoV. Subsequently, whole-genome sequencing was performed using the Illumina MiSeq platform, followed by phylogenetic analysis.

RESULTS:

Phylogenetic analysis incorporating publicly available sequences from GenBank revealed that HKU2-related CoVs are divided into two major clades. One clade, which includes SADS-CoV, contained multiple sublineages that independently clustered with HKU2-related CoVs from China, suggesting ongoing dynamic viral

Abstract No.: 203

Poster Code: : B-0014

A new reverse genetic system to engineer SARS-CoV-2 variants for live virus neutralising antibody assays

First Author: Federico Pehuen Pereyra Gerber, University of Cambridge

Co-Author(s): Isobel Ramsay-Stubbs, Abi Lamikanra, Heli Harvala, Lise Estcourt, David Roberts

BACKGROUND OBJECTIVES:

Infection with SARS-CoV-2 or vaccination against the viral Spike protein induces neutralising antibodies (NAbs), protecting against COVID-19. We previously developed a high-throughput live-virus assay to quantify NAbs in serum and plasma, whilst relying on reference laboratories for the isolation, propagation and sequencing of emerging variants. In practice, delays associated with the generation and distribution of those new viral stocks make it difficult to keep up with viral evolution. The objective of this study was to streamline the process, whilst retaining the advantages of gold-standard live-virus assays.

METHODS:

Mutations that promote escape from NAbs are found only in the <4kb gene encoding Spike, which mediates virion attachment to target cells. We have therefore developed a simplified reverse genetic system to incorporate relevant Spike genes or mutations into a parental SARS-CoV-2 isolate already in use in our laboratory. This Golden Gate Assembly-based approach uses five conventional bacterial plasmids encoding fragments of the viral genome to generate chimeric SARS-CoV-2 viruses with a wild-type backbone and either a new or a mutated Spike gene.

RESULTS:

Our new reverse genetic system allows the generation of engineered SARS-CoV-2 genomes in less than 24 hours and the amplification of new viral stocks in under a week. Careful optimisation of the design enables the reconstitution of any publicly available Spike variant (e.g. the $\approx 1,000$ plasmids in Addgene), the editing of Spike using site-directed mutagenesis, and the incorporation of Spike genes synthesised de novo. Engineered viruses display unaltered replication kinetics and may be used directly in assays for NAbs. As expected, NAb titres determined with those viruses correlate tightly with NAb titres of their cognate authentic

viruses, across a representative panel of serum samples.

CONCLUSIONS:

We have developed a simplified reverse genetic system to incorporate new Spike genes or mutations into a parental SARS-CoV-2 isolate, avoiding the use of BACS, YACS or error-prone polymerase reactions. In addition to live-virus NAb assays, the method also enables facile reverse engineering of SARS-CoV-2 for other applications.



Abstract No.: 204

Poster Code: : B-0015

A Graphical User Interface Platform for Semi-Supervised Prediction of Viral Receptor Usage From Nucleotide and Protein Sequences

*First Author: MinChan Kim, Chungbuk National University**Co-Author(s): JungHye Ji, Kang Min Chang, Kim Hye Kwon*

BACKGROUND OBJECTIVES:

Virus–host interactions are strongly influenced by viral receptor usage, which constrains host range and tissue tropism. However, virus–receptor binding evidence is incomplete and biased toward a limited number of experimentally reported interactions, leaving most virus–receptor combinations unreported (unknown). We developed a graphical user interface (GUI) platform to predict viral receptor usage from sequence inputs while explicitly accommodating unknown interaction space and enabling rapid candidate prioritization for downstream validation.

METHODS:

We curated a virus–receptor interaction matrix by cross-referencing publications to assign confident positive (P) and negative (N) labels and designated unreported interactions as unknown (U). The dataset comprised 65 receptors and 3,377 viral proteins (219,505 possible pairs) and was split into training (175,604 pairs) and validation (43,901 pairs) sets. Nucleotide inputs were automatically translated into protein sequences prior to tokenization and embedding. Paired virus and receptor embeddings were classified using a multilayer perceptron (MLP). In the current results reported here, predicted secondary-structure features were generated using a Chou–Fasman–based method and incorporated via structure-guided masking. To improve feature fidelity, we compared Chou–Fasman and S4pred against experimentally resolved secondary structures from proteins with known structures (PDB-derived), implemented an updated S4pred module, and are currently retraining and validating the model. The GUI provides training, checkpointing, and quality control, including monitoring of loss, AUROC/AUPRC, error profiling, and visualization (histograms and PCA plots). After training, the platform assigns probabilistic scores to P/N/U pairs, enabling export of high-confidence predicted

binders and non-binders ($U \rightarrow P$ or $U \rightarrow N$) and identification of high-confidence label-prediction discrepancies ($P \rightarrow N$).

RESULTS:

With the Chou–Fasman module, error profiling enabled identification of high-confidence discrepancies. For a selected checkpoint (epoch 28), no high-confidence false positives were observed under a stringent criterion (N-labeled pairs with predicted probability ≥ 0.9), while 12 high-confidence false negatives were flagged (P-labeled pairs with predicted probability ≤ 0.1). In a demonstration using a coronavirus spike sequence, receptor ranking prioritized angiotensin-converting enzyme 2 (ACE2). Performance results for the updated S4pred module will be reported after completion of ongoing retraining and validation.

CONCLUSIONS:

This GUI-based, semi-supervised framework enables scalable mapping of virus–host receptor interaction space from sequence data in the presence of pervasive unknowns. By integrating transparent quality control and automated candidate export, the platform can accelerate hypothesis generation and experimental prioritization. High-confidence predicted binders and non-binders, as well as high-confidence label-prediction discrepancies, will be validated using pseudovirus-based receptor binding/entry assays to confirm receptor usage and to test whether predicted non-binding pairs indeed lack functional interaction.



Abstract No.: 206

Poster Code: : B-0016

In Silico Sequence Mining (ISSM) : A Probe-Guided Computational Approach for Analyzing Next-Generation Sequencing Data

*First Author: HyeJi Jung, Chungbuk National University**Co-Author(s): MinChan Kim, MinChang Kang, HyeKwon Kim*

BACKGROUND OBJECTIVES:

Next-generation sequencing (NGS) technologies are increasingly used across research fields, but their analysis often requires complex bioinformatics pipelines and specialized computational expertise. We developed In Silico Sequence Mining (ISSM), a graphical user interface (GUI)-based tool for accessible, probe-guided screening of raw FASTQ and FASTA files using user-defined multi-probe sequences. This study aimed to introduce ISSM as a computational approach for identifying target-matched reads in NGS datasets, reducing technical barriers for non-specialist users, and facilitating practical application across diverse diagnostic and clinical research fields.

METHODS:

ISSM was implemented as a GUI-based tool that allows users to select input FASTQ or FASTA files, define probe sequences, and configure analysis parameters, including sequence-matching threshold and subsampling rate. The tool compares user-defined probe sequences with reads or sequences using a sequence-matching score-based approach and records matches in both original and reverse-complement orientations. ISSM was validated using 34,899 publicly available coronavirus genomes in FASTA format from the National Center for Biotechnology Information (NCBI) and in-house NGS data from Crandell-Rees feline kidney (CRFK) cells infected with feline coronavirus (FCoV), with a 119-probe set comprising coronavirus-related and control probes. The tool was further applied to environmental metagenomic NGS datasets obtained from the Sequence Read Archive (SRA) using the same 119-probe set, and to clinical human immunodeficiency virus (HIV)-related NGS datasets obtained from the SRA using 33 published HIV-1-targeting probes, including subtype-informative probes.

RESULTS:

The 119-probe set comprising coronavirus-related and control probes successfully identified the intended targets in the coronavirus FASTA analysis without false-positive detection. In CRFK cell NGS data, only beta-actin probes showed matched reads in the control group, whereas FCoV-infected samples showed matched reads for both FCoV-specific and universal coronavirus probes. Application to environmental metagenomic NGS datasets confirmed the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-matched reads not only in SARS-CoV-2-targeted sequencing datasets, but also in a subset of raw RNA sequencing datasets. In HIV-related NGS datasets, ISSM detected HIV-1 probe-matched reads across multiple datasets and supported preliminary subtype inference using subtype-informative probes.

CONCLUSIONS:

ISSM demonstrated probe-matched target identification at the single-read level and successful operation on untrimmed NGS data and FASTA files. Its GUI-based workflow enables users to perform probe-guided screening without specialized bioinformatics expertise and to flexibly configure probe combinations according to analytical needs. These features support ISSM for candidate primer and probe evaluation, pathogen detection, and broader applications in fields requiring sequence-based target screening, including oncology.



Abstract No.: 208

Poster Code: : B-0018

Isolation, Molecular Characterization, and Diagnostic Evaluation of Feline Coronavirus in Suspected Feline Infectious Peritonitis Cases

First Author: Hui-Wen Chen, National Taiwan University

Co-Author(s): Chao-Hsin Chen, Jimmy Chiu,

BACKGROUND OBJECTIVES:

Feline infectious peritonitis (FIP) is a fatal systemic inflammatory disease caused by feline coronavirus (FCoV), and its pathogenesis remains incompletely understood. Currently, the diagnosis of FIP relies on a combination of clinical signs, hematological and biochemical findings, and laboratory testing. FCoV is classified into two genotypes, type I and type II. Although FCoV nucleic acids or antigens are frequently detected in cats with suspected or confirmed FIP, successful viral isolation remains relatively rare in Taiwan. In laboratory settings, FIP diagnosis primarily depends on molecular detection methods and immunostaining techniques. In addition to virological findings, previous studies have shown that cats with FIP often exhibit elevated levels of acute phase proteins, particularly feline $\alpha 1$ -acid glycoprotein (fAGP).

METHODS:

In this study, we aimed to detect and isolate FCoV from 234 clinical samples collected from cats suspected of having FIP in Taiwan between September 2024 and November 2025. Enzyme-linked immunosorbent assay was used to evaluate fAGP levels in effusion samples, and potential factors associated with FIP were further analyzed.

RESULTS:

The results demonstrated that age younger than 2 years, purebred status, an A/G ratio of ≤ 0.4 , and fAGP levels were significantly associated with the occurrence of FIP. All 234 clinical samples were inoculated into the Fcwf-4 cell line for viral isolation, and 35 viral isolates were successfully obtained. Subsequent genotyping analysis revealed that type II FCoV was the predominant genotype ($n = 12$), followed by co-infection with type I and type II ($n = 9$), type I alone ($n = 4$), and untypable strains ($n = 10$).

CONCLUSIONS:

In conclusion, this study integrates virus isolation, molecular characterization, and clinical analysis to support the diagnosis of FIP and to provide valuable insights into the molecular epidemiology and virological characteristics of FCoV.

Abstract No.: 271

Poster Code: : B-0019

MERS-CoV Seroprevalence in Humans in Ethiopia: A Cross-Sectional Study 2021-2024

First Author: Marcel Müller, Charité - Universitätsmedizin Berlin

Co-Author(s): JOHANNA WILDEMANN, Minilu Dejene Yitna, Awgichew Terefe, Asaye Mitiku, Julia Tesch, Nicolas Heinemann, Andrea Sieberg-Riedel, John M. Drake, Michael L. Mann, Elisabeth Möncke-Buchner, Christian Drosten, Victor M. Corman, Lauren Carruth, Sally A. Lahm, Chirota Ayele Gizaw, Simenew Keskes Melaku, Amira A. Roess

BACKGROUND OBJECTIVES:

Middle East respiratory syndrome-Coronavirus (MERS-CoV) infections are associated with a severe respiratory disease (MERS) similar to COVID-19. MERS-CoV is transmitted from camels to humans with self-limiting human-to-human transmission. To date, 2,600 human cases with 900 deaths have been reported, mainly on the Arabian Peninsula. Multiple sero-surveillance studies from Africa show anti-MERS-CoV seropositivity rates between 0-25% in humans. MERS-CoV seroprevalence in humans in Ethiopia, home to an estimated 1.3 million camels, remains largely uncharacterized. Here, we determined the anti-MERS-CoV seropositivity in a population-based cross-sectional study to determine the MERS-CoV infection risks in different sub-populations in several regions.

METHODS:

Sera of n=405 participants were sampled between 2021-2024 across five samplings in Afar, Somali, and Oromia regions of Ethiopia. MERS-CoV-specific IgGs were detected by a commercial MERS-CoV S1 ELISA (Euroimmun) using an OD ratio (ODR) cut-off ≥ 0.3 for ELISA-positivity and a borderline range of 0.2-0.3. To confirm the ELISA results, positive and borderline samples were subsequently tested in a plaque reduction neutralization test (PRNT), if sufficient material was available.

RESULTS:

We identified MERS-CoV S1-based seropositivity in 36/405 (8.9%) individuals and borderline results for another 36/405 individuals. Seropositivity ranged from 2% in Oromia region in April 2023 to 23.7% in Afar in January 2023. For the sampling in Afar in January 2023, we excluded cross-reactivity with SARS-CoV-2-induced antibodies, Plasmodium-induced anti-His antibodies, and unspecific antibodies due to polyclonal B-cell activation. Of n=54 ELISA-positive and borderline samples, one sample showed a PRNT50 (1:80) for MERS-CoV EMC and four samples showed a PRNT50 for a Kenyan MERS-CoV strain (titers: 1x 1:320 and 3x 1:20).

CONCLUSIONS:

This study provides first evidence of human MERS-CoV seropositivity (4/405; 1%) in different sub-populations in Ethiopia, suggesting zoonotic transmissions from camels to humans. Further research is required to investigate the factors contributing to the observed variations in MERS-CoV seroprevalence across different regions and populations.



Abstract No.: 343

Poster Code: : B-0020

SARS-CoV-2 antibody immunity across three continents: the West Africa, West Indies, West London Consortium

First Author: David LV Bauer, The Francis Crick Institute

Co-Author(s): David Greenwood, Tiphaine Cayol, Oliver Hague, Eliza Mari Kwesi-Maliepaard, Shanice A Redman, Crick Serology Consortium, HERITAGE study team, Legacy Investigators, WINDFall Study Team, WWW Consortium Team, Flora Scott, Joshua J Anzinger, Gordon Awandare, Yaw Bediako, Edward J Carr, Christine VF Carrington, Adam Kucharski, Peter Quashie, Emma C Wall, Mary Y Wu

BACKGROUND OBJECTIVES:

The experience of the COVID-19 pandemic has differed across continents, with a striking lack of morbidity and mortality in some locations, such as West Africa. With flagship funding from the Wellcome Trust, we therefore established the WWW Consortium in Ghana, West Africa; Jamaica, West Indies; and West London. We hypothesized that regional differences might explain this observation, with a focus on differences in prior coronavirus exposure, exposure to malaria and its immunomodulatory effects, and differences in baseline immunoreactivity. Here, we describe the extent to which antibody immunity differs between these geographic locations.

METHODS:

The WWW Consortium harmonises across the HERITAGE (Accra, Ghana), WINDFall (Kingston, Jamaica) and Legacy (London, UK) studies, establishing sharing frameworks for samples, metadata, and data; related permissions and oversight; and associated physical and cloud infrastructure. With centralised testing, we performed serological assessments across all three locations longitudinally at four timepoints spanning 2024-2025 using high-throughput live virus neutralization against a panel of SARS-CoV-2 variants as well as seasonal coronaviruses HCoV-OC43 and HCoV-229E, including over 750 individuals.

RESULTS:

We found that across all sites most participants had detectable neutralising antibody titres against JN.1 and XEC – the predominant variants in 2024. There were site-related differences in immunity: vaccine-included SARS-CoV-2 strains were better neutralised by participants from the Legacy study – Ancestral, BA.5, XBB.1.5 initially, and JN.1 after a homologous booster in autumn 2024. For HERITAGE, neutralisation of both alpha- (HCoV-229E) and beta-coronaviruses (HCoV-OC43) was higher than WINDFall suggesting a cross-coronavirus

serological response in West Africa.

Finally, antigenic cartography identified two distinct antibody landscapes, with JN.1 and XEC antigenically distant in Legacy, but not in HERITAGE and WINDFall. Intriguingly, we also found that participants in HERITAGE had broader pan-coronaviridae neutralisation against HCoV-OC43 and HCoV-229E than WINDFall or Legacy.

CONCLUSIONS:

There is international heterogeneity in SARS-CoV-2 antibody immunity: our results suggest that both ancestry, environment, and pathogen exposure may continue to play a role in shaping immunity to coronaviruses in these populations — and also have contributed to shaping the experience of the COVID-19 pandemic in the past.

We continue to explore these factors in more detail through analysis of historical and ongoing longitudinal cohorts, mapping of cellular immunity using CyTOF panels, and examining antibody immunity in through more unbiased approaches such as PhIP-Seq and highly-multiplexed immunoassays. In the meantime, we suggest that global recommendations for vaccine strain selection would benefit from the incorporation of data from diverse populations to ensure accurate, equitable recommendations.



Abstract No.: 378

Poster Code: : B-0021

A quantitative real-world correlate of protection against SARS-CoV-2

First Author: Nicholas Matheson, University of Cambridge

Co-Author(s): Isobel Ramsay, Stephen Kaptoge, Spencer Keene, Matthew Walker, Amy McMahon, Charlotte Van Coeverden, Pehuén Pereyra Gerber, Emanuele Di Angelantonio

BACKGROUND OBJECTIVES:

Infection with SARS-CoV-2 or vaccination against Spike induces neutralising antibodies (NAbs) able to block viral replication. Observational studies and sub-cohorts from vaccine trials have shown that NAb titres correlate in general terms with protection against COVID-19. The objective of this study was to define the quantitative relationship between NAb titre against circulating SARS-CoV-2 variants and risk of viral infection.

METHODS:

We conducted a nested case-control study embedded within the UK TRACK-COVID study, which collected regular serum samples from a cohort of 19,036 NHS blood donors from the start of the COVID-19 pandemic. 360 cases with SARS-CoV-2 infection were matched to 1,061 uninfected controls, based on age, sex, geographical region and calendar time. Participants were infected with wildtype, Alpha, Delta and Omicron variants, and included a gender-balanced working-age population immunised with a mixture of vaccine types and schedules. Serum NAb titres at 50% inhibition (NT50s) against circulating SARS-CoV-2 variants were measured using a high-throughput, live-virus assay, calibrated using WHO International Standards.

RESULTS:

NAb titres were significantly lower in cases than controls ($p=0.001$), and the presence of any detectable NAb response was associated with a decreased risk of infection. NT50 thresholds corresponding to a 50% or 75% reduction in odds of infection were determined by age-adjusted conditional logistic regression analysis. Even at the highest NT50s, however, 100% protection from infection was not achieved.

CONCLUSIONS:

NAb titres against SARS-CoV-2 provide a quantitative real-world correlate of protection against SARS-CoV-2 infection. The information from our case-control study will inform the development of a risk-stratified clinical diagnostic test for COVID-19 immunity.

Abstract No.: 50

Poster Code: : C-002

Structural Divergence between the Unique RNA Proofreader in Highly Pathogenic Coronaviruses

First Author: Chang Liu, The Johns Hopkins University School of Medicine

Co-Author(s): Yu Li, Xiaocong Cao, Yang Yang

BACKGROUND OBJECTIVES:

Coronaviruses possess unique RNA proofreading exoribonucleases (ExoNs) that are critical for maintaining the fidelity of their unusually large RNA genomes and degrading double-stranded RNA intermediates formed during replication and transcription. These functions enable coronaviruses to escape detection by the host immune system. Although ExoNs play vital roles in the coronavirus life cycle, our understanding of their structural and catalytic diversity across different coronavirus species remains limited. This divergence may impact mutation rates, viral fitness, immune evasion, and resistance to antiviral therapies. The objective of this study is to characterize the structural and functional diversity of ExoNs among coronaviruses and to explore how these variations influence key aspects of viral biology.

METHODS:

In this study, we offer comparative structural (cryo-EM) and biochemical analyses of ExoNs from two major highly pathogenic human coronaviruses: Middle East Respiratory Syndrome Coronavirus (MERS-CoV) from the merbecovirus subgenus, and SARS-CoV-2 from the sarbecovirus subgenus.

RESULTS:

MERS-CoV ExoN exhibits significantly lower catalytic activity than SARS-CoV-2 ExoN, indicating divergence between merbecovirus and sarbecovirus ExoNs. Cryo-EM structures of MERS-CoV ExoN provide the first coronavirus ExoN structures outside the sarbecovirus group and reveal how it engages RNA substrates with various 3'-end base pairs or mismatches. Comparative analysis with SARS-CoV-2 ExoN structures elucidates the molecular basis of cross-subgenus divergence. Mutagenesis and enzymatic characterization identify two highly conserved

structural features that govern efficient excision of diverse nucleotides at the 3' terminus of RNA substrates.

CONCLUSIONS:

Functional and structural divergence between merbecovirus and sarbecovirus ExoNs contributes to differences in catalytic efficiency and RNA substrate engagement. The conserved features that enable efficient 3'-end nucleotide excision underpin a shared mechanism central to coronavirus RNA proofreading and immune evasion. These insights advance understanding of ExoN variability across coronaviruses and may inform strategies to target ExoN-mediated proofreading in antiviral development.



Abstract No.: 71

Poster Code: : C-0023

Non-Coding Viral Regulatory Elements Drive Differential Transcript and Protein Expression of SARS-CoV-2 Accessory Genes to Inhibit Antiviral Responses

First Author: Arkadeb Bhuinya, University of Saskatchewan

BACKGROUND OBJECTIVES:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused the largest documented coronavirus pandemic. While SARS-CoV-2 accessory proteins are not essential for viral genome replication or transcription, they play critical roles in viral pathogenesis and immune evasion. Despite their importance, the regulatory mechanisms that fine-tune accessory protein expression at both the transcriptional and translational levels remain very poorly understood. In this study, we identify virus-specific regulatory mechanisms governing the expression of SARS-CoV-2 accessory gene, Orf6.

METHODS:

We assessed the strength of accessory gene-specific non-coding regulatory regions using in vitro reporter assays combined with site-directed mutagenesis. A reverse-genetics platform was used for the generation of a SARS-CoV-2 mutant with reduced transcriptional activity, allowing us to evaluate the functional relevance of a key nucleotide within the corresponding viral promoter region. We validated these findings across three sarbecoviruses—SARS-CoV-2, a deer-adapted SARS-CoV-2 strain, and the bat-derived BANAL-236 using human cell systems, Syrian hamsters, clinical human samples, and both deer and bat cell culture models. A cell proliferation assay was conducted to evaluate the impact of accessory proteins on host cell cycle progression.

RESULTS:

Comparative genomic and functional analyses across >20 million SARS-CoV-2 sequences and known sarbecoviruses revealed that Orf6 generates markedly lower levels of sub-genomic RNA compared to other accessory genes. We discovered that the reduced transcript abundance of Orf6 is due to a missing

nucleotide within the Orf6 viral promoter region. Surprisingly, despite its low sub-genomic RNA levels, Orf6 protein was produced at levels higher than other accessory proteins. We are investigating the virus-specific regulatory sequences that uniquely modulate the translation of accessory genes in the host cellular environment. These findings uncover a previously unrecognized dual-layer regulatory strategy in which non-coding viral elements decouple transcript abundance from protein expression. In addition, we demonstrate for the first time that heightened amounts of Orf6 protein in cells alters the cell cycle ultimately impacting overall cellular homeostasis.

CONCLUSIONS:

Our results highlight the importance of virus-specific promoter architecture in shaping coronavirus gene expression and pathogenicity using cutting-edge in vitro and in vivo models.



Abstract No.: 82

Poster Code: : C-0024

Molecular Mechanisms of Cell Tropism Expansion in Infectious Bronchitis Virus and Its Biological Implications

First Author: Ye Zhao, China Agricultural University

Co-Author(s): Yingfei Li, Xuehui Zhang, Rong Liang, Ruotong Li, Ruihua Yang, Liwei Zhang, Guozhong Zhang, Jing Zhao

BACKGROUND OBJECTIVES:

Infectious bronchitis virus (IBV), a gammacoronavirus, is a major poultry pathogen with strict avian host tropism. While the classical Beaudette strain can replicate in mammalian cells, the molecular determinants governing cell tropism expansion in contemporary field IBV strains remain largely undefined. In this study, a dominant QX-type IBV strain (SD) was serially passaged in alternating avian and mammalian cells, generating a cell-adapted variant capable of efficient infection in BHK-21 cells. Whole-genome sequencing of plaque-purified adapted clones revealed 14 conserved consensus mutations across viral nonstructural, spike, and envelope proteins.

METHODS:

Recombinant viruses carrying the 14 consensus mutations (rP20) and the isogenic wild-type virus (rSD) were generated via reverse genetics. A chimeric virus with the wild-type backbone replaced by the spike gene of the adapted strain was constructed to evaluate the contribution of the S protein to mammalian cell tropism. Key mutations associated with tropism were identified and functionally validated via membrane fusion assays, replication kinetics analysis, and cross-genotype rescue experiments.

RESULTS:

Substitution of the spike gene alone failed to confer mammalian cell tropism, demonstrating that the S protein is insufficient to overcome host restriction barriers. IBV cell tropism expansion depended on synergistic mutations in nsp4 (T53I), envelope protein (E10G), and the S2 subunit, rather than the S1 subunit. Mechanistically, S2 mutations augmented membrane fusion activity without enhancing early viral entry, thereby facilitating robust replication in mammalian

cells. Introduction of this minimal mutation set into a GI-1 genotype backbone supported stable replication in BHK-21 cells. In vivo experiments in SPF chickens showed that the adapted virus exhibited significantly attenuated pathogenicity, reduced viral loads, and blunted inflammatory responses, yet retained potent immunogenicity and full protective efficacy against wild-type IBV challenge.

CONCLUSIONS:

Our data reveal that IBV cell tropism expansion requires coordinated mutations in the S2 subunit plus additional structural and nonstructural proteins. Acquisition of mammalian cell tropism leads to pronounced viral attenuation while preserving protective immunity, establishing BHK-21 cells as a valuable system for the development of next-generation live-attenuated IBV vaccines.



Abstract No.: 107

Poster Code: : C-0025

Modification of the s2m RNA structure in the Avian Coronavirus Infectious Bronchitis Virus reduces clinical disease in vivo.

First Author: Adam Rayment, The Pirbright Institute

Co-Author(s): Guilia Dowger, Katalin Foldes, Chandana Tennakoon, Christine Monceyron, Torstein Tengs, Trevor Sweeney, Valeria Lulla, Erica Bickerton, Sarah Keep

BACKGROUND OBJECTIVES:

The Gammacoronavirus Infectious Bronchitis Virus (IBV) is the aetiological agent of Infectious Bronchitis, an economically significant disease of poultry. Infection results in reduced weight gain and egg quality and quantity, making IBV a concern for global food security. IBV is currently controlled through serotype specific live attenuated vaccines (LAVs) that are generated via serial passage of virulent isolates in embryonated hens' eggs. This is a time consuming, environmentally costly process that results in unpredictable genetic changes and variable attenuation levels. Targeted modification of the IBV genome offers a mechanism for rational attenuation for the rapid generation of strain specific LAVs. A 41 nucleotide hairpin stem loop RNA structure, known as the s2m, is present in the 3'untranslated region (UTR) of all strains of IBV. The high conservation of s2m, both in sequence and structure, presents s2m as an attractive target for rational attenuation.

METHODS:

In two representative strains of IBV: Beaudette, a heavily lab attenuated strain unable to cause clinical disease, and M41, a pathogenic strain, the canonical structure of the s2m was altered by removing a highly conserved GCC motif predicted to be important for maintaining structure. The 41 nucleotides corresponding to the s2m were also removed. Resulting recombinant IBVs (rIBV) were characterised in vitro, in ovo and in ex vivo tracheal organ cultures before pathogenicity of select rIBVs was assessed in vivo in the natural host.

RESULTS:

Deletion of the s2m in Beaudette resulted in a 36 nucleotide insertion consisting of tandem repeats from upstream and downstream sequence duplications;

rescue of a deleted s2m in M41 did not occur. Assessment of mutant s2m rIBVs showed that modification of s2m reduced replication in ex vivo tracheal organ cultures and reduced IB clinical signs in vivo. Tracheal ciliary activity, a marker of IBV pathogenicity, was increased when compared to the pathogenic parental control. Analysis of viral genome levels and infectious viral progeny suggests this phenotype is not a result of replication deficits in vivo.

CONCLUSIONS:

The inability to generate rIBV with a deletion of s2m without the presence of a compensatory insertion, suggests a requirement for nucleotide occupation and/or structure in the s2m genome location. Whether the 36 nucleotide insertion restores a similar structure to s2m is undetermined. Disruption of the canonical s2m structure reduced clinical signs in vivo, indicating s2m could be a pathogenicity factor. Further investigation is required to determine a potential role for s2m modification in rationally attenuated vaccine design.



Abstract No.: 114

Poster Code: : C-0026

Structural insights into small-molecule inhibition of coronavirus RNA polymerase

*First Author: Yang Yang, Iowa State University**Co-Author(s): Chang Liu*

BACKGROUND OBJECTIVES:

Coronaviruses pose significant global health threats, exemplified by the recent COVID-19 pandemic. Central to their replication machinery is the RNA-dependent RNA polymerase (RdRp), which catalyzes viral RNA synthesis and is highly conserved among coronaviruses. As such, RdRp is an attractive target for broad-spectrum antiviral drug discovery. However, a thorough mechanistic understanding of RdRp structure, function, and its interactions with inhibitors at high resolution is lacking, constraining rational therapeutics design. Our goal was to elucidate the molecular basis of inhibitor binding and inhibition, integrating structural and biochemical data to inform future antiviral strategies.

METHODS:

Recombinant coronavirus RdRp was purified to high homogeneity. The structure of the apo form of RdRp was determined using single-particle cryo-electron microscopy (cryo-EM) to achieve high-resolution details of the enzyme architecture. Building upon this structural platform, we prepared complexes of RdRp with a panel of chemically distinct nucleoside inhibitors. Cryo-EM was used to solve the complex structures, enabling direct visualization of inhibitor binding sites and induced conformational changes. To functionally validate our structural findings, we conducted a comprehensive set of in vitro biochemical assays, including RdRp polymerase activity assays and binding affinity measurements using fluorescence-based and enzymatic approaches, to quantitatively evaluate the inhibitory effects of each compound.

RESULTS:

Our study revealed the high-resolution structures of coronavirus RdRp in complex with various inhibitors at resolutions of 2.2–2.7 Å, highlighting critical features, including the polymerase active site and regulatory domains. Nucleoside inhibitors were observed occupying the active site, mimicking RNA substrates.

The biochemical assays quantitatively corroborated the structural observations, demonstrating varying degrees of inhibition and affinity, and revealing strong concordance between structural placement and functional potency of inhibitors.

CONCLUSIONS:

Our integrated structural and biochemical analysis provides comprehensive insight into the architecture and inhibition of coronavirus RdRp. The results reveal detailed molecular interactions and mechanisms that underlie inhibition by different classes of compounds and underscore the value of high-resolution structural information in guiding antiviral drug design. This work establishes a robust platform for rational discovery and optimization of next-generation RdRp inhibitors, advancing the development of targeted therapeutics against a broad range of coronaviruses and contributing to pandemic preparedness.



Abstract No.: 135

Poster Code: : C-0027

Establishment of a reverse genetics system for bovine coronavirus and rescue of a reporter gene-expressing recombinant virus

First Author: Yoshiro Sugiura, Gunma University

Co-Author(s): Tatsuki Takahashi, Kenta Shimizu, Sodbayasgalan Amarbayasgalan, Enkhjin Dorjsuren, Shunji Yasuki, Like Luo, Wataru Kamitani

BACKGROUND OBJECTIVES:

Bovine coronavirus (BCoV) causes respiratory and gastrointestinal diseases in cattle and calves. BCoV infection is associated with increased mortality in calves. BCoV causes significant economic losses in the livestock industry. It belongs to the -coronavirus genus of the Coronaviridae family and contains a viral genome of approximately 31 kb. It is genetically similar to human coronavirus-OC43. However, a reverse genetics system to rescue recombinant BCoV has not yet been established. Therefore, in this study, we aimed to establish a reverse genetics system for recombinant BCoV.

METHODS:

We constructed a bacterial artificial chromosome (BAC) DNA containing the full-length genome of the BCoV Kakegawa strain and replaced the open reading frame-2 gene of BCoV with the ZsGreen reporter gene. Additionally, we constructed spike (S) and hemagglutinin-esterase (HE) expression plasmids derived from the BCoV Kakegawa strain. BAC DNA was co-transfected with the S and HE plasmids into 293T cells, followed by co-culture with naïve HRT-18G cells for four days. The co-cultured samples were inoculated into naïve HRT-18G cells. Recombinant BCoV was successfully rescued, as confirmed via immunofluorescence assay and titration. Finally, viral properties and the roles of plasmid-derived S and HE proteins during viral rescue were analyzed using the 50% tissue culture infectious dose assay and quantitative polymerase chain reaction.

RESULTS:

Notably, the recombinant BCoV expressing the ZsGreen reporter gene exhibited a lower viral titer and N RNA expression than the wild-type Kakegawa strain. The

recombinant BCoV was successfully passaged in naïve HRT-18G cells. BAC DNA alone did not yield sufficient subgenomic RNA expression during viral rescue; however, S and HE co-expression significantly increased subgenomic RNA expression.

CONCLUSIONS:

Overall, co-expression of S and HE plasmids was necessary to rescue infectious recombinant BCoV, suggesting that these genes play a crucial role in recombinant BCoV production. Furthermore, our findings suggest that the open reading frame-2 gene promotes viral replication. Future studies should elucidate the detailed mechanisms by which S and HE proteins contribute to viral replication.



Abstract No.: 142

Poster Code: : C-0028

Nonstructural protein 16 methyltransferase affects the kinetics and coordination of coronavirus polyprotein processing

*First Author: Kira Schamoni-Kast**Co-Author(s): Fatema-Aqila Said, Charlotte Uetrecht*

BACKGROUND OBJECTIVES:

During coronavirus infection, the viral polyproteins pp1a and pp1ab are proteolytically processed into nonstructural proteins (nsps) that assemble into the replication-transcription complex (RTC). Although polyprotein cleavage and RTC assembly are essential steps in the viral life cycle and represent attractive antiviral targets, their mechanistic interplay remains poorly understood. Therefore, we employed our recently established native mass spectrometry (nMS) workflow to characterize the binding capacities of processing intermediates (proteoforms) from the nsp7-11 polyprotein region and methyltransferase nsp16 in vitro. The enzyme nsp16 forms a functional 2'-O-methyltransferase complex with nsp10. This complex plays a crucial role in viral immune evasion by modifying the viral RNA cap structure to mimic that of host mRNA, thereby preventing recognition by the host immune system. To elucidate the relationship between cleavage dynamics and RTC assembly, we compared the cleavage patterns of the main protease (Mpro) within the nsp7-11 region across all seven human coronaviruses (hCoVs), alongside an analysis of nsp16 complexation.

METHODS:

Our sensitive and label-free nMS workflow enabled quantitative extraction of kinetic rates from this multi-step reaction process in a time-resolved manner. Using this approach, we monitored and quantified the cleavage of the nsp7-11 region by including the structural context. Thus, we combined the monitoring of complex viral cleavage processes of nsp7-11 with subsequent binding processes of methyltransferase nsp16.

RESULTS:

Our data demonstrate that polyprotein processing is governed not only by primary sequence but also by higher-order structural features. Comparative analysis revealed both conserved features and species-specific variations in the cleavage patterns. The interaction studies suggest that polyprotein processing could act as a molecular switch activating enzymatic functions of the RTC. Notably, we observed binding of nsp16 to processing intermediates such as nsp9-11, which leads to altered processing kinetics suggesting that protein complex formation during processing is a regulatory factor. Furthermore, we observed distinct intermediate populations among the seven hCoVs.

