# 10th International Influenza Meeting

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University of Muenster (Castle)

# Book of Abstracts

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# Tropism of an H5N1 virus for bovine cells is determined by the HA2 subunit regulating membrane fusion

**Authors:** Jia-Yi Chan[1]; Emma Balibrea Roelans[1]; Lisa Butticaz[1]; Etori Moreira[1]; Gert Zimmer[1]

[1] *Institute of Virology and Immunology*

**Corresponding Authors:** etori.aguiarmoreira@ivi.admin.ch, gert.zimmer@ivi.admin.ch, jiayi.chan@ivi.admin.ch, lisa.butticaz@ivi.admin.ch, emma.balibrearoelans@ivi.admin.ch

In 2024, H5N1 highly pathogenic avian influenza (HPAI) viruses were first detected in dairy cows in Texas, USA. Unlike typical influenza infections, the virus replicated predominantly in mammary gland tissue, causing a mastitis-like disease associated with high virus titers in milk.
To identify viral determinants of bovine cell tropism, we compared the bovine H5N1Tex/24, with the avian H5N1Yam/04 and H5N1Hok/04 viruses in bovine macrophage (BoMac), bovine kidney (MDBK), canine kidney (MDCK), and human lung epithelial (Calu-3) cells. The polybasic HA cleavage sites of H5N1Tex/24 and H5N1Yam/04 were mutated into monobasic motifs, and reassortant viruses were generated by reverse genetics.
H5N1Tex/24 and H5N1Yam/04 replicated efficiently in BoMac and MDBK cells, whereas H5N1Hok/04 did not. However, all three viruses reached high titers in MDCK and Calu-3 cells. Replacement of genome segments 1, 2, 3, 5, and 8 of H5N1Hok/04 with those of H5N1Tex/24 failed to enhance replication in bovine cells. In contrast, replacement of segment 4 significantly increased viral replication, identifying HA as a major determinant of bovine cell tropism.
Serial passaging of H5N1Hok/04 in BoMac cells increased replication capacity and selected mutations in the HA2 subunit. These mutations enhanced HA-mediated membrane fusion, indicating that HA fusion activity is a key factor influencing influenza virus cell tropism.

**Keywords**:

**Professional status of the speaker**:

Graduate student

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-46

**Poster slam / 142**

# A Vector-Based Influenza Vaccine Reveals the HA Stalk as a Dual Humoral and Cellular Immune Target

**Authors:** Max Geißler[1]; Konrad Merz[1]

**Co-authors:** Julian Volk [1]; Annika Schönsiegel [1]; Ralf Amann [2]; Oliver Planz [1]

[1] *Institute of Immunology Tübingen*

[2] *Fraunhofer IGB*

**Corresponding Authors:** max.geissler@student.uni-tuebingen.de, ralf.amann@ifiz.uni-tuebingen.de, konrad.merz@student.uni-tuebingen.de, julian.volk@uni-tuebingen.de, oliver.planz@uni-tuebingen.de

Universal influenza vaccine strategies predominantly exploit the conserved hemagglutinin (HA) stalk as a target for broadly neutralizing antibodies. Whether the HA stalk also contributes to protective T cell immunity remains poorly understood. We therefore investigated the underappreciated immunological capacity of HA stalk in shaping CD4+ T cell immunity within our Orf virus–based universal influenza vaccine candidate (ORFV-Flu).
Mice were immunized with ORFV-Flu expressing chimeric HA constructs. Overlapping HA stalk peptide libraries of multiple influenza A virus strains were screened by ELISpot and multiparametric flow cytometry to identify HA stalk–specific $CD4^+$ T cell responses.
ORFV-Flu elicited potent HA stalk-specific $CD4^+$ T cell responses, establishing the stalk as a dual humoral and cellular immune target through the discovery of immunogenic regions containing candidate MHC class II epitopes.
These findings expand the immunological concept of chimeric HA–based vaccination beyond antibody induction by identifying the HA stalk as a dual-function antigen capable of eliciting both humoral and $CD4^+$ T cell immunity. Combined with the potent HA stalk–specific antibody responses and conserved antigen-specific $CD8^+$ T cell immunity elicited by ORFV-Flu, these findings complete a coordinated immune framework integrating humoral, helper, and cytotoxic T cell responses for next-generation influenza vaccination.

**Keywords**:

**Professional status of the speaker**:

Graduate student

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-87

**Poster slam / 159**

# FluPrep: Identifying novel risk markers for emerging zoonotic influenza A viruses

**Author:** Lioba Böhm[1]

**Co-authors:** Stephan Ludwig [2]; Linda Brunotte [3]

[1] *Institute of Virology (IVM), University Münster, Immunology of Respiratory Tract Infections, Department of Medicine V, Justus-Liebig University Giessen*

[2] *Institute of Virology (IVM), University Münster*

[3] *Immunology of Respiratory Tract Infections, Department of Medicine V, Justus-Liebig University Giessen*

**Corresponding Author:** lioba.f.boehm@bio.uni-giessen.de

The FluPrep consortium aims to establish a reliable, multipronged risk assessment pipeline to evaluate the health risk and pandemic potential of emerging influenza A viruses (IAVs) for humans. This is critical as avian H5Nx IAVs of clade 2.3.4.4b have caused widespread outbreaks and spillover events in wild birds and mammals, facilitating genetic reassortment and stepwise adaptations to mammalian hosts. Consequently, zoonotic H5Nx strains have emerged with poorly defined risk profiles for humans.
This project seeks to characterize the immunopathological signatures of these emerging IAVs to define new risk criteria for integration into the FluPrep risk assessment. By employing human lung epithelial cells and *ex vivo* human lung explants, we will examine strain-specific phosphorylation of host immune regulatory kinases, specifically focusing on the TRIM28 pathway to identify patterns in immune signalling activation of emerging IAVs. To evaluate the intensity of the immune response, we will further analyse the transcription and secretion profiles of pro-inflammatory cytokines. Additionally, we will investigate the immune regulatory functions of the viral non-structural protein 1 (NS1) and the impact of specific mutations on the host immune response.
Ultimately, we aim to identify novel risk markers for emerging zoonotic IAVs by using native human lung tissue, thereby improving the predictions of disease severity and pandemic risk within FluPrep.

**Keywords**:

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-57

**Poster slam / 138**

# Influenza A virus NS1 targets the host ubiquitin ligase ZNF598 to promote innate immune evasion

**Author:** Théo Massart[None]

**Co-authors:** Justine Petit ; Melissa Havuz ; Samanta Chouela ; Lionel Tafforeau

**Corresponding Authors:** melissa.havuz@student.umons.ac.be, samanta.chouelambouwe@student.umons.ac.be, justine.petit@umons.ac.be, theo.massart@umons.ac.be, lionel.tafforeau@umons.ac.be

RIG-I-mediated sensing of viral RNA initiates type I interferon (IFN-I) expression, which is essential for innate immune defense. Because aberrant activation of this pathway can lead to autoimmune disorders, RIG-I signaling is tightly regulated. The E3 ubiquitin ligase ZNF598 indirectly suppresses IFN-I expression by conjugating ubiquitin D (FAT10) to RIG-I, thereby inhibiting its activation and attenuating the antiviral response. During its infectious cycle, Influenza A virus (IAV) exploits ubiquitin-mediated mechanisms to promote viral replication. The non-structural protein NS1 plays a key role in counteracting the host antiviral response. Preliminary work identified ZNF598 as a conserved interactor of NS1 from multiple IAV strains. Here, we characterize the interaction between ZNF598 and NS1 from three distinct IAV strains. We also investigate the impact of NS1 on ZNF598-dependent RIG-I FAT10ylation.

**Keywords**:

ZNF598, NS1, RIG-I, FAT10

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-94

**Poster slam / 144**

# Functional characterization of NS1 phosphorylation in pandemic H1N1 influenza A virus (pdm09) reveals site specific modulation of virus–host interactions

**Authors:** Matthias Weiler[None]; Yvonne Börgeling[1]; Stephan Ludwig[1]

**Co-authors:** Darisuren Anhlan [1]; Wolfgang Nacken [1]; Carmen Musholt [1]

[1] *Institute of Virology Muenster*

**Corresponding Authors:** nacken@uni-muenster.de, carmen.musholt@ukmuenster.de, ludwigs@uni-muenster.de, borgelin@uni-muenster.de, anhlan@uni-muenster.de, m_weil09@uni-muenster.de

The non-structural protein 1 (NS1) of pandemic H1N1 influenza A virus (pdm09) is a multifunctional virulence factor that antagonizes innate immunity and regulates multiple virus-host cell interactions. Despite its central role during infection, the contribution of site-specific phosphorylation remains poorly understood. We analyzed phospho-ablative and phospho-mimetic NS1 mutants using recombinant viruses and viral minigenome, IFN-β promoter, and transcriptional read-through reporter assays together with a six-site NS1 variant associated with restored CPSF30 binding. Replication kinetics of phosphorylation mutants were comparable to wild type. The phospho-mimetic S83D+T86D mutant markedly reduced viral polymerase activity, whereas the corresponding phospho-ablative mutant showed only minor effects, supporting a regulatory role of phosphorylation at these residues. All mutants retained robust IFN-β antagonistic activity, with S213E showing the strongest inhibition. The six-site NS1 variant increased transcriptional read-through, while viral polymerase activity and IFN-β antagonism remained largely unchanged. Together, these findings demonstrate that NS1 phosphorylation selectively modulates distinct viral functions without substantially affecting viral replication, whereas naturally occurring sequence variation primarily alters transcriptional read-through, providing new insights into the functional regulation of pdm09 NS1.

**Keywords**:

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

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INF26-97

**Poster slam / 145**

# Genetic basis for animal influenza A virus escape from domestic mammalian interferon-induced Mx1 restriction factors

**Authors:** Théotime Brehon[1]; Stéphane Descorps-Declère[2]; Sylvain Paisant[1]; Olivier Leymarie[3]; Catherine Isel[1]; Nadia Naffakh[1]

[1] *RNA Biology of Influenza Virus Unit, Institut Pasteur, Université Paris Cité, CNRS UMR3569, Paris, France.*

[2] *Institut Pasteur, Université Paris Cité, Hub Bioinformatique et Biostatistique, Paris, France*

[3] *Molecular Mechanisms of Multiplication of Pneumoviruses Unit (M3P), Institut Pasteur, Université Paris-Saclay, Université de Versailles St. Quentin, Université Paris Cité, UMR 1173 (2I), INSERM; Paris, France*

**Corresponding Authors:** theotime.brehon@pasteur.fr, nadia.naffakh@pasteur.fr

Since 2020, highly pathogenic avian H5N1 influenza A viruses (IAV) of clade 2.3.4.4b have expanded their host range to include domestic mammals such as dairy cattle and American mink. The human Mx1 interferon-induced protein is a major host-jump barrier, as Mx1-escape mutations on the viral nucleoprotein (NP) often incur a fitness cost. Swine Mx1 is less potent than human Mx1, facilitating the selection of mutations that promote escape from both. We investigate whether other domestic mammals, like swine, could serve as gateways for the pre-adaptation of avian IAV to humans through the selection of Mx1-escape mutations.

To this end, we use a Eurasian avian-like swine H1N2 virus (EAsw) as a zoonotic IAV model and we develop three complementary approaches: i) minigenome assays; ii) viral growth assays in cells stably expressing Mx1; and iii) a mutagenic screen of surface-exposed NP residues to identify combinations of mutations that can confer resistance to mammalian and human Mx1 proteins. To address biosafety for ii) and iii), we generated single-cycle, hemagglutinin (HA)-defective EAsw viruses that replicate only in transcomplementing MDCK-HA cells. Minigenome assays and growth curves reveal marked species-specific differences in anti-IAV activity.

These approaches should contribute to a better understanding of mammalian-adaptive mutations, which is essential for improving genomic surveillance and preventing the emergence of IAV with zoonotic and pandemic potential.

**Keywords**:

Influenza A virus, Mx1, domestic mammals, viral escape, nucleoprotein, host-jump barriers

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-98

**Poster slam / 150**

# Mapping the Molecular Determinants of Influenza Virus RNA Synthesis Initiation and Termination

**Authors:** Leander Witte[1]; Ervin Fodor[1]

**Co-author:** Amina Qadir [1]

[1] *University of Oxford*

**Corresponding Authors:** ervin.fodor@path.ox.ac.uk, leander.witte@path.ox.ac.uk

Influenza A viruses contain a segmented genome of eight negative-sense, single-stranded RNAs (vRNAs), each packaged with a viral RNA polymerase and nucleoprotein into viral ribonucleoprotein complexes (vRNPs). These vRNPs undergo both transcription and replication, producing (I) capped, polyadenylated mRNAs and (II) uncapped, full-length vRNAs via a complementary RNA (cRNA) intermediate. Transcription is primed by host-derived capped RNAs and terminates by polyadenylation at a polyuridine stretch upstream of the 5'terminus. In contrast, replication is triggered by interaction of the vRNP with a second polymerase and the host factor ANP32, initiating RNA synthesis de novo and replicating the RNA end to end.
However, important mechanistic details governing initiation and termination remain poorly understood. To address these gaps, we combine an in vitro reconstitution system with long-read Nanopore sequencing to directly profile terminal RNA sequences. This approach promises to reveal fundamental mechanisms underlying initiation and termination of influenza virus RNA synthesis.