CONCLUSIONS:

Together, our results suggest that hCoVs have evolved distinct polyprotein processing strategies, in which the complex formation of nsp16 constitutes a potential mechanism for processing regulation and thereby modulating RTC assembly. Our work provides a quantitative framework for dissecting the dynamic interplay between proteolysis, folding, and complex formation. It establishes nMS as a powerful platform for gaining mechanistic insights and for identifying new targets for antiviral intervention.



Abstract No.: 145

Poster Code: : C-0029

Packaging a 30kb RNA Genome: Lessons from HCoV-OC43

First Author: Catarina Nunes, The Francis Crick Institute

Co-Author(s): Harriet Mears, James Boot, Anob Chakrabarti, David LV Bauer

BACKGROUND OBJECTIVES:

At the final stages of viral replication, viral progeny must selectively package full-length genomes (gRNA) prior to egress. In coronaviruses, which possess large polycistronic RNA genomes, the mechanisms ensuring specific gRNA packaging remain incompletely understood. Several candidate packaging signal (PS) regions have been identified across coronaviruses, but their precise sequences, structural features, recognition mechanisms, and sufficiency for selective packaging remain debated. Using the human betacoronavirus HCoV-OC43, this study aims to characterise the molecular determinants of RNA packaging and map the genome-wide binding profiles of the Nucleocapsid (N) and Membrane (M) proteins.

METHODS:

We used Cross-linking Immunoprecipitation Sequencing (CLIP-seq) to characterise interactions between N and M proteins and genomic RNA. In parallel, we employed a novel reverse genetics system to generate HCoV-OC43 mutants targeting a candidate PS region analogous to that previously described in Mouse Hepatitis Virus (MHV). We then assessed viral replication kinetics and infectivity, and evaluated the efficiency and selectivity of genome packaging using sequencing-based approaches.

RESULTS:

CLIP-seq analysis revealed discrete and reproducible binding patterns of N and M proteins across the HCoV-OC43 genome, rather than a uniform association.

Enriched binding was observed at candidate PS regions, including a site analogous to the MHV PS, and additional novel putative packaging elements were identified. Functional examination of this region using a targeted mutant (OC43 silPS) showed no impairment in viral growth in vitro, suggesting the existence of compensatory mechanisms. However, RNA-seq analysis of virion-associated RNA revealed reduced packaging selectivity, with increased incorporation of subgenomic RNAs into viral particles.

CONCLUSIONS:

Our findings provide new insight into the conservation of RNA packaging mechanisms across coronaviruses, supported by the identification of a conserved PS in HCoV-OC43 analogous to that described in other betacoronaviruses. We further suggest that genome packaging is not driven by a single dominant signal, but instead relies on multiple cis-acting elements that collectively ensure selective incorporation of genomic RNA. Together, these results refine our understanding of coronavirus RNA encapsidation and reveal an additional layer of complexity in virion assembly.



Abstract No.: 178

Poster Code: : C-0030

High-temperature tolerance of bat and pangolin sarbecoviruses versus SARS-CoV-2

*First Author: Luo CHEN, The University of Tokyo**Co-Author(s): Ziyi Guo, Yukun Zhu, Keiya Uriu, Kei Sato*

BACKGROUND OBJECTIVES:

Understanding the mechanisms underlying host adaptation in emerging viruses is essential for future pandemic preparedness. Data published in 2021 suggest that thermosensitivity influences the viral fitness of SARS-CoV-2 in humans, with later omicron subvariants showing lower replication at elevated temperatures than earlier strains. The later-emerging strain of the omicron XBB variant, EG.5.1, showed enhanced spike-angiotensin-converting enzyme 2 interactions at lower temperatures than earlier omicron XBB subvariants. These findings suggest that SARS-CoV-2 is progressively adapting to replication at lower temperatures characteristic of the human upper respiratory tract. Although several sarbecoviruses, which represent a subgenus of coronaviruses including SARS-CoV-2, have been identified in wildlife, their thermosensitivity remains unaddressed. Therefore, we compared the in-vitro infection dynamics of ancestral SARS-CoV-2, BANAL-20-236, and pCoV-GD at 33, 37, and 40°C.

METHODS:

Vero E6-TMPRSS2 cells were infected with these three viruses at a multiplicity of infection of 0.01 and inoculated at 33, 37, or 40°C. We collected supernatants 1–3 days post-infection and measured the extracellular viral RNA levels by real-time quantitative PCR and viral titres as the 50% tissue culture infectious dose.

RESULTS:

At 33°C, all three viruses showed increasing extracellular viral RNA levels and infectious titres over time, but the replication level at 33°C was generally lower than that at 37°C for all viruses (appendix p 4). The replication of BANAL-20-236 and pCoV-GD at 40°C was generally maintained at levels similar to those at 37°C (appendix p 4), whereas WK-521 displayed markedly lower infectious titres than those observed at 37°C despite continued increases in viral RNA levels (appendix p 4). These results suggest that the replication of bat-derived and pangolin-

derived coronaviruses was less affected by high temperature, implying a higher degree of thermal tolerance.

CONCLUSIONS:

Our results suggest that BANAL-20-236 and pCoV-GD are more thermotolerant than WK-521. The core temperature of bats, which are natural reservoirs of sarbecoviruses, can reach approximately 40°C during flight. Such elevated temperatures can impose selective pressure, favouring viruses that better retain infectivity under febrile-like conditions. Generally, in pangolins, lower body temperatures, ie, approximately 33°C, are maintained; thus, strong selection for adaptation to higher temperatures is less likely in pangolin sarbecoviruses. In this context, the observed thermotolerance in pCoV-GD might reflect inherited traits from a bat-origin coronavirus lineage. Finally, our results reveal that SARS-CoV-2 (WK-521) showed reduced resistance to high temperature, but the underlying basis for this phenotype remains unclear. Further characterisation of thermosensitivity across sarbecoviruses could clarify the underlying determinants and improve the understanding of their transmission dynamics.



Abstract No.: 213

Poster Code: : C-0031

Initiation of synthesis in Coronaviruses

First Author: Ashleigh Shannon, Laboratoire AFMB, CNRS-Aix Marseille University

Co-Author(s): Véronique Fattorini, Barbara Selisko, Camille Falcou, Karine Alvarez, Françoise Debart, Jean-Jacques Vasseur, Mathieu Noel, François Ferron, Bruno Canard

BACKGROUND OBJECTIVES:

How viruses from the Coronaviridae family initiate viral RNA synthesis is unknown. We have previously show that the SARS-CoV-1 and -2 NiRAN domain on nsp12 can uridylylate the viral cofactor nsp8, forming a UMP-Nsp8 covalent intermediate that subsequently primes RNA synthesis from a poly(A) template. Interestingly, in parallel, the RdRp active site of nsp12 synthesizes a pppGpU primer, which primes (-)ssRNA synthesis at the precise genome-poly(A) junction. However, following several contradictory reports on the ability of the NiRAN to NMPylate different cofactor proteins, we have now further probed this protein-priming activity and NMPylation specificity.

METHODS:

NiRAN-mediated NMPylation and RdRp polymerisation activities were followed using radiolabeled nucleotide substrates and analysed through SDS-PAGE and UREA sequencing gels.

RESULTS:

Mutagenesis studies revealed nsp8 is labeled at the primary amine of a non-native glycine residue, present at the N-terminus of the expression construct (following cleavage with TEV protease), and negating the biological relevance of our previous findings. Surprisingly, removal of this single residue, eliminates all labeling and protein-primed activity, stressing the importance for using proteins with native N- and C- termini. Later studies have now revealed nsp9 to be the true target for both NMPylation and RNAlation - important in the viral RNA capping pathway. With the exception of our finding that nsp8 can be used to prime-synthesis, albeit by a non-native residue, there was no other evidence for protein-priming until recently. In October, 2023, Schmidt et al., published the finding that nsp9 is covalently linked to the 5' end of positive- and negative-sense RNA produced during SARS-CoV-2 infection. This linkage was found to be regulated by

its interaction with the host protein, staphylococcal nuclease domain-containing protein 1 (SND1), which was found to specifically recognize the 5' end of negative-sense RNA and be important for viral RNA synthesis. Although it is highly frustrating to have spent several years focused on the wrong protein, this finding supports the notion that protein-priming is occurring in CoVs, and opens up a plethora of options for further mechanistic and structural studies. Of note, Schmidt et al. also showed that nsp9-linkage on the (-)sense strand mapped roughly between the genome and poly(A) tail. Intriguingly, we also found a similar specificity for this region. Here we show that the SARS-CoV mRTC (nsp12-nsp7-nsp8) can initiate synthesis through a de novo, NiRAN independent pathway, through the synthesis of a pppGpU primer. This dinucleotide is complementary to the last two nucleotides of the SARS-CoV-2 genome, located precisely at the genome-poly(A) junction. De novo initiation was found to directly compete with the (artificial) nsp8-poly(U) protein-primed synthesis. This shows that the poly(A) tail and 3' genomic RNA sequence elements guide the positioning of the mRTC to the true 3'-end of the genome.

CONCLUSIONS:

The specific details behind initiation of RNA synthesis, including the role of SND1 and potential coordination between protein-priming and/or de novo induction remains to be studied. Synthesis initiation, and the precise role of the NiRAN therefore appears to be a complicated story, which remains to be fully elucidated.



Abstract No.: 218

Poster Code: : C-0032

Coronavirus Genome Packaging is Supported by a Network of RNA-protein Interactions

First Author: Harriet Mears, University of Bristol

Co-Author(s): Catarina Nunes, Ziyi Yang, James Boot, Kristijonas Raibuzis, Ciaran Gilbride, Anob Chakrabarti, David Bauer

BACKGROUND OBJECTIVES:

The final step in the coronavirus life cycle is the packaging of newly synthesised genomes into virus particles. Genome packaging in the model betacoronavirus Mouse Hepatitis Virus (MHV) is driven by a packaging signal (PS), located within a large RNA stem-loop structure in the genomic RNA (gRNA). Inclusion of the PS in heterologous RNAs promotes their uptake into virions, while mutation of the PS results in misincorporation of viral subgenomic RNAs. However, this does not completely abolish gRNA packaging, nor attenuate the growth of MHV in vitro, suggesting other regions of the genome may contribute to packaging.

METHODS:

Since recent studies suggest a wider network of RNA-protein interactions may be crucial for achieving packaging specificity, we sought to map the RNA-protein interaction network in purified virions. Using MHV and its close relative human coronavirus OC43 as model systems, we employed individual nucleotide-resolution cross-linking and immunoprecipitation to determine binding sites for the viral Nucleocapsid (N) and Membrane (M) proteins. By mapping protein crosslinking sites to parallel RNA structural data, we designed a panel of coding-silent mutations to disrupt local RNA structures in putative packaging signals, for phenotypic characterisation.

RESULTS:

We show that N binds non-uniformly across the genome. Alongside binding to the canonical PS, we observed large binding peaks within the nsp13, NS2 and M coding regions, which we hypothesise may constitute additional packaging signals. By contrast, there were fewer binding peaks for the viral Membrane (M), consistent with weak or indirect RNA binding activity. Mutation of N binding sites in isolation did not affect viral growth in vitro and genome packaging was comparable to wildtype virus. However, when combined with mutation of the canonical PS, N binding site mutants showed severe packaging defects and

reduced fitness.

CONCLUSIONS:

Our data support an additional layer of complexity to the classical model of coronavirus genome packaging, in which multiple genomic loci coordinate with the PS to promote specific gRNA encapsidation. In the future, our experimental approach will allow us to analyse other coronavirus genera, aiming to identify divergent packaging signals and establish principles of genome packaging family-wide.



Abstract No.: 284

Poster Code: : C-0034

Genome-wide CRISPR Screen Reveals the MDH1 as a Critical Host Factor for Infectious Bronchitis Virus Infection

*First Author: Ran Jing, Huazhong Agricultural University**Co-Author(s): Mengjia Zhang, Wentao Li*

BACKGROUND OBJECTIVES:

Infectious bronchitis (IB) is a highly contagious disease caused by the infectious bronchitis virus (IBV). As the prototypical member of the gamma-coronavirus, IBV causes respiratory and renal disorders, leading to substantial economic losses. Deciphering virus-host interaction networks is essential to reveal how IBV causes disease and to develop effective control strategies. In this study, we used a genome-wide CRISPR/Cas9 library screen to identify host factors for IBV infection. Malate dehydrogenase1 (MDH1) was one of the most significantly enriched factors. Knockout of the MDH1 gene in different cell lines significantly inhibited IBV infection.

METHODS:

After knocking out MDH1 with CRISPR/Cas9 system, we used indirect immunofluorescence (IFA), tissue culture infectious dose (TCID50) and reverse transcription quantitative polymerase chain reaction (RT-qPCR) to pinpoint the IBV replication stage affected by MDH1. Complementation with wild-type and active-site mutant MDH1 revealed key residues for IBV replication, and we further evaluated the broad-spectrum antiviral activity of MDH1 against different coronaviruses.

RESULTS:

MDH1 knockout eliminated IBV infection in Hela cells and reintroducing the MDH1 restored susceptibility to IBV. Comparing the growth kinetics of IBV found the virus titer peaked at 36 hours post-infection in wide-type cells, while few progeny virus was detected in knockout cells. Transfection with siRNA, followed by IBV infection, reduced viral N gene mRNA levels in DF-1 cells and CEFs. Binding and entry assays

revealed that MDH1 knockout did not affect IBV attachment or entry, indicating MDH1 is not required for viral entry. Immunofluorescence staining for double-stranded RNA, a marker of RNA viral replication, showed that MDH1 knockout significantly inhibited IBV replication. Further research has discovered that genetic rescue with wild-type MDH1, but not its catalytically inactive mutant, restored cellular susceptibility to IBV. Knockout of the MDH1 in PK-1 cells, followed by infection with different coronaviruses, confirmed that MDH1 inhibits infection by multiple coronaviruses.

CONCLUSIONS:

The identification of MDH1 as a key host factor influencing the IBV. Future work should aim to define the specific molecular interactions between MDH1 and the viral replication complex. A deeper understanding of how MDH1 supports viral replication will enhance our knowledge of IBV pathogenesis and pave the way for novel antiviral therapies.



Abstract No.: 352

Poster Code: : C-0035

Exploring the functional impact of ORF8 in SARS-CoV-2 infection

First Author: Yana Chen, Imperial College London

Co-Author(s): Wilhelm Furnon, Riona Pye, Ann-Kathrin Reuschl, Vanessa Cowton, Nicole Upfold, James Bazire, Maia Kavanagh-Williamson, Andrew D. Davidson, Arvind Patel, Massimo Palmarini, Lucy Thorne

BACKGROUND OBJECTIVES:

COVID-19, caused by SARS-CoV-2, remains a global health concern. The emergence of more transmissible SARS-CoV-2 variants of concern (VOCs) throughout the pandemic reflected selective pressure on the virus to adapt to its new human host and overcome innate and adaptive immunity. Interestingly, while VOCs convergently evolved to better antagonise human innate immunity through upregulation of key innate immune antagonists (e.g. ORF6, N, ORF9b), multiple VOCs independently evolved mutations to delete or downregulate ORF8. For example, Alpha and Omicron XBB.1.5 evolved premature stop codons in ORF8, while BA.5 had decreased ORF8 subgenomic RNA (sgRNA) and protein levels. Notably, multiple Omicron subvariants, most recently BA 3.2, deleted the entire ORF7a/b and ORF8 coding regions. Although ORF8 has been reported to support viral replication through processes including regulation of spike maturation and modulation of innate immunity, its repeated loss during viral evolution suggests that its function in virus infection and human adaptation remains unclear.

METHODS:

To further explore its role as an innate antagonist in the context of infection, we generated a reporter virus and reverse genetics mutants to either truncate or downregulate ORF8 expression through naturally-occurring mutations in different variants. Molecular techniques including virus growth kinetics, western blot and reporter assays were employed for characterisation on viral replication and host cellular responses.

RESULTS:

We found that the reporter virus with a deletion in ORF8 coding region induced greater innate immune activation in airway epithelial cells. We also engineered the Alpha truncated ORF8 (Q27STOP) into a wave 1 Wuhan-Hu-1 background and characterised it in a range of airway epithelial cell lines, finding cell-type specific differences in replication. Strikingly, we found that introducing a single nucleotide change into the BA.1 ORF8 transcriptional regulatory sequence (TRS), as evolved in BA.5, was sufficient to significantly downregulate ORF8 sgRNA. This highlights the flexibility of the virus to independently modulate the level of its accessory proteins through single TRS point mutations.

CONCLUSIONS:

Our ongoing goal is to use these mutants to understand the impact of ORF8 loss on the host response and ultimately viral transmission. The findings from the study will provide new insights into SARS-CoV-2 evolution and its mechanisms of immune evasion.



Abstract No.: 356

Poster Code: : C-0036

Make it make sense - a sensitive, quantitative and reproducible multiplex RT-qPCR assay for coronaviruses

First Author: Rebekah Penrice-Randal, University of Liverpool

Co-Author(s): Maximillian Erdmann, Leandro X. Neaves, Edward Emmott

BACKGROUND OBJECTIVES:

Coronaviruses generate distinct RNA molecules throughout their lifecycle, including genomic, anti-genomic and sub-genomic mRNAs (sgmRNA). Detection and quantitation of such RNAs can give insight into replication and transcription kinetics. For viruses such as the alphaviruses a clear link between regulated proteolysis and replication of RNA synthesis has been demonstrated. While proteomic methods are becoming available to study proteolysis; no readily available RNA methods exist to distinguish between sense and antisense RNA species. To support the study of proteolytic cleavage and its role in RNA synthesis and viral replication, we developed a multiplex strand-specific RT-qPCR to monitor sense (genome) and anti-sense (anti-genome) synthesis relative to a housekeeping gene. Additionally, we established amplicon-based sequencing approaches to support the study of viral mutations, sgmRNA expression.

METHODS:

RNA standards for genomic and antigenomic RNA were produced via in-vitro transcription using T7 RNA polymerase. Reverse transcription of standards representing 1012 copies was conducted using a gene-specific-primer (GSP) with a non-viral tag. cDNA was diluted, and qPCR was performed using GSPs, probes and primers complimentary to the non-viral tag. Viral infections of MHV, NL63, 229E, OC43 and IBV were carried out in 17 clone 1, LLC-MK2, Huh7, Mv.1.Lu and Vero cells respectively. After infection cultures were treated with DMSO or nirmatrelvir to inhibit the activity of nsp5. RNA was extracted and analysed by qPCR. Cellular RNA was concentration normalised prior to first-strand synthesis using the tagged-GSP. Multiplex qPCR was then used quantify the abundance of genome and anti-genome, which was normalised to GAPDH of the relevant viral host. In addition, sequencing primers were developed for MHV, NL63, 229E, OC43 and IBV using primal scheme. Sequencing libraries were prepared as per the artic protocol and sequenced on Oxford Nanopore to confirm consensus sequences, mutational spectra and sgmRNA expression.

RESULTS:

No self- or false priming was observed in standard curves from primer sets, and single-plex and multi-plex assays showed similar performance. The standard curve was repeated independently 8 times, where 101 copies to 108 copies of RNA provided a C_q value, with low intra-assay variability. Nirmatrelvir treatment of infected cells resulted in a significant reduction in anti-sense RNA synthesis.

CONCLUSIONS:

This centralised RNA toolkit for the quantification of different RNA species generated during coronavirus replication, provides the community with a resource to study viral RNA synthesis.



Abstract No.: 445

Poster Code: : C-0115

Molecular Architecture of the Double-Membrane Vesicle Pore Complex From Equine Arteritis Virus – a Minimal Nidovirus Model

*First Author: Wenxin Zhang, The University of Hong Kong**Co-Author(s): Tao Ni, Tingting Yang*

BACKGROUND OBJECTIVES:

Positive-strand RNA viruses of the order Nidovirales – which include coronaviruses and arteriviruses – remodel host membranes into double-membrane vesicles (DMVs) as replication organelle to shield viral RNA synthesis from immune sensors. In coronaviruses, newly synthesized viral RNA is exported from these vesicles through a large pore complex formed by viral nonstructural proteins. However, whether this architecture is unique to coronaviruses or conserved across divergent nidoviruses remains unclear. This study aims to determine the structure of the double-membrane vesicle pore complex in Equine arteritis virus, a prototype arterivirus with a small genome, and to compare its architecture with that of coronaviruses.

METHODS:

We combined in situ cryo-electron tomography with single-particle cryo-electron microscopy to visualize arterivirus-induced replication organelles and resolve the structure of the double-membrane vesicle pore complex in its native membrane environment. Structural analysis was integrated with replicon-based functional assays to evaluate conserved architectural features and their roles in viral RNA replication.

RESULTS:

We resolved the structure of the Equine arteritis virus double-membrane vesicle pore complex at 3.0 Å. The complex is formed by a 12:12 stoichiometry of nonstructural protein 2 and nonstructural protein 3 protomers, organized into four stacked concentric rings across the double membrane. Despite limited sequence similarity to coronavirus pore proteins, the arterivirus pore preserves key

architectural principles, including oligomeric organization, membrane-spanning topology, and a central positively charged channel compatible with viral RNA transport. Compared with the larger coronavirus pore complex, the arterivirus pore displays a more minimal architecture but retains conserved features essential for replication organelle function. Replicon-based assays further support the functional importance of these conserved structural elements in viral RNA replication.

CONCLUSIONS:

These findings reveal that nidovirus replication organelles share a conserved structural signature at the atomic level, despite major differences in genome size, virion morphology, and protein sequences. The arterivirus pore represents a minimal form of the nidovirus replication pore complex and supports the concept that double-membrane vesicle-based replication organelles are unified by a conserved RNA export machinery. This conserved architecture may provide a foundation for developing broad-spectrum antiviral strategies targeting nidovirus replication.



Abstract No.: 90

Poster Code: : D-0038

Identifying Human-Adaptive Coronavirus Entry Features

*First Author: Enya Qing, Loyola University Chicago**Co-Author(s): Tom Gallagher*

BACKGROUND OBJECTIVES:

Several animal CoVs (coronaviruses) species have adapted to stable maintenance in human populations. Intermediates in human (h) CoV adaptation are lacking for the currently endemic hCoVs 229E, NL63, OC43, and HKU1. However, SARS-CoV-2 changes are well-documented, particularly in the entry-directing S (spike) glycoproteins. Some of these S protein adaptations modify specific virus entry stages, including binding to distinct host cell ligands, subsequent virion conformational transitions, membrane fusion potentials, and entry kinetics. It remains unclear which of these entry features enable hCoV endemicity. Our objectives are to correlate specific virus entry stages with stable virus maintenance in humans. We expect that these correlations can be discerned by comparing endemic hCoVs to more recently introduced hSARS-CoV-2 variants in a series of entry assays.

METHODS:

We evaluate CoV – host interactions using our well-established reductionistic system with CoV VLPs (virus-like particles) and host cell-derived EVs (extracellular vesicles). Successful S-mediated “entry” is assessed using nanoluciferase fragments, HiBiT in VLPs and LgBiT in EVs, which complement to form measurable luminescence after VLP-EV fusion. Using this sensitive and modular system, we perform several comparative assessments of hCoV VLP interactions with a variety of EV-associated host factors.

RESULTS:

We first established VLP-EV systems for hCoVs 229E, NL63, HKU1. We then produced EVs containing, in addition to the known CoV entry receptors, three predicted

classes of entry cofactors. (1) Sialic acids: With TMPRSS2 receptors on EVs, HKU1 VLPs mediated membrane fusion, and fusion was enhanced with additional sialic acid enrichment on EVs. Sialic acids enrichment did not enhance NL63 or SARS-CoV-2, and actually inhibited 229E VLP membrane fusions. (2) Lectins: The lectin DC-SIGN rendered EVs specifically hypersensitive to fusion with NL63 VLPs, but not other hCoV VLPs. Conversely, the related DC-SIGNR enhanced membrane fusion of all tested endemic hCoVs except SARS-CoV-2 BJ.1. Notably, SARS-CoV-2 XBB recombinants derived from BJ.1 and BM.1.1.1 inherited DC-SIGNR hypersensitivity from BM 1.1.1. (3) Neuropilins: NRP2 but not NRP1 enhanced fusion of NL63 and all SARS-CoV-2 lineages tested.

CONCLUSIONS:

The results clearly demonstrate divergence in hCoV cofactor recognition. The diversity of DC-SIGNR utilization amongst concomitantly circulating SARS-CoV-2 strains indicates that cofactor selection is a feature of CoV evolution – particularly during transitions from zoonotic introduction to endemic human circulation. Our study highlights adaptive utilization of entry cofactors as plausible enablers of hCoV endemicity. Our findings also underline these entry cofactors as potential pan-hCoV antiviral targets.



Abstract No.: 143

Poster Code: : D-0040

Elucidating the receptor and entry barriers for hedgehog merbecoviruses

First Author: Victoria Jefferson, Washington State University

Co-Author(s): Min Jin, Zhe Zhao, Nicholas Catanzaro, Ralph Baric, Craig Wilen, James Rini, Michael Letko

BACKGROUND OBJECTIVES:

Merbecoviruses, a subgenus of betacoronaviruses closely related to Middle East respiratory syndrome coronavirus (MERS-CoV), have been identified in several species of bat, pangolins, mink, camels, humans, and hedgehogs. Merbecoviruses found in hedgehogs (*Erinaceus* sp.), or “ErinCoVs,” have been discovered across Europe and China, with detection rates of up to 60% of animals in Germany and Italy. Understanding the risk ErinCoVs pose to other species, including humans, begins with host receptor compatibility. Cell entry of coronaviruses is mediated by the interaction of the receptor binding domain (RBD) of the virus spike glycoprotein with host cell receptors. We have shown that the diverse RBDs of Merbecoviruses allow for their organization into at least four different clades that correlate to their receptor use: clade 1 containing MERS-CoV with DPP4, and clades 2 and 3 with ACE2. ErinCoV RBDs form the distinct clade 4, and our objective is to characterize their receptor.

METHODS:

We used VSV-based pseudotype systems, protein binding assays, and electron cryomicroscopy to identify the receptor for ErinCoVs. To further assess the spillover risk of ErinCoVs to other species, we screened APN orthologues from 30 other species and used point mutagenesis to identify key residues for ErinCoV RBD:APN binding and entry barriers.

RESULTS:

Merbecoviruses identified in both European and Chinese hedgehogs use aminopeptidase N (APN) as an entry receptor. We found that ErinCoV entry is species-restricted and identified several mechanisms preventing ErinCoV entry in other species.

CONCLUSIONS:

Our results shift the paradigm of known receptor use in betacoronaviruses by demonstrating the novel use of APN and provide a preliminary assessment of disease risk of ErinCoVs to humans and other species.

Abstract No.: 156

Poster Code: : D-0041

Generalists vs Specialists: What bat sarbecovirus host range and antigenicity tells us about the risk of spillover.

First Author: Nazia Thakur, The Pirbright Institute

Co-Author(s): Joseph Newman, Dongchun Ni, Jeffrey Seow, Abigail L Hay, Yeonjae Lee, Ayush Upadhyay, Babatunde E Ekundayo, John A Hammond, Thomas P Peacock, Kelvin Lau, Katie J Doores, Dalan Bailey

BACKGROUND OBJECTIVES:

Sarbecoviruses, including SARS-CoV and SARS-CoV-2, exhibit broad tropism for mammalian angiotensin-converting enzyme 2 (ACE2) receptors. Understanding the relationship between coronavirus host range and antigenicity is critical for assessing the zoonotic potential of related bat sarbecoviruses. We aimed to characterise host range of bat sarbecoviruses and examined how this correlates with antigenicity, with the goal of evaluating spillover risk and potential cross-protective immunity.

METHODS:

The host range of multiple bat sarbecoviruses was assessed using receptor-binding domain (RBD)-based binding assays and pseudotype entry assays across a diverse panel of mammalian ACE2 proteins. Antigenic relationships were evaluated using convalescent sera from SARS-CoV-2-infected individuals in pseudotype-based neutralisation assays. Additionally, monoclonal antibodies derived from vaccinated individuals with breakthrough infections were analysed to identify epitope classes and key RBD residues associated with cross-neutralisation.

RESULTS:

Broad ACE2 usage (a generalist phenotype) was most common in clade 1 sarbecoviruses, including SARS-CoV-2 and the BANAL isolates from Laos. In contrast, clade 3 (e.g., RhGB07) and 5 (e.g., Rc-o319) sarbecoviruses exhibited more restricted ACE2 usage (a specialist phenotype). The impact of the continued evolution of SARS-CoV-2 in humans was also examined, identifying an expanding pattern of generalism, particularly for the Omicron variant and its sub-lineages. Antigenic analysis revealed that generalist sarbecoviruses, especially those closely related to SARS-CoV-2, demonstrated higher levels of cross-neutralisation by convalescent sera, although limited cross-neutralisation was observed across

the entire subgenus. Monoclonal antibody analysis identified specific epitope classes and RBD residues that contribute to broader neutralisation of bat sarbecoviruses.

CONCLUSIONS:

These findings demonstrate a link between host range breadth and antigenicity in bat sarbecoviruses, with generalist viruses showing greater susceptibility to cross-neutralising immunity. The results highlight the importance of monitoring mutations in bat sarbecovirus spike proteins and maintaining surveillance of bat reservoirs to better anticipate and mitigate future zoonotic spillover events.



Abstract No.: 169

Poster Code: : D-0042

The impact of clade B lineage 5 MERS coronaviruses spike mutations from 2015 to 2023 on virus entry and replication competence

First Author: Ray So, The University of Hong Kong

Co-Author(s): Kenrie P. Y. Hui, John C. W. Ho, Kaman K. M. Lau, Ziqi Zhou, Michael C. W. Chan, Leo L. M. Poon, Malik Peiris

BACKGROUND OBJECTIVES:

MERS-CoV is an emerging coronavirus posing an ongoing pandemic threat. Dromedary camels in the Arabian Peninsula endemically circulate the virus and serve as primary zoonotic sources of human infections. More than 2600 human cases have been reported globally, with a case-fatality ratio as high as 36%. Nosocomial outbreaks have contributed over 50% of the cases. In contrast to earlier years with hundreds of cases reported annually, the case numbers sharply declined since 2019. The reason for the reduction of cases is unclear and may be multifactorial. We hypothesized that mutations accumulating in the virus spike protein may have reduced zoonotic potential.

METHODS:

We investigated the impact of recently emerged virus spike-protein mutations on virus entry and replication competence using pseudoviruses and replication-competent recombinant viruses by reverse genetics respectively. Host cell binding of spike S1 domain was evaluated by flow cytometry. We further measured the potential immune escape on the spike proteins by testing the neutralization titres against camel sera samples using our established pseudovirus neutralization assay.

RESULTS:

We found that virus spike variants detected in 2019 and some from 2023 show reduced cell entry, lower viral replication and reduced fitness in human primary alveolar epithelial cells and in multiple cell lines. All spikes tested showed similar

S1/S2 cleavage efficiency and utilized the cell-surface TMPRSS2 route for cell entry. Mechanistically, we showed the V530A mutation in the 2019 spike sequence had a reduced human DPP4 binding phenotype. We observed no more than 2-fold differences in the neutralization titres among different spike proteins against clade B seropositive camel sera.

CONCLUSIONS:

MERS-CoV spike mutations that arise in dromedary camels can modulate viral fitness in human cells and provide new insights to understand recent MERS epidemiology. Continuous surveillance in both human and camels will be important to keep track the virus evolution and update phenotypic data for zoonotic assessment.



Abstract No.: 254

Poster Code: : D-0043

Deletion of Furin Facilitates Efficient and Versatile Pseudotyped Particle Generation

First Author: Pengcheng Wang, Cambridge Institute of Therapeutic Immunology & Infectious Disease

Co-Author(s): Sam J. Wilson, Suzannah J. Rihn

BACKGROUND OBJECTIVES:

Coronavirus spike-bearing pseudotyped particle systems are indispensable tools for studying coronavirus entry and evaluating neutralizing antibodies and entry inhibitors. However, pseudotyped particle titers can be low when packaged particles bear coronavirus spikes that contain the furin cleavage site, and this has been a limitation for pseudotyped particle production applications. We therefore investigated whether CRISPR-Cas9-mediated knockout of endogenous furin in producer cells could improve the production of functional pseudotyped particles bearing spikes containing the furin cleavage site, and whether this strategy would provide broader utility than deletion of the furin cleavage site itself.

METHODS:

We generated furin knockout HEK293T producer cells and produced HIV-based pseudotyped particles bearing the spike proteins of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Middle East respiratory syndrome (MERS-CoV) and murine hepatitis virus strain MHV-JHM. Pseudotyped particle titers were measured on cells exogenously expressing the corresponding receptors. We also assessed spike-induced cell-cell fusion, spike integrity, and the effects of direct furin cleavage site mutation compared with endogenous furin knockout. In addition, high-titer SARS-CoV-2 pseudotyped particles were used in entry inhibition and neutralization assays.

RESULTS:

Furin knockout greatly increased MHV-JHM pseudotyped particle titers by approximately 30-fold. Furin deficiency also increased SARS-CoV-2 and MERS-CoV pseudotype titers by approximately 15-fold and 5-fold, respectively. Upon furin knockout, transfection of MHV-JHM spike no longer induced cell-cell fusion,

indicating that deletion of furin prevents exposure of the fusion peptide in MHV-JHM. In furin-deficient cells, spike proteins were detected predominantly in their full-length form, indicating that spike integrity was preserved during production. For SARS-CoV-2, endogenous furin knockout and furin cleavage site removal had comparable effects, whereas for MHV-JHM and MERS-CoV, furin knockout was more effective at raising particle titers than furin cleavage site removal. High-titer SARS-CoV-2 pseudotyped particles yielded stable and reproducible inhibition curves and supported neutralization assays.

CONCLUSIONS:

Deletion of endogenous furin provides a simple, effective, and broadly applicable strategy to improve coronavirus pseudotyped particle systems by eliminating furin-mediated pre-activation. This approach enables the generation of high titer pseudotyped particles without the need for viral sequence modification and facilitates downstream applications in viral entry studies, neutralization assays, and antiviral screening.



Abstract No.: 282

Poster Code: : D-0044

Cooperative Role of the Spike N-Terminal Domain in Sarbecovirus Entry and Immune Escape

*First Author: Hua Guo, Huazhong Agriculture University**Co-Author(s): Mengjie Qin, Jingwen Mo, TianYi Dong, Zishuo Guo, Zhengli-Shi*

BACKGROUND OBJECTIVES:

Of the hundreds of sarbecovirus strains identified in global wildlife, the spike (S) glycoprotein exhibits remarkable sequence diversity, particularly in the N-terminal domain (NTD) and receptor-binding domain (RBD). While the RBD is well-characterized for its role in host entry, the functional significance of NTD variation across the sarbecovirus subgenus remains largely enigmatic.

METHODS:

To address this knowledge gap, we systematically analyzed diverse sarbecovirus spike NTDs using a pseudovirus platform to assess their roles in cell entry and immune evasion.

RESULTS:

Our findings demonstrate that the NTD is not a functionally isolated entity; rather, it acts in concert with the RBD and other spike regions to orchestrate critical steps, including the efficiency of spike trimer incorporation into virions, overall spike stability, receptor engagement, protease dependency, and membrane fusion—processes that collectively determine viral entry efficiency. Furthermore, the NTD also influences the neutralization efficacy of RBD-specific antibodies, revealing a critical NTD-RBD interplay in sarbecovirus evolution and immune evasion.