**Keywords**:

**Professional status of the speaker**:

Graduate student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-100

**Poster slam / 157**

## Molecular Mechanisms of Deletion-containing Viral Genome Formation and Immune System Interaction in Influenza Viruses

**Authors:** Jana Mareike Schmitz[1]; Josua Janowski[None]; Petya Berger[None]; Rexford Mawunyo Dumevi[None]; Saskia Hinse[None]; Pia Leipe[None]; Sriram Kumar[None]; Duygu Merve Çalişkan[None]; Christopher Margraf[None]; Alexander Mellmann[None]; Stephan Ludwig[None]; Shazeb Ahmad[None]; Redmond Smyth[None]; Linda Brunotte[None]

[1] *Justus-Liebig-Universität Gießen*

**Corresponding Author:** jana.schmitz-1@uni-giessen.de

Highly pathogenic avian influenza viruses (HPAIVs) occasionally cross the species barrier and often induce excessive host inflammation in humans. Deletion-containing viral genomes (DVGs) arise through internal deletions during error-prone Influenza A virus (IAV) replication and can have immune stimulatory potential. We hypothesize that virus-specific DVG landscapes contribute to HPAIV pathogenicity and could further serve as prognostic markers for pandemic risk assessment. By characterizing DVG signatures across seasonal and highly pathogenic IAV strains, this study identified a range of HPAIV-specific small viral RNAs (mvRNAs) derived from several genome segments. Results from PRR-immunoprecipitations demonstrated that selected HPAIV-derived mvRNAs act as potent PKR-specific immune activators, potentially driving virus-induced host inflammation. Furthermore, distinct RNA secondary structures and selective DVG packaging suggest that specific structural motifs govern both their encapsidation and immune recognition. This project aims to unravel the molecular determinants of DVG formation, characterizing DVG-specific RNA structural elements and dissecting the role of the viral polymerase complex in DVG biogenesis. It further investigates DVG-driven immune activation, and finally, the therapeutic potential of engineered DVGs and mvRNAs as antiviral and immunomodulatory tools.

**Keywords**:

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-64

**Poster slam / 132**

# DEVELOPMENT OF A SURFACE-BASED ASSAY FOR MONITORING INFLUENZA VRNP REPLICATION

**Authors:** Ervin Fodor[1]; Tomas Shimkus[None]

[1] *Dunn School of Pathology, University of Oxford*

**Corresponding Author:** tomas.shimkus@lincoln.ox.ac.uk

Influenza A virus (IAV) is a globally significant respiratory pathogen responsible for seasonal epidemics and occasional pandemics associated with substantial morbidity and mortality. Its high mutation rate and frequent genetic reassortment enable rapid escape from existing antiviral therapies, underscoring the need for new therapeutic strategies. Current high-throughput assays for screening inhibitors of the influenza polymerase rely predominantly on cell-based systems. As a result, compounds that directly target the native viral replication machinery but lack activity in a cellular context may be overlooked, despite their potential as starting points for antiviral development. Here, we describe a surface-based fluorescence assay that measures the replicative activity of the viral polymerase within virion-derived viral ribonucleoprotein complexes (vRNPs). By preserving the native architecture and function of the viral replication machinery while eliminating the complexities of cell-based assays, this platform enables direct measurement of influenza polymerase activity in a multi-well format compatible with high-throughput screening.

**Keywords**:

**Professional status of the speaker**:

PhD student

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No, I am not a junior scientist.

**Registration ID**:

INF26-82

**Poster slam / 107**

# ANTI-INFLUENZA PROPERTIES OF PRIMULA VERIS AQUEOUS EXTRACT IN VITRO

**Author:** Miroslav Metodiev[1]

**Co-authors:** Lora Simeonova [1]; Mariana Kamenova-Nacheva [2]; Plamena Staleva [2]; Zhanina Petkova [2]

[1] *1 Department of Virology, Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

[2] *2 Laboratory Organic Synthesis and Stereochemistry, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*

**Corresponding Authors:** losimeonova@gmail.com, mnmetodiev@microbio.bas.bg

Influenza viruses remain a major public health challenge due to continuous antigenic evolution, increasing antiviral resistance, and limitations of current vaccines, highlighting the need for novel antivirals with alternative mechanisms of action. Natural products represent a promising source of antiviral compounds. We investigated the cytotoxicity, antiviral activity, combined therapy with oseltamivir, virucidal activity, and mode of action of Primula veris aqueous flower extract against influenza A viruses in vitro. HPLC-MS phytochemical analysis identified 16 major compounds. Antiviral activity was evaluated in MDCK cells against H3N2, H1N1, and H7N7 strains by cytopathic effect reduction microscopically and neutral red uptake assay. The extract showed low cytotoxicity (CC50 2.18 mg/ml; MTC 1.0 mg/ml) and moderate inhibition of all strains (IC50 0.346–0.368 mg/ml; SI 5.92–6.30). Combined treatment with oseltamivir produced additive and synergistic effects. Time-of-addition experiments demonstrated antiviral activity at both early (0–2 h) and late (10–12 h) stages of replication, reducing viral infectivity by 2.0 Δlg. The extract exhibited complete virucidal activity against H3N2, with a 5.0 Δlg reduction after 45 min of contact. These findings demonstrate the promising anti-influenza potential of P. veris aqueous extract and support further investigation of its antiviral mechanism.

**Keywords**:

Influenza, Primula veris

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-52

**Poster slam / 124**

## Characterizing Pre-Existing Humoral Immunity to Emerging Influenza A Viruses

**Author:** Anja Heuhsen[None]

**Co-authors:** Andreas Radbruch ; Andrey Kruglov ; Caroline Tizian ; Dorottya Bőgér ; Duygu Sert ; Frederik Heinrich ; Gabriela Maria Guerra ; Gülşah Gabriel ; Jan Licha ; Jenny Kirsch ; Katrin Lehmann ; Marco Witkowski ; Marie Knufinke ; Marina Bondareva ; Mir-Farzin Mashreghi ; Nico Neumann ; Pawel Durek ; Robin Mesnage ; Stephanie Stanelle-Bertram

**Corresponding Author:** anja.heuhsen@drfz.de

The Influenza A virus causes up to 1 billion infections and up to 500.000 deaths per year, representing a persistent global threat. Even though the virus evades the human immune system through antigenic drift and shift of the surface proteins hemagglutinin and neuraminidase, vaccination is still the best way to protect individuals from a severe outcome. Nevertheless, individual vulnerability to the virus is shaped by immunological imprinting, influencing the pool of long-lived plasma cells that secrete neutralizing antibodies to provide immediate protection upon reinfection.
This project aims to investigate potential cross-reactivity of these neutralizing antibodies imprinted by circulating subtypes, which is essential for modeling pandemic preparedness against novel subtypes and guiding future vaccination strategies. Utilizing a cross-sectional cohort with diverse immunological experience, including infection and vaccination, enables us to assess the pre-pandemic status. Moreover, we want to determine how changes in the nutritional state modulate humoral

immunity towards different subtypes, potentially enhancing vaccine responsiveness.
To address this, we established a cell-based assay, where we express respiratory viral surface proteins and acquire serum IgG and IgA levels via flow cytometry. The analysis of our cohort will allow us to assess further if factors like sex, age or body-mass-index have an impact on respiratory virus specific antibody secretion.

**Keywords**:

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26- 34

**Poster slam / 126**

## Neurotropism Distinguishes U.S. Human-Origin H5N1 from European Avian-Origin H5N5 Virus Infection in the Ferret Model

**Authors:** Marta Czechowicz[1]; Juliane Lang[2]; Maryna Kuryshko[2]; Elsayed M. Abdelwhab[2]; Reiner Ulrich[1]

[1] *Institute of Veterinary Pathology, Faculty of Veterinary Medicine, Leipzig University, An den Tierkliniken 33, 04103 Leipzig, Germany*

[2] *Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany*

**Corresponding Author:** marta.czechowicz@vetmed.uni-leipzig.de

Highly pathogenic avian influenza A viruses (HPAIV) of the clade 2.3.4.4b H5Nx lineage continue to emerge globally, posing a growing threat to animal and human health. The ferret model is one of the most reliable animal models for predicting the pathogenicity and virulence of HPAIV in humans.
This study compared the light microscopic lesions and viral tropism in ferrets infected with U.S. human-origin H5N1 by ocular inoculation or with European avian-origin H5N5 by intranasal inoculation.
H5N1 virus infection was characterized by necrotizing encephalitis accompanied by limited lesions in the respiratory tract. Bone marrow necrosis was also observed, indicating systemic dissemination. In contrast, H5N5 virus infection induced mild lesions in the respiratory tract without evidence of encephalitis and was associated with lymphoid necrosis within the pulmonary lymph nodes. Immunohistochemistry for the influenza A virus matrix protein confirmed viral antigen in affected tissues and closely correlated with the distribution of lesions.
Overall, our findings demonstrate distinct differences in lesion profiles and viral tropism in the ferret model. While the U.S. human-origin H5N1 virus exhibited pronounced neurotropism and systemic spread, the European avian-origin H5N5 virus remained largely restricted to the respiratory tract.

**Keywords**:

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-75

**Poster slam / 127**

# Respiratory Viral Infections in Lusaka, Zambia: Clinical Burden and Risk Factors in a high HIV-burden Setting

**Author:** Seke GY Muzazu[1]

**Co-authors:** Ralitza Dekova [2]; Daniel Siameka [3]; Tina Kayumba [3]; Muuka Muleya [3]; Ivonne Morales [2]; Ankur Gupta-Wright [4]; Monde Muyoyeta [3]; Claudia Denkinger [2]

[1] *1. TB & Other Respiratory Diseases Department, Centre for Infectious Disease Research in Zambia, Lusaka, Zambia 2. Heidelberg University Medical Faculty Heidelberg, Department of Infectious Disease and Tropical Medicine, Heidelberg University Hospital, Heidelberg, Germany*

[2] *Heidelberg University Medical Faculty Heidelberg, Department of Infectious Disease and Tropical Medicine, Heidelberg University Hospital, Heidelberg, Germany*

[3] *TB & Other Respiratory Diseases Department, Centre for Infectious Disease Research in Zambia, Lusaka, Zambia*

[4] *1. Heidelberg University Medical Faculty Heidelberg, Department of Infectious Disease and Tropical Medicine, Heidelberg University Hospital, Heidelberg, Germany 2. Department of Infectious Disease, Imperial College London, London, UK 3. Department of Infectious Diseases, Bristol NHS Foundation Trust, Bristol, England, UK*

**Corresponding Author:** seke.muzazu@cidrz.org

Respiratory infections dominate outpatient consultations in primary healthcare (PHC), yet their causes are rarely established in low-income settings where empirical antibiotic use predominates. This fuels inappropriate prescribing, undermines diagnostics, and weakens pandemic preparedness.

We report preliminary findings from a cross-sectional surveillance study in two PHC facilities in Lusaka, Zambia, enrolling adults (≥18 years) with influenza-like illness (ILI) between May 2025–May 2026. ILI was defined as fever (≥39 ℃ or self-reported) with cough or sore throat lasting 2–7 days. Sociodemographic, medical, and clinical data were collected, and nasopharyngeal swabs tested for influenza, RSV, and other pathogens using RT-PCR. Logistic regression identified predictors of viral positivity.

Of 313 participants, 83(28.5%) were respiratory virus positive: 26 rhinovirus, 12 influenza A/B, 4 SARS-CoV-2, and 1 RSV. Median age was 32 years; 59.7% female, 25.6% HIV-positive, and 53.7% vaccinated against COVID-19. Common symptoms included cough, rhinorrhoea, sneezing, sore throat, and headache. Sneezing and hot dry season were linked to lower odds of viral positivity, while rhinorrhoea increased odds.

In this high HIV-burden, non-temperate setting, viral positivity was substantial. Findings emphasize reducing unnecessary antibiotic use and highlight the need for rapid, affordable point-of-care diagnostics to strengthen PHC, improve patient care, and bolster pandemic readiness.

**Keywords**:

Influenza, RSV, HIV, surveillance

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-76

**Poster slam / 162**

## Functional Interactions Between Human, Bacterial Neuraminidases and Influenza A Viruses

**Author:** Laurine Payre[1]

**Co-authors:** Clément Fage [1]; Olivier Terrier [1]; Vanessa Escuret [1]

[1] *Université Claude Bernard Lyon 1*

**Corresponding Authors:** laurine.payre@univ-lyon1.fr, vanessa.escuret@chu-lyon.fr, clement.fage@univ-lyon1.fr, olivier.terrier@univ-lyon1.fr

Respiratory tract infections are responsible for 3 to 5 million deaths each year worldwide. Respiratory co-infections, involving multiple pathogens simultaneously or sequentially, involve complex host-pathogen interactions whose mechanisms remain poorly understood. In particular, the role of sialic acids and microbial neuraminidases has been little explored in viral/bacterial co-infections. This study investigate the role of the human neuraminidases NEU1 and NEU3 and the bacterial neuraminidases NanA and NanI in influenza A(H1N1) and A(H3N2) virus infection. Using different infection scenarios in the A549 cells line, we identified functional interactions between influenza A viruses and NEU1/NEU3. Furthermore, experiments performed in a reconstituted human airway epithelium model revealed a dual role for bacterial neuraminidases during influenza co-infection. Indeed, administration of NanA or NanI recombinant proteins before or early after infection inhibits viral production, whereas post-infection treatment promoted A(H1N1) spread. Our findings highlight the role of human and bacterial neuraminidases throughout the influenza A virus life cycle and suggest multiple functional interactions between virus, bacteria, and host during co-infection. These observation provide new insights into respiratory co-infection mechanisms and open new avenues for the development of broad-spectrum antimicrobial strategies.

**Keywords**:

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

NF26- 109

**Poster slam / 91**

## INFLUE-AIR: Understanding and Modelling the Airborne Spread of Highly Pathogenic Avian Influenza Viruses

**Author:** Esteban Suls[None]

**Co-authors:** Pierre Hostyn [1]; Xavier Simons [1]; Bénédicte Lambrecht [1]; Claude Saegerman [2]; Andy Delcloo [3]; Mieke Steensels [1]

[1] *Sciensano*

[2] *University of Liège*

[3] *Belgian Royal Meteorological Institute*

**Corresponding Author:** esteban.suls@sciensano.be

Highly pathogenic avian influenza (HPAI) H5 viruses continue to threaten poultry production, wildlife, and public health worldwide. Although direct contact is considered the primary transmission route, increasing evidence suggests that airborne spread may contribute to local dissemination. The INFLUE-AIR project aims to improve understanding of airborne HPAI transmission and its implications for surveillance and control. Laboratory and field investigations will identify and quantify key determinants of airborne spread, including viral excretion in aerosols and faeces, viral stability under different ambient temperature and humidity conditions, and infectivity of aerosolised virus. Air and dust sampling will be evaluated experimentally and during field outbreaks as a cost-effective alternative or complement to conventional individual bird swabbing for early HPAI detection in poultry flocks. Experimental and field data will feed epidemiological and atmospheric dispersion models to simulate viral emission, airborne dispersion, environmental persistence, and infection risk from infected poultry farms and contaminated wild bird faeces. The models will generate dynamic risk maps to support outbreak management and targeted mitigation strategies. By integrating all these virological, epidemiological, and meteorological data, the final model will support risk assessment, outbreak management, and targeted prevention strategies for the rising threat of HPAI viruses.

**Keywords**:

High Pathogenic Avian Influenza (HPAI), H5N1, field sampling, parameter identification, modelling

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-14

**Poster slam / 102**

## Discovery of a novel influenza A virus inhibitor targeting a distinct pocket at the viral nucleoprotein dimer interface

**Author:** Benjamin Van Loy[1]

**Co-authors:** Stijn Verschoren [1]; Nelleke Cloet [2]; Chang-Soo Yun [3]; Hugo Klaassen [2]; Rana Abdelnabi [1]; Nam-Chul Cho [3]; Pieter Leyssen [4]; Soo Bong Han [3]; Patrick Chaltin [2]; Johan Neyts [4]; Bert Vanmechelen [1]; Koert Stittelaar [1]

[1] *The VirusBank Platform, Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium*

[2] *CISTIM Leuven vzw, Leuven, Belgium*

[3] *Korea Research Institute of Chemical Technology, Daejeon, Republic of Korea*

[4] *Virology, Antiviral Drug & Vaccine Research Group, Department of Microbiology, Immunology and Transplantation, Rega Institute, KU Leuven, Leuven, Belgium*

**Corresponding Author:** benjamin.vanloy@kuleuven.be

The Global Viral Infection Research Center (G-VIC), a TOP TIER project of the National Research Foundation of Korea, aims to develop novel antivirals and vaccines through international collaboration. As part of this effort, the VirusBank Platform screened ~100,000 compounds from the Korea

Research Institute of Chemical Technology (KRICT) to identify inhibitors of influenza virus using a MUNANA assay on A549 cells.
Here we report compound R91, a novel hit with potent anti-influenza activity (EC50 = 1.3 μM, CC50 > 25 μM). R91 reduced vRNA levels by 5-log10 in the supernatant of MDCK cells infected with A/PR/8/1934 (H1N1) at 6.4 μM. Notably, the activity was restricted to the PR8 strain, with no detectable effects against other influenza A or B viruses.
Selection of resistant variants identified a P283S substitution in the viral nucleoprotein (NP), completely abrogating antiviral activity and identifying NP as the target. Structural analysis of the NP protein placed P283 within a druggable pocket at the NP dimer interface, adjacent to the binding site of nucleozin, a known NP-targeting influenza inhibitor. Interestingly, NPP283S viruses showed increased sensitivity to nucleozin. Moreover, time-of-addition assays revealed similar profiles for R91 and nucleozin, with loss of efficacy when added after ~4 h p.i.
These findings suggest that R91 interferes with NP–NP interactions, impairing ribonucleoprotein complex assembly and thereby inhibiting viral replication and transcription.

**Keywords**:

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-42

**Poster slam / 103**

# Madin-Darby canine kidney cells express multiple transmembrane serine proteases supporting trypsin-independent replication of various influenza viruses

**Authors:** Emma Balibrea Roelans[1]; Jia-Yi Chan[1]; Lisa Butticaz[1]; Etori Aguiar Moreira[1]; Gert Zimmer[1]

[1] *Institute of Virology and Immunology*

**Corresponding Authors:** gert.zimmer@ivi.admin.ch, jiayi.chan@ivi.admin.ch, lisa.butticaz@ivi.admin.ch, etori.aguiarmoreira@ivi.admin.ch, emma.balibrearoelans@ivi.admin.ch

Hemagglutinin (HA) is a key determinant of influenza A virus (IAV) virulence. HA mediates host cell binding and membrane fusion, the latter requiring proteolytic cleavage into HA1 and HA2. Most IAVs contain a monobasic cleavage site recognized by trypsin-like proteases expressed only in specific cell types.

This study compared IAV replication in various cell lines, including MDCK-I, MDCK-II and MDCK-NBL2, with and without exogenous trypsin. All MDCK sublines supported trypsin-independent replication, although with delayed kinetics and reduced titers compared to trypsin-dependent replication. MDCK-NBL2 supported multiple IAV strains, including human H1N1, porcine H3N2, and avian H5Nx, H7N7, and H9N2 viruses. Analysis of H5Nx viruses revealed marked differences in the proteolytic activation despite identical HA cleavage motifs.

Serine protease inhibitors blocked trypsin-independent replication in MDCK-NBL2 cells, indicating involvement of endogenous serine proteases. RT-PCR identified TMPRSS1, TMPRSS2, TMPRSS4, and TMPRSS11D (HAT) expression in MDCK cells. Canine TMPRSS2 and TMPRSS4 were found to support the activation of H1N1 HA. Other canine TMPRSS proteases are under investigation.

Overall, these findings identify MDCK-NBL2 cells as a convenient cellular system for influenza virus isolation, screening of protease inhibitors as potential antiviral compounds, and vaccine production without the need for exogenous animal-derived trypsin.

**Keywords**:

**Professional status of the speaker**:

Graduate student

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-48

**Poster slam / 105**

## Defining the PKR Interactome in Immune-activated A549 cells to Identify Modulators of PKR function

**Author:** Pia Madeleine Leipe[1]

**Co-authors:** Linda Brunotte [2]; Sriram Kumar [2]; Stephan Ludwig [3]

[1] *Universtity Münster, Institute for Virology*

[2] *Universitätsklinikum Gießen and Marburg (UKGM) Justus-Liebig-Universität (JLU) Gießen*

[3] *nstitute of Virology Muenster*

**Corresponding Authors:** piamadeleine.leipe@ukmuenster.de, ludwigs@uni-muenster.de, linda.brunotte@uni-giessen.de, sriram.kumar@uni-giessen.de

Infections with seasonal influenza A viruses (IAVs) typically trigger a balanced antiviral response, whereas highly pathogenic avian influenza viruses (HPAIVs) often lead to systemic inflammation and severe disease. The molecular determinants driving these aberrant reactions remain poorly defined. Our previous research identified a specific signaling cascade comprising protein kinase R (PKR), p38, and MSK1 that mediates the phosphorylation of the immune regulatory transcriptional co-repressor TRIM28. This pathway is uniquely activated by HPAIVs and is linked to the excessive expression of proinflammatory cytokines.
While HPAIV-derived viral RNAs are suspected drivers of PKR activation, the role of protein-protein interactions remains largely unexplored. To investigate this, we characterized the PKR interactome in immune-activated A549 cells using affinity-based protein enrichment and LC-MS. This approach identified several interferon-stimulated genes (ISGs) with proven antiviral activities as highly confident PKR binding partners. Western blot analysis verified our findings and further suggest that PKR could serve additional immune modulatory roles beyond the canonical function of inhibition of protein translation.

**Keywords**:

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-8

**Poster slam / 111**

# Longitudinal characterisation of cell-mediated immune responses following influenza A(H5N8) vaccination

**Author:** Ulla Mikkonen[1]

**Co-authors:** Emilia Peltoniemi [1]; Sophie Winter [2]; Arttu Reinholm [1]; Milja Belik [1]; Alina Iakubovskaia [3]; Arja Pasternack [3]; Olli Ritvos [3]; Oona Liedes [4]; Anu Haveri [4]; Saimi Vara [4]; Merit Melin [4]; Pekka Kolehmainen [2]; Laura Kakkola [5]; Ilkka Julkunen [2]

[1] *Institute of Biomedicine, University of Turku, Turku, Finland*

[2] *Institute of Biomedicine, University of Turku, Turku, Finland; InFlames Research Flagship Center, University of Turku, Turku, Finland*

[3] *Department of Physiology, University of Helsinki, Helsinki, Finland*

[4] *Finnish Institute for Health and Welfare, Helsinki, Finland*

[5] *Institute of Biomedicine, University of Turku, Turku, Finland; Clinical Microbiology, Turku University Hospital, Turku, Finland*

**Corresponding Author:** ulla.e.mikkonen@utu.fi

In 2023, Finland experienced a major outbreak of highly pathogenic avian influenza caused by clade 2.3.4.4b influenza A(H5N1) virus, with transmission from wild birds to fur farms. In June 2024, Finland became the first country to vaccinate occupational risk groups using the MF59-adjuvanted influenza A(H5N8) vaccine (A/Astrakhan/3212/2020, Seqirus). We aimed to characterise the quality and durability of cellular immune responses induced by this novel vaccine. Peripheral blood mononuclear cells were collected before vaccination and at 3 weeks, 6 months and 12 months after the second vaccine dose. Antigen-specific T-cell responses were analysed using an activation-induced marker assay following stimulation with overlapping H1, H5 and N8 peptide pools. Vaccine-induced B-cell responses were evaluated using ELISpot assay and flow cytometric detection of H5-specific memory B cells. Robust H5/N8-specific $CD4^+$ T-cell responses and relatively weak $CD8^+$ T-cell responses were observed. Cellular responses remained detectable 12 months after vaccination. Analyses also indicated induction of H5-specific memory B cells, with declining kinetics during follow-up. The influenza A(H5N8) vaccine induces durable cellular immune responses in individuals at occupational risk of avian influenza exposure. This research provides important information on the durability of influenza A(H5N8) vaccine -induced immunity, which supports future pandemic preparedness and avian influenza vaccine development.

**Keywords**:

Avian influenza, Cellular immunity, Influenza A(H5N8) vaccine

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26- 56

**Poster slam / 128**

# Predicting avian Influenza spillover through innovative tools to support public health decision-making

**Author:** Hassane Rolland[1]

**Co-authors:** Antoine Gerodez [1]; Bénédicte Lambrecht [1]; Killian De Geest [1]; Mieke Steensels [1]; Steven Van Borm [1]

[1] *Sciensano*

**Corresponding Authors:** mieke.steensels@sciensano.be, killian.degeest@sciensano.be, antoine.gerodez@sciensano.be, benedicte.lambrecht@sciensano.be, hassane.rolland@sciensano.be, steven.vanborm@sciensano.be

Highly pathogenic avian influenza (HPAI) H5N1 viruses from clade 2.3.4.4b represent a major threat to animal and public health. Since 2021, these viruses have undergone unprecedented ecological and epidemiological changes characterized by global dissemination and expansion of the range of susceptible bird and mammal species. Also, sustained mammal-to-mammal transmission has been documented in multiple settings and sporadic human infections raise concerns about their pandemic potential.

In this context, we are developing an integrated pipeline to assess the risk of HPAI H5N1 spillover into humans by combining genomic analyses with a panel of complementary in vitro assays designed to characterize viral adaptation to human. Specifically, these assays evaluate the capacity of the virus to replicate in human airway models, including air-liquid interface cultures and respiratory organoids, as well as key determinants of host adaptation, such as hemagglutinin receptor-binding preference, the pH threshold for HA-mediated membrane fusion, and viral polymerase activity in human cells.