CONCLUSIONS:

These results establish that the NTD acts as a key regulatory module, dynamically coordinating with the S1 and S2 subunits to influence both the initial stages of host cell entry and the evasion of humoral immune responses. This study offers new insights and plausible explanations for the evolutionary flexibilities and variation of the spike NTD and underscores its essential consideration in the design of broad-spectrum vaccines and therapeutics against emerging sarbecoviruses.

Abstract No.: 319

Poster Code: : D-0045

Investigation the role of GRP78 in SARS-CoV-1, SARS-CoV-2, and HCoV-NL63 entry

First Author: Bingjie HU, The University of Hong Kong

Co-Author(s): Yixin He, Zijiao zou, Madeline Hartnoll, Jasper Fuk-Woo Chan, Maozhou He, Hin Chu

BACKGROUND OBJECTIVES:

Despite SARS-CoV-1, SARS-CoV-2, and HCoV-NL63 all utilize ACE2 as their primary entry receptor, SARS-CoV-1 and SARS-CoV-2 are dramatically more pathogenic than HCoV-NL63, suggesting that unknown host or viral determinants, beyond ACE2 may impact the pathogenicity of the three viruses. Here, we dissected the functional role of GRP78 in virus entry of SARS-CoV-1, SARS-CoV-2, and HCoV-NL63 and found that the three viruses engaged GRP78 with distinct virological outcomes-promoting SARS-CoV-1 and SARS-CoV-2 entry and replication while negatively modulating the HCoV-NL63 entry and replication. We further investigated the underlying mechanism of the differential usage of GRP78 by HCoV-NL63 when compared with SARS-CoV-1 and SARS-CoV-2 and found that GRP78 competes with ACE2 bind to HCoV-NL63 spike proteins, whereas GRP78 can bind to spike proteins of SARS-CoV-1/2 together with ACE2. Overall, our work provides an explanation that may partly contribute to the discrepant tropism, tissue replication fitness, and pathogenicity of SARS-CoV-1, SARS-CoV-2, and HCoV-NL63.

METHODS:

All experiments with infectious SARS-CoV-1 and SARS-CoV-2 were performed in a BSL-3 facility at HKU following approved safety protocols. Animal studies were conducted in accordance with HKU's Committee on the Use of Live Animals in Teaching and Research. Six- to eight-week-old male golden hamsters were intranasally inoculated with 3×10^5 PFU of SARS-CoV-1, SARS-CoV-2, or HCoV-NL63. Virus replication in nasal turbinates and lung tissues was quantified by plaque assay and qRT-PCR, and histopathology was assessed by H&E and immunohistochemistry. Primary human nasal epithelial cells (hNECs) were cultured at air-liquid interface (ALI) and infected at an MOI of 2. VSV-based pseudoviruses bearing spike proteins of SARS-CoV-1, SARS-CoV-2, or HCoV-NL63 were generated in 293T cells, and entry assays were performed in 293T-hACE2 cells. Recombinant spike proteins and GRP78 were expressed and purified from 293F cells, and spike-GRP78 interactions were analyzed by cryo-EM single particle analysis.

RESULTS:

Using the hamster model, we found that while all three viruses efficiently replicated in the nasal turbinate tissues of the infected animals, only SARS-CoV-1 and SARS-CoV-2 but not HCoV-NL63 were readily found in the lung tissues, leading to dramatically lower pro-inflammatory cytokine levels in the lung tissues of HCoV-NL63-infected hamsters. In keeping with the *in vivo* findings, we found that while all three viruses replicated in primary human nasal epithelial cells (hNECs), only SARS-CoV-1 and SARS-CoV-2 replicated in the human lung Calu3 cell-line. Subsequent analyses found that HCoV-NL63 also utilized TMPRSS2 as a key protease during virus entry, similar to SARS-CoV-1 and SARS-CoV-2, which indicate that receptor and entry protease usage are not the dominant determining factors for the observed discrepant pathogenicity between the three viruses. Next, we evaluated the usage of potential accessory entry factors by the three viruses and found that glucose-regulated protein 78 (GRP78) was differentially utilized. We showed that the spike protein of all three viruses could bind to GRP78. Surprisingly, while GRP78 facilitated the entry and replication of SARS-CoV-1 and SARS-CoV-2, it negatively impacted the entry and replication of HCoV-NL63. We further demonstrated that both NTD and RBD of NL63 spike can bind to GRP78, while only the RBD of SARS-CoV-1/2 spikes can bind to GRP78. Then, using AlphaFold, we found that the binding models of GRP78/ACE2 with spike proteins of the three viruses are different. For SARS-CoV-1 and SARS-CoV-2, GRP78 and ACE2 can bind to spike proteins simultaneously. However, for HCoV-NL63, GRP78 competes with ACE2 for binding to spike proteins. These predicted binding models were supported by co-IP experiments involving GRP78, ACE2, and spike proteins. When we increased the amount of GRP78, the binding between the NL63 spike and ACE2 was affected. In contrast, the binding between the SARS-CoV-1/2 spike and ACE2 was not affected by increased GRP78. Overall, these results highlighted the potential role of GRP78 in differentiating the tropism and replication of SARS-CoV-1 and SARS-CoV-2 from that of HCoV-NL63.

CONCLUSIONS:

GRP78 oppositely modulated the entry and replication of SARS-CoV-1 and SARS-CoV-2 when compared to HCoV-NL63, driven by distinct binding models of GRP78/ACE2 with spike proteins of SARS-CoV-1/2 and HCoV-NL63. This differential virological outcome of GRP78 usage in part contributes to the discrepant tropism and pathogenicity of SARS-CoV-1 and SARS-CoV-2 when compared to HCoV-NL63.

Abstract No.: 324

Poster Code: : D-0046

Selective neutralisation of cell-free virus and cell-to-cell spread by antibodies to SARS-CoV-2 Spike

First Author: Nicholas Matheson, University of Cambridge

Co-Author(s): Isobel Ramsay, Kata Csiba, Pehuén Pereyra Gerber

BACKGROUND OBJECTIVES:

Infection with SARS-CoV-2 or vaccination against the viral Spike protein induces neutralising antibodies (NAbs), protecting against COVID-19. Some NAbs target the Spike receptor-binding motif (RBM), directly blocking its interaction with ACE2. Other NAbs target distant Spike receptor-binding domain (RBD) or non-RBD epitopes. The objective of this study was to better understand the mechanism of action of those antibodies, and what they can teach us about how SARS-CoV-2 is transmitted between cells.

METHODS:

We previously developed a high-throughput live-virus assay to quantify NAbs against SARS-CoV-2. We have now adapted that assay to selectively measure neutralising activity against cell-free infection and cell-to-cell spread. Results are validated using high-content automated microscopy and a modified Plaque Reduction Neutralisation Test (PRNT), in which plaque number and plaque size serve as proxies for different modes of infection. Finally, genome-wide CRISPR/Cas9-based knockout screening is used to elucidate the cellular machinery specifically required for cell-to-cell spread of SARS-CoV-2.

RESULTS:

NAbs targeting the RBM inhibit both cell-free infection and cell-to-cell spread of SARS-CoV-2. Conversely, other NAbs display differential activity, with antagonism of cell-free infection or cell-to-cell spread corresponding to distinct Spike epitopes. Strikingly, antibodies directed against the Spike N-terminal domain (NTD) selectively inhibit cell-to-cell spread. On the other hand, certain RBD antibodies are only effective against cell-free infection. The reciprocal activities of these antibodies are independent and additive, and distinct host factors are required for cell-to-cell spread. Amongst patient samples, relative neutralising activity against the different modes of infection correlates with protection against COVID-19.

CONCLUSIONS:

SARS-CoV-2 is able to replicate by both cell-free infection and cell-to-cell spread. NAbs directed against particular Spike epitopes are able to differentially inhibit these modes of infection, which require distinct host factors. Therapeutic antibodies, small molecules and immunogens targeting both modes of infection may therefore be required for maximum therapeutic effect.



Abstract No.: 347

Poster Code: : D-0047

Identification of Cross-species Transmission and Receptor Usage Distribution of MERS-related Coronaviruses in Bats

*First Author: Jing Chen, Guangzhou National Laboratory**Co-Author(s): Chunguang Wu*

BACKGROUND OBJECTIVES:

The Merbecovirus subgenus comprises four species: Betacoronavirus cameli (MERS-related coronavirus, MERSr-CoV), Betacoronavirus erinacei (EriCoV), Betacoronavirus pipistrelli (HKU5-CoV), and Betacoronavirus tytonycteridis (HKU4-CoV). MERS-CoV belongs to Betacoronavirus cameli, that also includes viruses circulating in vespertilionid bats. However, a phylogenetic gap persists among these diverse merbecoviruses and the potential spillover risk of animal merbecoviruses to humans remains to be investigated.

METHODS:

Here, we report several newly identified MERSr-CoV genome sequences. By integrating these with publicly available sequences, we investigate the evolutionary dynamics of these viruses and their potential cross-species and cross-regional transmission routes. We also performed the Cryo-EM structural analysis and structure-guided mutagenesis analysis to figure out the interaction mechanism of the receptor-binding domain (RBD) of BatCoV-8842 (from the MERSr-CoV HKU25 clade) and ACE2 receptor.

RESULTS:

Bat MERSr-CoVs efficiently utilize ACE2 or DPP4 as functional receptors and employ human host proteases for cell entry, indicating a potential cross-species transmission risk. The complex between the RBD of BatCoV-8842 and ACE2 from its natural host, *Vespertilio sinensis*, revealed a distinct binding interface compared to other ACE2-utilizing Betacoronavirus cameli viruses, although it shared a similar footprint on *Pipistrellus abramus* ACE2 with the ACE2-using HKU5-CoV from Betacoronavirus pipistrelli. MERSr-CoV HKU25 clade 8842 can adapt to ACE2 receptors from different species.

CONCLUSIONS:

SARS-CoV-2 is able to replicate by both cell-free infection and cell-to-cell spread. NAbs directed against particular Spike epitopes are able to differentially inhibit these. Our study provides new insights into the differential receptor usage among genetically diverse bat MERSr-CoVs and highlights the potential zoonotic risk posed by these viruses.



Abstract No.: 377

Poster Code: : D-0048

Q1020R in MERS-CoV spike proteins from recent human cases and mutations at L507 selected in vitro and occurring in vivo augment resistance to soluble human DPP4

First Author: Nianzhen Chen, German Primate Center - Leibniz Institute for Primate Research

Co-Author(s): Hannah Kleine-Weber, Lu Zhang, Anna-Sophie Moldenhauer, Khaled Alkharsah, Michael Winkler, Asisa Volz, Marcel A. Müller, Victor Corman, Christian Drosten, Markus Hoffmann, Stefan Pöhlmann

BACKGROUND OBJECTIVES:

Human cases of Middle East respiratory syndrome predominantly occur in Arabia despite MERS-coronavirus (MERS-CoV) circulation in both Arabian and African dromedary populations. MERS-CoV enters target cells via its spike (S) glycoprotein by engaging dipeptidyl peptidase 4 (DPP4) as its cellular receptor. DPP4 is present in both membrane-bound and soluble forms, the latter of which can act as a decoy receptor and inhibit viral entry. We investigated spike-mediated entry to identify factors that may contribute to the increased zoonotic spillover risk on the Arabian Peninsula and investigated resistance development in response to soluble DPP4.

METHODS:

We used pseudotyped particles bearing MERS-CoV S proteins to compare entry determinants of Arabian (Saudi Arabia, Oman) and African (Burkina Faso, Morocco, Nigeria, Kenya, Ethiopia, Egypt) camel-derived MERS-CoV variants, using the human isolate EMC/2012 as a reference. To examine adaptation to sDPP4, we performed serial passage of a replication-competent VSV chimera encoding MERS-CoV S (VSV-MERS-S) in the presence of human sDPP4.

RESULTS:

Cell entry driven by the S proteins from MERS-CoV from Arabian camels was less susceptible to inhibition by sDPP4 as compared to entry mediated by the S proteins from African variants. In contrast, entry into human cells, sensitivity to

interferon-induced transmembrane proteins and neutralizing antibodies was comparable. The differential sDPP4 resistance correlated with the Q1020R spike mutation, present in Arabian camels and recent human clinical isolates but absent in African MERS-CoV lineages. Further, indirect evidence was obtained that sDPP4 in human serum can inhibit MERS-CoV S-driven cell entry. Cultivation of VSV-MERS-S chimeras in the presence of sDPP4 selected for additional resistance mutations (L507H/I and T533P) in the receptor-binding domain. Notably, mutations at residue L507, located close to the DPP4-binding interface, were also observed in naturally occurring human MERS-CoV isolates (L507F/R/P), suggesting convergent evolutionary pathways. Mutations of L507 significantly reduced DPP4 binding affinity while enhancing resistance to sDPP4 inhibition, yet remained compatible with robust entry into target cells expressing high levels of DPP4. Collectively, these findings reveal that both naturally occurring and experimentally selected spike mutations can promote adaptation to sDPP4 pressure.

CONCLUSIONS:

Our findings support the idea that sDPP4 may act as a natural barrier to human MERS-CoV infection, which Arabian camel viruses may overcome more readily than African camel viruses due to the Q1020R mutation in the spike protein. These results may help to explain regional differences in zoonotic spillover risk. We further show that MERS-CoV may adapt to sDPP4 pressure by reducing receptor affinity without losing infectivity for high DPP4-expressing cells, which might have implications for the development and use of DPP4-based therapeutics.



Abstract No.: 384

Poster Code: : D-0049

Context-dependent effects of the furin cleavage site on sarbecovirus spike phenotype

*First Author: Igor Santos, The Pirbright Institute**Co-Author(s): Nazia Thakur, Dalan Bailey*

BACKGROUND OBJECTIVES:

The rise in wildlife-to-human zoonotic virus transmission demonstrates the importance of identifying mechanisms driving host jumps. The coronavirus family (CoVs), particularly sarbecoviruses, exemplify this potential with their broad host adaptability. The acquisition of the furin cleavage site (FCS, RRAR) in SARS-CoV-2 spike protein (S) was an essential adaptation for efficient transmission in humans. While the role of FCS has been extensively studied in SARS-CoV-2, its impact in related sarbecoviruses backgrounds remains under explored. Here, we evaluate whether FCS acquisition alone, or in combination with adaptive mutations, promotes phenotypes that could explain its importance to future pandemic potential.

METHODS:

Full length S proteins from SARS-CoV-2 B, B.1, and the bat sarbecoviruses Banal 20_103 (103) and Banal 20_236 (236) were transfected in 293T-ACE2 or A549-ACE2 TMPRSS2 (A549-AT) cells, including S variants with FCS insertion and/or D614G. Spike localisation was observed by immunofluorescence confocal microscopy and cell surface protein was quantified by flow cytometry. Lentiviral pseudotyped viruses were used to quantify viral entry and receptor usage, and live SARS-CoV-2 infection served as a benchmark for S localization and cell surface expression.

RESULTS:

Pseudotyped lentiviruses carrying each sarbecovirus S were able to infect both cell lines. However, 103 and 236 containing FCS produced lower-titre pseudoviruses despite their detection in supernatants. Notably, FCS insertion in 103 and 236 reduced entry in cells expressing ACE2 and ACE2/TMPRSS2 relative to their wild type spikes. Combining FCS with D614G enhanced receptor usage up to two-fold

in A549-AT cells, with no effect in 293T-ACE2 cells, which suggest the D614G is essential to allow entry in the context of TMPRSS2-expressing cells. D614G alone had minimal impact on entry, while FCS deletion from the B.1 backbone reduced entry in both cell lines. Furthermore, wild-type 103 and 236 spikes were less fusogenic than SARS-CoV-2 B and B.1, but the addition of FCS and D614G increased cell-cell fusion two- to three-fold compared to their wild-type counterparts, reaching similar levels to SARS-CoV-2 variants. Spike subcellular localisation was also mutation-dependent: FCS increased surface expression in 103 and 236 backbones, D614G had no statistical impact, while their combination altered intracellular localisation while maintaining high surface presence.

CONCLUSIONS:

Overall, our data indicate that FCS impact is mutation dependent, with D614G required to enable efficient entry, fusion, and spike trafficking in TMPRSS2-expressing cells not only for SARS-CoV-2, but their related sarbecoviruses. This further emphasizes the epistatic interaction that likely facilitated SARS-CoV-2 emergence.



Abstract No.: 111

Poster Code: : E-0050

The Spike Protein of an Apathogenic Strain of Infectious Bronchitis Virus Confers Attenuation in a Tropism Independent Manner.

First Author: James Kirk, The Pirbright Institute

Co-Author(s): Adam Rayment, Amber Tappin, Harry Francis, Thomas Peacock, Isobel Webb, Andrew Davidson, Paul Britton, Erica Bickerton, Sarah Keep, Sam Jones

BACKGROUND OBJECTIVES:

Infectious Bronchitis Virus (IBV) causes an economically damaging disease of poultry. Live attenuated vaccines are used to control IBV, which are generated through serial passage in embryonated hens' eggs. Attenuation occurs through unknown molecular mechanisms with minimal consensus level mutations, presenting a short route for reversion to virulence. Modifying IBV genomes to achieve rational attenuation allows for the rapid generation of safer vaccines with quicker response to emerging strains. The spike (S) glycoprotein of Beaudette, an attenuated strain, can confer in vivo attenuation and the ability to replicate in Vero cells, which are licensed for vaccine manufacturing, to the closely related pathogenic strain M41. The objectives were to establish whether the Beaudette S glycoprotein can confer attenuation in vivo, and Vero cell tropism in vitro to the distantly related nephropathogenic IBV strain D388.

METHODS:

We used the recombinant IBV D388-Beau(S), where the S ectodomain sequence of D388 has been replaced with the equivalent sequence from Beaudette. D388 and Beaudette share 82% S amino acid identity and belong to different genotypes, GI-19 and GI-1 respectively. D388-Beau(S) replication was assessed in vitro, in ovo and in ex vivo tracheal organ cultures. Pathogenicity was assessed in vivo and compared to the pathogenic control D388. Viral RNA and infectious virus were quantified in harvested tissues. Beaudette was not included in the in vivo study as it is well documented to be attenuated and to exhibit highly restricted replication in vivo.

RESULTS:

D388-Beau(S) exhibited comparable replication kinetics to Beaudette in vitro, in ovo and ex vivo. The ability to replicate in Vero cells was conferred. D388-Beau(S) infected chickens retained ciliary activity in the trachea, a marker of attenuation, and exhibited reduced IBV associated clinical signs compared to the D388 pathogenic control group. Analysis of primary and secondary sites of replication highlighted comparable levels of viral RNA between D388-Beau(S) and D388 infected groups. Analysis of infectious virus however demonstrated reduced levels of D388-Beau(S).

CONCLUSIONS:

The Beaudette S can be inserted into a distantly related IBV strain conferring extended tropism in vitro and attenuated in vivo phenotypes, highlighting a potential avenue of rational attenuation universally applicable to any IBV strain. The identification of comparable in vivo tropism, with D388-Beau(S) mirroring D388 and not Beaudette, suggests a tropism-independent manner of attenuation.



Abstract No.: 285

Poster Code: : E-0051

Engineering a mouse-adapted SADS-CoV and establishing a neonatal mouse model to study its infection

First Author: Mengjia Zhang

BACKGROUND OBJECTIVES:

Swine acute diarrhea syndrome coronavirus (SADS-CoV), an emerging bat-origin alphacoronavirus, causes severe disease in neonatal piglets, with significant economic losses to the swine industry. This study developed a mouse infection model using a murinized mutant virus to address limitations with piglet use.

METHODS:

A murinized SADS-CoV mutant was generated by targeted RNA recombination technology, confirmed by IFA using mAbs against SADS-CoV N protein and MHV S protein. To assess the characteristics of the chimeric virus in murine cells, LR7 cells were infected with the virus at 0.01 MOI and supernatants were harvested at indicated time points for viral titration. Ten 2-day-old SPF-grade BALB/c mice were intraperitoneally inoculated with mSADS-CoV at of $7 \times 10^{6.2}$ PFU/mL (1 ml per mouse). Clinical symptoms were recorded daily and fixed tissues were for histopathology and immunohistochemistry analysis. The tissue distribution was analyzed using RT-qPCR.

RESULTS:

Typical syncytia were observed on LR7 cells 24 h post-electroporation, consistent with cytopathic effect (CPE) induced by MHV. IFA staining using mAbs anti SADS-CoV N protein and MHV S protein confirmed successful rescue of mSADS-CoV. Growth kinetics on LR7 cells demonstrated efficient replication of mSADS-CoV in murine cells. Inoculation of 2-day-old BALB/C mice with mSADS-CoV resulted in mortality at 5-6 dpi, with affected large intestines displaying a reduction in goblet cell numbers and disorganized epithelial cell architecture, accompanied by inflammatory cell infiltration in the mucosal layer. Various tissues tested positive for mSADS-CoV by qRT-PCR, with viral loads exceeding 104.0 copies/mg, peaking at around 108.0 copies/mg in lungs and hearts. Immunohistochemical staining revealed viral presence in the alveolar walls of the lungs and the basal layers of the large and small intestines.

CONCLUSIONS:

In summary, the SADS-CoV mouse model presented in this study provides a valuable tool for investigating the molecular mechanisms of SADS-CoV replication,

in particular for the screening of antiviral drugs targeting viral replicase genes and for validating host factors involved in coronavirus replication.



Abstract No.: 323

Poster Code: : E-0052

Variations of accessory gene Ns7 from bat swine acute diarrhea syndrome-related coronavirus attenuate viral pathogenicity

*First Author: Xi Liu, Guangzhou Laboratory**Co-Author(s): Li-Na Ji, Tian-Yi Dong, Yun Luo, Zheng-Li Shi*

BACKGROUND OBJECTIVES:

The swine acute diarrhea syndrome coronavirus (SADS-CoV) is an emerging coronavirus of bat origin and causes severe diarrhea in piglets. Previous studies revealed that the partial deletion in the accessory gene Ns7 of SADS-CoV attenuate viral pathogenicity in piglets. In this study, we characterized the variations of Ns7 in bat SADS-related CoVs and analyze their roles in viral pathogenesis in vitro and in vivo. We constructed four SADS-CoV recombinant viruses (rSADS-CoV) carrying different Ns7 variants identified from bat SADSr-CoV or SADS-CoV genomes. These included a virus with a full-length Ns7 deletion (rSADS- Δ Ns7); a virus with muted ORF7b (rSADS- Δ Ns7b); a bat-derived Ns7 variant naturally muted ORF7b (rSADS-BtNs7); and a mutant containing a previously reported 58-nucleotide deletion in Ns7 (rSADS- Δ 58nt) that emerged during SADS-CoV culture. We then evaluated their pathogenicity in suckling mice, as well as their replication dynamics in swine cell lines and organoids. All four SADS-CoV Ns7 variants resulted in a 40.26-71.42% decrease in mortality and a reduction in viral load in the brain of infected mice. Notably, the pathogenicity of rSADS- Δ Ns7 and rSADS- Δ Ns7b mutants was completely attenuated in suckling mice, causing no mortality and eliciting a diminished inflammatory response. These two mutants also exhibited lower replication efficiency in swine cell lines and organoids, along with reduced cytokine-related pathway activation in intestinal organoids. Together, our findings reveal that Ns7b is a critical virulence factor and provide a strategy for the live-attenuated vaccine design of SADS-CoV and SADSr-CoVs.

METHODS:

Biosafety and ethics statements All animal experiments were conducted in an animal biosafety level 2 (BSL-2) facility, with approval from institutional biosafety committees following the country's biosafety regulations. All animal experiments strictly followed the guidelines for the care and use of laboratory animals established by the International Council for Laboratory Animal Science (ICLAS).

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee. Cells and viruses Caco-2 and Huh7 cells were kindly provided by Prof Lin-Fa Wang (Duke-NUS Medical School). IPEC-J2 and Vero cells were generously supplied by Prof Jing-Yun Ma (South China Agricultural University). All cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco, C11995500BT) supplemented with 10% fetal bovine serum (Gibco, A10033), and were routinely confirmed to be free of mycoplasma contamination. Viruses were propagated in DMEM with 10% tryptose phosphate broth (TPB, Caisson, TBL01) and 5µg/mL trypsin (Gibco, 25200). Viral titers were determined by the 50% cell culture infectious dose (TCID₅₀) assay in Huh7 and calculated using the Reed-Muench method. Construction and recovery of recombinant SADS-CoV Ns7 variants A yeast-based reverse-genetics platform were used to generate SADS-CoV Ns7 variants. The SADS-CoV genome (GeneBank No.MG557844) was divided into eight overlapping cDNAs fragments and cloned into the pGEM-T-EASY vector, as previously reported(34). Since the wild-type Ns7 gene is located within the eighth subclone, all genetic modifications were engineered directly into this fragment. To generate the Ns7 mutant, a green fluorescent gene(mNeonGreen) was synthesized and used to replace the wildtype Ns7 gene in the plasmid using ClonExpress® II One Step Cloning Kit (Vazyme, C112). To construct the Ns7b mutants, synonymous mutations were introduced to disrupt the initiation codons ATG and the subsequent three downstream ATG sites of Ns7b. To replace the wildtype Ns7, an orthologous gene from a bat SADS-related CoV (Sample ID: RaGD2025_8505) was amplified by PCR and then used to substitute the original Ns7 gene. To generate the cell adaptation strain, a previously reported 58nt deletion in Ns7 was introduced into the eighth subclone. All modified subclones were sequenced to confirm the presence of the intended mutations in the Ns7 gene. Eight fragments were assembled into a full-length plasmid via transformation-associated recombination in yeast(41, 42). The circular YACs were then isolated, purified, and subsequently used for viral rescue through transfection into Huh7 cells as described previously(34, 43). The rescued viruses were harvested after blind passage in Huh7 cells. Analysis of viral subgenomic RNA Total RNA was extracted from virus-infected Huh7 cells using the FastPure cell/tissue total RNA isolation kit (Vazyme, RC112). cDNA was synthesized using M-MLV reverse transcriptase (Promega, M1701) in accordance with the manufacturer's instructions. To amplify subgenomic mRNAs, a semi-nested PCR approach was employed. The first round of amplification was performed with forward primer Sub-SADS-F (5' -GTCCTTATCTTTTAGACTTTCCAGTC-3'), which anneals to the

leader sequence near the 5' end of the viral genome, and reverse primer Sub-SADS-R1 (5' -CATACGAGGTGTGACGGCTCTTC-3'), complementary to the 3' UTR. The second round of amplification utilized the same forward primer (Sub-SADS-F) together with an internal reverse primer, Sub-SADS-R2 (5'-CATGTAGTTAACTGCGTTGGTCTATAGG-3'), targeting the terminal region of the Ns7 gene. The PCR products were separated by electrophoresis on a 1% agarose gel, and the shortest bands, corresponding to the target sgRNA encompassing the variable region, were excised and confirmed by Sanger sequencing. Infection in suckling mice Twelve of pregnant female mice were randomly divided into six groups and observed daily to record the age of suckling mice. All 3-day-old mice ($n \geq 10$) were infected by intragastric injection with 4×10^5 50% TCID₅₀ viruses or an equal volume of DMEM as control. The weight change, survival, and clinical symptoms were monitored daily. Another twelve of pregnant female mice were randomly divided into six groups and the suckling mice were infected as above. The brain, lung, stomach, intestine tissues were collected at 1, 3, 5, 7dpi and detected viral load by RT-PCR using HiScript II One Step qRT-PCR SYBR Green Kit (Vazyme, Q221). Histopathological analysis and pathological score Brain, lung and intestine of suckling mice at 5dpi were collected and fixed in 4% paraformaldehyde (PFA, Servicebio, G1101) for one week. Tissues were cut into appropriate sizes and embedded in wax blocks and then cut into 3.5-mm sections, which were stained by hematoxylin-eosin (H&E) and indirect immunofluorescence (IFA) to show the pathological injury and detect the SADS-CoV antigen. Section scanning and pathological score were completed by Servicebio and the pathological scores are calculated based on the criteria shown in the supplementary Table.1 (44, 45). Host mRNAs expression analysis by RT-PCR Tissue samples (10–20 mg) or organoids were lysed in 500 μ L of Buffer RL. Total RNA was extracted using the FastPure® Cell/tissue total RNA isolation kit v2 (Vazyme, RC112) according to the manufacturer's instructions. Genomic DNA was digested with gDNA remover, and cDNA was synthesized using ABScript Neo RT Master Mix (Abclonal, RK20433) for qPCR template. The expression levels of host mRNAs encoding interferon-related genes (IFN- α , IFN- β , ISG15, and MX1) and pro-inflammatory cytokines (TNF- α , IL-6, CCL7, and CXCL10) were quantified using Taq Pro Universal SYBR qPCR Master Mix (Vazyme, Q712). The corresponding primer sequences(46-50) are provided in the supplementary Table 2. Swine lung and ileum organoids infection Swine lung and ileum organoids were established, maintained, and differentiated as previously described(34). To analyze replication kinetics, organoids were infected with SADS-CoV and recombinant SADS-CoV Ns7 variants at an MOI of 0.1 The supernatants and cell lysates were harvested

at the indicated time points for viral RNA extraction, RT-qPCR, and TCID50 assays. All TCID50 assays were performed in Huh7 cells and calculated using the Reed-Muench method. For the immunostaining assay, organoids were challenged with a higher viral titer (MOI=1) and fixed at 48hpi. Then, the immunofluorescence was determined with the antibodies mentioned below and visualized with a Leica Stellaris 8 confocal microscope. The details of antibodies used are as follows: Mouse anti-E-cadherin (catalog number 610181, 1:50) was acquired from BD Biosciences), Cy3-conjugated Goat Anti-Mouse IgG (SA00009-1, proteintech). Rabbit anti-HKU2-NP antibodies were prepared in-house. Growth kinetics Cell lines and organoids were infected with SADS-CoV mutant strains and the wild-type at an MOI of 0.1, as described previously (34). The supernatant was harvested from 3hpi to 144hpi. The viral titer of supernatant was determined by 50% cell culture infectious dose (TCID50) and calculated using the Reed-Muench method. RNA-seq and data analysis RNA was extracted from ileum organoids infected with SADS-CoV Ns7 variants or subjected to mock-infection at 72hpi (3 replicates each) and subjected for RNA-seq analysis. After mapping clean reads to GRCh38.p13 with HISAT2 v2.1.0 and converting the file formats with samtools v1.10-24, we used stringtie v2.1.0 to assemble and quantitate transcripts. The Gene count matrix was imported into R v4.2.2 for subsequent analyses. Data preprocessing and sorting were performed using the tidyr package v1.3.1 and dplyr package v1.1.4. Differential gene expression analysis and read count normalization were conducted using DESeq2 v1.38.3. The condition was set at $\log_2\text{FoldChange} > 2$ and $p\text{-value} < 0.05$. The volcano plots describing between-group difference were generated using ggplot2 package v3.4.2. Differentially expressed genes were subsequently submitted to MetaScape (<https://metascape.org/gp/index.html#/main/step1>) for pathway enrichment analysis. scRNA-seq preparation of ileum organoids Ileum organoids were infected with SADS-CoV Ns7 variants at an MOI of 0.1. At 48 hours post-infection, all organoids were gently scraped, detached from the wells, and then digested with a 1:1 mixture of trypsin and TrypLE containing 0.1 mg/mL DNase (11284932001; Roche) at 37°C for 6 mins. The resulting suspension was filtered through a 40- μm cell strainer to remove debris. The cells were then counted using trypan blue (0.2%) exclusion. We used the DNBelab C Series High-throughput Single-cell RNA Library Preparation Set V3.0 (MGI, 940-001988-00) for single-cell droplet generation and subsequent library preparation according to the manufacturer's instruction. The cDNA library products were circularized using the MGIEasy Circularization Kit (MGI, 1000005260). All of the libraries were sequenced on an DNBSEQ-T7. scRNA-Seq data analysis Raw sequencing data were processed using DNBelab C Series™ HT Single-Cell

Analysis Software (DNBC4tools v2.1.2) for upstream analysis. Reads were aligned to the Sscrofa11.1, which was obtained from Ensembl (<https://www.ensembl.org/>). Further analyses were performed using Seurat v4.3 in R v4.4(51). Cells with less than 500 detected genes, more than 8,000 detected genes or with mitochondrial gene expression exceeding 20% were excluded. Genes expressed in fewer than ten cells were also filtered. Gene expression matrices were normalized using NormalizeData method. The top 2,000 variable features were identified using the FindVariableFeatures function with the "vst" method for downstream analysis. Data were scaled and centered using ScaleData. Principal component analysis (PCA) was performed on the scaled data, and the number of principal components used for downstream analyses was selected using ElbowPlot. Samples were integrated based on PCA embeddings using the function RunHarmony of the R package harmony v1.2.3(52). Cells were clustered using FindNeighbors and FindClusters (resolution = 0.8) function using the selected principal components. Two-dimensional visualization of cell clusters was performed using Uniform Manifold Approximation and Projection (UMAP) with the function RunUMAP. Marker genes were identified using the FindAllMarkers function with the Wilcoxon rank-sum test, applying Benjamini-Hochberg (BH) correction for multiple testing. Cell clusters were annotated based on the expression of known marker genes and published single-cell datasets. To minimize false-positive assignments, cells were stringently classified as SADS-positive only when at least two SADS genes were detected within the same cell. Differential gene expression analysis between experimental conditions was conducted using the Wilcoxon rank-sum test implemented in Seurat. Genes with an adjusted P value < 0.05 and an absolute log2 fold change > 0.25 were considered significantly differentially expressed. Gene Ontology (GO) pathway analyses were performed using clusterProfiler v4.12.6(53). A P value of less than 0.05 was considered statistically significant. Cytotoxicity assay. Supernatants were collected at 72 hpi from lung or ileum organoids infected with SADS-CoV Ns7 variants. Cell death was then quantified by measuring lactate dehydrogenase (LDH) release using a commercial LDH Cytotoxicity Assay Kit (Beyotime, C0017) according to the manufacturer's instructions. Generation of the mutant of SADS-CoV replicon. To generate the SADS-CoV Ns7b mutant replicon, we used a previously established wild-type (WT) replicon as the backbone(54). In this backbone, the S/3a and E/M open reading frames had been replaced with the neomycin phosphotransferase (Neo) and NanoLuc luciferase (Nluc) genes, respectively. We then introduced three synonymous mutations to disrupt Ns7b in this backbone, generating the Δ Ns7b mutant replicon in a single step as described above. Subsequently, Nluc expression was quantified in HEK293, IPEC-J2 and IPI21 cell

lines following transfection with the respective replicon plasmids. Statistical analysis Data from at least three independent experiments are presented as mean $\pm$ SEM. Statistical analyses were performed using GraphPad Prism software (version 10.0). For comparisons among multiple groups, a one-way ANOVA (or two-way ANOVA, as appropriate) was conducted, followed by [Specify the Post-hoc Test] for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

RESULTS:

Rescue and characterization of recombinant SADS-CoV carrying Ns7 variants We indentified three distinct types of Ns7 from 131 bat SADSr-CoV genomes, including one which has full-length sequence of Ns7 but without open reading frame (ORF), 76 genomes harboring a full-length of Ns7 with ORF7a and muted ORF7b due to mutations in the initiation ATG of Ns7b, and 54 genomes containing a full-length sequence of Ns7 with ORF7a and 7b similar as SADS-CoV. Based on the variations in Ns7 genes, we constructed four recombinant SADS-CoVs (rSADS-CoV) carrying different Ns7 variants: a variant in which Ns7 was replaced with a green fluorescent protein gene (mNeonGreen), designated rSADS- Δ Ns7; a variant in which Ns7b expression was silenced by mutating the initiation codon (ATG) and the three downstream ATGs of the Ns7b gene without affecting Ns7a expression, designated rSADS- Δ Ns7b; a variant carrying a naturally occurring Ns7b mutation from a bat SADS-related CoV (sample ID: RaGD2015_8505), designated rSADS-BtNs7; and a variant with a 58-nucleotide deletion in Ns7 (rSADS- Δ 58nt) that emerged during virus culture (Fig 1A). All recombinant viruses were successfully rescued in Huh7 cells, and viral nucleocapsid (N) protein expression was verified by immunofluorescence assays (IFA). Strong mNeonGreen signals were additionally detected in rSADS- Δ Ns7-infected cells (Fig 1B). As expected, we detected subgenomic RNAs (sgRNAs) of the four rSADS-CoV Ns7 variants and SADS-CoV as anticipated (Fig 1C). The accuracy of the leader and coding segments was further validated by Sanger sequencing (Fig 1D).