The pipeline is currently being benchmarked using seasonal human and representative avian influenza viruses to define reference phenotypic profiles associated with human and avian adaptation. Ultimately, this framework aims to strengthen the early identification of H5N1 viruses with increased zoonotic potential and to provide experimental evidence supporting public health risk assessment and preparedness.

**Keywords**:

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26- 17

**Poster slam / 130**

# Ability of Influenza A virus non-structural 1 protein to inhibit the innate immune pathways

**Authors:** Beda Anttila[1]; Ulla Mikkonen[1]; Rickard Lundberg[1]; Sini Huuskonen[2]; Pamela Österlund[2]; Pekka Kolehmainen[1]; Ilkka Julkunen[1]; Laura Kakkola[1]

[1] *University of Turku*

[2] *University of Helsinki*

**Corresponding Authors:** laura.kakkola@utu.fi, pamela.osterlund@helsinki.fi, bemaan@utu.fi, ilkka.julkunen@utu.fi, pejukol@utu.fi, ulla.e.mikkonen@utu.fi, rickard.lundberg@utu.fi

Influenza A viruses (IAVs) are pathogens that cause mainly respiratory infections in humans, but they can also infect variety of animals. IAVs are also responsible for seasonal epidemic outbreaks in humans, and the zoonotic strains pose a possible pandemic threat. In recent years, the H5N1 avian influenza virus, IAV subtype, has caused unprecedented bird mortality and infections in mammals around the world, raising concerns about adaptation to humans and highlighting the pandemic threat. The objective of this research is to investigate the differences in the IAV non-structural protein 1 (NS1), a multifunctional virus protein primarily affecting the immune response of the host, between different human and avian IAV strains, with a focus on the strains from the H5N1 subtype. The inhibitory effect of thirteen NS1 proteins on the retinoic acid inducible gene 1 (RIG-I) pathway was determined with a luciferase assay, indicating that all NS1 proteins inhibit the pathway. However, some differences between examined NS1 proteins were observed and the biological relevance of these differences is being further studied by revealing the interactions of NS1 proteins with host cell proteins by proximity-dependent labeling method and pull-down assay. This research offers valuable insights into the interactions between NS1from different IAV strains and the host cell. Characterizing the function of the NS1 protein is important to better understand how IAV strains adapt to new host species.

**Keywords**:

influenza A virus, interactome, NS1, RIG-I

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-59

**Poster slam / 137**

# Deciphering the immune mechanisms of influenza vaccines on the innate immune response

**Authors:** Josel Huch[1]; Julia Voges[1]; Sebastian Knab[1]; Rebecca Hasseli-Fräbel[2]; Ricarda Mittrowann[1]; Johannes Roth[1]

[1] *Institute of Immunology, University of Münster*

[2] *Institute of Immunology, University of Münster, Section of Rheumatology and Clinical Immunology, University Hospital of Münster*

Rising public skepticism toward vaccination highlights the need for evidence of influenza vaccine effectiveness. Individuals with rheumatoid arthritis (RA) receiving immunomodulatory therapy represent a population for whom vaccine-mediated protection is especially relevant. This project investigates the mechanistic effects of three licensed influenza vaccines on innate immune function in healthy controls and RA. To characterize vaccine-driven innate immune reprogramming, primary human monocytes, HoxB8 monocytes and HEK Blue reporter cell lines were used. Vaccine-associated activation was interrogated using Toll-like receptor (TLR)-specific ligands followed by cytokine profiling. This approach aims to identify the TLRs engaged by each vaccine and their downstream signaling pathways. Pre-treatment with Efluelda, Influsplit, and Influvac reprogrammed monocytes, resulting in enhanced cytokine responses upon restimulation with the TLR7/8 agonist R848 and the TLR2 agonist LTA compared to monocytes not pre-treated with influenza vaccines. These effects were stimulus-specific, as vaccine pre-treatment did not alter responses to other TLR agonists. This framework will be applied to compare monocyte responses in RA patients with and without immunotherapy before and after influenza vaccination. These data will clarify how immunotherapy alters vaccine-induced innate immune signaling and support improved vaccination strategies for immune-mediated inflammatory diseases.

**Keywords**:

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

91

**Poster slam / 163**

# Avian Influenza Surveillance in Central Asia: A Possible Reporting Gap for Uzbekistan Relative to Kazakhstan

**Author:** Malika Kurbaniyazova[1]

**Co-author:** Nilufar khamzaeva [2]

[1] *Assistant, Department of Epidemiology, Tashkent State Medical University, Tashkent, Uzbekistan*

[2] *PhD, Associate Professor, Department of Epidemiology, Tashkent State Medical University, Tashkent, Uzbekistan*

**Corresponding Authors:** m.kurbaniyazova@tashmeduni.uz, n.xamzaeva@tashmeduni.uz

Highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b has been repeatedly reported in Central Asia. WOAH data show 27 outbreaks in Kazakhstan during 2005-2023 (20 poultry, 7 wildlife), plus detections in southern Siberia. Uzbekistan lies on the same Central Asian flyway, linking Siberian breeding areas with South/Southwest Asian wintering grounds, where wild bird migration and duck density have been linked to HPAI risk. Despite comparable exposure, our structured English-language search of WOAH/WAHIS, FAO EMPRES-i, WHO risk assessments, and peer-reviewed literature found no comparable dataset for Uzbekistan - an absence that may reflect limited indexing, language barriers, or a true surveillance gap.
Objective: To characterise the Kazakhstan-Uzbekistan reporting asymmetry along the shared flyway and its implications for regional HPAI surveillance.
Methods: Structured review of WOAH/WAHIS, FAO EMPRES-i, WHO risk assessments, and peer-reviewed English-language literature, cross-checked against regional surveillance reviews.
Findings: Kazakhstan has publicly accessible documentation of 27 outbreaks; no equivalent Uzbekistan dataset was identified. Evidence cannot yet distinguish under-ascertainment, limited data accessibility, and a true surveillance gap.
Conclusion: Improving access to national surveillance data and strengthening cross-border collaboration within a One Health framework would support fuller assessment of HPAI circulation along this flyway.

**Keywords**:

Avian influenza (H5N1); Central Asian flyway; Uzbekistan; Kazakhstan; Surveillance gap; One Health

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-110

**Session 1: Virus host cell interaction I / 169**

# Keynote: MHC-II entry competence as zoonotic trait of influenza A viruses

**Session 1: Virus host cell interaction I / 93**

# Structural and functional analysis of the binding interface of the H18N11 bat Influenza A virus and the proteinaceous receptor MHCII

**Authors:** Jonathan Robert[1]; Maria K. Osman[1]; Nico J. Halwe[2]; Valeria Calvaresi[3]; Anna Kotanska[4]; Jan Gradon[4]; Leon Michalski[4]; Stanley Sau[4]; Stephen Martin[4]; Eamonn Reading[5]; Martin Beer[2]; John J. Skehel[4]; Steve J. Gamblin[4]; Martin Schwemmle[1]; Kevin Cimninski[1]; Antoni G. Wrobel[3]; Peter Reuther[1]

[1] *Institute of Virology, University Medical Centre of Freiburg, Germany*

[2] *Institut für Virusdiagnostik (IVD), Friedrich-Loeffler-Institut, Greifswald - Insel Riems, Germany*

[3] *Department of Biochemistry, University of Oxford, UK*

[4] *Structural Biology of Disease Processes Laboratory, The Francis Crick Institute, London, UK*

[5] *Department of Chemistry, Britannia House, King's College London, London, UK*

**Corresponding Author:** peter.reuther@uniklinik-freiburg.de

The discovery of influenza A virus (IAV) subtypes H17N10 and H18N11 in New World bats challenged the paradigm that viral entry depends on hemagglutinin (HA) binding to sialic acid. Instead, these bat IAV use the protein receptor MHC class II (MHCII). MHCII from diverse species, including humans, mediates infection, and conserved residues within MHCII are critical for interaction with H18. Here, we structurally characterized the MHCII binding site on H18.

We purified soluble H18 homotrimers and MHCII heterodimers and demonstrated direct interaction by bio-layer interferometry. Soluble MHCII efficiently blocked H18N11 infection, confirming its essential role in entry. Using hydrogen-deuterium exchange mass spectrometry and cryo-EM, we mapped the MHCII binding interface to a discrete region in the H18 head domain, distinct from the classical sialic acid binding site. Mutational analysis identified key residues required for membrane fusion triggered by the H18-MHCII interaction. Viruses carrying mutations in this interface showed severely impaired growth. Passaging of the mutant viruses led to a compensatory mutation near the binding site that restored membrane fusion and viral replication to wildtype levels.

These findings provide the first structural insights into the H18–MHCII interface and identify critical residues mediating this interaction.

**Keywords**:

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26- 31

**Session 1: Virus host cell interaction I / 131**

# MHC-II functions as pH-dependent membrane fusion switch for bat influenza A virus

**Author:** Petr Chlanda[1]

**Co-authors:** Sarah Peterl ; Jonathan Robert ; Maria Osman ; Rebecca Haines ; Konstantin Fischer ; Richard Langi ; Kevin Ciminski ; Martin Schwemmle ; Peter Reuther

[1] *Heidelberg University Hospital*

**Corresponding Author:** petr.chlanda@bioquant.uni-heidelberg.de

Influenza A virus hemagglutinin is a prototypical class I viral fusion protein that binds sialylated glycans and is activated by low pH in endosomes. In contrast, bat-derived IAV subtypes H17N10 and H18N11 use major histocompatibility complex class II (MHC-II) as an entry receptor, but how this receptor contributes to membrane fusion remains unknown. We find that MHC-II-dependent hemagglutinin subtypes H17, H18, and H19 possess an increased negative net charge relative to canonical HAs. Using cryo-electron tomography, we demonstrate that H18N11 morphology remains stable and H18 is in prefusion conformation at strongly acidic pH. Remarkably, H18 undergoes fusion-relevant conformational changes only when both MHC-II binding and low pH are present. By reconstitution of H18N11 fusion with liposomes and purified MHC-II, we show that receptor engagement is required to trigger the fusion activity of H18. These findings identify MHC-II as a receptor that directly triggers membrane fusion and reveal a previously unrecognized receptor-dependent mechanism of influenza virus entry.

**Keywords**:

Bat influenza A virus, H18N11, Hemagglutinin, MHC-II, membrane fusion, cryo-electron tomography

**Professional status of the speaker**:

Professor

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26- 16

**Session 1: Virus host cell interaction I / 108**

# Probing an evolutionary bifurcation to host shut-off

**Authors:** Jacob Neeves[1]; Garance Meyer[2]

**Co-authors:** Miki Uchima [2]; Thomas Courty [2]; Stuart McKellar [2]; Ruth Harvey [3]; Jon Wilson [3]; Michael H. Malim [4]; Hannah E. Mischo [4]

[1] *Department of Infectious Diseases, King's College London, UK*

[2] *1Department of Infectious Diseases, King's College London, UK*

[3] *The Francis Crick Institute, London, UK*

[4] *King's College London*

**Corresponding Authors:** michael.malim@kcl.ac.uk, hannah.mischo@kcl.ac.uk, garance.meyer@kcl.ac.uk

To infect its host, influenza A virus (IAV) critically depends on its ability to shut-off host cell gene expression. When infected, host cells activate defence-gene transcription whose protein-products

interfere with viral propagation and induce an anti-viral state in neighbouring cells. A major contributor to IAV-induced host-cell shut-off is the non-structural protein 1 (NS1), which interferes both with gene activation and mRNA 3'end cleavage. The latter inhibits mRNA polyadenylation and nuclear export of cellular mRNAs thereby effectively preventing translation of newly induced genes, including those required to warn bystander cells. How - when host shut-off is active - can infected cells signal to bystander cells? We have identified a set of transcripts that escape host shut-off, some of which encode secreted messengers with the potential to establish the innate immune response to IAV infection.

NS1-mediated shut-off through disrupted transcription termination is well-characterised in human adapted strains but is absent in most studied zoonotic strains. Conversely, during adaptation of the zoonotic A/California/ 07/2009(H1N1) (pdmH1N1) to the human population, NS1 gained the ability to interfere with cellular transcription termination. How - if not through NS1-action - is host shut-off achieved in zoonotic IAV strains? We will present a hitherto unidentified mechanism of host shut-off that appears to be active in a range of zoonotic strains of the H5N1 clade 2.3.4.4.b.

**Keywords**:

host shut-off, host pathogen interaction, host adaptation, mechanisms of gene expression control, innate immunity, bystander versus infected cells.

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

1615-6264

**Session 2: Vaccines** / **170**

## Keynote: Preventive vaccination against highly pathogenic avian influenza in duck farms in France

**Session 2: Vaccines** / **115**

## Pre-clinical efficacy of a universal influenza vaccine candidate inducing complementary humoral and cellular immunity

**Author:** Julian Volk[1]

**Co-authors:** Annika Schönsiegel [2]; Carina Metz [2]; Melanie Müller [3]; Ralf Amann [4]; Oliver Planz [2]

[1] *Institute of Immunology*

[2] *Institute of Immunology Tübingen*

[3] *Prime Vector GmbH*

[4] *Fraunhofer IGB*

**Corresponding Author:** julian.volk@uni-tuebingen.de

Current influenza vaccines primarily induce strain-specific antibodies against the variable hemagglutinin (HA) head while eliciting limited T cell immunity, resulting in suboptimal protection against drifted and emerging zoonotic influenza viruses. We developed an Orf virus-based influenza vaccine

(ORFV-Flu) combining chimeric HAs with conserved influenza T cell epitopes to induce coordinated broadly reactive humoral and cellular immunity. The ORFV platform has already demonstrated clinical safety in humans, supporting rapid clinical translation.
Following systemic prime and respiratory boost immunization ("prime-and-pull") of HLA-A*02-transgenic mice, ORFV-Flu provided complete protection against lethal heterologous influenza challenge and reduced pulmonary viral loads by up to 99%, clearly outperforming seasonal vaccination. Remarkably, ORFV-Flu exclusively induced pulmonary tissue-resident memory T cells, together with broad HA stalk-specific IgG, mucosal IgA, and robust helper and cytotoxic T cell responses targeting the HA stalk and conserved influenza virus antigens. Collectively, these findings identify ORFV-Flu as a clinically translatable universal influenza vaccine candidate with the potential to protect against seasonal, drifted, and emerging zoonotic threats, including H5N1.

**Keywords**:

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-47

**Session 2: Vaccines** / **154**

## Granger-causality analysis reveals defective viral genomes with antiviral potential

**Author:** Mia Le[None]

**Co-authors:** Giulia Saresini ; Jens Lohmann ; Nils Petersen ; Sophie Duraffour ; Jan Baumbach ; Sara Grundel ; Tanja Laske

**Corresponding Authors:** sara.grundel@htwk-leipzig.de, mia.le@uni-hamburg.de, giulia.saresini@leibniz-liv.de, tanja.laske@leibniz-liv.de, jens.lohmann@uni-hamburg.de, jan.baumbach@uni-hamburg.de, duraffour@bnitm.de, nils.petersen@bnitm.de

During viral infection, replication errors can generate defective viral genomes (DVGs) that may interfere with virus replication and reduce infectious virus concentration. Identifying DVGs responsible for these effects is important because of their potential as antiviral candidates. However, experimentally screening all candidates is time- and resource-intensive, creating a need for computational prioritization. We therefore applied Granger-causality analysis to time-series data from continuous influenza A virus (IAV) infection to prioritise DVGs whose abundance predicts changes in infectious virus concentration.

We analysed next-generation sequencing counts of 1,968 IAV DVGs alongside infectious virus concentrations in infected cell cultures. For each DVG, we compared two ordinary least squares models: a restricted model using infectious virus concentration alone and a full model additionally including DVG counts. We also tested the reverse relationship, enabling classification as Granger-causing, Granger-caused, bidirectional, or unrelated.

After Benjamini-Hochberg correction, 255 DVGs showed Granger-causing or bidirectional relationships with infectious virus concentration. Previously validated defective interfering particle candidates also received plausible Granger labels. These findings suggest that Granger-based classification reflects antiviral activity and can help prioritise DVGs for experimental validation.

**Keywords**:

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-104

**Poster viewing / 135**

# Avian H5N1 spillover into mammals in Finland: Evolutionary pathways and early signs of human adaptation

**Authors:** Veronica Lehtinen[1]; Eda Altan[1]; Pamela Österlund[1]

**Co-authors:** Laura Kakkola [2]; Miao Jiang [1]; Riku Fagerlund [1]; Tarja Sironen [1]

[1] *University of Helsinki*

[2] *University of Turku*

**Corresponding Authors:** tarja.sironen@helsinki.fi, laura.kakkola@utu.fi, veronica.lehtinen@helsinki.fi, riku.fagerlund@helsinki.fi, eda.altan@helsinki.fi, pamela.osterlund@helsinki.fi, miao.jiang@helsinki.fi

Avian influenza A/H5N1 is driving a severe panzootic, with increasing spillover from avian reservoirs to wild mammals. Outbreaks in fur farms in Spain and Finland (2022–23) demonstrated high susceptibility of these mammals to avian influenza, while infections in dairy cattle in the USA highlighted the virus's expanding host range. These developments emphasize the need to understand mechanisms of cross-species transmission.
This project aims to investigate avian H5N1 and seasonal H3N2 virus infections and host immune responses in human upper airway epithelial and immune cells. Recent Finnish H5N1 isolates (2021–23) from fur farms and wildlife were propagated for study. Human primary nasal epithelial (HNE) cells were differentiated at air-liquid interface (ALI), providing a physiologically relevant model for early virus–host interaction.
Phylogenetic analysis showed the Finnish isolates clustered into three distinct branches, indicating divergent evolutionary paths. Network analysis revealed close genetic relationships between viruses from wild birds and fur animals within the 2023 epizootic, while strains from different farms were more distant, suggesting multiple introductions. Despite mutations between avian and mammalian isolates, no differences in replication were observed in human dendritic cells. However, mammalian-origin H5N1 showed slightly reduced replication in HNE cells compared with seasonal H3N2. Further studies on early virus–host interactions are ongoing.

**Keywords**:

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-90

**Poster viewing / 139**

# Avian influenza virus detection and genetic characterization in domestic duck flocks through slurry analysis.

**Author:** Sebastien SOUBIES[1]

**Co-authors:** Jean-Luc Guérin [1]; Guillaume Croville [1]; Maxime Fusade-Boyer [1]; Laura Lebouteiller [1]; Clément Castille [1]

[1] *IHAP, Université de Toulouse, INRAE, ENVT, Toulouse, France*

**Corresponding Authors:** maxime.fusade-boyer@envt.fr, guillaume.croville@envt.fr, laura.lebouteiller@envt.fr, jean-luc.guerin@envt.fr, clement.castille@envt.fr, sebastien.soubies@envt.fr

Avian influenza viruses (AIVs) circulate widely in domestic and wild birds and pose major risks to animal and public health, in particular those of high pathogenicity (HP). Ducks play a key role in AIV ecology due to frequent subclinical infections and high viral shedding. Efficient environmental surveillance approaches are needed to complement costly individual testing, especially in the context of large-scale anti-H5 vaccination as implemented in France. We evaluated the slurry produced in duck fattening units as a matrix for AIV detection and optimized molecular protocols for molecular detection and genetic characterization. Slurry samples (n = 172) were collected from 37 duck farms in southwestern France between May 2024 and February 2025. Among several extraction strategies, RNA extraction from the solid fraction using TRIzol LS followed by magnetic bead purification yielded the highest sensitivity. Using this protocol, 82% of samples tested positive for AIV RNA, with 30% of positives being H5-positive; no H7 viruses were detected. Whole-segment RT-PCR and sequencing were successful for shorter genomic segments, enabling subtype determination. Virus isolation consistently failed. These findings demonstrate that slurry is a promising matrix for AIV detection, providing valuable molecular data. Slurry-based surveillance may therefore complement individual testing and improve early warning capacities for influenza surveillance in a One Health perspective.

**Keywords**:

avian influenza, duck, environmental virus detection

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-43

**Poster viewing / 147**

# Respiratory Virus Coinfections in Influenza A and B Cases Detected by Multiplex NAATs: A Two-Season Study from Slovenia

**Author:** Katarina Prosenc Trilar[1]

**Co-author:** Nataša Berginc [1]

[1] *National Laboratory for Health Environment and Food, Slovenia*

**Corresponding Authors:** katarina.prosenc@nlzoh.si, natasa.berginc@nlzoh.si

The increasing affordability of syndromic multiplex testing has led to its widespread use for diagnosing respiratory infections, enabling improved detection of viral coinfections. We analyzed respiratory specimens collected in Slovenia from primary care and hospital patients during the 2024/25 and 2025/26 respiratory virus seasons. Specimens were tested using nucleic acid amplification tests (NAATs) for influenza A (InfA), influenza B (InfB), respiratory syncytial virus (RSV), parainfluenza viruses (PIV), adenoviruses (AdV), human metapneumovirus (hMPV), human coronaviruses (hCoV), enteroviruses/rhinoviruses (EV/RV), and SARS-CoV-2. Among 74,658 specimens, positivity rates were 7.7% for InfA, 1.3% for InfB, 18.3% for EV/RV, 7.4% for SARS-CoV-2, 4.0% for hCoV, 3.5% for PIV, 3.1% for hMPV, and 2.9% each for RSV and AdV. Coinfections were detected in 0.8% of InfA-positive and 0.15% of InfB-positive specimens. EV/RV was the most common copathogen (0.48% and 0.11%, respectively). In InfA-positive specimens, coinfections with SARS-CoV-2 (0.25%), hCoV (0.18%), AdV (0.09%), PIV (0.06%), RSV (0.05%), hMPV (0.03%), and InfB (0.01%) were observed. In InfB-positive specimens, hMPV (0.04%), RSV and AdV (0.03% each), and PIV (0.01%) were detected, whereas no InfB/SARS-CoV-2 coinfections were identified. Overall, influenza virus coinfections were rare despite extensive multiplex testing, with EV/RV representing the predominant copathogen.

**Keywords**:

influenza, respiratory viruses, confections, multiplex NAAT, surveillance

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26- 60

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# Did the influenza A(H3N2) antigenic variant K infect other age groups than preceding A(H3N2) viruses?

**Author:** Olav Hungnes[1]

**Co-authors:** Torstein Aune [1]; Andreas Rohringer [1]; Even Fossum [1]; Karoline Bragstad [1]

[1] *Norwegian Institute of Public Health*

**Corresponding Authors:** torstein.aune@fhi.no, andreas.rohringer@fhi.no, even.fossum@fhi.no, karoline.bragstad@fhi.no, olav.hungnes@fhi.no

The seasonal influenza virus subtypes and lineages differ in their impact in different age groups. Influenza A(H3N2) viruses have been regarded as affecting the elderly particularly hard. Consistent with this, Norwegian virological surveillance has for many years shown that the A(H3N2) viruses have been disproportionately represented among elderly patients compared with other subtypes and lineages. However, in recent seasons we have seen a trend with less pronounced over-representation of elderly persons among H3N2 cases.

In late summer 2025, a new H3N2 antigenic drift variant, subclade K, emerged and spread rapidly, causing unusually early outbreaks in many northern hemisphere countries, including Norway where this variant was clearly predominant among characterised H3N2 viruses. The K variant harbours mutations at several antigenically important sites in the viral haemagglutinin, which may differentially affect immunity in older and younger population groups, given their distinct histories of exposure to previous H3N2 antigenic variants.

In the present study, we investigate whether the age profile of H3N2 subtyped cases during the

variant K-dominated 2025/2026 season differs from the H3N2 age profile prior to the emergence of subclade K.

**Keywords**:

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-73

**Poster viewing / 152**

# NATURAL PRODUCTS FROM PLANT BIODIVERSITY AS A SOURCE OF ANTIVIRAL AND PRO-RESOLVING COMPOUNDS AGAINST INFLUENZA A VIRUS

**Author:** José Hugo de Sousa Gomes[1]

**Co-authors:** Felipe Rocha da Silva Santos [2]; Fernão Castro Braga [2]; Eike R. Hrincius [3]; Stephan Ludwig [3]

[1] *UKM, Institute fur Virologie*

[2] *UFMG*

[3] *UKM*

**Corresponding Author:** josehugoufmg@gmail.com

Influenza A virus (IAV) remains a major global health challenge due to its high genetic variability, limited therapeutic options, and the emergence of antiviral resistance. Natural products are a promising source of antiviral agents. However, their antiviral and immunomodulatory mechanisms remain poorly understood. In particular, their potential to promote the resolution of virus-induced inflammation remains largely unexplored. In this context, our group has investigated two Brazilian species, Terminalia glabrescens (Tg) and Terminalia phaeocarpa (Tp), in in vitro models of Zika virus (ZIKV) and SARS-CoV-2 infection. Their antiviral activity has been associated with distinct classes of secondary metabolites, including the terpene β-sitosterol from Tg, the ellagitannins 1-O-α-galloylpunicalagin and punicalagin, and the flavonoids quercitrin and afzelin isolated from Tp leaves. Based on these findings, we investigated whether these natural products also exhibit antiviral and pro-resolving activity against IAV. While no cytotoxicity was observed, antiviral assays demonstrated that all tested compounds significantly reduced IAV replication. Further studies are evaluating their antiviral potency and investigating their capacity to modulate virus-induced inflammatory responses as pro-resolving agents. Collectively, these findings highlight plant-derived compounds as promising antiviral agents for IAV therapy.