Pathogenicity evaluation of SADS-CoV Ns7 variants in suckling mice In a previous study, we demonstrated that suckling mice were highly susceptible to SADS-CoV infection and exhibited severe morbidity and mortality (21). To assess the pathogenicity of these recombinant viruses in vivo, 3-day-old C57BL/6 mice were challenged with SADS-CoV and with four rSADS-CoV Ns7 variants (rSADS- Δ Ns7, rSADS- Δ Ns7b, rSADS-BtNs7 and rSADS- Δ 58nt). Survival and body weight were monitored over 12 days (Fig. 2A). Mice infected with wild-type SADS-CoV had a survival rate of less than 25% by day 12, whereas all four recombinant virus groups

exhibited survival rates ranging from 50% to 100% (Fig. 2A, left panel). In contrast, both rSADS-BtNs7 and rSADS- Δ 58nt led to partially attenuated phenotypes, with slower weight gain and a 71.42% and 40.26% decrease in mortality, respectively (Fig 2A). Viral RNA was detected in the brain, lung, and small intestine of all infected mice. There were no difference in viral loads in lung and small intestine of the infected groups, but significantly different in the brain between the wild-type SADS-CoV and the four Ns7 variants. In wild-type-infected mice, viral RNA in the brain remained at a high level from 5 to 7 dpi. In contrast, very low levels of viral RNA were detected in the brains of mice infected with rSADS- Δ Ns7, rSADS- Δ Ns7b, and rSADS-BtNs7. Although rSADS- Δ 58nt exhibited similar viral loads to SADS-CoV at 5dpi, the virus was cleared in some individuals at 7dpi (Fig 2B). Viral N protein expression was consistent with viral RNA loads across all groups (Fig. 2C). Histopathological examination of the brains revealed that wild-type infection caused neuronal necrosis, neuronophagia, and acute neuronal swelling in the medulla of the cerebellum. In contrast, milder pathological lesions were observed in mice infected with the four Ns7 variants, particularly in the rSADS- Δ Ns7 and rSADS- Δ Ns7b groups (Fig. 2D-E, Table S1). In the small intestine, wild-type SADS-CoV infection induced far more severe damage, including pronounced villus rupture and inflammatory cell infiltration. Infection with rSADS- Δ 58nt or rSADS-BtNs7 resulted in cellular vacuolization and separation of the epithelium and lamina propria, whereas only mild pathological changes were observed in mice infected with rSADS- Δ Ns7 or rSADS- Δ Ns7b (Fig 2D-E, Table.S1). In the lungs, limited lesions, such as hemorrhage and inflammatory cell infiltration, were observed across all infected groups. Wild-type SADS-CoV, rSADS-BtNs7, and rSADS- Δ 58nt led to more severe alveolar wall thickening compared to rSADS- Δ Ns7 and rSADS- Δ Ns7b groups. The relatively mild lung damage is consistent with the known enteric tropism of SADS-CoV (1-3). Collectively, these results demonstrate that both Ns7a and Ns7b contribute to SADS-CoV virulence, with Ns7b playing a particularly critical role, justifying their selection for further mechanistic analysis.

Innate immune response induced by SADS-CoV Ns7 variants in mice Early interferon responses are associated with reduced mortality in MERS and COVID-19(22, 23), whereas sustained strong expression of inflammatory cytokines and insufficient or delayed IFN response lead to severe disease outcomes(24). Accessory proteins of coronaviruses play important roles as virulence factors and are involved in modulating host innate immune response(12, 13). Our previous study demonstrated that the inflammatory and interferon responses are associated with the severe pathology in the brain caused by SADS-CoV(21). Based on these findings, we hypothesized that the Ns7a/7b accessory protein of

SADS-CoV may function as virulence factors that influence the host innate immune response. To evaluate differences in the innate immune response to SADS-CoV Ns7 variants *in vivo*, we measured the expression of host mRNAs encoding interferon (IFN)-related genes (IFN- β , IFN- λ , ISG15 and Mx1) and pro-inflammatory cytokines (TNF- α , IL-6, CCL7 and CXCL10) in the brain and small intestine at 3, 5, and 7dpi (Fig 3A–B). In the brain, the IFN-related gene response was limited. IFN- β , IFN- λ , and ISG15 showed comparable expression levels between wild-type and Ns7-deletion mutants at 3, 5, and 7 dpi, while Mx1 was modestly higher in wild-type-infected mice at 7 dpi (Fig 3A). In contrast, pro-inflammatory cytokines were robustly upregulated: TNF- α , CCL7, and CXCL10 were significantly higher in the wild-type group compared to both Ns7 mutants, with peak induction at the late infection stage (Fig 3A). These data suggest that the brain mounts a predominantly pro-inflammatory, rather than a robust antiviral IFN, response to SADS-CoV infection. In the small intestine, a markedly different pattern was observed. Both IFN-related genes (IFN- β and IFN- λ) and pro-inflammatory cytokines (TNF- α , IL-6, and CCL7) were significantly upregulated at 7 dpi in mice infected with wild-type SADS-CoV (Fig 3B). In contrast, the Ns7 mutants showed earlier induction of ISG15, Mx1, and CXCL10 at 5 dpi, even in the absence of robust IFN- β /IFN- λ upregulation, suggesting that the expression of these ISGs might be regulated independently of *de novo* IFN production (25–27). Thus, mutation of Ns7 shifts the intestinal immune response toward an earlier antiviral state, while wild-type infection leads to a delayed but exaggerated inflammatory response in the late stage. Collectively, these findings indicate that the SADS-CoV Ns7a/7b accessory protein contributes to virulence by differentially regulating interferon and pro-inflammatory responses *in vivo*.

Replication dynamics of SADS-CoV Ns7 variants in cells and organoids SADS-CoV Ns7 variants exhibited reduced replication efficiency *in vivo* compared to the wild-type strain. To further clarify whether the absence of Ns7a/7b affects viral replication *in vitro*, human Caco2 cells and porcine IPEC-J2 cells were infected with the wild-type SADS-CoV and the four rSADS-CoV Ns7 variants at an MOI=0.1, and viral titers in the supernatant were measured (Fig 4A). Our results showed that rSADS- Δ Ns7, rSADS- Δ Ns7b and rSADS-BtNs7 had lower replication efficiency in the IPEC-J2 and Caco2 cell lines than SADS-CoV and rSADS- Δ 58nt. Furthermore, rSADS- Δ 58nt exhibited reduced replication efficiency in IPEC-J2 cells relative to wild-type SADS-CoV, whereas no significant difference was observed between rSADS- Δ 58nt and wild-type virus in Caco-2 cells. To better mimic the *in vivo* microenvironment, we further tested susceptibility and replication efficiency of rSADS- Δ Ns7 and rSADS- Δ Ns7b in swine lung and intestinal organoids. As shown in

Fig 4B, SADS-CoV resulted in substantially increase viral loads, whereas rSADS- Δ Ns7 and rSADS- Δ Ns7b exhibited significantly reduced replication efficiency in lung and ileum organoids. Consistent with the viral replication kinetics, the expression levels of the SADS-CoV N protein in lung organoids infected with wild-type virus was significantly higher than those infected with rSADS- Δ Ns7 and rSADS- Δ Ns7b (Fig 4C). Given that previous studies have reported significant cell death induced by SADS-CoV infection, we further quantified virus-induced cell death triggered by rSADS- Δ Ns7, rSADS- Δ Ns7b and SADS-CoV infection by measuring the release of lactate dehydrogenase (LDH) in the supernatant of infected organoids. We observed that SADS-CoV infection induced a higher LDH release both in swine lung and ileum organoids compared to that induced by the mutant strains (Fig 4E). Taken together, these findings demonstrate that the absence of Ns7a/7b attenuates SADS-CoV replication and reduces virus-induced cytotoxicity, which is consistent with the in vivo attenuation phenotype observed earlier.

Different expression of cytokine-related pathway and host metabolic induced by SADS-CoV Ns7 variants in organoids To further understand the biological processes affected by different SADS-CoV Ns7 variants, we performed bulk RNA sequencing (RNA-seq) on ileum organoids infected with wild-type SADS-CoV, rSADS- Δ Ns7, or rSADS- Δ Ns7b at an MOI of 0.1 at 48 hpi. The gene expression volcano plot showed that wild-type infection triggered a robust transcriptional alteration, with 401 upregulated and 294 downregulated genes. In contrast, both rSADS- Δ Ns7 and rSADS- Δ Ns7b mutants induced only minimal gene expression differences (Fig 5A). While extracellular matrix organization was a common GO term enriched among all infection groups, “negative regulation of cell population proliferation” was not enriched in rSADS- Δ Ns7- or rSADS- Δ Ns7b-infected groups (Fig 5B). This result aligns with our previous observation that these mutant strains induce milder clinical symptoms compared to wild-type SADS-CoV. We further profiled the host response in virus-infected swine organoids using scRNA-seq at an MOI of 0.1 at 48 hpi. Following data processing, we performed cell type identification, which revealed major intestinal cell types, including proliferative epithelial cells, regenerative enterocytes, enterocytes, goblet cells, and tuft cells (Fig 5C). This confirms that our organoid system accurately recapitulates the primary cellular composition of the in vivo intestinal epithelium(28). Although viral infection was detectable in multiple cell types, SADS-CoV and its Ns7 variants exhibited a pronounced tropism for enterocytes (Fig 5D), consistent with in vivo findings. Virus-infected cells were first defined by the detection of at least two distinct SADS-CoV genes. Subsequently, we determined the transcriptional profiles of these cells. This

comparison identified 8 commonly upregulated genes (RPS5, TXNIP, OLFM4, IER3, TEX14, ZAN, SCAMP2, ACTG1) and 8 commonly downregulated genes (MRPL57, PHLDA2, ENSSSCG00000024388, FASN, SCD, FADS2, ASCL2, IGFBP2), with the most pronounced dysregulation occurring during wild-type SADS-CoV infection (Fig 5E). Among the upregulated genes, TXNIP, IER3, and OLFM4 are known to be involved in inflammatory and stress responses (Fig 5F). Conversely, the downregulated genes included multiple fatty acid synthesis genes (FASN, SCD, FADS2), suggesting a disruption of lipid metabolism, consistent with previous reports on coronavirus infection (Fig 5F). GO enrichment revealed that the upregulated gene modules in the wild-type-infected group were enriched for several inflammatory response pathways, including leukocyte activation and transforming growth factor beta (TGF- β) production. In contrast, the downregulated modules were predominantly associated with energy metabolism-related pathways, such as ATP biosynthesis, glucose metabolism, and long-chain fatty acid transport (Fig 5G). However, the expression of genes involved in these pathways remained largely unchanged in cells infected with the rSADS- Δ Ns7b or rSADS- Δ Ns7 mutant (Fig 5H). Importantly, we found that the expression levels of cytokine-related pathway genes, including interleukin (IL), chemokine, and IL receptor genes, were significantly upregulated in the wild-type-infected group, whereas rSADS- Δ Ns7 and rSADS- Δ Ns7b infection failed to induce the expression of these genes (Fig 5I). In addition, comparison of wild-type SADS-CoV and rSADS- Δ Ns7b infection revealed that wild-type virus induced more pronounced activation of the Wnt signaling pathway and a stronger cell adhesion response (Fig 5J and 5K).

CONCLUSIONS:

In this study, we found that the loss of Ns7 or Ns7b expression significantly attenuates SADS-CoV, completely abolishing its lethality in suckling mice. Ns7 mutants also exhibited lower replication efficiency in swine cell lines and organoids, along with reduced cytokine-related pathway activation in intestinal organoids. These findings suggest that some bat SADSr-CoVs harboring a natural Ns7b deletion likely have low intrinsic pathogenicity. Therefore, the targeted deletion of virulence determinants represents a promising strategy for developing live-attenuated vaccines against SADS-CoV and bat SADSr-CoVs.



Abstract No.: 363

Poster Code: : E-0053

SARS-CoV-2 Omicron EG.5 and JN.1 induce enhanced pathogenicity in K18-hACE2 mice compared with the early Omicron subvariants

First Author: Tsoi Man Mandy Ho, The University of Hong Kong

Co-Author(s): Nigeer Te, Kenrie Pui Yan Hui, Rachel Hiu Ha Ching, John Chi Wang Ho, Samuel Mo Sheung Cheng, John Malcolm Nicholls, Michael Chi Wai Chan, Leo Lit Man Poon, Alex Wing Hong Chin

BACKGROUND OBJECTIVES:

The K18-hACE2 transgenic mouse model has long been a cornerstone of our efforts to study SARS-CoV-2 pathogenesis and evaluate medical countermeasures. However, as the virus evolved, early Omicron subvariants like BA.1 and BA.2 exhibited significantly reduced pathogenicity in these mice, rendering the model less effective for critical research. Recognizing this limitation in the context of the endemic phase of COVID-19, our work characterizes the pathogenicity of newer Omicron lineages—specifically EG.5 and JN.1—in the K18-hACE2 model. We aimed to determine if these subvariants could restore the model's utility. By comparing their virulence to ancestral and early Omicron strains, we sought to provide the scientific community with an updated, reliable infection model capable of supporting the ongoing development of vaccines and antiviral therapies.

METHODS:

To investigate this, 6-8-week-old K18-hACE2 mice were intranasally infected with 10^4 PFU of the ancestral strain (WT) or various Omicron subvariants, including BA.2, BA.2.86, JN.1, and EG.5, using PBS as a mock control. We conducted daily monitoring for 10 days, assessing clinical severity such as weight loss and respiratory function. To quantify viral kinetics and tissue-specific impact, we harvested lung, brain, and nasal turbinate tissues at 2 and 5 days post-infection (dpi). We determined viral titers through TCID₅₀ assays and assessed histopathological damage via hematoxylin and eosin staining. Furthermore, we have done immunohistochemistry to detect SARS-CoV-2 nucleocapsid protein and performed RT-qPCR to measure mRNA expression of key inflammatory markers, including IFN β , IL-6, RANTES, and TNF, in both lung and brain tissues to evaluate

the immune response.

RESULTS:

Our study revealed marked differences in pathogenicity among the lineages. Mice infected with the ancestral WT strain exhibited severe disease, including significant weight loss and uniform lethality within 7 days. In contrast, the BA.2 and BA.2.86 subvariants resulted in minimal clinical impact. Interestingly, while JN.1 caused moderate illness, the EG.5 lineage demonstrated high pathogenicity remarkably similar to the ancestral WT strain. Mice infected with EG.5 experienced substantial weight reduction and 100% mortality by 8 dpi. Our data further showed that EG.5-infected mice maintained higher viral titers in the lungs at 5 dpi and exhibited neurotropism, evidenced by viral presence in the brain. This heightened virulence was mirrored at the molecular level, as we observed significantly upregulated cytokine and chemokine expression in the brain tissues of EG.5-infected animals, directly correlating with the severe clinical outcomes we documented.

CONCLUSIONS:

Based on our findings, we concluded that the pathogenicity of circulating Omicron subvariants is not uniform. Specifically, the EG.5 lineage represents a significant evolution in virulence within the K18-hACE2 model. By demonstrating that EG.5 induces severe, lethal disease comparable to ancestral strains, we have validated its potential as an updated and effective model for future SARS-CoV-2 research. We believe this is a crucial development, as it allows researchers to once again utilize the K18-hACE2 mouse platform to reliably test the efficacy of current and next-generation vaccines and therapeutics. Our work highlights the necessity of continuous monitoring of viral evolution and emphasizes how specific subvariants can restore the relevance of established laboratory models. Ultimately, we hope this model will facilitate the ongoing efforts to address the endemic threats posed by SARS-CoV-2 variants.



Abstract No.: 397

Poster Code: : E-0054

Attenuated Pathogenicity and Transmission of Pangolin-Derived Sarbecoviruses Compared with SARS-CoV-2

First Author: Mei-Qin Liu, Guangzhou National Laboratory

Co-Author(s): Hao-Feng Lin, Ren-Di Jiang, Li-Na Ji, Qian-Chun Gong, Jin-Wei Zhang, Jian-min Li

BACKGROUND OBJECTIVES:

Sarbecoviruses related to SARS-CoV-2 have been detected in Malayan pangolins, but their pathogenic potential and transmission risk remain unclear. Previous studies showed that pangolin-derived viruses MpCoV-GD and MpCoV-GX can use human ACE2 as a receptor and replicate in human cells. However, no comprehensive comparison of these two viruses with SARS-CoV-2 has been performed. Therefore, this study aimed to compare MpCoV-GD, MpCoV-GX, and SARS-CoV-2 in terms of cellular entry mechanisms, infectivity in human cell lines and organoids, pathogenicity and transmission in K18-hACE2 transgenic hamsters, and cross-protection by existing SARS-CoV-2 immunity and therapeutics.

METHODS:

This study compared MpCoV-GD, MpCoV-GX, and SARS-CoV-2 using multiple approaches. Cell-cell fusion assays measured spike-mediated membrane fusion via split GFP and luciferase. Attachment and internalization were tested by binding at 4°C and pronase treatment, followed by RT-qPCR. Protease dependency was evaluated by overexpressing furin, TMPRSS2, and cathepsin L in 293T-hACE2 cells and treating with specific inhibitors. Virus replication was assessed by one-step growth curves in VeroE6, Calu-3, Caco-2, and Huh7 cells. Human nasal, lung, intestinal, and colon organoids were infected and analyzed by immunofluorescence and viral titration. In vivo pathogenicity was tested in K18-hACE2 hamsters intranasally inoculated with 1×10^5 TCID₅₀ of each virus, monitoring body weight, mortality, tissue viral loads by plaque assay, and lung histopathology. Direct contact transmission was assessed by co-housing infected index hamsters with naïve hamsters. Aerosol transmission was tested using separate mesh cages with directional airflow. Antiviral efficacy of remdesivir and molnupiravir was measured

by qRT-PCR. Neutralizing activity of convalescent sera, vaccine-immune sera, and monoclonal antibodies (S309, REN10933, REN10987) was determined by plaque reduction neutralization test (PRNT₅₀).

RESULTS:

Cell-cell fusion assays showed SARS-CoV-2 spike mediated the strongest membrane fusion, followed by MpCoV-GD, then MpCoV-GX. Attachment and internalization assays revealed SARS-CoV-2 had the highest attachment capacity, and MpCoV-GD showed higher internalization than MpCoV-GX. One-step growth curves demonstrated SARS-CoV-2 and MpCoV-GX replicated more efficiently than MpCoV-GD. Protease overexpression and inhibition assays indicated all three viruses required host proteases for entry. Human organoid infection and titration showed SARS-CoV-2 replicated most efficiently, with MpCoV-GX replicating better than MpCoV-GD. K18-hACE2 hamster infection, weight monitoring, plaque assays, and histopathology showed MpCoV-GD caused severe lung pathology and 100% mortality similar to SARS-CoV-2, while MpCoV-GX caused mild inflammation and delayed death. Direct contact transmission experiments showed all three viruses transmitted via direct contact. Aerosol transmission experiments showed SARS-CoV-2 transmitted efficiently, but pangolin viruses showed limited aerosol transmission. Antiviral treatment with remdesivir and molnupiravir inhibited all three viruses. Plaque reduction neutralization tests showed convalescent and vaccine sera neutralized both pangolin viruses; S309 showed broad neutralization, while REN10933/10987 neutralized only MpCoV-GD.

CONCLUSIONS:

Both pangolin-derived sarbecoviruses can infect human cells and organoids but with lower efficiency than SARS-CoV-2. MpCoV-GD exhibits pathogenicity comparable to SARS-CoV-2 in K18-hACE2 hamsters, while MpCoV-GX is milder. Both pangolin viruses transmit via direct contact but have significantly reduced aerosol transmissibility. Existing SARS-CoV-2 vaccines and antivirals provide partial cross-protection against these viruses. Continuous surveillance of pangolins and other wildlife is warranted to prevent future zoonotic spillover events.



Abstract No.: 398

Poster Code: : E-0055

Comparative pathology of SARS-CoV-1 and SARS-CoV-2 infection in the golden Syrian hamster model

First Author: Javier Salguero, UK Health Security Agency

Co-Author(s): Yper Hall, Kevin Bewley, Lizzie Davies, Ines Ruedas-Torres, Laura Hunter, Kathryn Ryan

BACKGROUND OBJECTIVES:

Severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) and SARS-CoV-2 are responsible of high-consequence respiratory disease for the 2002-2004 SARS outbreak and the COVID19 pandemic respectively. The golden Syrian hamster model has been a very valuable tool to investigate the pathogenesis of these coronavirus diseases and carry out preclinical testing of vaccines or antiviral therapies. In this study, we evaluate and compare the respiratory pathology induced by both viruses in this animal model.

METHODS:

We have infected Syrian golden hamsters via intranasal route with different SARS-CoV-1 and SARS-CoV-2 strains/variants. Animals were culled at different time points after challenge (between 1 and 28 dpc). Samples from the lower and upper respiratory tract, including lung, trachea and nasal turbinates, were fixed in buffered formalin and embedded in paraffin wax. Tissue sections were stained with H&E for histopathological analysis, ISH-RNAScope for the detection and quantification of virus RNA and mRNA of pro-inflammatory cytokines and IHC multiplex IF to evaluate the inflammatory cell populations participating in the development of the lesions (macrophages, T cells, B cells and heterophils).

RESULTS:

Similar lesions were found for both SARS-CoV-1 and SARS-CoV-2 infected with highly virulent variants. Initial (1-3 dpc) pulmonary lesions consisted of bronchiolar epithelial necrosis associated with infiltration of inflammatory cells within the alveolar and airway spaces. Inflammatory cells were mainly polymorphonuclear heterophils at the beginning followed by the development of a severe broncho-

interstitial pneumonia (4-6dpc), with abundant macrophages and T cells within the lesioned area. Pulmonary lesions were still marked at 7 dpc, while virus RNA was almost cleared by that time point. Only minimal residual lesions were observed at 28dpc. Similar evolution was found in the upper respiratory tract, with severe necrosis followed by inflammatory cell infiltration and severe exudates, that persisted for 7 days after challenge but were cleared at 28dpc. Interestingly, there was no virus RNA detected in nerve terminations within the olfactory mucosa or the rostral brain. Other low pathogenic SARS-CoV-2 variants showed milder lesions and presence of virus RNA in the lower and upper respiratory tract.

CONCLUSIONS:

The golden Syrian hamster is a highly valuable model to study pathogenic coronavirus disease, mimicking the main respiratory lesions observed in acute severe infections in humans.



Abstract No.: 422

Poster Code: : E-0056

Human respiratory tract organoids reveal the divergent pathogenesis between human coronaviruses OC43 and SARS-CoV-2

First Author: Jingshu Zhang, Erasmus MC

Co-Author(s): Anna Z. Mykytyn, Sem Koornneef, Tim I. Breugem, Debby Schipper, Petra B. van den Doel, Corine H. GeurtsvanKessel, Rory D de Vries, Theo Bestebroer, Bas B. Oude Munnink, Berend-Jan Bosch, Mart M. Lamers, Robbert Rottier, Bart L. Haagmans

BACKGROUND OBJECTIVES:

The emergence of SARS-CoV-2 in 2019 led to a global pandemic, and its persistence in the human population is anticipated for the foreseeable future. The common cold human coronavirus OC43, on the other hand, has been circulating in the human population for more than 100 years. Despite both viruses having zoonotic origins and targeting the human respiratory tract, they cause very different clinical outcomes, with OC43 causing only mild symptoms and occasional small outbreaks. The factors driving the differential pathogenesis between SARS-CoV-2 and OC43 have not yet fully been understood.

METHODS:

To investigate the respiratory tract replication competence and cellular tropism of OC43 compared to SARS-CoV-2, clinical OC43 strains were isolated from patient nasal swabs using human bronchiolar organoid-derived cultures. Human nasal organoids and human alveolar type 2 (AT2) organoids were used to assess viral replication in the upper and lower respiratory tract, respectively.

RESULTS:

OC43 replicated efficiently in human nasal organoids, albeit to a lesser extent and with greater donor-specific variations compared to SARS-CoV-2. Similar to SARS-CoV-2, OC43 mainly targeted ciliated cells in the nasal and bronchiolar organoids. OC43 did not replicate in human AT2 organoids, whereas SARS-CoV-2 did show robust replication. Histochemical analysis of human respiratory tract tissues showed a high abundance of OC43 receptor expression in the nose, trachea, and bronchus, but not in the alveoli. This differential receptor distribution likely accounts for the restricted permissiveness of the alveolar epithelium to OC43 infection.

CONCLUSIONS:

Our results elucidate the differential tissue tropism of OC43 within the human respiratory tract compared to highly pathogenic SARS-CoV-2. The limited permissiveness of the alveolar epithelium to OC43 may be linked to its mild pathogenesis.



Abstract No.: 429

Poster Code: : E-0057

SARS-CoV-2 induced reprogramming of retinal glucose metabolism: mechanisms of ocular pathogenesis and therapeutic implications

First Author: Yuanchen LIU, The University of Hong Kong

BACKGROUND OBJECTIVES:

About 10% of people recovering from SARS-CoV-2 infection have symptoms lasting more than three months. These post-acute sequelae of COVID-19 (PASC, or long COVID) increasingly involve the eye. Retinal complications occur during acute infection and in long COVID, but their mechanisms remain unclear. In this study, we established a SARS-CoV-2 infection model using human retinal organoids (ROs). Integrated transcriptomic and metabolomic analyses revealed that infection with the SARS-CoV-2 Omicron variant caused markedly more severe infection in ROs than the ancestral wild-type strain. Omicron infection was associated with a marked reduction in glucose levels in the culture supernatant. These findings are indicative of metabolic reprogramming toward enhanced glycolysis. The retina is a highly metabolically active neural tissue that depends on tightly regulated glucose metabolism and mitochondrial function to sustain normal physiological activity. Disruption of retinal metabolic homeostasis can trigger oxidative stress, inflammatory responses, cellular injury, and visual dysfunction. Although retinal abnormalities have been observed in patients with COVID-19, the mechanistic link between coronavirus infection and retinal damage, particularly from a metabolic perspective, has not been systematically elucidated. Clarifying the metabolic basis of virus-induced retinal injury is therefore of both fundamental scientific interest and clear clinical relevance. This study aims to delineate the roles of glycolytic reprogramming in retinal infection and to assess whether metabolic interventions can attenuate viral replication and protect retinal cells. Our findings will deepen understanding of virus-host metabolic interactions and may help identify potential therapeutic strategies for ocular complications associated with viral infection.

METHODS:

1. Virus. Ancestral SARS-CoV-2 and Omicron subvariants (e.g., BA.5.2, XFG) were isolated from COVID-19 patients in Hong Kong. Viruses will be propagated and titrated using plaque assays on VeroE6-TMPRSS2 cells as previously described (33). Viral stocks will be aliquoted and stored at -80°C until use.

2. Quantitative RT-PCR (qRT-PCR). Total RNA will be extracted from retinal organoids and hamster retinal tissues using TRIzol reagent. Reverse transcription will be performed with the Transcriptor First Strand cDNA Synthesis Kit (Roche). qRT-PCR will be conducted using LightCycler 480 master mix (Roche) to quantify viral RNA (SARS-CoV-2 RdRp gene), proinflammatory cytokines (e.g., IL-6, TNF- α), interferon-stimulated genes (ISGs), and metabolic enzymes. Relative expression levels will be normalized to housekeeping genes (e.g., β -actin) and analyzed by the $\Delta\Delta C_t$ method.

RESULTS:

We first established retinal organoids from human embryonic stem cells (hESCs) as an experimental platform to investigate SARS-CoV-2 infection in retinal tissue. The organoids expressed key retinal markers, including OTX2, RCVRN, and TFAP2A, and RNA-sequencing analysis further confirmed retinal identity by showing reduced expression of pluripotency-associated genes together with robust expression of retinal cell-specific markers. Using this model, we found that both SARS-CoV-2 WT and Omicron BA.5.2 efficiently infected and replicated in retinal organoids, with BA.5.2 showing a slightly higher replication level. Infection by either strain induced a strong pro-inflammatory response, as indicated by elevated levels of TNF- α , IFN- α , IL-6, IL-8, IL-12, and CCL2. Transcriptomic profiling revealed that infected organoids were enriched for pathways associated with neuroinflammation, pyruvate metabolism, oxidative phosphorylation, COVID-19, and type I diabetes mellitus, while genes related to diabetic retinopathy and inflammatory responses were also differentially expressed across mock- and virus-infected groups. Further multi-omics analyses showed that SARS-CoV-2 infection caused marked metabolic reprogramming in retinal organoids. In particular, pathways involved in glucose, pyruvate, and lactate metabolism were significantly altered, together with broader metabolic programs including glycolysis, the TCA cycle, and oxidative phosphorylation. Metabolomic analysis supported these findings by identifying dysregulation of metabolites involved in glucose utilization, amino acid catabolism, and TCA cycle activity. Notably, these infection-associated metabolic features resembled aspects of the metabolic dysfunction observed in age-related macular degeneration. To validate these observations in vivo, we used a Syrian hamster model and detected both viral RNA and infectious virus in retinal tissues after intranasal infection, confirming that SARS-CoV-2 can reach and infect the retina. Infected animals showed reduced expression of the

photoreceptor-associated marker RCVRN, persistent upregulation of inflammatory cytokines, and structural disruption of retinal layers, indicating sustained retinal injury. Importantly, treatment with Paxlovid reduced ocular viral replication and attenuated the enrichment of metabolic pathways linked to the Warburg effect, pyruvate metabolism, and lactate signaling, suggesting that antiviral intervention can partially reverse the metabolic abnormalities associated with SARS-CoV-2-induced retinal pathology.

CONCLUSIONS:

In conclusion, our study establishes hESC-derived retinal organoids as a relevant model for investigating SARS-CoV-2 retinal infection. We show that both WT and Omicron BA.5.2 can infect retinal tissue, triggering persistent inflammation and marked metabolic reprogramming. These alterations, which were further validated in vivo in hamsters, are associated with retinal structural and functional damage. Importantly, Paxlovid treatment reduced viral burden and alleviated infection-associated metabolic disturbances. Together, our findings suggest that SARS-CoV-2 contributes to retinal pathology through inflammatory and metabolic mechanisms, highlighting metabolic reprogramming as a potential therapeutic target.

Abstract No.: 34

Poster Code: : F-0058

Cell death and innate immunity in coronavirus infection

First Author: Hin Chu, The University of Hong Kong

Co-Author(s):

BACKGROUND OBJECTIVES:

Infection by coronaviruses including SARS-CoV-1, MERS-CoV, and SARS-CoV-2 result in substantial cell death in cell culture and in patient samples, suggesting a potential link between cell death and coronavirus pathogenesis. In our earlier studies, we showed that coronavirus infection triggers apoptosis, which contributes to virus-induced lung damage. We further revealed that coronaviruses exploit a component of the host apoptosis cascade, caspase-6, to enhance virus replication. More recently, we found that coronavirus infection concurrently activates both apoptosis and necroptosis but the role of necroptosis in coronavirus biology remains unknown.

METHODS:

We investigated the contribution of necroptosis in coronavirus-induced cell death, pro-inflammatory response, and pathogenesis upon the infection of SARS-CoV-1, MERS-CoV, and SARS-CoV-2 using cell lines, ex vivo human lung cultures, and different animal models.

RESULTS:

We demonstrated that coronavirus infection activates both apoptosis and necroptosis in the infected cells. We found that both pathway contributes to coronavirus-induced cells death and that simultaneous inhibition of both apoptosis and necroptosis is required to optimally inhibit coronavirus-induced cell damage. In parallel, coronavirus-triggered pro-inflammatory response is mediated by necroptosis but not apoptosis. Necroptosis inhibition alone significantly promoted the survival of coronavirus-infected animals.

CONCLUSIONS:

Coronavirus-induced necroptosis contributes to pathogenesis and disease severity. Targeting coronavirus-induced necroptosis should be explored as a novel strategy against coronavirus infection.

Abstract No.: 38

Poster Code: : F-0059

A Single-Cell Transcriptomics Approach to Compare Coronavirus Infection in Human and Camelid Primary Airway Epithelial Cells

First Author: Vera Flück, Institute for Virology and Immunology

Co-Author(s): Mitra Gultom, Annika Kratzel, Sarah Ochsenbein, Corinne Gurtner, Horst Posthaus, Patrik Zanolari, Christian Drosten, Simon Schröder, Jan Felix Drexler, Marcel Müller, Ronald Dijkman, Volker Thiel, Jenna Kelly

BACKGROUND OBJECTIVES:

MERS-CoV is a zoonotic coronavirus that can lead to severe respiratory illness in humans, but only causes mild respiratory illness in dromedary camels, which are a major reservoir host for MERS-CoV. To identify species-specific host factors that may influence disease outcomes in humans and camelids, airway epithelial cell (AEC) culture models were developed for *C. bactrianus* and *L. glama*.

METHODS:

Single-cell transcriptomics was performed on human, camel, and llama AEC cultures infected with MERS-CoV, HCoV-229E, and DcCoV-229E. HCoV-229E belongs to the common cold coronaviruses and causes mild symptoms in humans, while DcCoV-229E is a phylogenetically related virus, which was isolated from dromedary camels. To compare the response to CoV infection among different host species, we established an extensive bioinformatics pipeline in R, that is compatible with camelid species and allowed functional analysis.

RESULTS:

Using this workflow, we were able to annotate not only the human airway epithelial cells in our samples but also the main cell types of the camelid species. Additionally, we investigated how host cell tropism and viral entry receptor expression presented under uninfected and virus-infected conditions. Intriguingly, we found that viral entry receptor expression changes upon MERS-CoV and dcCoV-ACN4 infection and that these changes might play a major role in the manifestation of the host cell tropism for both viruses. Furthermore, we assessed the differential gene expression patterns between infected and uninfected cells

in each species and airway epithelium cell type. We discovered that several genes involved in the unfolded protein response network, cilium organization and early anti-viral response were upregulated in MERS-CoV-infected ciliated cells in both human and camelids.

CONCLUSIONS:

Notably, this work represents the first steps in generating a comprehensive framework to analyze and identify the fundamental characteristics of virus-host interactions in the respiratory epithelium of well and less-studied species with single-cell resolution.



Abstract No.: 40

Poster Code: : F-0060

MERS-CoV infection dynamics, immune responses and viral persistence in an insectivorous bat model

First Author: Arinjay Banerjee, University of Saskatchewan

Co-Author(s): Kaushal Baid, Sauhard Shrivastava, Akarin Asavajaru, Arkadeb Bhuinya, Arianna Hurtado, Kim R. Chiok, Shane Seheult, Darryl Falzarano, Paul A. Faure

BACKGROUND OBJECTIVES:

Middle East respiratory syndrome coronavirus (MERS-CoV) is a zoonotic virus first identified in Saudi Arabia in 2012 and is associated with severe respiratory disease in humans. Vesper bats, which harbor betacoronaviruses, are hypothesized to be ancestral reservoirs of MERS-CoV. Despite the identification of MERS-CoV-related viruses in vesper bats, the infection dynamics of MERS-CoV in these species remain poorly understood. In this study, we experimentally infected *Eptesicus fuscus*, an insectivorous vesper bat, with MERS-CoV to investigate viral replication, shedding, clinical symptoms, pathology, and the development of antiviral immune responses.

METHODS:

E. fuscus bats were infected or mock-infected with 10^5 TCID₅₀/mL of MERS-CoV. Bats were sampled at 2-, 4-, 7-, and 14-days post-infection. Clinical parameters, including body weight and temperature were monitored throughout the study. Tissues were examined for gross pathology. We evaluated antiviral gene expression using qPCR and bulk RNA sequencing. Virus-specific neutralizing antibody titers were assessed by micro-neutralization assays, and we confirmed viral protein detection using immunohistochemistry (IHC) and whole tissue section scans.

RESULTS:

Infected bats showed no signs of weight loss or elevated body temperature during the study period. MERS-CoV RNA was detected in samples from infected bats, confirming viral replication. Gene expression analyses revealed upregulation of interferon-related genes, indicating activation of an early antiviral response.

No gross lung pathology or overt tissue damage was observed in infected animals, although IHC identified distinct areas of virus infection. Infected bats developed virus-specific B cell responses, including the production of neutralizing antibodies.

CONCLUSIONS:

MERS-CoV can productively infect *E. fuscus* bats without inducing clinical disease or lung pathology, despite evidence of viral replication and antiviral immune activation. These findings support the use of *E. fuscus* as a viable experimental model for studying MERS-CoV and MERS-CoV-related viruses, especially to study infection dynamics, tissue tropism, and host immune response. This model may provide critical insights into mechanisms of viral tolerance and persistence in reservoir hosts.



Abstract No.: 67

Poster Code: : F-0061

Cell Type-Dependent Genetic Stability of EGFP in Recombinant HCoV-229E

First Author: Aikeda AISHAJIANG, The University of Tokyo

Co-Author(s): Yuki Kitai, Shohei Kojima, Rina Hashimoto, Miyuki Kawase, Hiroshi Katoh, Kazuya Shirato, Kazuo Takayama, Makoto Takeda

BACKGROUND OBJECTIVES:

Human coronavirus 229E (HCoV-229E) is a positive-sense RNA virus belonging to the family Coronaviridae and causes common cold symptoms in humans. Its genome encodes the conserved open reading frames (ORF1ab, S, E, M, and N) and an accessory gene, ORF4. To study viral replication, we generated a recombinant HCoV-229E expressing enhanced green fluorescent protein (EGFP) by inserting the EGFP gene with a 2A peptide between ORF4 and the S gene. This system enables visualization of viral replication in different cell culture models. However, deletion mutations in the EGFP gene were observed during propagation in certain cell types, suggesting that the stability of foreign gene insertion in the HCoV-229E genome may depend on the host cell environment. Therefore, we investigated the genetic stability of the recombinant virus in multiple cell culture systems.

METHODS:

To evaluate the stability of the inserted EGFP gene, the recombinant HCoV-229E/1121-EGFP virus was propagated in multiple cell culture systems, including human airway epithelial cells cultured at air-liquid interface (ALI), human airway organoids, LLC-MK2 cells, and BHK cells. EGFP expression and viral replication were monitored over time, and the integrity of the EGFP region was analyzed after serial passage or long-term culture.

RESULTS:

Deletion mutations in the EGFP gene occurred rapidly when the recombinant virus was propagated in human primary respiratory epithelial cells under ALI

culture conditions. In contrast, in human airway organoids, EGFP expression remained stable for more than 30 days, and deletions began to appear only after prolonged culture (around day 40 in a 56-day experiment). When the virus was serially passaged up to ten times in LLC-MK2 cells (approximately 30 days in total), no deletion was detected and viral replication efficiency was maintained. However, deletion mutations in the EGFP gene appeared after only one passage in BHK cells. These results indicate that the genetic stability of the inserted EGFP sequence varies markedly depending on the host cell type.

CONCLUSIONS:

These findings demonstrate that the stability of foreign gene insertions in recombinant HCoV-229E is highly dependent on the cellular environment. While the recombinant virus remains genetically stable in organoids and certain cell lines, other cell types promote rapid genomic deletion. Further analysis using additional cell systems may help clarify the mechanisms regulating coronavirus genome stability. This recombinant virus system provides a useful tool for investigating the interaction between coronavirus genome integrity and host cell factors.



Abstract No.: 72

Poster Code: : F-0062

A mouse model of prolonged SARS-CoV-2 infection reveals viral evolution and therapeutic efficacy

First Author: Tomokazu Tamura, Kyushu University

Co-Author(s): Takaya Ichikawa, Lei Wang, Shuhei Tsujino, Rigel Suzuki, Kento Mori, Saori Suzuki, Masumi Tsuda, Masaaki Murakami, Naganori Nao, Shinya Tanaka, Takasuke Fukuhara

BACKGROUND OBJECTIVES:

Prolonged SARS-CoV-2 infection is increasingly recognized in severely immunocompromised patients, such as those with hematologic malignancies or receiving immunosuppressive therapy. These patients may shed infectious virus for extended periods, creating clinical management challenges and providing a potential environment for viral evolution and resistance mutations. However, the mechanisms underlying viral persistence and optimal treatment strategies remain poorly understood due to the limited availability of appropriate experimental models. The objective of this study was to establish a small-animal model that recapitulates prolonged SARS-CoV-2 infection and to investigate host responses, intra-host viral evolution, and the efficacy of antiviral treatments during persistent infection.

METHODS:

To generate a model of prolonged infection, wild-type BALB/c mice, BALB/c-nu/nu (nude) mice, and C.B-17/1cr-scid/scid (SCID) mice were intranasally inoculated with mouse-adapted SARS-CoV-2 (MA10). Viral loads and infectious titers in lung tissues were monitored over time. Host responses were analyzed by serum cytokine/chemokine profiling and lung transcriptome analysis using RNA sequencing. To assess intra-host viral evolution, viral genomes from infected lung tissues were analyzed by deep sequencing using a Nanopore platform. Finally, the utility of this model for therapeutic evaluation was examined by testing the antiviral efficacy of remdesivir and the neutralizing monoclonal antibody tixagevimab.

RESULTS:

SCID mice, which lack both B and T cells, maintained high levels of infectious virus in the lungs for up to 56 days post infection, whereas virus was cleared

within weeks in immunocompetent BALB/c mice and nude mice. Transcriptomic analyses revealed sustained activation of innate immune response pathways in the lungs of SCID mice during the late phase of infection, while systemic inflammatory responses were limited. Deep sequencing demonstrated a progressive increase in viral genetic diversity during prolonged infection, with several dominant mutations emerging at later time points. Therapeutic evaluation showed that remdesivir treatment reduced viral loads but failed to achieve viral clearance even after extended administration. In contrast, the neutralizing antibody tixagevimab provided strong prophylactic protection and eliminated infectious virus when administered therapeutically, provided the infecting variant remained susceptible to the antibody.

CONCLUSIONS:

We established a robust mouse model that reproduces key features of prolonged SARS-CoV-2 infection observed in immunocompromised patients. Our findings reveal persistent innate immune activation and progressive viral diversification during prolonged infection and demonstrate that neutralizing antibody therapy can effectively achieve viral clearance. This model provides a valuable platform for studying the molecular mechanisms of viral persistence and evaluating therapeutic strategies against prolonged SARS-CoV-2 infection.



Abstract No.: 83

Poster Code: : F-0063

Characterization of Nucleolar Localization Signals in the Avian Infectious Bronchitis Virus Nucleocapsid Protein and Their Critical Role in Viral Replication

First Author: Ye Zhao, China Agricultural University

Co-Author(s): Liwei Zhang, Yingfei Li, Jun Zhao, Rong Liang, Jing Zhao, Guozhong Zhang

BACKGROUND OBJECTIVES:

The nucleocapsid (N) protein of infectious bronchitis virus (IBV) is the most abundant viral protein in infected cells and is essential for virion assembly and for modulating host cellular processes. Although previous studies have reported nucleolar accumulation of IBV N, a comprehensive mapping of its nucleolar localization signals (NoLSs) is lacking. In this study, we systematically mapped functional NoLSs within the IBV N protein and evaluated their contributions to viral replication.

METHODS:

We analyzed the subcellular distribution of IBV N during infection and after transient expression. EGFP-tagged N protein truncation mutants were constructed and analyzed to identify nucleolar localization sequences (NoLSs). Alanine-scanning mutagenesis was performed to map the functional NoLSs and key residues responsible for nucleolar targeting. Recombinant viruses carrying selected NoLS mutations were rescued by reverse genetics and evaluated by in vitro replication assays.

RESULTS:

The N protein exhibited a dual cytoplasmic-nucleolar distribution under both infection and transfection conditions. Truncation analysis identified four candidate NoLSs (NoLS1–4) and a nuclear localization signal (NLS). Alanine-scanning mutagenesis revealed that NoLS3 (aa 330–349) and NoLS4 (aa 356–368) functioned as the primary nucleolar targeting determinants in the full-length N protein. Basic residues R337, K339, R341, R345, R349 in NoLS3, and K356, R359 in NoLS4 were essential for nucleolar localization. Notably, the double mutant virus

rYN-356A/359A targeting NoLS4 abolished nucleolar accumulation of N protein and exhibited significantly attenuated replication.

CONCLUSIONS:

In summary, we systematically identified and characterized two functional nucleolar localization sequences (NoLS3 and NoLS4) within the IBV nucleocapsid protein. Specific basic residues in these regions are critical for directing N protein to the nucleolus. Furthermore, abrogation of nucleolar targeting significantly impairs viral replication. These results demonstrate that nucleolar localization of the IBV N protein is essential for optimal virus proliferation, providing new insights into the functional role of N protein subcellular localization during IBV infection.



Abstract No.: 85

Poster Code: : F-0064

SLiM-Based Benchmarking of Positive Selection Detection Methods in SARS-CoV-2 Within-Host Evolution

*First Author: Mengting Li, The University of Hong Kong**Co-Author(s): Haogao Gu, Leo L.M. Poon*

BACKGROUND OBJECTIVES:

Understanding the intra-host evolution of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is critical for interpreting viral adaptation and immune escape. During prolonged infections—particularly in immunocompromised patients—the viral Spike gene may experience rapidly changing selective pressures that promote adaptive evolution. Detecting such adaptive mutations is challenging because most analytical frameworks were designed to identify fixed substitutions across divergent lineages rather than the low-divergence, polymorphism-dominated populations typical of intra-host viral evolution. Consequently, the performance of commonly used positive selection detection methods under such conditions remains poorly characterized.

METHODS:

To benchmark these methods under intra-host evolutionary scenarios, we conducted forward-in-time population genetic simulations using the SLiM framework. Simulation parameters were informed by empirical estimates of SARS-CoV-2 intra-host evolution, including severe transmission bottlenecks, realistic mutation and recombination rates, and a range of selection coefficients. Tree-sequence simulations generated error-free multiple sequence alignments representing viral populations evolving within hosts. Using these datasets as ground truth, we evaluated four classes of methods: phylogenetic codon substitution models (Codeml, FEL, MEME, and FUBAR), divergence-polymorphism tests (McDonald-Kreitman and HKA), site-frequency spectrum statistics (Tajima's D and Fay & Wu's H), and haplotype-based approaches (EHH and iHS). Performance was assessed using confusion matrices, precision-recall curves, and ROC analysis.

RESULTS:

Across datasets with extremely low sequence divergence ($>99.9\%$ sequence identity) and strong linkage among sites, method performance varied substantially. Codon substitution models showed high specificity but limited power under low divergence. Divergence-polymorphism tests exhibited reduced sensitivity due to elevated background polymorphism. Site-frequency spectrum statistics detected recent selective events but showed reduced specificity under severe bottlenecks. Haplotype-based approaches more readily detected recent sweeps but produced elevated false-positive rates due to strong linkage effects.

CONCLUSIONS:

None of the evaluated frameworks consistently performed well under intra-host viral evolutionary conditions. These results highlight a mismatch between the assumptions of classical substitution models—designed to detect fixed substitutions over long evolutionary timescales—and the polymorphism-dominated dynamics of intra-host viral populations. Integrating multiple analytical signals or developing models that explicitly account for linkage and polymorphism dynamics may improve detection of adaptive variants in rapidly evolving viral populations.



Abstract No.: 89

Poster Code: : F-0066

Persistent SARS-CoV-2 infection drives viral evolution of innate immune evasion

*First Author: Ann-Kathrin Reuschl, Queen Mary University of London**Co-Author(s): Linran Wang, Lucy G. Thorne, Khadija Khan, Alex Sigal, Greg J. Towers, Clare Jolly*

BACKGROUND OBJECTIVES:

SARS-CoV-2 adaptation to humans has led to distinct genotypes that facilitate escape from host immune responses and enhance transmission frequency. We have previously shown that dominant and emerging SARS-CoV-2 Variants of Concern (VOCs) have independently evolved to better antagonise human innate immunity during airway epithelial cell infection by increasing viral RNA and/or protein levels of innate immune antagonist proteins, including Orf6, nucleocapsid (N), and Orf9b. How dominant variants evolve these features remains poorly understood. We hypothesise that persistent long-term infection of immunocompromised individuals, rather than continuous transmission chains, promotes adaptation and transmission advantages. However, it is unknown whether SARS-CoV-2 evolves enhanced innate immune evasion during these persistent infections.

METHODS:

Here, we compared several replication competent longitudinal SARS-CoV-2 isolates (ancestral, Beta, Delta) recovered from HIV-infected immunocompromised patients who remained infected for several months. We assessed replication and innate immune activation of these isolates during infection of highly permissive Calu-3 lung epithelial cells as well as primary human airway epithelial cells.

RESULTS:

Strikingly, several features associated with variant dominance emerged within a single host during persistent infection. For example, we identified VOC-like adaptation between time points, included the SGF-deletion in Nsp6 found in most VOCs, altered expression of viral innate antagonists at the RNA and protein

level (including Orf6, Orf9b and Orf7), and altered resistance to spike-targeting host restriction factors. We mechanistically link these changes to improved innate immune evasion using reverse genetics viral mutants. Intriguingly, we show that modulation of viral innate antagonist expression, host antiviral immune defenses and interferon-sensitivity during epithelial infection did not necessarily occur in a step-wise manner from isolate to isolate.

CONCLUSIONS:

Our work suggests that coronaviruses can sample innate immune evasion configurations which provides a putative mechanism for VOC evolution of innate immune evasion during persistent infection and underlines the importance of treating immunodeficiency wherever it occurs as a globally important health intervention.



Abstract No.: 108

Poster Code: : F-0067

Investigating the Impact of SARS-CoV-2 Lineage-Defining Mutations Outside of Spike on Replication and the Host Innate Immune Response

First Author: Andrew Davidson, University of Bristol

Co-Author(s): Isobel Webb

BACKGROUND OBJECTIVES:

SARS-CoV-2 has undergone rapid genetic diversification since its emergence, giving rise to multiple lineages and variants of concern (VOCs). While many mutations in the Spike (S) protein affect antibody recognition and receptor binding, the contribution of mutations outside of S to viral replication and host responses remains less well understood. This study aimed to define the impact of non-S lineage-defining mutations on viral replication, fitness, and the host innate immune response.

METHODS:

A panel of recombinant SARS-CoV-2 VOCs (rVOCs) expressing the same S gene from the ancestral B.1 virus (containing the D614G mutation (termed rB.1)) were generated, for the VOCs Alpha, Beta, Gamma, Delta, and Omicron (BA.1 and BA.5). Viral replication, plaque morphology, and fitness were assessed in VeroE6-TMPRSS2 (VTN) and Calu-3 cells. Competition assays were performed in Calu-3 cells to evaluate relative fitness. The role of innate immune signalling in Calu-3 cells was examined using the JAK-STAT inhibitor ruxolitinib in combination with growth curve and transcriptomic analysis. The replication of the rVOC-B.1-S viruses and their parental VOCs was examined in K18-hACE2 mice followed by transcriptomic analysis of lung tissue at 5 days post-infection.

RESULTS:

All rVOC-B.1-S viruses replicated comparably to rB.1 in VTN cells. In contrast, rBeta-B.1-S and rBA.5-B.1-S showed significantly attenuated replication in Calu-3 cells. rAlpha-B.1-S, rBeta-B.1-S and rBA.5-B.1-S formed smaller plaques in VTN cells, indicating reduced cell-to-cell spread. Competition assays confirmed reduced

fitness of rBeta-B.1-S and rBA.5-B.1-S, which were rapidly outcompeted by rB.1. Attenuated replication of rBeta-B.1-S and rBA.5-B.1-S was rescued by inhibition of JAK-STAT signalling, implicating the interferon response as a key restriction factor. Transcriptomic analysis of Calu-3 cells infected +/- ruxolitinib demonstrated induction of antiviral genes at lower viral RNA levels for rBeta-B.1-S, suggesting that additional mechanisms contribute to attenuation. The rBeta-B.1-S, rBA.1-B.1-S and rBA.5-B.1-S viruses replicated to significantly lower titres in the lung, nasal tissue and brain of K18-hACE2 mice than rB.1 and did not cause significant weight loss. Transcriptomic analysis of murine lung tissue is in progress to establish if comparable transcription patterns are observed in vitro and in vivo.

CONCLUSIONS:

Non-S lineage-defining mutations significantly influence SARS-CoV-2 replication, fitness, and the host innate immune responses. These findings highlight the importance of considering mutations outside of the S protein in understanding variant biology and underscore their potential impact on viral pathogenicity and evolution.



Abstract No.: 112

Poster Code: : F-0068

Antibody-mediated selection drives the emergence of spike haplotypes resembling variants of concern during persistent SARS-CoV-2 infection

First Author: Rui Galao, King's College London

Co-Author(s): Luke B Snell, Suzanne Pickering, Jeffrey Seow, Carl Graham, Michael H. Malim, Katie J Doores, Gaia Nebbia, Stuart JD Neil

BACKGROUND OBJECTIVES:

Persistent SARS-CoV-2 infection in immunocompromised individuals provides a unique environment for within-host viral evolution under sustained but often incomplete immune pressure. While such infections have been implicated in the emergence of variants of concern (VOCs), the mechanistic links between intra-host diversification, spike mutational trajectories, and phenotypic consequences remain incompletely defined. This study aimed to resolve full-length spike haplotypes during prolonged infection and to integrate genomic and functional data to understand how antibody-mediated selection shapes viral evolution and phenotypic escape.

METHODS:

Longitudinal respiratory samples were obtained from individuals with prolonged SARS-CoV-2 infection, including a case exceeding 500 days. We applied a sequencing strategy capable of reconstructing full-length spike haplotypes, enabling assessment of mutation linkage and co-evolution within individual viral genomes. Evolutionary dynamics were analysed through temporal tracking of haplotypes and identification of positively selected residues. Phenotypic consequences were evaluated by mapping mutations onto known functional domains and antibody epitopes, alongside neutralisation assays assessing sensitivity to autologous and heterologous sera and monoclonal antibodies.

RESULTS:

Persistent infection was characterised by the emergence of multiple co-circulating spike haplotypes, with rapid turnover driven by selection at key antigenic sites.

Mutations accumulated in regions associated with receptor binding and antibody recognition, including recurrent substitutions observed in circulating VOCs. Haplotype reconstruction revealed epistatic combinations of mutations that would not be apparent from consensus sequencing, highlighting coordinated evolutionary pathways. Phenotypically, these variants displayed progressive reductions in neutralisation sensitivity, with stepwise acquisition of resistance to both patient-derived and broadly neutralising antibodies. In some cases, ancestral viruses evolved spike proteins exhibiting antigenic profiles converging on Omicron-like immune escape. Despite enhanced antibody evasion, certain variants showed evidence of constrained fitness, suggesting trade-offs between immune escape and viral entry efficiency or stability. Overall, the data support a model in which sustained antibody pressure drives diversification, followed by selection of mutation constellations that optimise escape while maintaining sufficient functionality.

CONCLUSIONS:

These findings provide mechanistic insight into how prolonged SARS-CoV-2 infection fuels the emergence of immune-evasive variants through antibody-driven selection acting on linked spike mutations. Integration of haplotype-resolved genomics with phenotypic analyses reveals that intra-host evolution proceeds through structured, stepwise pathways involving functional trade-offs. Persistent infections therefore represent critical reservoirs for the generation of antigenically advanced variants, underscoring the importance of therapeutic intervention and genomic surveillance in immunocompromised populations to limit evolutionary opportunities for the virus.



Abstract No.: 121

Poster Code: : F-0069

Characterizing the Localization and Interactome of FLAG-Tagged SARS-CoV-2 Membrane Protein During Infection

First Author: Isobel Webb, University of Bristol

Co-Author(s): Chun-Hin San, Maximilian Erdmann, Kate Heesom, Andrew Davidson

BACKGROUND OBJECTIVES:

The SARS-CoV-2 membrane (M) protein is the most abundant structural protein in the viral envelope and is essential for viral assembly and release. Despite its central role, its localisation and host interaction network during infection remain incompletely defined. This study aimed to generate recombinant viruses expressing tagged M proteins to enable detailed analysis of M protein localisation and interactions.

METHODS:

Recombinant SARS-CoV-2 viruses in a Wuhan-Hu-1 background with D614G spike were engineered to express either a FLAG epitope (rSARS-CoV-2-M-FLAG) or mNeonGreen (rSARS-CoV-2-M-NG) fused to the N-terminus of the M protein. Viruses were rescued by transfection of in vitro transcribed RNA. Viral replication, genetic stability, and growth kinetics were assessed in A549-ACE2-TMPRSS2 (AAT), VeroE6-TMPRSS2 (VTN), and Calu-3 cells. Protein localisation was examined by immunofluorescence assay (IFA), and M protein interactions were analysed by immunoprecipitation followed by quantitative mass spectrometry.

RESULTS:

rSARS-CoV-2-M-FLAG was successfully rescued and remained genetically stable after five passages, with growth kinetics and plaque morphology comparable to wild-type virus. In contrast, rSARS-CoV-2-M-NG failed to produce infectious virus, however, lysates from transfected cells enabled infection and intracellular replication, indicating a defect in viral release. FLAG-tagged M protein in rSARS-CoV-2-M-FLAG infected cells or lysates produced from them was detected by IFA, western blot, and immunoprecipitation. IFA pre- and post-permeabilization

identified both external whole virions coating the outside of cells and internal M protein colocalised to a GM130 Golgi marker. M protein was also found to colocalise with the nucleocapsid protein. Immunoprecipitation and mass spectrometry identified high-confidence host interaction partners currently undergoing validation.

CONCLUSIONS:

The rSARS-CoV-2-M-FLAG virus provides a robust tool for studying M protein localisation and interactions during infection. These findings highlight the importance of the M protein in viral assembly and release and establish a platform for identifying host factors interacting with M protein during SARS-CoV-2 infection.



Abstract No.: 125

Poster Code: : F-0070

SARS-CoV-2 Omicron EG.5 and JN.1 induce enhanced pathogenicity in K18-hACE2 mice over the early Omicron subvariants

First Author: Alex Wing Hong Chin, The University of Hong Kong

Co-Author(s): Mandy Tsoi Man HO, Nigeer TE, Leo Lit Man POON

BACKGROUND OBJECTIVES:

Transgenic mice expressing human ACE2 (K18-hACE2 mice) is a widely used small animal model for investigating the pathogenesis of SARS-CoV-2 and evaluating vaccines and antivirals. The ancestral SARS-CoV-2 strain and certain variants including Alpha, Beta and Delta variants are lethal to K18-hACE2 mice. However, early Omicron subvariants are generally less pathogenic in K18-hACE2 mice, rendering this animal model less suitable for assessing vaccine and antiviral efficacy against these subvariants. In this study, we aim to compare the pathogenicity of several e Omicron emerging subvariants in K18-hACE2 mice to identify a suitable animal infection model for the Omicron variant.

METHODS:

To compare the pathogenicity of the Omicron subvariants and the ancestral (WT) SARS-CoV-2 virus in mice, K18-hACE2 mice were intranasally inoculated with WT and Omicron subvariants BA.2, BA.2.86, JN.1 and EG.5 at 10^4 PFU/mouse. The change in body weight was observed after infection. Any mouse showing more than 25% weight loss or showing severe clinical signs will be humanely euthanized. The viral titre in lungs and brains was also determined. In addition, the cytokine and chemokine expression of the infected lungs and brains were also compared among the mice infected with different viral strains.

RESULTS:

Among the tested Omicron lineages, JN.1 and EG.5 induced significant weight loss in the infected mice. All EG.5-infected mice died within 8 days after infection, similar as the WT control. They showed delayed viral clearance and more severe pathology in lungs and viral tropism to brains. The of IFN, IL-6, RANTES and TNF

expressions of the brains were also elevated in the EG.5-infected mice. In contrast, early Omicron subvariants BA.2 and BA.2.86 showed low pathogenicity in mice with mild weight loss with no observable clinical signs and lethality.

CONCLUSIONS:

We report an enhanced pathogenicity of the Omicron JN.1 and EG.5 viruses in K18-hACE2 mice compared to their ancestral Omicron subvariants. The pathogenicity of the EG.5 lineage is comparable to the WT SARS-CoV-2, suggesting a potential application of this Omicron lineage in the mouse infection model for antiviral drug and vaccine assessment.



Abstract No.: 149

Poster Code: : F-0071

Development and Characterisation of Human and Porcine Primary Cell Air-Liquid Interface Cultures to Study Influenza-Coronavirus Coinfections

*First Author: Teagwen Cameron, The University of Edinburgh**Co-Author(s): Rosemary Blake, Paul Digard, Christine Tait-Burkard*

BACKGROUND OBJECTIVES:

Respiratory coronaviruses and influenza viruses co-circulate globally in both human and pig populations, posing a significant public health concern. Coinfections can exacerbate disease severity, increasing morbidity and mortality whilst imposing economic burdens. Despite their importance, the mechanisms and outcomes of coinfections remain poorly understood and vastly understudied. Both viruses primarily target the respiratory epithelial layer, making it a critical site for investigating viral infections. To better understand the dynamics and consequences of influenza-coronavirus coinfections, there is a need for robust in vitro models, both human and porcine, that accurately replicate the structural and functional complexities of the respiratory epithelium.

METHODS:

To mimic the physiology, polarity, and cellular complexity of the respiratory epithelium we have established human (HBECs) and porcine primary bronchial epithelial cells (PBECs) grown at the air-liquid interface (ALI). Whilst methods and cells for HBEC-ALI culture are well established and many reagents commercially available, porcine systems are still in their early stages. Here, we developed methods for the isolation and culturing of PBECs and their differentiation at ALI to form a pseudostratified columnar epithelium, reminiscent of the respiratory epithelium in vivo with ciliated, goblet, and basal cells present.

RESULTS:

HBECs were commercially obtained, and existing protocols were adapted for their expansion in submerged monoculture and subsequent differentiation at ALI. Differentiation of HBEC-ALI cultures have been confirmed via transepithelial

electrical resistance (TEER) readings, brightfield microscopy, observation of beating cilia and mucus production, and immunostaining with confocal. Furthermore, HBEC-ALI cultures have been shown to be permissive to infection with human coronavirus 229E and to support viral replication as previously shown.

To develop a suitable porcine in vitro model, we established a protocol to isolate and expand PBECs from lung tissue across different age groups. A suitable expansion medium was identified to expand and generate low-passage PBEC stocks. Building upon our knowledge of HBEC-ALI cultures, we then identified an optimised basolateral differentiation medium and established a protocol for the differentiation of PBEC-ALI cultures. We confirmed suitable differentiation of PBEC-ALI cultures as described above.

CONCLUSIONS:

Both HBEC- and PBEC-ALI showed rapid differentiation (within 2-3 weeks) and susceptibility to relevant respiratory pathogens. By applying BEC-ALI cultures, we have developed suitable human and porcine in vitro models of the respiratory epithelium enabling the study of influenza-coronavirus coinfections and allowing for comparisons between the two species.



Abstract No.: 152

Poster Code: : F-0072

Novel African Rhinolophus bat ACE2 sequences reveal the determinants of Afro-Eurasian sarbecovirus entry

First Author: Yueying ZHANG , The University of Tokyo

Co-Author(s): Shigeru Fujita, Masahiro Kajihara, Katendi Changula, Bernard Mudenda Hang'ombe, Shusuke Kawakubo, Jumpei Ito, Akinori Kanai, Yutaka Suzuki, Ayato Takada, Kei Sato, Spyros Lytras

BACKGROUND OBJECTIVES:

As putative natural reservoirs of sarbecoviruses, including SARS-CoV and SARS-CoV-2, Rhinolophus bats play a critical role in the emergence of zoonotic coronaviruses, making it essential to understand their interactions with these viruses for future pandemic preparedness. While Asian Rhinolophus bats have been relatively well studied, African species remain underrepresented, highlighting the need for further investigation. In this study, we aimed to investigate the Angiotensin-converting enzyme 2 (ACE2) in Rhinolophus bats collected in Zambia, Africa, while testing their susceptibility to various Sarbecovirus Spike (S) protein.

METHODS:

ACE2 sequences from African Rhinolophus bats were cloned and sequenced, followed by phylogenetic analysis to determine their evolutionary relationships with previously reported ACE2 orthologs. Cytochrome b (CYTB) sequences were also obtained from RNA-seq data and analyzed phylogenetically to further identification of species and comparison. To assess receptor function, the African bat ACE2 sequences were introduced into HOS-TMPRSS2 cells to establish stable cell lines, which were then used in pseudovirus infectivity assays. In parallel, ACE2 variants were generated by site-directed overlap extension PCR, and HEK293T cells transiently expressing ACE2 variants were generated by transfection and used for pseudovirus infectivity assays.

RESULTS:

For the obtained ACE2 sequences, ACE2 polymorphism was observed among Rhinolophus simulator individuals, revealing the presence of multiple ACE2

genotypes within the sampled population. Despite this sequence variation, none of the *R. simulator* ACE2 variants supported infection by the clade 3 Afro-Eurasian sarbecoviruses tested in this study. In contrast, two clade 3 viruses, RhGB01 and BM48-31, were able to utilize *Rhinolophus blasii* ACE2, but not *R. simulator* ACE2, indicating species-specific differences in receptor usage. Further mutational analyses demonstrated that residues at positions 31 and 41 of ACE2 play critical roles in modulating spike-ACE2 interactions, with combined substitutions leading to reduced viral entry efficiency.

CONCLUSIONS:

This study reports the ACE2 sequences of *R. simulator* and *R. blasii*, and provides new insights into the molecular interactions between African *Rhinolophus* species ACE2s and the S protein of sarbecoviruses circulating in Africa and Europe. These findings expand our understanding of the molecular determinants of sarbecovirus host range and support improved surveillance and risk assessment of emerging coronaviruses.



Abstract No.: 153

Poster Code: : F-0073

Modulation of SARS-CoV-2 replication by Co-Infections: Insights from Experimental and community-based Surveillance data

First Author: Manel Essaidi-Laziosi, University of Geneva

Co-Author(s): Francisco Javier Pérez-Rodríguez, Ana Rita Goncalves Cabecinhas, Catia Alvarez, Patricia SUTER BOQUETE, Tania SPEDALIERO, Nicolas Hulo, Laurent Kaiser, Isabella Eckerle, Manel Essaidi-Laziosi

BACKGROUND OBJECTIVES:

Early in the COVID-19 pandemic, clinical evidence regarding the impact of co-infections on SARS-CoV-2 was limited due to sparse outpatient data and non-pharmaceutical interventions (NPIs) that suppressed the activity of other respiratory viruses. During the last years, we combined experimental and population surveillance approaches to assess whether seasonal respiratory viruses modulate SARS-CoV-2 replication and subsequently validate our predictions based on clinical data.

METHODS:

We conducted experimental co-infection experiments involving SARS-CoV-2 and other viruses, such as rhinovirus (RV), influenza A (IAV), and influenza B (IBV), using reconstituted human airway epithelia (HAE). Viral replication was assessed through real-time RT-qPCR to quantify viral RNA and infection titers via focus-forming units. Following the lifting of non-pharmaceutical interventions, we examined respiratory viral co-detections from the Swiss surveillance system, utilizing data from 5,369 symptomatic outpatient nasopharyngeal specimens (August 2022-July 2024). These specimens were tested for 16 respiratory viruses using multiplex (RT-)PCR to characterize co-detections, age patterns, and viral loads.

RESULTS:

In HAE models, primary infection with RV, IAV, or IBV significantly impaired SARS-CoV-2 replication in an interferon-dependent manner. Community surveillance revealed co-detections in 9% of positive samples (year-round, mostly dual), predominantly in children and involving RV, SARS-CoV-2, bocavirus, and

adenovirus. Co-detection of SARS-CoV-2 with another virus was associated with significantly lower viral loads of the index pathogen, particularly in early to middle-aged adults.

CONCLUSIONS:

HAE models predicted the suppression of SARS-CoV-2 by seasonal viruses, and community data post-NPI relaxation corroborated altered viral loads in co-infections. Our findings highlight that co-infection patterns are dependent on host responses and age. These combined-approach studies highlight HAE as a robust model for recapitulating in vivo respiratory infections and predicting respiratory-virus dynamics for public health, while showing that surveillance could generate hypotheses to probe viral interactions and virus-host interplay.



Abstract No.: 160

Poster Code: : F-0074

Role of MOV10 helicase in SARS-CoV-2 pathogenesis

First Author: Maria Guzman, National Center of Biotechnology

Co-Author(s): Diego Muñoz-Santos, Jorge Ripoll-Gomez, Isabel Sola, Luis Enjuanes, Sonia Zuñiga

BACKGROUND OBJECTIVES:

Coronaviruses (CoVs) may cause life-threatening respiratory diseases in humans. SARS-CoV-2 emerged in 2019, causing more than 779 million infections and more than 7 million deaths worldwide. There are few approved antivirals against human CoV infections and none against host targets. Understanding CoV-host interactions may help to identify novel therapeutic targets. MOV10 is an RNA helicase involved in cellular RNA metabolism. We demonstrated that MOV10 interacts with human CoVs nucleocapsid (N) protein and has antiviral activity during highly pathogenic MERS-CoV and SARS-CoV-2, but not mild HCoV-229E, infections. These data suggested a role of MOV10 in the modulation of human CoVs pathogenesis.

METHODS:

Both MOV10 KO cells and mouse-adapted SARS-CoV-2 (SARS-CoV-2-MA) and BALB/c mice were used as a model to address the role of MOV10 in CoV pathogenesis. Since MOV10 $-/-$ mice are embryonically lethal, in vivo MOV10 knock-down was performed using LNA GapmeR technology, which is based in antisense oligonucleotides specifically designed for sustained silencing in vivo.

RESULTS:

We found that SARS-CoV-2 infection elicited a reduced interferon (IFN) response in MOV10 KO infected cells, compared with that in parental cells, while an increase was observed in the induction of pro-inflammatory cytokines. Preliminary tests in murine cell cultures indicated that one of the LNA GapmeRs efficiently silenced MOV10 and that SARS-CoV-2 replication levels were increased in these silenced cells, in agreement with our previous findings. These findings enabled the selection

of an LNAGapmeR for large-scale synthesis, necessary for in vivo use. For in vivo evaluation, the selected LNAGapmeR or a negative control one were intranasally administered to mice, and one day later mice were infected with a sublethal dose of SARS-CoV-2-MA. Clinical signs and survival were monitored, and samples were collected at different time points for analysis. Infected mice inoculated with the silencer showed greater weight loss compared to those inoculated with the negative control. Furthermore, all infected mice inoculated with the silencer died, while those inoculated with the control survived. Differences in lung damage and innate immune response were also found.