**Keywords**:

Antiviral agents; Pro-resolving agents; Influenza A virus; Brazilian biodiversity

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-69

**Poster viewing / 155**

# Nasally Administered Adenovector-Based Pandemic Influenza Vaccine

**Authors:** Pamela Österlund[None]; Riku Fagerlund[None]

**Corresponding Authors:** pamela.osterlund@helsinki.fi, riku.fagerlund@helsinki.fi

Zoonotic influenza viruses continue to pose a major pandemic threat, highlighting the need for rapidly adaptable vaccines that induce both mucosal and systemic immunity. We are developing an intranasal adenovirus serotype 5 (Ad5)-vectored influenza vaccine platform optimized for pandemic preparedness.

The vaccine combines complementary hemagglutinin (HA) immunogens: a strain-specific HA-head construct and a conserved HA-stem construct designed to induce broad cross-protective immunity. To enhance cellular responses and antibody durability, the antigens incorporate modular T-helper epitope domains. Influenza-specific antigen designs were generated and cloned into an established Ad5 vector platform previously validated for intranasal vaccination.

We have successfully generated Ad5 vaccine constructs expressing influenza HA-head and HA-stem immunogens. Initial studies are evaluating antigen expression, secretion, and processing in mammalian cells to identify lead candidates for preclinical development. Planned studies will assess intranasal immunization in mice, including mucosal IgA, systemic IgG, T-cell responses, and neutralization breadth against pre-pandemic influenza viruses, including H9N2.

This work establishes a flexible intranasal adenovector vaccine platform designed to provide broad and durable protection against influenza viruses with pandemic potential while supporting rapid vaccine adaptation to emerging threats.

**Keywords**:

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-89

**Poster viewing / 161**

# Quantum AI for developing models to study the response of vaccination of avian influenza H5N1 virus

**Author:** Gudrun Baersch[1]

**Co-author:** Sudhir Bhatia [1]

[1] *Genekam Biotechnology AG*

**Corresponding Author:** gbaersch@gmail.com

Artificial intelligent models are being used to do different tasks in medicine. Similarly quantum computers are being developed as these computers are in the position to conduct the tasks million times faster than super computers available today. Supercomputers can need 100 years to conduct the difficult task, but quantum computer can do the same task in a few minutes.

The aim of this study is to develop the concepts by using quantum computers and artificial intelligence tools to create models that simulate the immune response to the avian influenza virus based on immunological datasets. Experiments are then conducted to proof these generated concepts in laboratory setting.

These models should be in position to predict the kinds of antibodies being generated after the vaccination as well as other immunological responses, but indicating the unwanted affects, if any. In this way, quantum computers can be used to prepare for better and safer vaccines for pandemic pathogens like avian influenza H5N1 strains in advance as well as also predict diversity of human antibodies generated with the vaccines. This work is a future vision of immunology using Quantum AI.

**Keywords**:

Quantum computer, AI applications, Avian influenza H5N1 virus, Antibodies, Vaccine response

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

**95**

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# The antifungal agents Itraconazole and Isavuconazole exhibit antiviral activity during influenza A virus and Aspergillus fumigatus coinfection in vitro and ex vivo

**Author:** Sarah König[1]

**Co-authors:** Christina Ehrhardt [1]; Lisa Gelhausen [1]

[1] *Section of Experimental Virology, Institute of Medical Microbiology, Jena University Hospital*

**Corresponding Authors:** sarah.koenig2@med.uni-jena.de, christina.ehrhardt@med.uni-jena.de

Influenza-associated pulmonary aspergillosis remains a life-threatening complication with high mortality. Although combined antiviral and antifungal therapy appears to be a rational approach, a better understanding of its effects on both pathogens is needed. The antifungal agent itraconazole (ITC) is already known for its antiviral properties against influenza A viruses (IAVs). However, its use in invasive pulmonary aspergillosis is limited for example by its high risk of drug-drug interactions. Instead, voriconazole (VRC) and isavuconazole (ISA) are recommended as first-line treatment options. Furthermore, the antiviral effect of ITC was yet not examined under coinfection conditions.
Thus, this project investigates the antiviral properties of ITC, VRC and ISA against IAVs during mono- and coinfection with *Aspergillus fumigatus* in presence and absence of the antiviral drug Oseltamivir (OSV). In *in vitro* assays, titres and viral protein expression of IAV was reduced in ITC and ISA-treated samples, but not in VCZ-treated ones. In combination with OSV the antiviral effects of ITC and ISA were enhanced. Subsequently, these findings were reproduced in *ex vivo* coinfection experiments using precision-cut mouse lung slices.

Our findings indicate that both ITC and ISA exhibit antiviral activity against IAV during mono- and coinfection, which is further enhanced in the presence of OSV. Further experiments are required to elucidate the underlying mechanisms of these observations.

**Keywords**:

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26- 25

**Poster viewing / 116**

## Influenza A virus (IAV) NS1 mediated host gene shut off takes place in nuclear speckles

**Author:** Wolfgang Nacken[1]

**Co-authors:** Andre Schreiber ; Juliane Mayr ; Matthias Weiler ; Yvonne Börgeling ; Stephan Ludwig

[1] *Institut für Virologie Münster*

**Corresponding Authors:** matthias.weiler@uni-muenster.de, andre.schreiber@uni-muenster.de, nacken@uni-muenster.de, mayrj@uni-muenster.de, borgelin@uni-muenster.de, ludwigs@uni-muenster.de

IAV-NS1 proteins are known to inhibit host gene expression. In both transfection and infection experiments a strong attenuation of reporter gene expression is observed when NS1 proteins are fused to protein domains guiding NS1 exclusively to nuclear speckles (nsps). IAV H7N7 SC35M NS1 protein that is fused to domains, that guide it to nuclear or non-nuclear compartments other than nsps, show little or no ability to attenuate reporter gene expression. A nsp-localised NS1-effector domain is sufficient to inhibit gene expression. The protein SON is an essential component of nsps. In cells treated with siRNA targeted against SON the ability of NS1 to suppress the expression of a reporter gene is reduced relative to cells with fully functional nsps. Lastly, we demonstrate that the NS1 mediated suppression relies on transcriptional inhibition. Our data suggest that IAV-NS1 suppresses nuclear speckle promoted gene expression by inhibition of transcription.
Reportedly the f2f3 domain of CPSF30 binds to NS1. The f2f3 domain will be fused to domains that guide it to nuclear or non-nuclear compartments. In reporter gene assays we try to link subcellular localisation of the NS1 binding recombinant f2f3 domain with inhibition of host gene expression and/or APA (alternative polyadenylation of host transcripts) to investigate whether CPSF30-NS1 interaction takes place in nsps and how this will affect APA and reporter gene transcription.

**Keywords**:

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF24-45

**Poster viewing / 122**

# Can we blame the cranes? In-depth analyses of HPAIV H5N1 whole genome sequences in a densely poultry populated area

**Author:** Christine Bächlein[1]

**Co-authors:** Anne Wöhlke [1]; Christa Jeske [2]; Ole Stejskal [3]; Josef Diekmann [3]; Laura Zani [3]; Andreas Moss [4]; Jens Brackmann [4]; Louise Herms [1]; Katharina Ebhardt [1]; Susanne Rickling [1]; Martin Runge [1]; Anne Pohlmann [5]

[1] *Institute for Food Safety and Veterinary Public Health Braunschweig/Hannover, Lower Saxony State Office for Consumer Protection and Food Safety, Hannover, Germany*

[2] *3 Animal Disease Control and Task-Force Veterinary Services, Lower Saxony State Office for Consumer Protection and Food Safety, Oldenburg, Germany*

[3] *Animal Disease Control and Task-Force Veterinary Services, Lower Saxony State Office for Consumer Protection and Food Safety, Oldenburg, Germany*

[4] *Institute for Food Safety and Veterinary Public Health Oldenburg, Lower Saxony State Office for Consumer Protection and Food Safety, Oldenburg, Germany*

[5] *Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald, Germany.*

**Corresponding Author:** christine.baechlein@laves.niedersachsen.de

In 2025, HPAIV H5N1 led to massive losses among wild birds and poultry. Cranes were particularly affected, and in Lower Saxony, roughly 1.5 million poultry were lost due to HPAI. The aim of this study was to investigate the genetic relationships between H5N1 viruses detected in wild birds and those circulating in poultry. Illumina whole genome sequencing generated complete viral sequences from 60 wild birds and 85 poultry flocks.
All viral sequences were assigned to the H5N1 euDI.2.1 genotype. Notably, H5N1 viruses recovered from cranes formed a distinct monophyletic group, regardless of the geographic location of sampling. In contrast, no comparable species-specific clustering was observed among other wild bird species. Instead, phylogenetic analyses suggested a regional rather than host-associated clustering pattern. The same applied to poultry-derived viruses in municipalities with exceptionally high poultry densities.
To further investigate viral dissemination, phylogeographic analyses were conducted for both the crane-associated and other wild bird and poultry-associated clusters. The crane-associated cluster exhibited a broad geographic distribution across northern Germany. In contrast, a poultry-associated cluster was largely confined to the major poultry production area of western Lower Saxony. Reconstruction of viral migration pathways revealed numerous links among geographically proximate poultry holdings, indicating predominantly local transmission dynamics.

**Keywords**:

HPAVI H5N1, wild birds, poultry, genome sequencing, virus transmission pathways

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26- 72

**Poster viewing / 166**

# The expansion of the indication and occupational recommendation for seasonal influenza vaccination in Germany: an evidence review by the Standing Committee on Vaccination (STIKO)

**Authors:** Johanna Schlaberg[1]; Robin Schaefer[1]

**Co-authors:** Stefan Brockmann [2]; Ralf Dürrwald [3]; Thomas Harder [1]; Anja Kwetkat [2]; Beate Müller [2]; Marianne Röbl-Mathieu [2]; Julia Tabatabai [2]; Berit Lange [2]

[1] *Robert Koch Institute, Department 3 infectious disease epidemiology, unit 33: immunization/STIKO*

[2] *Member of Standing Committee on Vaccination*

[3] *Robert Koch Institute, Department 1 infectious diseases, unit 17: influenza viruses and other respiratory tract viruses*

**Corresponding Author:** schlabergj@rki.de

Background
Seasonal, porcine and avian influenza A viruses are endemic in many species and countries. Due to the potential of influenza viruses to exchange whole genome sequences, new influenza virus variants arise when animals or humans are at the same time infected with two different strains. New reassorted virus strains could have pandemic potential.

Methods
Following its standard procedure, STIKO reviewed evidence on disease burden and risk of co-infections of influenza viruses in humans. A nationwide survey was conducted across all 16 federal states of Germany on how many people in an occupational or private setting are exposed to animals (livestock, wild animals) which could be infected with avian or porcine influenza.

Results
The results from the survey showed that approximately 850,000 people occupied in companies working with poultry, swine or wild birds are potentially exposed to avian or porcine influenza virus. In Germany, between 2007 and 2020 six infections with porcine influenza have been described, none with avian influenza. In 2023/2024 16 spillover infections of the A(H5Nx)-variant have been recorded in mammals in Germany (e.g. foxes, racoons or seals).

Conclusion
To reduce risks of new reassorted influenza virus strains, STIKO decided in 2025 to extend its recommendation for seasonal influenza vaccination to people with prolonged and repeated private or occupational contact with animals potentially infected with avian or porcine influenza.

**Keywords**:

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-115

**Poster viewing / 100**

# A high-throughput high-content imaging screening platform for the identification of novel influenza virus inhibitors

**Author:** Stijn Verschoren[1]

**Co-authors:** Bert Vanmechelen [1]; Nelleke Cloet [2]; Thibault Francken [3]; Erik Nijs [1]; Benjamin Van Loy [1]; Rana Abdelnabi [1]; Johan Neyts [3]; Koert Stittelaar [1]

[1] *The VirusBank Platform, Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium*

[2] *CISTIM Leuven vzw, Leuven, Belgium*

[3] *Virology, Antiviral Drug & Vaccine Research Group, Department of Microbiology, Immunology and Transplantation, Rega Institute, KU Leuven, Leuven, Belgium*

**Corresponding Author:** stijn.verschoren@kuleuven.be

Developing novel influenza antivirals remains an urgent need due to the limitations of current options and the threat of future pandemics. Here we present the design and validation of a robust high-throughput screening (HTS) assay to facilitate the discovery of novel anti-influenza compounds.
We established cell-based assays using A549 and Calu-3 reporter cell lines expressing fluorescent markers (miRFP670 and mScarlet respectively). Using high-content imaging (HCI), this platform enables real-time monitoring of virus-induced cytopathic effect (CPE) and compound-associated cytotoxicity. The assays were optimized and scaled to both 96- and 384-well formats.
Both reporter cell lines were highly susceptible to influenza A and B virus infection, as indicated by a pronounced loss of fluorescent signal upon cell death. HCI facilitated precise optimization of key parameters, including cell density, viral input, and infection kinetics.
The assay demonstrated high robustness across multiple screening runs, achieving a Z'-factor > 0.5. Validation with reference antivirals, such as baloxavir, confirmed its suitability for large-scale compound screening. Notably, while the A549-based assay failed to detect antiviral activity of neuraminidase inhibitors, the Calu-3-based assay successfully captured this class of antivirals.
Together, this platform provides a robust and scalable approach for the identification of novel influenza antivirals, supporting future antiviral discovery efforts.