CONCLUSIONS:

Overall, our results highlight the modulation of SARS-CoV-2 pathogenesis by MOV10 protein and warrant further studies to elucidate the underlying molecular mechanisms.



Abstract No.: 167

Poster Code: : F-0075

Differential evolution of Rhinolophid bat antiviral restriction

First Author: Aaron Irving, Guangzhou National Laboratory

Co-Author(s): Xiaomeng Li, Yue Dong, Richard Sloan

BACKGROUND OBJECTIVES:

There are >1500 species of bats yet only some species are associated with hosting pathogenic coronaviruses. To identify if there are lineage-specific genetic adaptations associated with the hosts of SARSr-CoVs we utilized reference quality genomes to identify genes undergoing rapid adaptations or expansions compared to other bats and other mammals. We propose these adaptations confer a specific advantage to Rhinolophid bats, particularly Asian-lineage Rhinolophid bats, in controlling Sarbecovirus infection.

METHODS:

Through careful annotation, positive selection analysis and isoform identification, coupled with structural modelling we identified candidates across different Rhinolophid bat lineages to test for antiviral efficacy. Through synthesizing these gene constructs, functional validating and screening across a range of pathogens including coronaviruses with Pseudovirus and real virus infection, we interrogated antiviral function.

RESULTS:

Rhinolophid bats exhibit more effective ISG15, that regulates ISGylation intracellularly and blocks coronavirus infection. They also exhibit high baseline expression, multiple isoforms and enhanced IFITM3 antiviral capacity. Coupled with this they exhibit a modified Type-I IFN system, even containing a functionally deficient IFN α decoy.

CONCLUSIONS:

Rhinolophid bats are a diverse group of bats, evolutionary distant to each other yet with multiple evolved antiviral genes that confer enhanced antiviral protection, particularly against coronaviruses like SARSr-CoV's. We show distinct differences between Rhinolophus species that may impact viral spread and transmission and are important factors when predicting risk of zoonotic spillover to humans.

Abstract No.: 173

Poster Code: : F-0076

Identification and characterisation of novel SARS-CoV-2-related coronaviruses dynamically circulating in Southeast Asia

First Author: Wilaiporn Saikruang, The University of Tokyo

Co-Author(s): Spyros Lytras, Supaporn Wacharapluesadee, Arnon Plianchaisuk, Opass Puthachoen, Kei Sato

BACKGROUND OBJECTIVES:

The emergence of SARS-CoV-2 has underscored the urgent need to understand the biological characteristics and spillover risks of SARS-CoV-2-related coronaviruses (SC2r-CoVs) in their wildlife reservoirs, particularly horseshoe bats (*Rhinolophus*). Gaining in-depth insights into these virological traits is essential for pandemic preparedness, as it enables the identification of factors that could drive the emergence of new coronaviruses with pandemic potential.

METHODS:

In this study, we performed viral surveillance by sampling acuminate horseshoe bats (*Rhinolophus acuminatus*) from an artificial cave in Thailand. Using reverse genetics, we generated infectious virus from the RacCS20637 genome and compared its virological properties with those of SARS-CoV-2 and the previously identified bat SC2r-CoV, BANAL-20-236. A comprehensive set of approaches, including cryo-electron microscopy, pseudovirus and live virus assays, as well as hamster infection models, was employed to characterize these viruses. Additionally, phylogeographic and recombination analyses were conducted to investigate the geographic spread of the virus in relation to co-circulating lineages.

RESULTS:

Our sampling identifies the first co-infection of an ACE2- and a non-ACE2-using SC2r-CoV, suggesting possible interactions between viruses with different receptor tropisms. We demonstrate that RacCS20637—a representative virus of the novel clade—can effectively bind to human ACE2 and use this receptor for cellular entry. Using reverse genetics, we generated live virus from the RacCS20637 genome and compared its virological characteristics with those of SARS-CoV-2 and a previously reported bat SC2r-CoV, BANAL-20-236. Similar to BANAL-20-236 and unlike SARS-CoV-2, RacCS20637 exhibited reduced fusogenicity. Importantly,

both bat SC2r-CoVs did not replicate in human nasal and tracheal epithelial cells. Moreover, RacCS20637 was susceptible to SARS-CoV-2 small-molecule inhibitors and neutralizing sera.

Although this newly identified ACE2-using virus can bind to human ACE2, it shows reduced pathogenicity and transmissibility in hamsters compared to SARS-CoV-2, underscoring the distinct biological properties that differentiate SARS-CoV-2 from its bat-associated counterparts. Finally, we implemented a state-of-the-art phylogeographic approach to trace the movement of the bat SC2r-CoVs presented here across their evolutionary history. We showed that some of their genomic segments recently moved from Laos to Thailand, reinforcing the notion that SC2r-CoVs continuously travel across their host ranges in Southeast Asia.

CONCLUSIONS:

In our study, we identified novel bat coronaviruses circulating in Thailand, contributing to the assessment of spillover potential for this virus group. Continuous field surveillance is crucial for detecting and responding to emerging zoonotic threats.



Abstract No.: 199

Poster Code: : F-0077

Trypsin Promotes Cell-to-Cell Transmission of Human Coronavirus OC43 via Enhanced Cell Fusion

*First Author: Hye Won Bae, Chungbuk National University**Co-Author(s): Seong Sik Jang, Thi Van Lo, Hye Kwon Kim*

BACKGROUND OBJECTIVES:

Human coronavirus OC43 (HCoV-OC43), a low-pathogenic betacoronavirus, is widely used as a model for coronavirus research. Its spike protein mediates host entry and membrane fusion through proteolytic activation. Although proteases such as trypsin are known to enhance viral fusion, their role in modulating transmission patterns and driving spike adaptation in HCoV-OC43 remains unclear. This study aimed to investigate the effects of trypsin on HCoV-OC43 transmission and spike-mediated fusion.

METHODS:

HCoV-OC43-infected Vero-E6 donor cells were seeded in transwell inserts, while uninfected recipient cells were placed in the lower chamber to assess virion transfer through membrane pores. Cell-free transmission was evaluated under fetal bovine serum (FBS) or trypsin conditions using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and immunofluorescence (IF) analysis. Cell-to-cell transmission was assessed by co-culturing mCherry-expressing infected cells with non-fluorescent uninfected cells, and syncytia-like formations were observed. For adaptation analysis, the virus was serially passaged 15 times in trypsin-containing conditions, followed by spike gene sequencing and fusion activity assessment.

RESULTS:

Trypsin treatment significantly reduced viral replication in recipient cells in the transwell system, indicating suppression of cell-free transmission. In direct co-culture with mCherry-expressing infected cells, trypsin increased syncytia-like formation with non-fluorescent uninfected cells, suggesting enhanced cell fusion and cell-to-cell transmission. To assess viral adaptation, HCoV-OC43 was serially passaged 15 times in trypsin-containing conditions, resulting in three amino acid

mutations in the spike gene. Notably, these adapted viruses no longer exhibited increased fusion activity upon trypsin exposure.

CONCLUSIONS:

Trypsin modulates HCoV-OC43 transmission by suppressing cell-free spread while promoting spike-mediated cell fusion and cell-to-cell transmission. However, prolonged exposure to trypsin drives viral adaptation through acquisition of spike mutations, leading to loss of trypsin-dependent fusion enhancement. These findings indicate that the mutated spike regions are critical determinants of protease-mediated fusion and key sites for viral adaptation.



Abstract No.: 210

Poster Code: : F-0078

Structural investigation of the interplay between coronavirus replication, cellular oxygen sensing and iron homeostasis.

First Author: Peter Wing, University of Oxford

Co-Author(s): Yasmin Jiwa, Jiyeon Ha, Sri Chava, Kamal Nahas, Ivy Wang, Jessica Walker, Archana Jadhav

BACKGROUND OBJECTIVES:

The preservation of the cellular oxygen equilibrium is an essential component of cell physiology, necessitating the meticulous regulation of oxygen supply to sustain metabolic efficiency whilst circumventing potential toxicity. It is well documented that iron homeostasis is critical for several viral-associated diseases as well as providing essential co-factors for many oxygen-dependent enzymes. Notably, several bat species exhibit adaptations in iron metabolism, thought to be linked to their unique physiology. Recent work has revealed that coronaviruses encode a remarkably high abundance of metal-binding proteins and infections are associated with dysregulated iron metabolism and oxidative stress. We critically lack spatial and temporal information concerning how iron and other metal co-factors essential for enzymatic functions are distributed throughout the cell in response to cellular stressors such as hypoxia or viral infection. As a significant co-factor in several enzymes involved in oxidative phosphorylation, we hypothesised that hypoxia and coronaviral infection substantially disrupts iron homeostasis, given their respective impacts on mitochondrial morphology and function.

METHODS:

We employed a correlative, structural imaging workflow that unites cryo-SIM, cryo-soft X-ray tomography and nano-X-ray fluorescence (XRF) combined with live-cell imaging to investigate the interplay between iron homeostasis, hypoxia and coronaviral infection in human and bat cells.

RESULTS:

We established that hypoxia induces a rapid, yet transient uptake of iron, concentrating to discrete locations in the peri-nuclear region. Utilising X-ray absorption near-edge spectroscopy (XANES) and X-ray fluorescence (XRF) we

confirmed that rapid activation of cellular oxygen sensing induced distinct cytoplasmic distributions of iron, zinc and copper. Subsequent analysis by cryo-X-ray imaging, revealed a high concentration of iron filled vesicles adjacent to mitochondria in hypoxic cells. We applied these findings to viral infection, with mouse hepatitis virus (MHV) or SARS-CoV-2 resulting in pronounced redistribution of sub-cellular iron compared to uninfected cells. Further, infection perturbed expression of key iron transport proteins, disrupting cellular iron trafficking in response to hypoxia. Notably, bat-derived beta-coronaviruses enhanced transferrin receptor 1 expression and altered expression was noted in various bat cell lines compared to human cells, indicating species-specific regulation of iron uptake in response to coronaviral infection.

CONCLUSIONS:

Collectively, these findings provide the first spatially resolved, dynamic analysis of iron distribution in both hypoxia and coronaviral infection. The observed changes in iron homeostasis provide valuable new insights into the mechanisms by which coronaviruses manipulate the cellular environment to facilitate viral replication and pathogenesis.



Abstract No.: 211

Poster Code: : F-0079

Reverse Genetics-Driven Profiling of SARS-CoV-2 Nucleoprotein Interaction Networks

First Author: Lara-Marie Breul, Universitätsklinikum Hamburg-Eppendorf

Co-Author(s): Julia Berger, Neele Pekarek, Jonas Schröder, Kira Schamoni-Kast, Charlotte Uetrecht, Jens Bosse, Susanne Pfefferle

BACKGROUND OBJECTIVES:

Since the emergence of SARS-CoV-2, extensive research has focused on viral structural proteins and their roles in replication, transmission, and immune evasion. While the spike protein has received predominant attention, increasing evidence identifies the N (nucleocapsid) protein as a central determinant of viral fitness. Beyond its canonical role in viral ribonucleoprotein assembly, N contributes to replication efficiency, host adaptation, and modulation of innate immune responses. Owing to its multifunctionality, N is expected to participate in complex virus-host interaction networks that are tightly regulated in space and time. However, systematic characterization of these interactions under native infection conditions remains limited due to biosafety constraints. We hypothesize that the SARS-CoV-2 N protein forms a dynamic, spatiotemporally regulated host interactome and that naturally occurring N mutations reshape these interaction networks, thereby influencing viral replication, immune evasion, and virulence.

METHODS:

To address this, we established a reverse genetics platform based on CLEVER (Cloning-free and Exchangeable system for Virus Engineering and Rescue), enabling generation of recombinant SARS-CoV-2 variants differing exclusively in the N gene. The workflow integrates affinity purification-based proteomics, proximity-dependent labeling (e.g., for example miniTurboID), and advanced imaging approaches including split-GFP live-cell imaging and CLEM (correlative light and electron microscopy) to resolve the spatial and temporal organization of N protein complexes.

RESULTS:

The reverse genetics platform has been successfully implemented and enables systematic engineering of N variants under native infection conditions. CLEVER

fragments encoding tagged N proteins were generated and sequence-verified, including constructs carrying either a V5 epitope tag or a tandem Strep-tag with an engineered TEV protease cleavage site to facilitate complementary affinity purification strategies. Recombinant virus rescue for these tagged variants is ongoing. In parallel, the experimental pipelines required for interactome mapping have been established. The proximity-dependent labeling workflow is being implemented and stable cell lines required for miniTurboID-based interaction mapping are currently being generated. Together with affinity purification strategies enabled by the engineered tags, this system provides complementary approaches to capture both stable and transient N-associated protein complexes.

CONCLUSIONS:

Our platform enables systematic dissection of SARS-CoV-2 N protein interaction networks under native infection conditions. Ongoing analyses will reveal how naturally occurring N mutations reshape virus-host interaction landscapes and influence viral fitness and host responses.



Abstract No.: 212

Poster Code: : F-0079

The role of SARS-CoV-2 accessory protein ORF9b in the pathogenesis of severe COVID-19 through activation of the NLRP3 dependent inflammasome leading to the release of IL-1 β

First Author: Tin Long Yuen, The University of Hong Kong

Co-Author(s): Zi-wei Ye

BACKGROUND OBJECTIVES:

SARS-CoV-2 is responsible for the pandemic of COVID-19, which has been characterized by elevated cytokine levels and heightened immune response. One of the main causes of severe cases and death was found to be the cytokine storm, there are many cytokines that could possibly cause the cytokine storm and I will be focusing on one of the possible targets. By targeting components of the cytokine storm, we can potentially address the root cause and relieve patients of their severe symptoms. My project focuses on SARS-CoV-2 accessory protein ORF9b and its impact on the inflammasome. The inflammasome, a key component of the innate immune system, consists of a sensor protein, an adaptor protein, and an effector protein. Upon activation, it triggers the production of interleukin-1 beta (IL-1 β) and gasdermin D (GSDMD). My study will focus on the inflammasome pathway and the role of SARS-CoV-2 accessory protein ORF9B in this context.

METHODS:

Methods include tissue culture, western blot, ELISA, RNA extraction and qPCR.

RESULTS:

This includes verifying the interaction of ORF9B with the inflammasome structure, and also determining whether and how ORF9B co-localizes with ASC and NLRP3 proteins to promote ASC oligomerization. Furthermore, there is a need to investigate the mechanism by which ORF9B induces the release of IL-1 β , leading to an exaggerated immune response.

CONCLUSIONS:

In summary, my study will shed light on the intricate molecular choreography between ORF9B and the inflammasome during SARS-CoV-2 infection. Research on SARS-CoV-2 is very important as it has caused a major pandemic, and solving its influence on our innate immunity and the underlying mechanisms can not only help to prepare for another pandemic, but also improve our understanding of our immune defence against viruses. This study was supported by the Health and Medical Research Fund, grant number: HMRF 21200492

Abstract No.: 220

Poster Code: : F-0081

Viral Variants take control: SARS-CoV-2 nucleocapsid mutations reshape innate immunity through variant-specific host reprogramming

First Author: Julia Berger, University Medical Clinic Hamburg Eppendorf

Co-Author(s): Jonas Schröder, Neele Pekarek, Timothy Soh, Lara-Marie Breul, Patrick Blümke, Jiabin Huang, Jens B Bosse, Charlotte Uetrecht, Susanne Pfefferle

BACKGROUND OBJECTIVES:

The Nucleocapsid (N) and overlapping ORF9b gene of SARS-CoV-2 have accumulated mutations throughout the pandemic, yet their impact on virus-host interactions during native infections remains poorly understood. Most studies have utilised N-overexpression systems, leaving effects on the cellular interactome in an infectious context unexplored. Here, we engineered isogenic recombinant viruses differing only in their N/ORF9b sequences to systematically investigate variant-specific host reprogramming at transcriptomic and proteomic levels in infected human lung epithelial cells.

METHODS:

Recombinant SARS-CoV-2 carrying variant-specific N/ORF9b sequences and a luciferase reporter (ORF7) in a parental Wuhan-Hu-1 backbone were generated using reverse genetics (rN-Wuhan, rN-B.1.1.7, rN-B.1.617.2, rN-BA.2, rN-BA.2.86). Growth kinetics were assessed on interferon-deficient (Vero E6) and -competent (CaLu-3, A549-ACE2-TMPRSS2) cell lines, with viral replication monitored by plaque assay, RT-qPCR and/or luciferase activity. For multi-omics profiling, CaLu-3 cells were infected in biological triplicates. Host and viral protein expression was quantified by label-free LC-MS/MS, while transcriptomic changes were assessed by bulk RNA-sequencing (150 bp paired-end, >25M reads per sample).

RESULTS:

All recombinant viruses demonstrated retained replication ability and lytic infection on all tested cell lines, reaching comparable peak titres of 10⁶ PFU/ml by 72 hpi on Vero E6/CaLu-3 cells. No significant differences in growth rates of maximum titres were observed between recombinants, with similar replication patterns on A549-ACE2-TMPRSS2 cells, confirming that N/ORF9b mutations do not

impair viral fitness or cytopathic effects. Viral protein analysis revealed that while N protein abundance remained consistent across all variants, with a slightly lower abundance in rN-B.1.1.7, ORF9b expression showed striking differences: early pandemic variants (rN-Wuhan, rN-B.1.1.7) displayed high ORF9b levels, while Omicron variants (rN-BA.2, rN-BA.2.86) and rN-B.1.617.2 ORF9b levels were significantly reduced by 48 hpi in comparison. Parallel transcriptomic and proteomic analysis of the host response revealed systematic reprogramming that correlated with these viral protein differences, despite comparable viral RNA loads. Most pronounced variant-specific divergence occurred in innate immune pathways: rN-B.1.1.7 induced notably low expression of interferon-stimulated genes (Z-scores: -1.54 – -0.71) at both protein and transcript levels, while rN-B.1.617.2 exhibited the highest induction (Z-scores: 0.14 – 1.89). Interestingly, rN-BA.2 showed an intermediate pattern – transcriptional activation comparable to rN-B.1.617.2, but more restrained protein-level responses, suggesting post-transcriptional modification.

CONCLUSIONS:

Using isogenic recombinant viruses, we demonstrate that variant-specific N/ORF9b mutations drive distinct host reprogramming in infected lung epithelial cells. Coordinated changes in ORF9b expression and innate immune responses suggest that N/ORF9b evolution fine-tunes host-pathogen interactions through altered protein function rather than abundance.



Abstract No.: 224

Poster Code: : F-0082

SARS-CoV-2 intra-host recombination promotes epistatic spike interactions and temperature-dependent adaptation

*First Author: Mazharul Altaf, University of Cambridge**Co-Author(s): Bo Meng, Ravindra Gupta*

BACKGROUND OBJECTIVES:

The SARS-CoV-2 pandemic has been driven by successive variants carrying spike mutations that enhance transmissibility, fusogenicity and immune evasion. Temperature gradients across the human airway also influence viral entry and replication, yet their role in variant adaptation remains underexplored under physiologically relevant conditions. Chronic infections in immunocompromised individuals provide a setting for prolonged viral evolution and may generate mutations later seen in variants of concern. In this study, a previously uncharacterised spike mutational trio (S13I, W64G, P330S) that emerged during chronic pre-Omicron infection was investigated, assessing their effects on antibody escape, cell entry, spike cleavage and fusogenicity across airway-relevant temperatures.

METHODS:

This study was primarily a laboratory-based study using spike-presenting pseudotyped virus (PV) with mutations generated by site-directed mutagenesis. The sensitivity to vaccinee sera, monoclonal antibody, infectivity in a variety of model cell lines, spike cleavage, drug inhibitors, and fusogenicity were investigated.

Sensitivity to antibodies in serum was tested using sera collected from vaccinees as part of the Cambridge-NIHR Bioresource. The participants (average age: 67; age range: 61-73; 40% male) in this study were immunised with two doses of the ChAdOx1 (AZD1222) vaccine.

Where appropriate, this study was performed across 32°C, 37°C, and 39°C to mimic physiological temperature gradients encountered during a SARS-CoV-2 infection across different tissues and compartments.

For recombination detection, this study used primer/quality-trimmed BAMs of reads separately assembled from each sampling time-point and physiological

location that was aligned to the GenBank MN908947.3 sequence. From these BAMs, per-site alleles at spike positions were extracted using a pileup-based approach (SAMtools mpileup/HTSlib engine), applying base-quality ($Q \geq 20$) filters and excluding unmapped and secondary/supplementary alignments.

For molecular dynamics simulations and trajectory analysis, W64G mutation was introduced to the glycosylated spike protein with ACE2 (one RBD “up” state) in the D614G background. The mutation was introduced with UCSF Chimera followed by the protonation step. The system was simulated with Gromacs version 2021.3 utilizing CHARMM36 all-atom force field. Particle Mesh Ewald was applied with a grid spacing of 0.16 nm to account for electrostatics, alongside a van der Waals cutoff of 1.2 nm. V-rescale thermostat and Parrinello-Rahman barostat were applied to maintain the temperature at 310 K and pressure at 1 bar, respectively. The mutant system was simulated for 1 μ s. All structures were visualized and rendered using PyMOL. Analysis of trajectories (such as distance, RMSD/RMSF, and dihedral angle calculations) was performed in Python with the MDAnalysis library.

RESULTS:

Longitudinal sampling of a chronically infected immunocompromised individual with pre-Omicron ancestral SARS-CoV-2 identified the transient emergence of a rare spike mutational cluster comprising S13I, W64G and P330S, detected at high intra-host frequency in upper and lower respiratory tract samples. Global sequence analysis showed that co-occurrence of these substitutions was exceptionally uncommon, and read-level analysis provided evidence consistent with intra-host recombination linking the mutations.

Functional characterisation using pseudotyped virus (PV) assays demonstrated that the single mutants impaired cell entry, S1/S2 cleavage and syncytium formation, whereas the triple mutant restored these phenotypes to near wild-type levels, consistent with compensatory epistasis.

Neutralisation assays revealed temperature-dependent immune escape, with N-terminal mutations S13I and particularly W64G showing reduced susceptibility to vaccine sera and sotrovimab at 37°C, while P330S and the triple mutant showed

relatively greater escape at 32°C.

All mutants retained dependence on TMPRSS2-mediated entry, although inhibitor studies showed mutation-specific, temperature-sensitive modulation of fusion pathway usage.

Molecular dynamics simulations further showed that W64G induced local N-terminal domain rearrangements with distal perturbations at the S1/S2 cleavage loop, providing a structural basis for its pleiotropic effects.

Across mutants, S1/S2 processing strongly correlated with both cell-cell fusion and viral entry.

CONCLUSIONS:

The observations from this study suggest that intra-host evolution selects for multidimensional optimisation of spike behaviour, balancing stability, protease accessibility, and receptor engagement to maintain efficient viral fitness under restrictive environmental and immune pressures.

The findings highlight the continuing importance of sustained genomic surveillance, particularly in immunocompromised hosts, and supports integrative approaches that couple evolutionary virology with clinical and epidemiological monitoring. Such strategies are essential for anticipating the properties of future variants and informing the design of countermeasures capable of mitigating the risks posed by SARS-CoV-2 – a virus that continues to adapt across both environmental and host-specific landscapes.



Abstract No.: 230

Poster Code: : F-0083

Temperature-tuned RIG-I-MAVS signalling defines upper airway permissiveness to seasonal coronavirus OC43

First Author: Dongsheng Luo, University of Essex

BACKGROUND OBJECTIVES:

The study investigates why seasonal coronaviruses like HCoV-OC43 preferentially replicate in the cooler environment of the upper respiratory tract (33 °C) compared to the warmer lower respiratory tract (37 °C). Historically, it was unclear whether this was due to the virus's own enzymatic limitations or the host's immune efficiency. The researchers aimed to define the mechanistic basis for this temperature-dependent tropism by testing the efficiency of host innate immunity across physiological temperature gradients. Using transcriptomics and CRISPR-Cas9 genetic engineering, the objective was to determine if the RIG-I-MAVS signaling axis—the cellular “alarm system” that detects viral RNA—functions more robustly at core body temperatures to restrict infection. Ultimately, the study sought to prove that cooler nasal temperatures create a permissive niche for viral replication by attenuating the host's interferon-mediated antiviral defenses rather than by enhancing intrinsic viral fitness.

METHODS:

Virological and Pharmacological Assays

Temperature-Controlled Infection: Cells (fibroblasts and epithelial models) were infected with HCoV-OC43 and maintained at either 33 °C (nasal temperature) or 37 °C (core temperature). Viral growth was measured via titers and genome copy numbers over a set time course.

Pathway Perturbation: To test the role of the interferon (IFN) system, researchers pre-treated cells with recombinant IFN- α to induce an antiviral state. Conversely, they used Ruxolitinib, a JAK/STAT inhibitor, to pharmacologically block the signaling required to respond to interferon.

Molecular and Genetic Analysis

Transcriptomics (RNA-seq): The team stimulated cells with specific ligands—5'ppp-dsRNA (to trigger RIG-I) and poly(I:C) (to trigger MDA5)—at both temperatures. They then performed RNA sequencing to quantify the expression levels of Interferon-Stimulated Genes (ISGs).

CRISPR-Cas9 Gene Editing: To pinpoint the exact signaling nodes, researchers generated knockout (KO) cell lines. By deleting RIG-I, MDA5, or MAVS, they could observe whether the virus lost its temperature sensitivity when these specific immune “sensors” or “adaptors” were missing.

RESULTS:

The study found that HCoV-OC43 replication is significantly enhanced at 33 °C because the host’s innate immune response is “sluggish” at cooler temperatures, rather than due to any defect in the virus’s ability to replicate at 37 °C. Using RNA-seq and pharmacological inhibitors, the researchers demonstrated that the interferon (IFN) response is much more robust at core body temperature, effectively restricting viral growth. Crucially, CRISPR-Cas9 knockouts revealed that the RIG-I–MAVS signaling axis acts as a temperature-tuned sensor; while the absence of MAVS completely abolished temperature-dependent restriction, RIG-I deficiency specifically boosted viral levels at 37 °C. Ultimately, the cooler environment of the upper airway provides a permissive niche for seasonal coronaviruses by attenuating the cellular alarm system that would otherwise trigger a potent antiviral state.

CONCLUSIONS:

The study concludes that the upper respiratory tract’s susceptibility to seasonal coronaviruses is determined by temperature-tuned host immunity rather than intrinsic viral constraints. By identifying the RIG-I–MAVS axis as a critical temperature-sensitive signaling node, the researchers proved that warmer core temperatures (37 °C) enhance the efficiency of viral sensing and the subsequent interferon response, effectively “shielding” the lower lungs. Conversely, the cooler environment of the nasal cavity (33 °C) creates a permissive niche by attenuating this innate immune alarm system, allowing HCoV-OC43 to replicate with less interference. This discovery shifts the understanding of viral tropism from a virus-centric view to one centered on the thermal regulation of human antiviral defenses.



Abstract No.: 308

Poster Code: : F-0084

CRISPR/Cas9 engineered nasal organoid platform to dissect coronavirus host factors

First Author: Sophie Wilhelmina van Leur, Duke-NUS Medical School

Co-Author(s): Julie TS Chu, Marcus G Mah, Akanksha Rawat, Nicholas KW Yeo, Alan CY Hsu, Gavin JD Smith, Mart M Lamers

BACKGROUND OBJECTIVES:

Respiratory coronaviruses initiate infection in the upper respiratory tract. Deciphering host-virus interactions at the primary site of infection is a key strategy for identifying new antiviral targets. Yet, physiologically relevant upper respiratory tract models that enable the dissection of host factors remain scarce. Human nasal organoids, derived from healthy donor nasal basal stem cells, recapitulate native nasal epithelial architecture, including multiciliated cells, secretory lineages, and functional mucus barriers. However, robust CRISPR/Cas9 engineering workflows for nasal organoids remain limited, hindering functional interrogation of host factors. Here, we aimed to establish and validate a CRISPR/Cas9-engineered nasal organoid platform to dissect coronavirus host factors.

METHODS:

We established a human nasal air-liquid interface (ALI) model using basal cells derived from healthy donor nasal brushings and generated a cryopreserved collection of human nasal donors. Cells were expanded and differentiated at ALI for six weeks to form a pseudostratified epithelium before being used for experiments. All virus stocks were produced and titrated on nasal organoids. In parallel, we developed an integration-free CRISPR/Cas9 workflow in these cells, using transient editing at the basal stem cell stage. Edited knockout cell pools were differentiated at ALI to generate mature knockout nasal organoids, before being infected with SARS-CoV-2. SARS-CoV-2 replication was determined using confocal imaging, RT-qPCR, and live virus titrations.

RESULTS:

We characterized our human nasal ALI model by immunofluorescence staining and transcriptomic analysis, confirming cellular diversity and expression of key proteases and receptors for SARS-CoV-2 infection. As proof-of-concept, CRISPR knockout of ACE2 (primary receptor) and TMPRSS2 (key entry protease) in healthy

donor nasal organoids both resulted in significant reductions in SARS-CoV-2 replication. Critically, organoid architecture and differentiation markers remained unaffected by our CRISPR editing workflow.

CONCLUSIONS:

This CRISPR-editing platform enables rapid validation of candidate host factors directly in a physiologically relevant nasal epithelium model. It provides a robust system for studying host determinants and evaluating antiviral targets, and can be readily adapted to generate targeted panels for emerging respiratory viruses.



Abstract No.: 334

Poster Code: : F-0085

CATHEPSIN D IS A NOVEL BROAD-SPECTRUM HOST FACTOR PROMOTING CORONAVIRUS REPLICATION

*First Author: Yuansen SHI, Guangzhou National Laboratory**Co-Author(s): Wenjie Huang, Meiqin Liu, Jing Chen, Yutao Pan, Zhengli Shi*

BACKGROUND OBJECTIVES:

Coronaviruses (CoVs) represent an important class of pathogens responsible for major zoonotic outbreaks, including severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and coronavirus disease 2019 (COVID-19). The viral life cycle critically depends on host cellular factors. Systematic identification and characterization of these host factors are of fundamental scientific and translational importance, as they contribute to elucidating viral pathogenesis, assessing the risks of emerging coronaviruses, and developing broad-spectrum, host-directed antiviral therapeutics.

METHODS:

Based on integrated transcriptomic and proteomic analyses of COVID-19 patient lung tissues, we identified cathepsin D (CTSD) as a candidate host factor. Immunofluorescence of lung tissues from patients and infected mice showed altered CTSD expression, and in infected cells, CTSD co-localized with the viral nucleocapsid (N) protein. Functional studies using siRNA knockdown and plasmid-based overexpression demonstrated that CTSD promotes viral replication, as measured by viral protein and RNA levels. Moreover, CTSD supported replication in coronavirus replicon systems, indicating a broader role in related viral infections.

RESULTS:

Through multi-omics analyses, we identified one host factor, CTSD which was upregulated during SARS-CoV-2 infection. Consistently, immunodetection assays confirmed a significant upregulation of CTSD protein in post-mortem lung tissues of COVID-19 patients, as well as in a human angiotensin-converting enzyme 2 (ACE2) knock-in mouse model infected with SARS-CoV-2. A SARS-related CoV WIV1 infection in A549-hACE2 and HeLa-hACE2 cells also induced a significant increase in intracellular CTSD expression, and CTSD exhibited obvious colocalization with viral N protein in infected cells. To characterize the functional

role of CTSD during viral infection, we performed loss-of-function and gain-of-function assays. Small interfering RNA (siRNA)-mediated knockdown of endogenous CTSD significantly suppressed viral replication, whereas overexpression of CTSD in A549-hACE2 cells markedly enhanced viral replication. Further dissection of the viral life cycle revealed that CTSD knockdown had no effect on viral binding and internalization into host cells, but significantly inhibited viral genome replication. Notably, experiments using coronavirus replicon systems (including SARS-CoV-2, MERS-CoV, and WIV1) demonstrated that CTSD knockdown in A549-hACE2 cells markedly repressed the replication activity of these coronaviruses. Collectively, these findings demonstrate that CTSD is a critical host factor that supports the replication of coronaviruses, suggesting CTSD as a promising host-targeting candidate for the development of broad-spectrum anti-coronavirus therapeutics.

CONCLUSIONS:

CTSD is a novel host factor that promotes coronavirus replication, providing a new target for the development of antiviral drugs against coronaviruses.



Abstract No.: 337

Poster Code: : F-0086

Diverse Cell Entry Strategies of Porcine Coronaviruses and Diversity of these Viruses in Lower Saxony, Germany

First Author: Nicole Moor, German Primate Center GmbH

Co-Author(s): Luise Graichen, Jasmin Waness, Isabel Hennig-Pauka, Elise Opitsch, Baxolele Mhleke, Melanie M. Brinkmann, Stefan Pöhlmann, Markus Hoffmann

BACKGROUND OBJECTIVES:

Zoonotic viruses in animal reservoirs threaten human health. Swine harbor diverse coronaviruses (CoVs) from different genera, including the alphacoronaviruses transmissible gastroenteritis virus (TGEV), porcine respiratory coronavirus (PRCV), porcine epidemic diarrhea virus (PEDV), and swine acute diarrhea syndrome coronavirus (SADS-CoV), as well as the betacoronavirus porcine hemagglutinating encephalomyelitis virus (PHEV), and the deltacoronavirus porcine deltacoronavirus (PDCoV). Infections by these viruses range from being subclinical to severe enteric, respiratory, and neurological disease in pigs. We investigated coronavirus diversity in animal species in Lower Saxony, Germany, and examined virus-host interactions, focusing on viral entry mechanisms.

METHODS:

A pan-coronavirus PCR approach was used to screen pig samples and sympatric wild animal species. Viral entry and host range were assessed using vesicular stomatitis virus (VSV)-based pseudoviruses bearing spike proteins from TGEV, PRCV, PHEV, PEDV, PDCoV, and SADS-CoV, with porcine and human cell lines as targets. Receptor usage was investigated through transient expression of candidate receptors, while pH-dependence and the choice of spike-protein activating proteases were analyzed using specific inhibitors.

RESULTS:

PRCV was highly prevalent, indicating a major contribution to respiratory disease burden, co-infections, and production losses. PHEV was also frequently detected, supporting its circulation in swine populations. In contrast, screening of wildlife near pig farms (deer, wild boar, hare, pheasant) showed no evidence of coronavirus infection. Distinct cell tropism patterns were observed. Pseudotypes bearing PEDV, PHEV, and PDCoV spike proteins (PEDVpp, PHEVpp, PDCoVpp) entered human cells, indicating potential for cross-species transmission, whereas

entry of TGEVpp and PRCVpp was largely restricted to porcine cells. Analysis of receptor usage confirmed TGEV, PRCV, and PDCoV spikes utilize porcine aminopeptidase N (APN), while the PHEV spike engages dipeptidase 1 (DPEP1) for entry. SADS-CoVpp was inefficient, and no evidence for the use of a known coronavirus receptor was obtained. PEDVpp entered several cell lines efficiently, but also without utilizing a known coronavirus receptor. In Huh-7 cells, entry of PEDVpp and PDCoVpp depended on endosomal acidification and the activity of endosomal/ lysosomal cysteine cathepsins, whereas TGEVpp, PRCVpp, and particularly PHEVpp, exhibited reduced or independence on pH and cysteine proteases.

CONCLUSIONS:

These findings demonstrate high PRCV prevalence and its ongoing circulation in pigs. The ability of several porcine coronaviruses to enter human cells indicates their zoonotic potential and underscores the need for continued surveillance. Finally, the receptors used by PEDV and SADS-CoV spike proteins for cell entry remain to be identified.



Abstract No.: 400

Poster Code: : F-0087

Alternative Splicing Endows Oligoadenylate Synthetase 2 With Distinct Mechanisms of Restriction of Severe Acute Respiratory Syndrome Coronavirus 2

First Author: Yongtao He, University of Cambridge

Co-Author(s): Srikeerthana Kuchi, Toni Luise Meister, Yingxue Wang, Bo Meng, P. Pereyra Gerber, Pengcheng Wang, David L Robertson, Suzannah Rihn, Nicholas Matheson, Ravindra Gupta, Pfaender Stephanie, Sam J Wilson

BACKGROUND OBJECTIVES:

The oligoadenylate synthetase-ribonuclease L (OAS-RNase L) pathway is an important antiviral defence against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), but the relative contributions of individual OAS paralogues and their isoforms have not been fully defined. We aimed to define the antiviral activities and mechanisms of OAS proteins against SARS-CoV-2, determine their relevance in endogenous settings, and identify viral determinants associated with viral escape from OAS-mediated restriction.

METHODS:

Stable expression and gene knockout approaches were used to assess the effects of OAS proteins and RNase L on SARS-CoV-2 replication. Lipid-modification mutants were tested to determine the requirement for membrane targeting. Patient RNA sequencing datasets were analysed to examine associations between the relative expression of OAS2 isoforms and disease severity. Divergent SARS-CoV-2 variants and in vitro evolution experiments were used to examine the determinants associated with sensitivity or resistance to the OAS-RNase L system.

RESULTS:

Exogenous expression experiments indicated that OAS1 p46, OAS2 p69, and to a lesser extent OAS2 p71 restrict SARS-CoV-2 replication. OAS1 p46 and OAS2 p71 act through canonical RNase L activation, whereas OAS2 p69 inhibits viral replication through an RNase L-independent mechanism. Lipid modification is required for the anticoronaviral activity of OAS1 and OAS2. In CRISPR-modified cells, Loss of OAS2 increases SARS-CoV-2 replication both in the presence and absence of RNase L, further supporting an RNase L-independent antiviral role for OAS2 p69. The relationship between p69 expression, which strongly inhibits SARS-CoV-2 in vitro, and the severity of COVID-19 disease is currently under evaluation.

CONCLUSIONS:

Multiple lipid-modified OAS isoforms can mediate SARS-CoV-2 restriction through RNase L-dependent or RNase L-independent mechanisms. Moreover, specific SARS-CoV-2 lineages may have adapted to escape the OAS system.



Abstract No.: 427

Poster Code: : F-0088

Transmembrane serine proteases facilitate flavivirus infection through prM cleavage

First Author: Tianrenzheng Zhu, The University of Hong Kong

Co-Author(s): Wang Yang, He Yixin, Wang Lei, Zhang Xi, Hin Chu

BACKGROUND OBJECTIVES:

Zika virus (ZIKV) remains a major global health threat, yet its pathogenic mechanisms and host factors supporting viral replication are still not fully understood. In particular, the processes regulating viral maturation and infectivity require further investigation. This study aimed to identify key host determinants involved in ZIKV replication and to clarify the role of transmembrane serine proteases in flavivirus infection. Specifically, we sought to determine whether TMPRSS13 and related type II transmembrane serine proteases (TTSPs) facilitate viral maturation through prM cleavage, and to evaluate their potential as targets for broad-spectrum antiviral intervention.

METHODS:

The study combined transcriptomic profiling and siRNA screening in human macrophages and dendritic cells to identify host factors involved in ZIKV replication. Pharmacological inhibition was evaluated using the serine protease inhibitor Nafamostat in human organoids and in IFNAR1^{-/-} A129 mice. Subcellular localization and mechanistic analyses were performed to determine the role of TMPRSS13 in viral maturation, with a focus on prM cleavage in the Golgi apparatus. Reverse genetic approaches were further used to assess whether TMPRSS13 could restore the virulence of recombinant ZIKV carrying prM cleavage-resistant mutations that cannot be processed by Furin. In addition, a systematic analysis of other type II transmembrane serine proteases (TTSPs), including TMPRSS2, was conducted to evaluate their intracellular roles in the replication of diverse flaviviruses.

RESULTS:

TMPRSS13 was identified as a critical host factor that promotes efficient ZIKV replication. Mechanistic studies showed that TMPRSS13 localizes to the Golgi and cleaves prM, thereby promoting viral maturation and infectivity in a manner functionally comparable to Furin. Reverse genetic experiments demonstrated

that TMPRSS13 can restore the virulence of recombinant ZIKV carrying prM cleavage-resistant mutations that are not processed by Furin, indicating that TMPRSS13 serves as an alternative protease essential for prM processing and viral maturation. Beyond TMPRSS13, other TTSP family members, including TMPRSS2, were also found to facilitate the replication of multiple flaviviruses through conserved intracellular prM cleavage mechanisms.

CONCLUSIONS:

This study identifies TMPRSS13 as a previously unrecognized intracellular host factor that facilitates flavivirus maturation by mediating prM cleavage in the Golgi. It further establishes TTSPs as a broader regulatory framework supporting the replication of diverse flaviviruses through conserved prM processing. These findings not only deepen the understanding of flavivirus pathogenic mechanisms but also highlight TTSPs as promising targets for the development of broad-spectrum antiviral therapies.



Abstract No.: 440

Poster Code: : F-0089

Characterization of canonical and non-canonical antiviral responses in white-tailed deer cells infected with host-adapted SARS-CoV-2

First Author: Sophie-Marie Aicher, Univeristy of Toronto

Co-Author(s): Celine Tan (1,3), Briallen Lobb (4), Max Erdmann (5), Leandro X. Neves (5), Levi Klassen (3), Shyan Mascarenhas (4), Jonathon D. Kotwa (3), Yaejin Lee (1,3), Sowmya Thanikachalam (6), Lauren Crawshaw (7), Theo J. Moraes (6), Jeff Bowman (7), Edward Emmott (5), Andrew C. Doxey, Arinjay Banerjee (1,2), Samira Mubareka (1,3)

BACKGROUND OBJECTIVES:

Zoonotic viruses, including SARS-CoV-2, have increasingly caused public health emergencies, yet protective mechanisms in wildlife reservoirs remain poorly characterized. SARS-CoV-2 circulates subclinically in white-tailed deer (WTD) populations, where the deer-adapted B.1.641 strain emerged and subsequently demonstrated spillback transmission to humans, highlighting the critical need to understand viral tolerance mechanisms in reservoir hosts. Little is known about how WTD respond to SARS-CoV-2 or the specific factors influencing lack of clinical disease. We investigated the molecular basis of disease tolerance in WTD through comprehensive characterization of antiviral responses to host-adapted SARS-CoV-2.

METHODS:

We established novel WTD cellular models by generating ciliated nasal epithelia on air-liquid interface from WTD nasal brushes, recapitulating the primary infection site. Through bulk RNA-seq transcriptomics, we characterized viral replication kinetics and host responses to SARS-CoV-2 B.1.641 (deer-adapted) and Delta variants. Additionally, we analyzed transcriptomic data from naturally infected WTD nasal swabs collected through the Wildlife Emerging Pathogen Initiative (Wild EPI), providing complementary in vivo validation of cellular findings. Label-free quantitative mass spectrometry proteomics was performed to validate transcriptional findings at the protein level.

RESULTS:

We observed potent upregulation of both type I and III interferons alongside robust activation of canonical antiviral genes including BST2, MX1, RSAD2, and RTP4 in WTD cells, responses significantly stronger than those observed in human

control cells. Remarkably, SARS-CoV-2 Delta variant induced only moderate antiviral gene expression in WTD cells, instead triggering pronounced upregulation of genes involved in extracellular matrix remodeling and cilium assembly inhibition, suggesting variant-specific adaptation mechanisms. Proteomics analysis confirmed transcriptional upregulation of key antiviral effectors including MX1, MX2, and IFIT1, demonstrating that enhanced interferon signaling translates to protein-level changes. Comparative analysis between naturally infected animals and experimental cellular models showed consistent activation of antiviral immune pathways, validating our cellular systems as physiologically relevant models for reservoir host studies.

CONCLUSIONS:

Our data demonstrate potent antiviral immune responses in WTD upon SARS-CoV-2 infection. The variant-specific differential responses suggest adaptive mechanisms between SARS-CoV-2 and WTD. Our findings reveal that enhanced interferon responses, coupled with tissue remodeling mechanisms, constitute a multi-layered defense strategy that may contribute to viral tolerance in wildlife hosts. The novel WTD cellular models established here provide valuable tools for investigating the interplay between SARS-CoV-2 variants and potential novel wildlife reservoirs. Characterization of viral tolerance mechanisms in reservoir species will further our understanding of zoonotic spillovers and may identify targets for therapeutic intervention against emerging viruses.



Abstract No.: 117

Poster Code: : G-0090

Cross-species quantification of antiviral immune responses from large-scale RNA-seq data using ISG Profiler

First Author: Luca Nishimura, The University of Tokyo

Co-Author(s): Hiroaki Unno, Kyoko Kurihara, Mai Suganami, Junna Kawasaki, Spyros Lytras, Kaho Okumura, Edward C Holmes, Jumpei Ito, Kei Sato

BACKGROUND OBJECTIVES:

Many emerging infectious diseases originate from zoonotic viruses, highlighting the need for comprehensive viral surveillance across diverse animal species. While homology-based approaches are widely used for virus detection, they are computationally demanding and may fail to detect viruses with low sequence similarity. To address these challenges, our group is developing an integrated framework for large-scale viral surveillance from RNA-seq data, consisting of three steps: (1) quantification of host immune responses, (2) prediction of viral infection status based on host transcriptomic patterns, and (3) downstream virome analysis for viral discovery. In this presentation, we focus on the first step of this framework which enables rapid and cross-species quantification of interferon-stimulated gene (ISG) expression.

METHODS:

We developed ISG Profiler, a computational tool that rapidly quantifies the expression levels of evolutionally conserved core ISGs from animal RNA-seq data. We constructed a database including 59 core ISGs and 100 internal control genes (ICGs) derived from 398 amniota species. RNA-seq reads were mapped to this database using Salmon, and normalized ISG expression levels were calculated with ICGs. It enabled cross-species comparison without requiring reference genomes.

RESULTS:

ISG Profiler was validated with experimentally interferon induced datasets from ten animal species. The results showed strong correlation with conventional RNA-seq analysis and consistently higher ISG expression in interferon positive samples. Similar ISG upregulation patterns were observed in both experimentally and

naturally viral infected samples. Analysis of 170,000 RNA-seq data revealed that ISG expression patterns vary substantially depending on host animal taxonomic order and infecting viral families. For example, while RNA viruses generally induced ISG expression, notable exceptions were observed, and certain DNA viruses also triggered strong responses. We also observed that basal ISG expression levels varied substantially across host species, with relatively higher expression observed in Primates, Carnivora, and Artiodactyla, indicating inherent differences in antiviral immune states.

CONCLUSIONS:

ISG Profiler enables rapid and scalable quantification of antiviral host responses across diverse animal species. This approach provides a practical foundation for large-scale comparative analysis of immune responses and supports future efforts in infection detection and viral discovery.



Abstract No.: 139

Poster Code: : G-0091

Population Immunology: Building Immune Barriers to Confront the Global Challenges of Respiratory Pathogens

First Author: William Liu, Guangzhou National Laboratory

Co-Author(s): Jinmin Tian, George F. Gao

BACKGROUND OBJECTIVES:

Respiratory pathogens such as influenza viruses, respiratory syncytial virus, and SARS-CoV-2 continue to pose significant threats to global health. In recent years, unusual fluctuations in the circulation of these pathogens have been observed worldwide, particularly following the implementation and relaxation of non-pharmaceutical interventions during the COVID-19 pandemic. These patterns suggest that changes in population-level immunity play a critical role in shaping epidemic dynamics. This study aims to introduce the concept of Population Immunology and propose the framework of the Population Immune Barrier (PIB) to explain how collective immune characteristics influence the emergence, transmission, and evolution of respiratory pathogens.

METHODS:

We performed an integrative analysis combining current evidence from immunology, epidemiology, and virology with findings from the studies of SARS-CoV-2. We longitudinally analyzed convalescents infected by different variants and vaccinated donors to assess the dynamics of antibody escape and spike-specific T-cell responses. Subsequently, using serotype-based classification of SARS-CoV-2 variants, we systematically evaluated cross-reactive T-cell immunity across different serotypes and identified mutations enriched in T-cell epitope hotspot regions. These data were interpreted within the PIB framework and provided key evidence for the proposal of Population Immunology.

RESULTS:

We propose that PIB represents a dynamic immune landscape shaped by prior infections, vaccination coverage, population turnover, and viral evolution. Reduced pathogen exposure and waning immune memory may generate immune gaps, increasing susceptibility and contributing to post-pandemic rebounds, whereas pre-existing and cross-reactive T-cell immunity can reinforce

PIB and partially protect against emerging variants. We further describe three interaction patterns between PIB and pathogen transmission: (1) seasonal amplification associated with periodic circulation of viruses, (2) gradual strengthening of PIB during emerging epidemics that slows the emergence of new variants, and (3) rebound dynamics driven by immune gaps following prolonged suppression of pathogen circulation. The studies of SARS-CoV-2 have provided direct evidence supporting PIB. Longitudinal analyses showed that antibody immune escape remained over time, whereas T-cell responses were broadly preserved and cross-reactive across variants. In addition, cross-serotype T-cell immunity was stronger within the same serotype and remained relatively maintained between serotypes despite antibody escape, although it declined progressively with cumulative antigenic divergence.

CONCLUSIONS:

The Population Immunology framework highlights the importance of population-level immune dynamics in shaping respiratory pathogen transmission. Strengthening PIB through vaccination strategies, immune monitoring, and integrated pathogen surveillance may enhance pandemic preparedness and improve global responses to emerging respiratory pathogens-related threats.



Abstract No.: 144

Poster Code: : G-0092

A 10-year follow-up study of humoral immunity in MERS-CoV recovered patients

First Author: So-Hee Kim, Seoul National University

Co-Author(s): Jiwon Choi, Songhyeok Oh, Yujin Kim, Namhyuk Cho

BACKGROUND OBJECTIVES:

Understanding long-term adaptive immunity against human coronaviruses is important for pan-coronavirus vaccine development. Here, we extended follow-up of the 2015 Korean MERS cohort to 10 years post-infection to characterize long-term humoral and cellular immune responses and investigate the underlying B cell repertoire.

METHODS:

Longitudinal plasma samples from MERS-CoV-recovered individuals were analyzed up to 10 years post-infection. Antigen-specific IgG responses against MERS-CoV and SARS-CoV-2 spike proteins were measured by ELISA, and neutralizing antibody titers against MERS-CoV, SARS-CoV-2 wild-type (WT), and Omicron BA.5 were measured using neutralization assays. Participants were classified into responder and non-responder groups based on MERS S1-specific IgG seropositivity. T cell responses were assessed by IFN- γ ELISpot using MERS-CoV structural proteins. In addition, antigen-specific B cells were sorted from selected responders (7–9 years post-infection) using MERS-CoV spike and SARS-CoV-2 spike probes, followed by single-cell RNA sequencing and BCR repertoire analysis.

RESULTS:

MERS-CoV spike(S1)-specific IgG seropositivity increased during the COVID-19 pandemic period and gradually declined thereafter, allowing classification into negative and positive responder groups. Positive responders maintained higher anti-MERS spike IgG titers and neutralizing activity throughout the 10-year follow-up, with a modest rebound after approximately 7 years. By contrast, anti-SARS-CoV-2 spike IgG and neutralizing responses were induced during the pandemic period and gradually declined, although BA.5-specific neutralizing activity was relatively better maintained than WT responses. In addition, positive IFN- γ ELISpot responses against MERS-CoV structural proteins were detected based on healthy

control-defined positivity thresholds. Ongoing single-cell transcriptomic and BCR analyses identified IgG1-expressing memory B cell clones among both mono-reactive and cross-reactive antigen-specific populations.

CONCLUSIONS:

These findings demonstrate durable humoral and cellular immunity in MERS-CoV-recovered patients up to 10 years post-infection. Notably, pre-existing immune memory, particularly in positive responders, appears to be maintained long-term and further modulated following SARS-CoV-2 exposure. This study provides additional insight into long-term coronavirus immunity and may support pan-coronavirus vaccine development.



Abstract No.: 201

Poster Code: : G-0093

Cellular innate immune determinants of SARS-CoV-2 clearance and disease severity

First Author: Margarida Bonifacio, The Francis Crick Institute

Co-Author(s): Javier Alvarez de Miranda, Jamie Barrett-Rodger, Katja Finsterbusch, Andreas Wack, David LV Bauer

BACKGROUND OBJECTIVES:

SARS-CoV-2 (SCV2) infection results in a range of clinical outcomes. Human and animal studies show that a rapid innate immune response is critical for early viral control. Conversely, excessive recruitment of inflammatory monocytes and neutrophils, particularly at later stages, is associated with severe infection and lung injury. While the effects of adaptive immune cells have been well defined in SCV2 infection, the roles of innate immune cells, such as dendritic cells and monocytes, and how they limit lung injury are less understood. Here, we aimed to dissect the contributions of DCs and monocytes in the response to SCV2 infection.

METHODS:

Mice lacking conventional dendritic cells via deletion of the Fms-related tyrosine kinase 3 ligand gene (Flt3l^{-/-} mice) or with a severe defect in recruitment of inflammatory monocytes and in monocyte-derived macrophages via deletion of C-C chemokine receptor type 2 gene (Ccr2^{-/-} mice) were infected with mouse-adapted SCV2 (MA20). Lung, nasal tissue, and lung-draining mediastinal lymph nodes were collected at 3, 7, and 14 days post-infection. Infection was monitored by plaque assay, RT-qPCR, and immunohistochemistry. Immune responses were characterised by flow cytometry.

RESULTS:

MA20 infection in wild-type mice is cleared by day 7 post-infection. We observed an increase in migratory dendritic cell numbers in the mediastinal lymph nodes during early infection and an expansion of lymph node-resident dendritic cells, germinal centre B cells and T cell populations, including central memory and effector memory CD8⁺ T cells, and CD4⁺ T follicular helper (Tfh) cells, within the first week of infection. In contrast, Flt3l^{-/-} mice experienced more severe disease, delayed clearance, and reduced adaptive immune cell populations (including Spike-specific CD8⁺ T cells, germinal centre B cells, and Tfh cells) in the mediastinal lymph nodes. Similarly, Ccr2^{-/-} mice lost more weight during acute infection and

had higher viral loads in the lung and nasal compartment.

CONCLUSIONS:

Our results suggest that the presence of dendritic cells and CCR2-dependent recruitment of inflammatory monocytes is critical for early viral control. It is likely that inflammatory monocytes act as a two-edged sword, with their association with severity depending on the magnitude and temporal dynamics of their recruitment into the lung. Our results highlight the importance of a more detailed understanding of the role of innate cell subsets in infection and have motivated current efforts to map the roles of other cells, such as natural killer cells. Overall, this work provides an important basis for understanding immune cell interplay in SCV2 infection and mechanisms underlying the development of pathology following infection.



Abstract No.: 389

Poster Code: : G-0095

The role of SARS-CoV-2 Membrane protein antibodies in protective humoral immunity

First Author: Daniel Egan, University of Cambridge

Co-Author(s): Matteo Ferrari, George Carnell, Andrew Chan, Laurence Tiley, Helen Baxendale, Jonathan Heeney

BACKGROUND OBJECTIVES:

Betacoronaviruses have caused some of the largest epidemics in recent history and pose an ongoing threat to global health. Efforts to develop effective and durable vaccines are undermined by the rapid evolution of new viral variants which evade prior immunity. The SARS-CoV-2 Membrane protein, which is prevalent on the virion surface and is essential for viral replication and transmission, has undergone minimal mutation throughout the COVID-19 pandemic. This protein is thus a potential target for durable and broadly protective vaccine-induced immunity. The role of Membrane protein-specific antibodies in protecting against COVID-19 is not well understood. This study aimed to characterise the humoral immune response to the SARS-CoV-2 Membrane protein following natural infection in a varied cohort, and to correlate antibody levels and functions with clinical outcomes.

METHODS:

Humoral immune responses to SARS-CoV-2 Membrane were studied following natural infection in a comparative cohort of healthcare workers (uninfected and mild disease), hospitalised patients (severe disease), and immunodeficient individuals. Antibody levels were quantified using ELISA and flow cytometry. Antibody Fc effector functions were measured using reporter cell assays and flow cytometry. Correlations were made between SARS-CoV-2 Membrane protein antibody levels, antibody effector function, and clinical outcomes.

RESULTS:

Patients with severe COVID-19 disease showed higher levels of IgG antibodies towards Membrane protein compared to those with mild disease, while immunodeficient individuals demonstrated poor humoral responses. IgG antibodies produced were demonstrated to have potential to elicit ADCC and ADCP responses. Antibody levels to Membrane protein decayed slowly over time compared to Spike and Nucleocapsid-directed antibodies.

CONCLUSIONS:

Functional humoral immune responses towards the SARS-CoV-2 Membrane protein correlate with disease severity, are impaired in immunodeficient individuals, and persist longer than antibodies towards other SARS-CoV-2 proteins. Understanding the relative role of different components in protective immune responses is important for the generation of successful, variant-proof vaccines.



Abstract No.: 414

Poster Code: : G-0096

Differential Antibody Cross-Reactivity of mRNA and Inactivated SARS-CoV-2 Vaccines Against Seasonal Coronaviruses

First Author: Cheng Xiao, The University of Hong Kong

Co-Author(s): Nancy H.L. Leung, Carolyn Cohen, Xia Lin, Mark Zanin, Sophie Valkenburg, Malik Peiris, Ben Cowling, Sook-San Wong,

BACKGROUND OBJECTIVES:

Cross-reactivity between SARS-CoV-2 and seasonal coronaviruses has raised concerns about potential impacts on vaccine efficacy and immune responses.

METHODS:

We conducted a longitudinal observational study of 169 adults aged 18 and older receiving either BNT162b2 mRNA vaccine or SinoVac inactivated COVID-19 vaccine. Serum samples were collected at baseline, after first dose, and after second dose. We measured antibody responses (IgG, IgA, IgM) against SARS-CoV-2 and four seasonal coronaviruses (HCoV-OC43, HCoV-HKU1, HCoV-229E, HCoV-NL63) by the electrochemilum Meso Scale Diagnostics (MSD) platform. Functional assays including neutralization and FcR11a binding were performed on a subset of samples.

RESULTS:

Cross-reactive responses were comparable across all adult age groups without significant age-dependent differences, despite baseline variations in IgM antibodies level. BNT162b2 vaccination induced substantial cross-reactive antibody responses against betacoronaviruses, with up to 80% of participants showing elevated titers against HCoV-OC43 particularly. In contrast, SinoVac vaccination showed minimal cross-reactive effects, and cross-reactivity to alphacoronaviruses remained minimal for both vaccines. Importantly, despite elevated binding antibodies, functional assays revealed no significant increase in neutralization or FcR11a binding activity against seasonal coronaviruses.

CONCLUSIONS:

These findings will be important for future universal coronavirus vaccine development.

Abstract No.: 417

Poster Code: : G-0097

SARS-CoV-2 induced earlier host antiviral responses than SARS-CoV in human bronchial epithelial cells and Syrian hamsters

First Author: Chi Cheng Sou, The University of Hong Kong

BACKGROUND OBJECTIVES:

SARS-CoV and SARS-CoV-2 exhibit different transmissibility and pathogenicity in humans. We aim to compare viral replication kinetics and host responses in different in vivo and in vitro models of these genetically-related Sarbecoviruses.

METHODS:

Differentiated normal human bronchial epithelial cells (NHBE) were infected with SARS-CoV (strain: HK39849) or SARS-CoV-2 (strain: hCoV-19/Hong Kong/VM0001061/ 2020) at an MOI of 0.5 or MOCK-infected with PBS to determine infectious viral load and host response by bulk RNA sequencing. Male Syrian hamsters were intranasally-inoculated with 10^5 TCID₅₀ of SARS-CoV, SARS-CoV-2, or PBS. Nasal turbinates and lungs were collected on 1, 2, and 5 days post-inoculation (dpi) to determine viral load and host response. Gene-set enrichment analysis (GSEA) was used to identify pathways of significant biological functions. RT-qPCR was used to validate the RNAseq results.

RESULTS:

In NHBE, SARS-CoV-2 replicated to higher titers than SARS-CoV at 48- and 72-hours post-infection (hpi) while inducing more robust host responses, with 4,065 and 2,184 differentially expressed genes (DEGs) identified when compared to mock-infected cells, respectively. Specifically, SARS-CoV-2 infection significantly upregulated expression of specific pattern recognition receptors (PRRs) than SARS-CoV, including TLR2, TLR3, RIG-I, MDA5, LGP2, CLEC7A, NOD1, NOD2, AIM2, IFI16, and ZBP1. In Syrian hamsters, SARS-CoV-2 replicated more efficiently than SARS-CoV on 1 dpi in the nasal turbinates, but both reached comparable titers on 2 and 5 dpi. In the lungs, SARS-CoV-2 and SARS-CoV showed comparable replication at 1, 2, 5 dpi. Both viruses also showed comparable airborne transmission potential to naive hamsters. Despite of comparable replication of the two viruses in this host, SARS-CoV-2 induced earlier and more robust antiviral host response than SARS-CoV in the nasal turbinates (9197 vs 7865 DEGs) and in

the lungs (7831 vs 5523 DEGs). In particular, SARS-CoV-2 significantly induced higher expression of specific PRRs than SARS-CoV on 1 dpi in both nasal turbinates and the lungs, including TLR2, TLR6, TLR7, RIG-I, MDA5, LGP2, CLEC7a, CLEC4e, NOD2, NLRP3, NLRC4, and ZBP1. In the lungs, earlier expression of interferons and interferon-stimulated genes (ISGs) was validated by RT-qPCR in SARS-CoV-2 on 1dpi.

CONCLUSIONS:

SARS-CoV-2 induced earlier antiviral response than SARS-CoV in both NHBE cells and Syrian hamsters. We identified shared innate sensors that may facilitate an earlier induction of type I interferon antiviral responses after SARS-CoV-2 infection than SARS-CoV in two different hosts. Our results also suggest that specific viral protein encoded by SARS-CoV may have facilitated evasion of innate sensing than SARS-CoV-2. Elucidating these virus-specific immune evasion mechanisms may uncover novel therapeutic targets to promote early antiviral immunity and restrict viral replication.



Abstract No.: 417

Poster Code: : H-0098

The Characterization of Molecular Mechanism of Viral Inactivation on Copper Surface Coatings

*First Author: Chi Yeung Shuen, The University of Hong Kong**Co-Author(s): Chin Wing Hong, Poon Lit Man*

BACKGROUND OBJECTIVES:

Fomite-mediated transmission is a major indirect route for enveloped respiratory viruses such as SARS-CoV-2 and influenza viruses, enabling virions shed from infected individuals to contaminate high-touch surfaces and subsequently infect susceptible hosts. This study investigates copper oxides' viral inactivation mechanism against both pathogens under physiologically relevant conditions. Hence, we seek to develop possible novel interventions. By exploring the performance of inactivation and the factors involved, we aim to interrupt the fomite transmission chain and inform next-generation antiviral coatings.

METHODS:

Inactivation assay Active ingredients of copper-containing coatings are selected as the testing candidates, which are CuO(s), Cu₂O (s) and CuCl₂ (aq). Copper candidates are incubated with 1:1 volume of H₂O and wild type SARS-CoV-2 virus or PR8 for different time points and at different amounts/concentrations. After 60 minutes of incubation, mixtures involving powder will be centrifuged down before supernatant collection. 105ul of supernatants containing the virus is then titrated by TCID₅₀ assay to determine the infectious viral titre using VeroE6 TMPRSS2 cell or MDCK cell.

Inactivation factor assay 100ul of 20mM EDTA, BCS, Tiron, D-mannitol are introduced to the samples of inactivation assay respectively in order to determine the effect of soluble Cu(II) ions, that maybe leached out from the coating. Infectious viral titres will be compared between the experimental groups with or without chelators to determine the effect of soluble copper ions on virus inactivation.

Filter test CuO(s) and Cu₂O(s) are incubated with 200ul of H₂O and 100ul of 20mM BCS or Tiron for 60 minutes under 1500 rpm at 25°C. Mixtures were transferred to a filter column and spin under 130,000 rpm for 1 minute. Flow through were collected as Chelator-Copper-ion samples. Filtered copper oxide powder were collected as Chelator-treated-oxide samples. Both groups of samples were

examined by the inactivation assay.

RESULTS:

CuO, Cu₂O and CuCl₂ are effective in inactivating both SARS2 and PR8 viruses following time-dependent and amount/concentration-dependent trend. At 60 minutes, 300mg CuO, 200mg Cu₂O and 1mM CuCl₂ can reduce the viral titer more than 3 log(TCID₅₀/ml), which is around 99.9% viral reduction.

Decoupling test demonstrated that copper oxides primarily inactivate the viruses by physical contact since the supernatant from the oxides incubated with water did not provide viral reduction within 60 minutes while the powder leftover from the incubation did.

Then, we moved to investigate the factor involved in this direct-physical-contact-based viral inactivation. From our result, EDTA is able to protect the viral titer for both SARS2 and PR8 from the inactivation of the copper candidates representing copper ions are majorly involved in the mechanism of inactivation. However, the introduction of D-mannitol showed no effect on the virus inactivation suggesting hydroxyl radical-induced oxidation is primarily not involved in this experiment. Interestingly, BCS and Trion showed a degree of enhancement on the viral reduction by Cu₂O. BCS is able to further reduce the viral titer of SARS2 by around 1 log(TCID₅₀/ml) whereas Trion is able to enhance 0.5 to 1 log(TCID₅₀/ml) viral reduction for SARS2 and 1 log(TCID₅₀/ml) for PR8. It is suggested that BCS and Trion were complexed with the copper ions of the copper oxides, which allowed the ions to more readily react with the viral particles. RT-qPCR result also showed that the group of Trion adding to Cu₂O with PR8 has vRNA damaging. This might be caused by the generation of Cu³⁺ ion during the process of Fenton-like reaction. Complexation of BCS or Trion to the copper oxides is able to enhance the inactivation by introducing mobile copper ions to the solution to break viral proteins or generate ROS to the viruses.

CONCLUSIONS:

This study clarifies the antiviral mechanisms of copper-oxide-coating surface against SARS-CoV-2 and Influenza A Virus (PR8). While CuO, Cu₂O and CuCl₂ exhibited amount/concentration- and time-dependent inactivation, distinct pathways emerged. Copper oxides primarily acted via physical contact. Another mechanistic finding observed involves reactive copper complexes. BCS is able to

form highly unstable Cu(I)-BCS2 complexes on Cu₂O surfaces. These negatively charged species are able to target the RBD of SARS-CoV-2, breaking disulfide bonds and disrupting ACE2 binding by the copper ion additive in its structure. Similarly, Tiron is able to catalyze ROS generation via Haber-Weiss and Fenton-like reactions, producing hydroxyl radicals ($\bullet$ OH) and cupryl ions (Cu³⁺). These reactive oxidants can cause irreversible structural damage to the viral proteins and lipid envelopes of both viruses proven by studies, demonstrating that copper-induced redox chemistry is a dominant factor of the copper oxides' antiviral activity in this experiment. For both viruses, D-mannitol failed to protect against CuO or Cu₂O, indicating that physical disruption and disulfide bond cleavage was the main inactivation factor instead of oxidative lipid damage. Overall, this research highlights that the virucidal activity of copper oxides rely on the physical adsorption of viral particles on oxide surfaces and the possibility of labile ion-mediated redox reactions by complexation. These findings underscore the potential of engineered copper materials as broad-spectrum virucides.



Abstract No.: 176

Poster Code: : H-0099

COVID-19 vaccination induces cross-neutralisation of sarbecoviruses related to SARS-CoV-2

First Author: Grace West, University of Cambridge

Co-Author(s): Rebecca Morse, Benjamin Sievers, Adam Abdullahi, Kimia Kamelian, Mark Tsz Kin Cheng, Douglas Stewart, Divya Diamond, Mazharul Altaf, Lourdes Ceron-Gutierrez, Olga Sokolova, David Robertson, Spyros Lytras, Rainer Döffinger, Sam Wilson, Suzannah Rihn, Ravindra Gupta

BACKGROUND OBJECTIVES:

The combined threats of future sarbecovirus zoonosis and continually emerging SARS-CoV-2 variants of concern (VOCs) highlight the need to assess the breadth of existing SARS-CoV-2 vaccine-mediated protection. In particular, it remains unclear to what extent current vaccination strategies elicit cross-neutralising antibody responses against divergent sarbecoviruses.

METHODS:

Here we investigate a cohort of older individuals, who received four COVID-19 vaccine doses, for potential cross-neutralisation. Serum samples were assessed using lentiviral pseudoparticles bearing spike proteins from either Omicron VOCs or other sarbecoviruses, including SARS-CoV-1 and related animal coronaviruses, to evaluate neutralising antibody responses.

RESULTS:

Despite recent fourth bivalent mRNA vaccine doses (encoding SARS-CoV-2 Wu-1 and Omicron BA.1 spikes), neutralisation of Omicron lineage VOCs was reduced compared to Wu-1, consistent with an imprinted immune response. Similarly, particles bearing either SARS-CoV-1 or a SARS-CoV-1-related bat sarbecovirus spike were neutralised less efficiently than Wu-1. Unexpectedly, however, particles bearing spikes from two animal SARS-CoV-2-related viruses, BANAL-20-52 and a pangolin CoV, were more sensitive to serum neutralising antibodies, with NT50 values approximately 7.4-fold and 13.3-fold higher, respectively, than SARS-CoV-2 Wu-1.

CONCLUSIONS:

These surprising findings suggest that vaccine-mediated adaptive immunity may provide efficient cross-neutralisation, and potential protection, against certain animal sarbecoviruses.

Abstract No.: 91

Poster Code: : J-0101

SARS-CoV-2 Δ Mac1 induces robust cellular and humoral immunity with complete protection against both homologous and heterologous SARS-CoV-2 variants

First Author: Pradtahna Saenjamsai, University of Kansas

Co-Author(s): Debarati Chanda, Anthony R. Fehr, Rudragouda Channappanavar

BACKGROUND OBJECTIVES:

The continued emergence of SARS-CoV-2 variants poses a challenge to current vaccines, which primarily target spike protein and exhibit waning protection against infection. There is a critical need for live-attenuated vaccines (LAVs) that present a broader range of viral epitopes and induce robust immunity. Coronavirus uses its Mac1 domain to counteract host ADP-ribosylation and suppress innate immune responses. Previous work from our laboratory demonstrated that deletion of the Mac1 domain (Δ Mac1) results in a highly attenuated virus and induces strong interferon responses and interferon-stimulated genes (ISGs) during infection (Alhammad et al. 2023. PNAS). Here, we evaluate a Δ Mac1 mutant virus as a potential interferon-inducing LAV candidate.

METHODS:

We evaluated the safety and efficacy of the Δ Mac1 vaccine using the K18-hACE2 transgenic mouse model. Mice were immunized intranasally with Δ Mac1 and subsequently challenged with lethal doses of either wild-type (Wuhan) SARS-CoV-2 or variants including Delta and Gamma to assess protective efficacy. Safety was monitored through daily weight and clinical scoring. Efficacy was evaluated by measuring survival, viral clearance, and lung pathology following challenge. To characterize immune responses associated with protection, we assessed virus-specific polyfunctional CD4⁺ and CD8⁺ T cell responses in the lungs and spleen, representing local respiratory and systemic immunity, respectively, as well as cross-neutralizing antibody responses.