**Keywords**:

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-41

**Poster viewing / 104**

## FineInfect –Particulate Matter in Focus: Effects on molecular mechanisms during respiratory infections

**Author:** Christina Ehrhardt[1]

**Co-authors:** Johannes Jungwirth [1]; Sarah König [1]

[1] *Section of Experimental Virology, Institute of Medical Microbiology, Jena University Hospital*

**Corresponding Authors:** johannes.jungwirth@med.uni-jena.de, sarah.koenig2@med.uni-jena.de, christina.ehrhardt@med.uni-jena.de

Air pollution caused by fine particulate (PM) matter from industry, traffic, and the burning of fossil fuels poses a significant global health risk, particularly for immunocompromised individuals. Infections with influenza A viruses (IAVs) and/or the fungus *Aspergillus fumigatus* can cause severe, often

life-threatening pulmonary diseases. General research findings suggest that air pollution could further exacerbate the spread and severity of such infections. However, the impact of exposure to PM on the interplay between pathogens and the immune system remains poorly understood.
The aim of the project is to establish simple and complex *in vitro* cell culture models as well as an *ex vivo* mouse lung model to investigate the effects of environmental factors on pathogen-host interactions. The project focus on the impact of standardized, commercially available fine dust particles of different sizes on IAV infection and IAV-associated co-infections with *A. fumigatus*.
Here, we present initial results from infection experiments in various cell culture systems. While inhibition of IAV-induced cellular mechanisms, such as interferon-beta-mediated signaling was frequently observed, independent of the PM source and the cell culture model examined, effects on viral replication were dependent on the cell type and timing of PM incubation. Furthermore, we will provide first evidence of PM-mediated effects on cell cycle progression and the consequences for infection.

**Keywords**:

particulate matter, influenza A virus infection, pathogen-host interaction

**Professional status of the speaker**:

Professor

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-5

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# Zoonotic Risk Assessment of 2.3.4.4b H5Nx Avian Influenza-A Viruses Reveals Intermediate Host Systems as Key Determinants of Productive Replication in Primary Human Lung Tissues

**Authors:** Sriram Kumar[1]; Duygu Merve Çalışkan[2]; Darisuran Anhlan[2]; Anmari Christersson[2]; Saskia Hinse[1]; Pia Madeleine Leipe[2]; Susanne Köthe[3]; Vera Krüger[4]; Stefan Fischer[4]; Stephan Ludwig[2]; Timm Harder[3]; Martin Beer[3]; Linda Brunotte[1]

[1] *Immunology of Respiratory Tract Infections, University Hospital Gießen and Marburg, Germany*

[2] *Institute of Virology, University Hospital Münster, University of Münster, Germany*

[3] *Institute of Diagnostic Virology, Friedrich Loeffler Institute, Greifswald, Germany*

[4] *Department of Thoracic Surgery and Lung Support, Ibbenbüren Hospital, Germany*

**Corresponding Author:** sriram.kumar@uni-muenster.de

Clade 2.3.4.4b H5Nx highly pathogenic avian influenza viruses (HPAIVs) have diversified extensively during global spread, yet the determinants of their zoonotic potential remain incompletely understood. We characterized eleven genetically distinct H5Nx isolates collected in Germany between 2016 and 2021 using comparative genomics and physiologically relevant human respiratory models. Despite representing multiple reassortant lineages with some substitutions linked to mammalian fitness, none encoded canonical mammalian-adaptive markers PB2-E627K, PB2-D701N, or PB2-Q591K. All isolates replicated efficiently in avian and mammalian cell lines regardless of propagation history. In contrast, primary human lung explants and differentiated nasal epithelial air-liquid interface cultures revealed a propagation-dependent phenotype: Egg-derived viruses exhibited no infectivity, while the same isolates propagated in MDCK cells established productive infection in both upper and lower respiratory tract models. Repropagation in eggs restored the restricted phenotype without adaptive mutations, implying reversible host-dependent factors, potentially including glycosylation-related changes. All viruses exhibited susceptibility to oseltamivir. These findings identify propagation host as a key determinant of 2.3.4.4b H5Nx infectivity in human respiratory tract, highlighting

the value of primary human respiratory models for improving zoonotic risk assessment beyond genomic surveillance alone.

**Keywords**:

H5Nx Viruses, Zoonotic Potential, Avian Influenza, Human Lung, ex vivo Infection

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

Inf26-0027

**Poster viewing / 109**

# Advancing influenza virus treatment: in vitro and ex vivo studies of PI3K inhibitor-loaded lipid nanoparticles

**Author:** Josefine Schroeder[None]

**Co-authors:** Jana Ismail [1]; Caroline T. Holick [1]; Johannes Jungwirth [2]; Laura Klement [3]; Stephanie Hoeppener [1]; Christian Kosan [4]; Michaela Schmidtke [2]; Bettina Löffler [5]; Christine Weber [1]; Ulrich S. Schubert [1]; Carsten Hoffmann [3]; Stephanie Schubert [1]; Christina Ehrhardt [2]

[1] *Laboratory of Organic and Macromolecular Chemistry (IOMC), Friedrich Schiller University Jena, Jena, Germany*

[2] *Section of Experimental Virology, Institute of Medical Microbiology, Jena University Hospital, Jena, Germany*

[3] *Institute of Molecular Cell Biology, Center for Molecular Biomedicine, Jena University Hospital, Jena, Germany*

[4] *Department of Biochemistry, Center for Molecular Biomedicine (CMB), Friedrich-Schiller-University Jena, Jena, Germany*

[5] *Institute of Medical Microbiology, Jena University Hospital, Jena, Germany*

**Corresponding Author:** josephine.schroeder@med.uni-jena.de

Influenza A viruses (IAVs) continue to pose a major global health threat. Annual vaccination provides only limited protection against emerging subtypes, and rising resistance to approved antivirals highlights the need for alternative therapeutic strategies. Host-directed therapies, such as targeting phosphatidylinositol 3-kinases (PI3Ks) exploited by IAVs during replication, offer promising alternatives. At the same time, advances in nanotechnology have established lipid nanoparticles (LNPs) as effective carriers that enhance drug stability, bioavailability, and targeted delivery. This study explores the combination of the PI3K inhibitor pictilisib with LNPs as a potential antiviral strategy. To improve circulation and distribution, LNPs commonly include "stealth"polymers. Here, established polyethylene glycol (PEG) Lipids were compared to poly(2 oxazoline) (POx) Lipids. PEG is widely used but associated with immunogenicity, whereas POx offers similar shielding with lower risk of hypersensitivity. Using both systems, pictilisib was efficiently encapsulated and retained antiviral and anti inflammatory activity comparable to the free drug in vitro. Notably, ex vivo experiments showed that POx-LNPs loaded with pictilisib were more effective than free pictilisib, indicating improved stability and local availability. These findings suggest that PI3K targeted LNP formulations may provide a promising approach for further development of future IAV intervention strategies.

**Keywords**:

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

53

**Poster viewing / 148**

## Signaling networks in influenza virus assembly and budding

**Author:** Juliane Mayr[None]

**Co-authors:** Matthias Weiler [1]; Darisuren Anhlan [1]; Carmen Musholt [1]; Fabian Herrmann [2]; Alexander Mellmann [3]; Petr Chlanda [4]; Stephan Ludwig [1]; Yvonne Börgeling [1]

[1] *Institute of Virology, University of Münster*

[2] *Institute of Pharmaceutical Biology and Phytochemistry*

[3] *nstitute of Hygiene, University Hospital Münster*

[4] *BioQuant Center for Quantitative Biology, Heidelberg University*

**Corresponding Author:** mayrj@uni-muenster.de

Assembly and budding of influenza A virions is a tightly regulated process orchestrated by matrix protein M1. We showed that phosphorylation of M1 at Y132 drives efficient virus assembly and budding. Here, we identify signaling pathways involved in M1-induced assembly and budding and aim to pin-point signaling networks decisive for virion morphology. M1 phospho-site mutants were generated via reverse genetics. Virus budding phenotypes and morphological changes were evaluated using atomic force microscopy (AFM) and cryo-electron tomography (cryo-ET), respectively. To examine host cell signaling, kinase activity profiling of cells infected with M1 mutant viruses or wild type strains of spherical, filamentous or mixed morphology was performed using PamGene technology. M1 mutant virions changed to a more filamentous morphology or retained a predominant spherical shape with slight differences in size. Kinome activity analysis revealed a strong activation of S/T kinases by filamentous and viruses of mixed morphology during budding that was not observed with spherical strains and cannot be attributed to virus replication efficiencies. Overall, activity of Y kinases was increasingly reduced during late stages of budding independent of virion morphology. However, single M1 mutations were sufficient to induce very low if any activity changes of Y kinases compared to uninfected cells. Our data indicate that M1 possibly determines virion morphology via orchestrating S/T kinase signaling.

**Keywords**:

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-99

**Poster viewing / 160**

# Nascent transcript sequencing reveals cap-snatching specificity of influenza A virus

**Authors:** Jane Sharps[None]; Ervin Fodor[None]; Nicholas Proudfoot[None]

**Co-authors:** Inna Zukher ; Maud Dupont [1]; Rui Sousa Luis

[1] *Sir William Dunn School of Pathology*

**Corresponding Author:** maud.dupont@path.ox.ac.uk

Influenza A virus mRNAs are synthesised in the nucleus by the viral RNA-dependent RNA polymerase (FluPol). To initiate viral transcription, FluPol binds host RNA polymerase II (RNAPII). The endonuclease subunit of FluPol then cleaves nascent transcript, producing a short capped RNA fragment, which primes viral mRNA synthesis. Previous studies suggested this cap-snatching process is largely dependent on transcript level, though others reported gene-specificity. However, these studies were based on sequencing Flu mRNAs and thus limited by small 12 nt primers, which are hard to uniquely map to the human genome.

Here, we present a complimentary approach of identifying the downstream portion of the host cap-snatched transcripts using polymerase intact nascent transcript (POINT) technology. We use native immunoprecipitation of RNAPII elongation complexes to isolate associated nascent RNAs and prepare 5′-end specific libraries, with reads long enough for unique mapping.

This approach allowed us to identify cap-snatching events directly from the 5′ ends of RNAPII-associated transcripts. We report a previously overlooked preference for variant spliceosomal U1 snRNAs and avoidance of the more abundant U1 snRNAs. These findings suggest that in addition to transcript abundance, other factors are regulating influenza A virus co-transcriptional cap-snatching and potentially support a model in which FluPol exhibits a higher degree of substrate specificity than previously appreciated.

**Keywords**:

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-105

**Poster viewing / 165**

# Clade 2.3.4.4b H5N1 influenza A virus exhibits high infectivity in human respiratory tract models

**Authors:** Anna Löwa[1]; Matthias Budt[2]

**Co-authors:** Anita Balázs [3]; Victor Sikora [1]; Mark Melzer [4]; Annika E Pfeiffer [2]; Mara Fischer [1]; Maren Mieth [1]; Katharina Hellwig [1]; Emanuel Wyler [5]; Mario Tönnies [6]; Torsten T Bauer [6]; Mareike Graff [7]; Jens Neudecker [8]; Jens C Rückert [8]; Achim D Gruber [9]; Markus Landthaler [5]; Lisa Buchauer [1]; Stefan Hippenstiel [10]; Thorsten Wolff [11]; Andreas C Hocke [1]

[1] *Department of Infectious Diseases, Respiratory Medicine and Critical Care, Charité - Universitätsmedizin Berlin, Berlin, Germany*

[2] *Unit 17 "Influenza and other Respiratory Viruses", Robert Koch Institute, Berlin, Germany.*

[3] *Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine, Charité –Universitätsmedizin Berlin, Berlin, Germany*

[4] *Department of Infectious Diseases, Respiratory Medicine and Critical Care, Charité - Universitätsmedizin Berlin, Berlin, Germany.*

[5] *Berlin Institute for Medical Systems Biology (BIMSB), Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC) and IRI Life Sciences, Institute for Biology, Humboldt Universität zu Berlin, Berlin, Germany*

[6] *HELIOS Clinic Emil von Behring, Department of Pneumology and Department of Thoracic Surgery, Chest Hospital Heckeshorn, Berlin, Germany*

[7] *Department for Thoracic Surgery, DRK Clinics, Berlin, Germany*

[8] *Department of General, Visceral, Vascular and Thoracic Surgery, Charité - Universitätsmedizin Berlin*

[9] *Institute of Veterinary Pathology, Freie Universität Berlin, Berlin, Germany*

[10] *Department Infectious Diseases and Respiratory Medicine; Charite - Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany*

[11] *Unit 17 "Influenza and other Respiratory Viruses", Robert Koch Institute, Berlin, Germany*

**Corresponding Authors:** stefan.hippenstiel@charite.de, m.graff@drk-kliniken-berlin.de, mara.fischer@charite.de, torsten.bauer@helios-gesundheit.de, anita.balazs@charite.de, emanuel.wyler@mdc-berlin.de, katharina.hellwig@charite.de, andreas.hocke@charite.de, lisa.buchauer@charite.de, maren.mieth@charite.de, jens.neudecker@charite.de, mark.melzer@charite.de, jens-c.rueckert@charite.de, anna.loewa@charite.de, pfeiffera@rki.de, achim.gruber@fu-berlin.de, victor.sikora@bih-charite.de, wolfft@rki.de, markus.landthaler@mdc-berlin.de, mario.toennies@helios-gesundheit.de, budtm@rki.de

**Introduction**: Bovine-transmitted clade 2.3.4.4b H5N1 viruses have raised concerns about mammalian adaptation and pandemic potential. Primary human respiratory models enable direct assessment of viral replication and host responses. **Goal**: To characterize replication, tropism and pathogenicity of a bovine clade 2.3.4.4b H5N1 virus in human respiratory models. **Materials & Methods**: Primary nasal air-liquid interface cultures (nALI), organoid-derived bronchial epithelium and ex vivo human lung tissue (aHuLu) were infected with H5N1bov/2024 and compared with H5N1/2004, H3N2/1999 and H5N8/2016. Viral replication, tropism, epithelial injury and host responses were analysed. **Results**: H5N1bov/2024 replicated efficiently in all models, comparable to H5N1/2004. Bronchial cultures produced viral titres ~2 $\log_{10}$ higher than nasal and alveolar tissues. In ex vivo lung tissue, infection predominantly targeted alveolar type II cells. At 72 h post infection, infected ATII cells detached, accompanied by loss of occludin and increased caspase-3 activation. RNA sequencing demonstrated a transcriptional response resembling H5N1/2004, with strong induction of interferons, ISGs and inflammatory chemokines. **Summary**: H5N1bov/2024 efficiently infects human respiratory tissues, induces robust innate immune responses and causes epithelial injury. These findings support the use of complementary human respiratory models for influenza pathogenesis studies and pandemic risk assessment.