RESULTS:

Primary infection with Δ Mac1 was highly attenuated, causing minimal clinical disease or weight loss. Vaccination elicited robust polyfunctional CD4⁺ and CD8⁺ T cell responses targeting multiple structural (S, M) and accessory (ORF3a) proteins in both the lungs and spleen, indicating strong local and systemic cellular immunity. Furthermore, Δ Mac1 induced high-titer antibodies capable of

cross-neutralizing wild-type, Delta, Gamma and Omicron variants. Following lethal challenge, vaccinated mice exhibited 100% survival and complete protection, with significantly enhanced viral clearance from the lungs compared to controls.

CONCLUSIONS:

These findings demonstrate that targeted attenuation through Mac1 deletion safely elicits broad cellular and humoral immunity. The Δ Mac1 candidate effectively protects against diverse SARS-CoV-2 variants, supporting interferon-inducing live-attenuated vaccines as a promising strategy for controlling the continued evolution and spread of SARS-CoV-2.



Abstract No.:137

Poster Code: : J-0102

A single-dose amber codon carrying live attenuated vaccine elicits pan-sarbecovirus immunity and blocks SARS-CoV-2 transmission

First Author: NIGEER TE, The University of Hong Kong

Co-Author(s): Jiangchuan Li, Alex Wing Hong Chin, Haogao Gu, Yi Huang, Mandy Tsoi Man HO, Tom Chi Yeung Shuen, Samuel Mo Sheung Cheng, Chee Wah Tan, Linfa Wang, Kenrie Pui Yan Hui, Michael Chi Wai Chan, Bo Feng, Malik Peiris, Leo Lit Man Poon

BACKGROUND OBJECTIVES:

Existing COVID-19 vaccines exhibit clear limitations against the ongoing emergence of variants of concern, as their efficacy is undermined by cumulative mutations in the spike or its RBD, the primary antigenic target. In addition, the intramuscular route of administration is suboptimal for inducing robust mucosal immunity, an important factor in preventing respiratory tract infection and transmission. To overcome these challenges, we aim to develop a novel mucosal vaccine with enhanced immunogenicity.

METHODS:

We invented a live attenuated vaccine (AC_ORF) by introducing amber codons (UAG) into the coding region of SARS-CoV-2 genome. Specifically, multiple lysine codons of ORF3a, 6 and 7a were mutated to UAG, disrupting the translation of these accessory proteins in the normal cells. Nevertheless, the virus can be efficiently propagated in an engineered cell line expressing an orthogonal translational system, which, in the presence of a supplied unnatural amino acid N6-Carbobenzyloxy-L-lysine, facilitates read-through of the UAG codons to produce full-length, functional proteins.

RESULTS:

AC_ORF shows attenuation both in vitro and in vivo. A single AC_ORF immunization protects mice against antigenically diverse SARS-CoV-2 variants and even SARS-CoV-1. The sera from the vaccinated mice demonstrated neutralizing titres against the SARS-CoV-2 strains of the ancestral, early VoCs, circulating VoCs, and a Pangolin sarbeco-CoV. Moreover, immunization with the mutant virus efficiently blocked SARS-CoV-2 transmission in the hamster model.

CONCLUSIONS:

These findings establish AC_ORF as a true pan-sarbecovirus mucosal vaccine that confers broad immunity against both antigenically distinct VoCs and heterologous SARS-CoV-1, offering a promising defense against future sarbecovirus pandemics.

Abstract No.:138

Poster Code: : J-0103

Broad beta-CoV immunity and transmission blockade by a single-dose live-attenuated vaccine with atypical codon usage

First Author: NIGEER TE, The University of Hong Kong

Co-Author(s): Alex Wing Hong Chin, Haogao Gu, Janice Zhirong Jia, Yi Huang, Samuel Mo Sheung Cheng, Chee Wah Tan, Jincun Zhao, Kenrie Pui Yan Hui, Michael Chi Wai Chan

BACKGROUND OBJECTIVES:

Current COVID-19 vaccines have saved countless lives but primarily aim to induce immunity to the spike or its RBD protein and often fail to confer broad or durable protection against rapidly evolving variants and prevent transmission. In this study, we have developed novel live attenuated SARS-CoV-2 mutant variants that retain all critical immunogens, exhibiting potential for vaccine implementation.

METHODS:

We developed SARS-CoV-2 vaccine candidates by modifying the codon usage pattern of the viral genome, resulting in attenuated viral replicons without altering encoded amino acid sequences. Using a well-established reverse genetics technique, we successfully generated infectious clones of these mutants. We evaluated their replication and genetic stability in cell lines and chose the most attenuated mutant virus, cb1, to assess its virulence, immunogenicity, and protective efficacy against both wild-type (WT) SARS-CoV-2 and the latest widely circulating Omicron variants, as well as other unrelated betacoronaviruses in vivo.

RESULTS:

A single intranasal dose of cb1 vaccine elicits remarkably broad and potent immunity that overcomes existing parenteral vaccines' limitations. cb1 induced robust neutralizing antibody and T cell responses that translated into complete protection in animal models, including prevention of viral transmission to unvaccinated contacts. Notably, cb1 provided cross-protection not only against diverse SARS-CoV-2 variants of concern but also against more divergent SARS-CoV-1 and hCoV-OC43, a breadth of immunity unparalleled by current vaccines.

CONCLUSIONS:

Our findings highlight the potential of cb1 to address urgent needs for next-generation COVID-19 vaccines that elicit mucosal immunity with broad, long-lasting efficacy, eliminating the necessity for frequent updates.

Abstract No.:154

Poster Code: : J-0104

Prefusion engineering of Embecovirus spike proteins enables broad coronavirus antigen design

First Author: Jelle Melchers, Utrecht University

Co-Author(s): Jarek Juraszek, Ruben Hulswit, Daan van Overveld, Lam Le, Frank van Kuppeveld, Daniel Hurdiss, Berend-Jan Bosch, Hans Langedijk, Mark Bakkers,

BACKGROUND OBJECTIVES:

The persistent threat of zoonotic coronavirus spillovers into the human population underscores the need for broad, adaptable vaccine design strategies. The spike (S) glycoprotein, the key antigen on the viral envelope, is the primary component of coronavirus vaccines, yet its metastability presents challenges for vaccine efficacy and manufacturability. In this study we aim to lock the S protein in its prefusion conformation to design a suitable coronavirus vaccine antigen. We integrate rational design with an AI/ML algorithm (ReCaP) to identify stabilizing substitutions, using the OC43 S protein as a prototype antigen. To provide stabilization strategies that are broadly applicable to other coronavirus spikes we assess the potential of translating these mutations to spikes of related coronaviruses Equine coronavirus (ECoV) and human coronavirus HKU1 (HCoV-HKU1).

METHODS:

We assess the effect of the selected amino acid substitutions on expression and thermal stability of the spike protein by size-exclusion analysis via UHPLC of supernatants of transiently transfected Expi293 cells. We continue to purify and characterize selected designs harbouring the best performing substitutions by SEC-MALS and DSF. Finally, we resolve cryo-EM structures of the designed antigens to interpret the mechanism by which the substitutions confer improved prefusion stability.

RESULTS:

We identify a panel of mutations in the OC43 S which combined greatly improve expression and thermal stability of the prefusion spike. A majority of the panel also translate to related embecovirus S proteins like ECoV and HCoV-HKU1 and enable straightforward design of prefusion stabilized antigens for these viruses. The structural analysis of the substitutions by cryo-EM inform on the functionality of these spikes and how regions of instability are conserved. Key stabilizing substitutions arrested release of the fusion peptide or improved the polar

interactions of a bound free fatty acid at the interprotomer S1B interface to lock the spike in the 'down' state.

CONCLUSIONS:

Using a combination of rational design, phylogenetic analysis, and the machine-learning model ReCaP, we describe a broadly applicable approach for the design of prefusion stabilized embecovirus spike proteins. Using the approach we were able to design well expressing prefusion stabilized antigens for OC43, ECoV and HKU1 spikes, and elucidate the first available structure of the ECoV prefusion spike protein. Overall, the panel of stabilizing substitutions that we identified provide a framework for the design of stabilized antigens of related embecoviruses.



Abstract No.:158

Poster Code: : J-0105

An Attenuated Influenza-Vectored Vaccine Platform for Mucosal Delivery of SARS-CoV-2 RBD Confers Broad Protection Against SARS-CoV-2 and Heterologous Influenza Strains

First Author: Yuner Gu, The University of Hong Kong

Co-Author(s): Prof. Leo L.M. Poon, Dr. Alex W.H. Chin

BACKGROUND OBJECTIVES:

Since the 1918 flu pandemic, the global community has faced recurring public health crises driven primarily by respiratory pathogens such as influenza and coronaviruses. The fact that the majority of these outbreaks resulted in widespread morbidity and profound socio-economic disruption highlights the urgent need for vaccine platforms capable of inducing broad, durable immunity. Integrating historical lessons with recent advances in COVID-19 and influenza vaccinology, this study explores the design of a novel influenza virus-based platform that combines innovative delivery strategies with targeted antigen engineering

METHODS:

Using an attenuated recombinant influenza H1N1 viral vector, we engineered two distinct vaccine candidates: one that displays the wild-type SARS-CoV-2 Receptor Binding Domain (RBD) on the virion surface via fusion with neuraminidase (NA), and another that drives cell-surface expression of the RBD using a transmembrane anchor. Both two candidates demonstrated significant attenuation in mammalian cells and murine models without compromising antigen expression. To optimize the immune response, we also introduced a G502E mutation in RBD to abrogate the RBD-hACE2 interaction, a modification designed to minimize receptor-mediated interference and focus the induction of potent neutralizing antibodies.

RESULTS:

Our results demonstrate that the G502E mutation significantly outperforms the wild-type RBD, eliciting higher RBD-specific neutralizing antibody titers that remain stable for at least six months post-immunization. In lethal challenge mouse models, both vaccine constructs provided robust protection against the

homologous H1N1 strain, while also eliciting strong cross-protection against heterologous H3N2 and H5N1 viral challenges. Furthermore, beyond influenza, this platform successfully conferred protective immunity against wild-type SARS-CoV-2 in a mouse model.

CONCLUSIONS:

Collectively, these findings validate an influenza virus-based RBD delivery strategy capable of eliciting broad-spectrum immunity against both coronaviruses and influenza viruses. The combination of rational antigen design and vector-based delivery offers a promising approach for developing broadly protective vaccines against continuously evolving respiratory viral threats.



Abstract No.:281

Poster Code: : J-0106

Identification of Monoclonal Antibodies Targeting the SARS-CoV-2 Spike Protein for the Therapeutic Treatment of Omicron Infections

First Author: Yun-Sang Tang, The University of Hong Kong

Co-Author(s): David SC Hui, Ka-Pun Mok, Yuanxin Sun

BACKGROUND OBJECTIVES:

SARS-CoV-2 is a rapidly evolving virus that is responsible for COVID-19 infections. Despite that recent SARS-CoV-2 variants in general lead to milder symptoms, they retain enhanced transmissibility, immune escape, and can still cause severe diseases in high-risk groups such as the elderly and the immunocompromised. Currently, most of the available monoclonal antibodies are not reactive against emerging variants, and there is thus a need to identify novel antibodies that can cross-react with multiple variants of concern for therapeutic purpose. In this study, we aimed to identify cross-reactive anti-SARS-CoV-2 antibodies that targeted the spike protein and could neutralize multiple variants of concern.

METHODS:

The single B-cell receptor repertoire of 20 individuals vaccinated with wild-type BNT162b2 mRNA vaccine was sequenced, from which a library of paired antibody heavy chains and light chains were obtained. These antibodies were then expressed in the Expi293F system. We characterized the neutralization potency of these antibodies against various SARS-CoV-2 variant strains by enzyme-linked immunosorbent assay (ELISA) and the surrogate virus neutralization test (sVNT). We also determined the protective potency of the selected antibodies in mice against wild-type (WT) or EG.5.1 challenge.

RESULTS:

From the paired heavy chain and light chain sequences of vaccinated individuals, we reconstructed 95 antibodies to the receptor-binding domain (RBD) and 42 antibodies to the N-terminal domain (NTD). A total of 39 RBD-specific antibodies were positive in WT-RBD binding, while 36 of them were also cross-reactive to Omicron BA.1-RBD. One NTD-specific antibody was found to bind to both WT and BA.1 NTD. Using sVNT, we identified RBD-specific antibodies that could neutralize

WT, BA.1, BA.2, BA.4/5, EG.5.1 and JN.1 variant RBDs to different extents, among which two (RBD-7 and RBD-94) showed broad neutralization activities across different variant strains. RBD-7 could protect mice against lethal WT SARS-CoV-2 challenge either applied alone or in combination with NTD-34, an NTD-specific antibody. RBD-94 could protect mice from lethal EG.5.1 SARS-CoV-2 challenge in both prophylaxis and treatment schemes. Upon antibody administration, infected mice showed significantly reduced lung viral titre and improved survival compared to vehicle control.

CONCLUSIONS:

From the paired heavy chain and light chain sequences of vaccinated individuals, we reconstructed 95 antibodies specific to the receptor-binding domain (RBD) and 42 antibodies specific to the N-terminal domain (NTD). A total of 39 RBD-specific antibodies were positive in WT-RBD binding, while 36 of them were also cross-reactive to Omicron BA.1-RBD. One NTD-specific antibody was found to bind to both WT and BA.1 NTD. Using sVNT, we identified RBD-specific antibodies that could neutralize WT, BA.1, BA.2, BA.4/5, EG.5.1 and JN.1 variant RBDs to different extents, among which two (RBD-7 and RBD-94) showed broad neutralization activities across different variant strains. RBD-7 could protect mice against lethal WT SARS-CoV-2 challenge either applied alone or in combination with NTD-34, an NTD-specific antibody. RBD-94 could protect mice from lethal EG.5.1 SARS-CoV-2 challenge in both prophylaxis and treatment schemes.



Abstract No.:314

Poster Code: : J-0107

Cross-reactive T cells to Betacoronaviruses

First Author: Zhirong Jia, Pasteur Research Pole

Co-Author(s): Prathanporn Kaewpreedee, Carolyn A Cohen, Faraz Ahmed, Ahmed Abdul Quadeer, Matthew R McKay, Nancy H L Leung, Malik Peiris

BACKGROUND OBJECTIVES:

Viral variants that can escape neutralizing antibodies can undermine immune mediated protection, and is a major challenge for effective vaccines. T cell responses elicited by SARS-CoV-2 exposure target multiple conserved antigens of the virus other than just the highly mutable spike protein targeted by antibodies. This advantage underlies the possibility of universal vaccine against multiple coronaviruses. Yet reports on the T cell responses conferred by SARS-CoV-2 exposure to other subgenera of betacoronaviruses is limited. We compared the T cells responses to betacoronaviruses from donors with different SARS-CoV-2 exposure history to determine the extent of cross-reactive T cells responses to inform future universal vaccine development.

METHODS:

In our study we used peripheral blood mononuclear cells (PBMCs) samples from pre- and post-COVID blood donors, BNT162b2 and CoronaVac vaccinees, and people with and without long-COVID symptoms to evaluate the T cell responses to a peptide panel of coronaviruses.

RESULTS:

T cell responses were observed with RaTG13, HKU3, MERS, and SARS2 viruses for spike and non-spike peptides. In vitro recalled IFN γ CD4+T cell responses to RaTG13, HKU3 and SARS2 spike stimulations were observed, but not to MERS, in the BNT162b2 vaccinees. IFN γ CD8+T cell responses recalled in vitro were only to SARS2 spike in the BNT162b2 vaccinees. Whilst there was no recalled IFN γ CD8+T cell responses to any of non-spike stimulations in all groups. Vaccinations also increased IFN γ CD4+T cells cross reactive to RaTG13 spike over time.

CONCLUSIONS:

CD4+ T cell plays a key role in vaccination induced cross-reactivity among betacoronaviruses, which extend to RaTG13 and HKU3 viruses for spike in the BNT162b2 vaccination and to non-spike proteins by CoronaVac vaccination. Thus, T cell cross-reactivity to related coronaviruses is increased by vaccination and type.

Abstract No.:327

Poster Code: : J-0108

Efficacy Evaluation of an Oral DNA Vaccine Against Porcine epidemic diarrhea virus Infection in Pigs

First Author: Frank, Chia-Jung Chang, Reber Genetics Co., Ltd.

Co-Author(s): Carol, Pei-Yin Wu, Tzu-Po Luo

BACKGROUND OBJECTIVES:

Since the emergence of highly infectious porcine epidemic diarrhea (PED) G2 virus in 2010, it has caused great economic losses in swine industry worldwide. It infects pigs of all age and mucosal immunity has been proven important for protection against PEDV infection. Here an oral PEDV DNA vaccine was developed and its ability to induce PEDV-specific immune responses, by itself and in conjunction with a commercially available subunit vaccine, and efficacy against PEDV infection was evaluated.

METHODS:

3-week-old piglets were vaccinated at 3 and 6 weeks old with oral PEDV DNA vaccine, subunit vaccine (subunit) via intramuscular injection or equal volume of saline. For combined prime-boost trial, 3-week-old piglets were vaccinated with either oral DNA vaccine or subunit vaccine at 3 weeks old, followed by vaccination at 6 weeks old with subunit vaccine. Blood samples were collected at 0-, 3-, and 6-weeks post vaccination to evaluate antibody response. Experiment piglets were challenged at 9 weeks old with Taiwan indigenous PEDV G2 virus. Clinical/fecal scores were recorded daily after challenge and pigs were weighed before and after challenge to evaluate growth performance during challenge period.

RESULTS:

Vaccination with either oral vaccine or subunit vaccine, followed by a booster with subunit vaccine can induce both PEDV-specific IgG response in pigs, while only the former (primed with oral vaccine) induces PEDV-specific IgA response. Vaccination with either oral DNA vaccine or subunit vaccine or in all combination tested significantly reduce clinical/fecal scores after PEDV challenge. Vaccinated pigs also exhibit higher average daily weight gain than the control pigs during

challenge period.

CONCLUSIONS:

Results from present study showed that vaccination with a novel PEDV DNA vaccine could provide protection in piglets against PEDV infection. Oral DNA vaccine can induce PEDV-specific IgA while intramuscular vaccination of a commercially available PEDV subunit vaccine can induce PEDV-specific IgG. A vaccination program including both can induce both IgG and IgA responses. With a 2-shot vaccination program, this novel oral PEDV DNA vaccine provides flexibility to the farmers. reactivity to related coronaviruses is increased by vaccination and type.



Abstract No.:44

Poster Code: : K-0109

Rational Discovery of Escape-Resistant Therapeutics against Coronaviruses

*First Author: Matthew Zirui Tay, A*STAR*

Co-Author(s): Wei Shen Ho, Louis DeFalco, Ching-Wen Huang, Han Kee Ong, Weikang Nicholas Lin, Yuling Huang, Ajayanandan Yadunandan, Chia Yin Lee, Rabiatal Adawiyah Minhat, Bei Wang, Yuansheng Yang, Roland Huber, Cheng-I Wang

BACKGROUND OBJECTIVES:

Viral evolution is a key consideration in developing antiviral therapeutics. Target diversification due to evolution or immune pressure is a common feature of many viruses, and can result in loss of therapeutic efficacy. In this study, I outline an approach towards the development of escape-resistant therapeutics, using coronaviruses as an exemplar system. A key insight to this process is that the antigenic diversity across a viral species group provides insights into the evolutionary space within which a viral strain can operate. Consequently, resistance to future evolutionary drift of a viral target can be engineered by developing therapeutics that have sufficient breadth to cover other antigenically distant virus targets within the same viral group. This strategy was applied towards discovery and optimization of an ACE2 receptor decoy designed to neutralize all strains of SARS-CoV-2, as well as other ACE2-using alphacoronaviruses and betacoronaviruses.

METHODS:

Key interaction residues and mutations showing favourable binding energetics against multiple coronavirus species using computational homology modeling were selected and synthesized in a mammalian library display format for screening. Hits were identified, and their breadth and potency were confirmed biochemically via biolayer interferometry and functionally via pseudovirus neutralization assays and live virus neutralization assays in vitro.

RESULTS:

ACE2 decoys were identified with simultaneously increased potency across all tested SARS-CoV-2 variants, as well as other human and animal sarbecovirus, merbecovirus, and setracovirus subgenera. These

CONCLUSIONS:

The improved ACE2 decoy molecules not only offer a promising avenue for broad-spectrum coronavirus neutralization but also provide valuable insights into the ACE2-virus interaction dynamics. Our findings highlight the adaptability and efficacy of combining computational and high-throughput screening approaches to identify future-proof therapeutics in response to emerging and diverse viral threats.



Abstract No.:184

Poster Code: : K-0110

Development of bispecific inhibitor to simultaneously target coronavirus entry and replication.

First Author: Vivian Shuai, The University of Hong Kong

BACKGROUND OBJECTIVES:

Coronaviruses have caused three major endemics in the past two decades. Alarmingly, recent identification of novel zoonotic coronaviruses that caused human infections suggests the risk of future coronavirus outbreak caused by spillover infection from animal reservoirs remains high. Therefore, development of alternative therapeutic options with broad-spectrum anti-coronavirus activities are urgently needed.

METHODS:

We then screened an in-house de novo synthesized chemical library consisting of over 5000 peptidomimetic candidates to identify one lead compound which simultaneously suppressed the enzymatic activities of the host protease TMPRSS2 and the viral protease Mpro. We evaluated the antiviral efficacy of the stepwise structure-optimized candidate TMP1 in the in cell lines, primary human airway organoids and transgenic mice and hamsters. Crystallography, pseudovirus assays and cell-cell fusion assays, enzymatic assays with site-directed mutagenesis were carried out to illustrate the mode of action of the bispecific inhibitor.

RESULTS:

TMP1 shows broad-spectrum protection not only against different SARS-CoV-2 variants but also against multiple human-pathogenic coronaviruses in vitro. By using the K18-hACE2 transgenic mouse, hDPP4 knock-in mouse and golden Syrian hamster models, we demonstrate TMP1 cross-protects against highly-pathogenic coronaviruses (SARS-CoV-1, SARS-CoV-2 and MERS-CoV) in vivo and efficiently abrogates SARS-CoV-2 transmission. Through structural and mutagenesis studies, we confirm the direct interaction of TMP1 with Mpro and TMPRSS2, and pinpoint the key sites of interactions. Importantly, TMP1 inhibits the infection of nirmatrelvir-resistant SARS-CoV-2 escape mutants.

CONCLUSIONS:

Our findings demonstrate the antiviral potential of the bispecific Mpro/TMPRSS2 antiviral design against human-pathogenic coronaviruses and other emerging coronaviruses.

Abstract No.:277

Poster Code: : K-0111

Establishment of In Vitro and In Vivo Models for Bovine Coronavirus Antiviral Evaluation

First Author: Changcheng Liu, Huazhong Agricultural university

BACKGROUND OBJECTIVES:

Bovine coronavirus (BCoV) is a major global veterinary pathogen that causes calf diarrhea, respiratory infections, and winter dysentery in adult cattle, posing a significant threat to the livestock industry. Despite its impact, comprehensive research has been hindered by the subtle cytopathic effects (CPE) observed in traditional cell lines, such as HRT-18, and a persistent lack of robust, reproducible animal models. This study aims to engineer a chimeric "murinized" BCoV (mBCoV) to facilitate high-throughput visual screening, while simultaneously optimizing a virulent wild-type (WT) infection model to provide a platform for physiological validation.

METHODS:

Utilizing targeted RNA recombination, we generated a recombinant BCoV (mBCoV) in which the native spike glycoprotein ectodomain was substituted with that of Mouse Hepatitis Virus (MHV). A panel of antiviral compounds was screened to evaluate their inhibitory potential against mBCoV in vitro.

Two-day-old SPF BALB/c mice (n=8 per group) were assigned to three groups: Infection group: Mice was performed via i.p. injection with wtBCoV at a lethal dose (10^{6.5} TCID₅₀); Treatment group: Mice was administered GC376 via i.p. injection at a dosage of 25 mg/kg/day.; Control group : Injected with an equal volume of sterile saline.

Survival, clinical signs, and body weight were recorded daily. Post-mortem, major organs and intestinal tissues were harvested for viral quantification via RT-qPCR and histopathological evaluation using H&E and IHC staining.

RESULTS:

mBCoV serves as an efficient tool for in vitro antiviral screening. Inoculation of LR7 cells with mBCoV induces typical visual syncytium formation within 24–36 hpi.

Furthermore, the 3C-like protease inhibitor GC376 shows equivalent antiviral efficacy against both mBCoV and wild-type BCoV.

Intraperitoneal injection of wtBCoV leads to robust viral replication and 100% mortality at 5 days post infection. Treatment with GC376 drastically reduced viral genome copies by 4.5 logs, effectively conferring 100% protection against lethal challenge. Treated mice remained asymptomatic and exhibited normal growth kinetics throughout the observation period.

CONCLUSIONS:

We successfully established a comprehensive in vitro and in vivo screening platform to evaluate antivirals targeting the BCoV replication stage. This workflow validated by the strong efficacy of GC376, our recombinant mBCoV and lethal neonatal mouse model provide a powerful system for testing future therapeutic candidates.



Abstract No.: 283

Poster Code: : K-0112

Establishment of a Mouse Model for Porcine Epidemic Diarrhea Virus

First Author: Mengdi Zhang, Huazhong Agricultural University

BACKGROUND OBJECTIVES:

Porcine Epidemic Diarrhea (PED) is an acute and highly contagious enteric disease caused by the Porcine Epidemic Diarrhea Virus (PEDV). Characterized by vomiting, diarrhea, and dehydration, it primarily affects neonatal piglets, with mortality rates reaching 100%. Due to the limitations of viral entry barriers, PEDV exhibits restricted infectivity in non-swine species, resulting in a critical shortage of susceptible, cost-effective, and high-efficiency small animal models for research. This study established a susceptible and lethal murine model for PEDV by utilizing a chimeric recombinant virus (mPEDV) expressing the MHV S protein, thereby providing a rapid and cost-effective in vivo platform for antiviral drug evaluation and pathogenesis research.

METHODS:

AG129 mice (4-5 weeks old, n=10) were randomly assigned to infection or control groups. Infection was performed via i.p. injection (10^6 TCID₅₀/mouse). Survival, clinical signs, and body weight were monitored daily. Tissues (heart, liver, spleen, lungs, kidneys, intestines, and brain) were harvested for viral load quantification (RT-qPCR) and pathological analysis (H&E and IHC).

RESULTS:

To evaluate mouse susceptibility, various mouse strains were inoculated with the maximum cultured viral titer (10^6 TCID₅₀/mouse). A lethal mouse model was successfully established in AG129 mice. The mice began to die at 4 days post infection (dpi), and reached 100% mortality at 6 dpi. Necropsy revealed that the main gross lesions included hepatomegaly with necrotic foci and splenomegaly. HE staining showed disordered hepatic cords, sinusoidal congestion, and extensive hepatocyte degeneration and necrosis, accompanied by inflammatory cell infiltration. The lungs exhibited inflammatory infiltration, edema, and thickening of the alveolar septa. Furthermore, IHC analysis demonstrated widespread expression of PEDV N protein in the liver, spleen, lungs, kidneys, and intestines.

CONCLUSIONS:

We have successfully developed a novel lethal mouse model for PEDV using a chimeric mPEDV strain in AG129 mice. The model effectively overcomes species-specific entry barriers and induces 100% mortality with systemic organ involvement within 6 days. This study provides a highly efficient and stable animal platform for the in vivo evaluation of vaccines and antiviral therapeutics.



Abstract No.: 322

Poster Code: : K-0113

Neutralizing Antibodies Targeting the S1B Domain of Porcine Deltacoronavirus with Protective Efficacy in Embryonated Chicken Egg

First Author: Jiaru Zhou, Huazhong Agricultural University

Co-Author(s): Mengjia Zhang, Wentao Li

BACKGROUND OBJECTIVES:

Porcine deltacoronavirus (PDCoV) is an emerging enteric pathogen that causes severe diarrhea, vomiting, and dehydration in piglets and a recent human infection report raises concerns about its zoonotic potential. The S1 subunit of the PDCoV spike protein is the primary target of neutralizing antibodies. This study aimed to generate and characterize potent neutralizing monoclonal antibodies against PDCoV and evaluate their protective efficacy in vivo preliminarily.

METHODS:

The recombinant PDCoV S1 protein fused with a mouse Fc tag and expressed in a HEK293F-based eukaryotic expression system was used as the immunogen for antibody production. Neutralizing activity was evaluated using a recombinant luciferase-expressing PDCoV in Huh7 cells, and the 50% inhibitory concentration (IC₅₀) values were calculated. Biolayer interferometry was used to assess antibody binding kinetics, APN receptor binding inhibition, and antibody binding competition. For in vivo evaluation, embryonated chicken eggs (ECEs) were used as a PDCoV challenge model. ECEs were randomly assigned to experimental groups and inoculated with PDCoV at a lethal dose (10^{6.5} TCID₅₀), followed by administration of mAbs at 10 IC₅₀. Embryo survival and viral loads were monitored.

RESULTS:

Five monoclonal antibodies (3E9, 4A8, 5F8, 11D9 and 13D5) targeting the S1B domain exhibited neutralizing activity in vitro. All five antibodies inhibited viral infection in a dose-dependent manner, with 3E9 showing the highest potency (IC₅₀: 0.17 µg/mL). Mechanistic analysis demonstrated that these antibodies interfered with the interaction between the PDCoV S protein and its receptor APN. Competitive binding assays revealed that 13D5 recognized a distinct non-overlapping epitope, whereas 3E9, 4A8, 5F8, and 11D9 competed with each

other for a shared or overlapping epitope within S1B. Furthermore, these antibodies exhibited cross-reactivity with S1B proteins from human- and avian-origin deltacoronaviruses, indicating recognition of conserved antigenic sites. In the embryonated chicken egg model, all five neutralizing antibodies improved survival following PDCoV challenge. Antibody 3E9 provided complete protection, while the others delayed or reduced mortality.

CONCLUSIONS:

We identified five potent neutralizing monoclonal antibodies targeting the S1B domain of PDCoV. These antibodies block viral entry by inhibiting APN receptor binding and exhibit cross-reactivity with deltacoronaviruses from human and avian. Their protective efficacy in the ECE model highlights their potential as broadly applicable therapeutic candidates.



Abstract No.: 128

Poster Code: : M-0114

Evolution of receptor usage plasticity in merbecoviruses through a computational approach

First Author: Jarel Elgin Tolentino, The University of Tokyo

Co-Author(s): Victoria Jefferson, Nicholas J. Catanzaro, Ralph S Baric, Michael C. Letko, Spyros Lytras, Kei Sato

BACKGROUND OBJECTIVES:

Merbecoviruses within the Betacoronavirus genus exhibit remarkable plasticity in receptor usage that affects their host range and zoonotic potential. This group includes Middle East respiratory syndrome coronavirus (MERS-CoV) and several bat-associated viruses, such as HKU5-related coronaviruses (HKU5r-CoVs), some of which have recently been detected in farmed mink. Understanding how merbecoviruses evolve new receptor specificities is critical for anticipating spillover risk and guiding emergence preparedness. Two complementary studies in merbecovirus evolution is presented here. The main aim of these studies is to dissect the molecular and evolutionary events underlying receptor usage plasticity in merbecoviruses.

METHODS:

Using large-scale phylogenetic reconstruction, recombination analysis, molecular dating, and AI-guided structural modeling, the results obtained suggest that receptor adaptation can arise through both genomic recombination and localized structural plasticity within the Spike protein.

RESULTS:

In the first study, we demonstrate that MERS-CoV acquired its dipeptidyl peptidase 4 (DPP4) usage through a recombination event involving the Spike gene. Although the MERS-CoV genome is most closely related to ACE2-using bat viruses such as NeoCoV and PDF-2180, recombination analyses reveal that its receptor-binding domain (RBD) clusters with DPP4-using merbecoviruses. This finding explains the evolutionary origin of MERS-CoV and indicates that receptor switching might have occurred in bats prior to spillover into camels and humans. In the second study, we investigated the newly-detected HKU5r-CoV in mink and identified the RBD loop2 region as a key determinant of ACE2 usage and host tropism. Structural

modeling and recombination analyses show that loop2 exhibits substantial structural and sequence plasticity. Notably, a single amino acid substitution (R548S) is sufficient to enable human ACE2 usage showing the latent zoonotic potential of HKU5r-CoVs circulating at the wildlife–livestock interface.

CONCLUSIONS:

Together, our results demonstrate that receptor usage in merbecoviruses is governed by recombination and structural changes within the Spike protein, particularly in the RBD region. From the recombination-driven emergence of MERS-CoV to the loop2-mediated adaptation of HKU5r-CoVs in mink, both cases show how minor genetic events can unlock new host ranges and zoonotic potential. These findings highlight the importance of continued efforts to dissect the evolutionary drivers of merbecoviruses, as understanding these events is critical for predicting and mitigating their emergence.



Abstract No.: 448

Poster Code: : M-0116

High MERS-CoV endemicity and co circulation of other coronaviruses in camels with evidence of zoonotic spillover to camel workers, Saudi Arabia

First Author: Abdullah Algaissi, Jazan University, Saudi Arabia

Co-Author(s): Alaa Mofaa, Nader Kameli, Aisha Ghazwani, Anwar M Hashem

BACKGROUND OBJECTIVES:

MERS CoV remains a WHO priority pathogen with dromedary camels as the primary reservoir. The Jazan region, a cross border camel trade hub in southwestern Saudi Arabia, lacks active surveillance data. We conducted a One Health cross sectional study to assess MERS CoV infection and seroprevalence in camels, co circulation of other coronaviruses, and evidence of zoonotic spillover to humans.

METHODS:

Methods: From December 2025 to April 2026, nasal swabs and serum were collected from 300 camels (farms, markets, slaughterhouses) and serum from 100 occupationally exposed camel workers and 100 community controls. Camel nasal swabs were tested by RT qPCR for MERS CoV targeting upE and ORF1a; samples with $Ct < 35$ for both targets were considered positive. Non MERS coronaviruses were detected using pan CoV RdRp primers. Partial Spike sequencing (615 bp) was performed on high titer ($Ct < 25$) MERS CoV positive samples. Camel and human sera were screened by ELISA and confirmed by pseudovirus neutralization (pVNT, $IC_{50} \geq 1:20$). Logistic regression identified risk factors for human seropositivity.

RESULTS:

Camel MERS CoV seroprevalence was 84.6% (254/300; 95%CI 80.1–88.3%). Active MERS CoV RNA was detected in 18.7% (56/300; 95%CI 14.6–23.5%). Among RNA positive camels, 67.9% (38/56) were co infected with non MERS coronaviruses. Sequencing of 20 samples identified clade B5 (98.9–99.7% identity to Arabian Peninsula strains). Neutralizing antibodies were found in 11.0% (11/100) of camel workers vs. 1.0% (1/100) of controls ($p < 0.001$). Independent risk factors were daily camel contact (aOR 3.1, 95%CI 1.4–6.9, $p = 0.004$), raw camel milk consumption (aOR 2.7, 95%CI 1.2–6.1, $p = 0.015$), and age > 40 years (aOR 2.3, 95%CI 1.1–4.8, $p = 0.028$).

CONCLUSIONS:

This first Jazan surveillance documents high MERS CoV endemicity in camels, frequent non MERS CoV co infection, and confirmed zoonotic spillover to occupationally exposed workers. Targeted behavioural interventions in camel trade networks are urgently needed.