**Keywords**:

H5N1; human 3D models; pandemic potential.

**Professional status of the speaker**:

Professor

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-113

**Poster viewing / 167**

## A comprehensive evidence review to inform a potential recommendation on the use of human influenza A(H5) vaccines in Germany

**Authors:** Mona Askar[1]; Wiebe Külper-Schiek[1]

**Co-authors:** Johanna Schlaberg [1]; Martin Beer [2]; Stefan Brockmann [3]; Ralf Dürrwald [4]; Timm Harder [2]; Anja Kwetkat [3]; Beate-Sigrid Müller [3]; Marianne Röbl-Mathieu [3]; Julia Tabatabai [3]; Robin Schäfer [1]; Berit Lange [3]

[1] *Robert Koch Institute - Unit 33*

[2] *Friedrich Löffler Institute*

[3] *STIKO member*

[4] *Robert Koch Institute - Unit 17*

**Corresponding Authors:** kuelperw@rki.de, askarm@rki.de

Background
Unprecedented avian influenza A(H5) activity occurred among wild birds, poultry and some wild mammals across Europe in 2025/26. Given pandemic risks from zoonotic infections, the German Standing Committee on Vaccination (STIKO) reviewed evidence to inform possible national use of A(H5) vaccines.
Methods
Following its standard procedure, STIKO reviewed surveillance data on disease burden and conducted a systematic review (SR) on safety/efficacy of EU-approved A(H5) vaccines. Experiences from countries with existing programmes informed acceptability and implementation considerations.
Results
In 2025/26, >240 A(H5)-outbreaks in poultry and >3,100 -detections in wild birds were reported in Germany. 110 human infections were confirmed globally in occupationally exposed individuals in 2024/25, none in Germany or the EU. Three human A(H5) vaccines are licensed in the EU. The SR screened 2,085 records and included one study showing 54-80% seroconversion after two doses of CELLDEMIC, with mild-to-moderate transient reactions. Vaccine uptake in Finland and Canada was challenged by low-risk perception and mistrust.
Conclusions
Substantial A(H5) activity in Europe, and possible (severe) human infections and viral adaptation with pandemic potential support the relevance of a recommendation for at-risk groups. The evidence-review provided valuable insights for assessment of emerging pathogens and implementation of potential recommendations for pandemic preparedness in Germany.

**Keywords**:

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-116

**Poster viewing / 140**

# Reviewing Mechanism of Action of MF59-Adjuvanted Influenza Vaccine for Enhancing Innate and Adaptive Immune Responses

**Authors:** Ashraf[None]; Chang[None]; Ciglia[None]; Sara Baniameri[None]; Elfassy[None]; Fortanier[None]; Music[None]

**Corresponding Author:** sara.baniameri@seqirus.com

Enhanced influenza vaccines, including MF59-adjuvanted formulations, help to overcome immunosenescence by improving immunogenicity compared to standard formulations. MF59 (an oil-in-water

emulsion adjuvant) orchestrates both innate and adaptive immune functions, thereby amplifying, broadening, and prolonging immune responses. This work describes the pathways by which MF59-adjuvanted vaccines enhance immune responses. A framework of the mechanism of action of MF59 has been developed based on established immunology literature. Mechanisms were described using evidence from preclinical and clinical studies. MF59 promotes the recruitment of monocytes, macrophages, and granulocytes at the injection site, and stimulates the production of chemokines and inflammatory cytokines. This transient immune-competent microenvironment promotes antigen uptake, supports the differentiation of APC precursors toward DCs, enhances germinal center activity, and drives broader T- and B-cell activation, including increased memory B-cell generation, improved antibody quality and diversity for both HA and NA, and, in some settings, cross-reactivity to drifted strains. MF59-adjuvanted influenza vaccine targets various components of the immune system to enhance immune responses and overcome immunosenescence. MF59-adjuvanted vaccines provide multifaceted orchestration of innate and adaptive immunity and support improved and more durable protection against influenza in older adults.

**Keywords**:

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26- 50

**Session 3: Viral Pathogenesis** / **97**

# Emerging H5N5 clade 2.3.4.4b viruses with enhanced fitness in birds and mammals

**Authors:** Maryna Kuryshko[1]; Elsayed Abdelwhab[None]

[1] *Friedrich-Löffler-Institut Bundesforschungsinstitut für Tiergesundheit*

**Corresponding Authors:** maryna.kuryshko@fli.de, el-sayedmohammed.abdel-whab@fli.de

H5N5 HPAIV clade 2.3.4.4b have increased in prevalence across Eurasia and North America and were associated with human infection in the US. Their evolution and zoonotic potential remain insufficiently characterised.
We analysed global H5N5 prevalence (2000–2026) and assessed 4 representative viruses in chickens and ducks. Zoonotic potential was evaluated in airway epithelial (ALI) cultures and ferrets. NA activity, replication, antigenicity, and antiviral susceptibility were compared.
H5N5 viruses show intercontinental spread with distinct Eurasian and North American lineages and multiple introductions into Canada and Japan. Recent strains after 2023 form a cluster with NA stalk deletions and mammalian markers. These viruses exhibit significantly increased NA activity since 2020, while remaining susceptible to NA inhibitors.
Recent isolates were more virulent and transmissible, with higher replication efficiency in chickens and ducks than earlier H5N5 viruses. In ferrets, they caused severe systemic disease with neurological signs and weight loss. Replication was observed in ALI cultures.
Our findings demonstrate H5N5 clade 2.3.4.4B HPAIV acquired enhanced NA activity, expanded host range, and capacity for severe disease in mammals. Increasing prevalence in wild birds, poultry, and mammals, combined with intercontinental spread and preserved antiviral susceptibility, supports inclusion in pandemic preparedness. Recent H5N5 mammalian potential warrants urgent risk assessment.

**Keywords**:

H5N5, clade 2.3.4.4B, highly pathogenic avian influenza, ferret model, zoonotic risk, neuraminidase activity, intercontinental spread

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26- 38

**Session 3: Viral Pathogenesis / 119**

## Microbiota dysbiosis in aging weakens gut–lung axis defense against influenza A virus

**Author:** Christoph Wernike[1]

**Co-authors:** Antje Häder ; Yasmina Reisser [1]; Kiana Garakani [2]; Franziska Hornung [1]; Bettina Löffler [1]; Claude Jourdan Le Saux [2]; Stefanie Deinhardt-Emmer [1]

[1] *Jena University Hospital*

[2] *University of California San Francisco*

**Corresponding Author:** antje.haeder@med.uni-jena.de

Aging alters the intestinal microbiota, reducing diversity, metabolic capacity, and antiviral defense along the gut–lung axis. This study investigated whether aging-associated microbial dysbiosis affects susceptibility to influenza A virus (IAV) and whether short-chain fatty acid (SCFA) supplementation can restore antiviral responses during cellular senescence.
Using young and aged mice, we analyzed microbiota composition and metabolic function before and after IAV infection. Shotgun metagenomics, RNAseq, flow cytometry, and plaque assays were used to assess microbial, transcriptomic, cytokine, and viral changes. *In vitro*, senescent primary human lung fibroblasts were infected with IAV and treated with acetate.
Aging caused microbial shifts, including reduced *Akkermansia muciniphila* and *Faecalibaculum rodentium*, reduced carbohydrate-fermenting enzymes, and impaired SCFA synthesis. IAV infection further worsened these changes. Senescent lung fibroblasts were more susceptible to IAV infection, while acetate reduced viral replication and inflammatory cytokine expression via GPR41/43 activation and histone acetylation.
Our findings show that aging-associated microbial dysbiosis weakens antiviral defense, whereas acetate restores responses in senescent cells.

**Keywords**:

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26- 67

**Session 3: Viral Pathogenesis** / **156**

# Exploring the role of Klebsiella pneumoniae and bacteria-derived outer membrane vesicles (OMVs) in viral pneumonia using primary human lung models

**Author:** Saskia Sophie Hinse[1]

**Co-authors:** Yannick Teschke [2]; Lukas Skerbs [3]; Christian Rüter [2]; Andreas Margraf [4]; Klaus Schughart [3]; Marcel Trautmann [5]; Vera Krüger [6]; Stefan Fischer [6]; Rainer Wiewrodt [6]; Stephan Ludwig [3]; Petra Dersch [2]; Linda Brunotte [1]

[1] *Department of Medicine V: Immunology of Respiratory Tract Infections Gießen*

[2] *Institute of Infectiology Münster*

[3] *Institute of Virology Münster*

[4] *Department of Anesthesiology Münster*

[5] *Gerhard-Domagk-Institute of Pathology Münster*

[6] *Clinic for Thoracic Surgery and Lung Support Ibbenbüren*

**Corresponding Author:** saskia.hinse@uni-giessen.de

Bacterial co-infections are a common complication in respiratory viral infections. ICU patients with influenza and COVID-19 frequently develop co-infections with Gram-negative bacteria, particularly *K. pneumoniae*. Beyond direct bacterial infection, host lung tissue is exposed to extracellular outer membrane vesicles (OMVs), which modulate pulmonary immune responses. Using native distal lung explants, alveolar type 2 (AT-2) organoids, and isolated alveolar macrophages, we show that both bacteria and OMVs elicit distinct immune responses in human lung tissue. While live bacteria induce a rapid pro-inflammatory cytokine response already within 1 hour post infection, accompanied by an early type I IFN response, OMVs triggered a delayed but sustained immune response. Transcriptomic profiling revealed that OMVs alone promoted an IFN-dominated, antiviral intracellular response, while simultaneously suppressing epithelial defense and regenerative pathways and attenuating neutrophil recruitment. Analysis of individual cell systems identified macrophages as the major drivers of the inflammatory response. Together, these findings demonstrate that *K. pneumoniae* OMVs alone are sufficient to reshape immune responses in human lung tissue and establish an antiviral-like transcriptional state, highlighting their potential role as modulators of pulmonary immunity that may influence host responses during bacterial–viral co-infections.

**Keywords**:

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-102

**Session 3: Viral Pathogenesis** / **125**

# The Eye as a Gateway: Ocular Tropism, Host Responses and Transmission of Clade 2.3.4.4b H5 Viruses

**Authors:** Juliane Lang[1]; Maryna Kuryshko[1]; Marta Czechowicz[2]; Annett Petrich[1]; Jules Spooren[1]; Juliette Gross[1]; Ahmed M. Elsayed[3]; Luis Martinez-Sobrido[3]; Reiner Ulrich[2]; Axel Karger[1]; Elsayed M. Abdelwhab[4]

[1] *Friedrich Loeffler Institute, Institute of Molecular Virology and Cell Biology (IMVZ)*

[2] *Institute of Veterinary Pathology, Faculty of Veterinary Medicine, Leipzig University*

[3] *Texas Biomedical Research Institute, San Antonio, TX 78227, USA*

[4] *Friedrich-Loeffler-Institut*

**Corresponding Authors:** axel.karger@fli.de, lmartinez@txbiomed.org, aelsayed@txbiomed.org, jules.spooren@fli.de, juliette.gross@fli.de, marta.czechowicz@vetmed.uni-leipzig.de, reiner.ulrich@vetmed.uni-leipzig.de, sayed.abdelwhab@fli.de, annett.petrich@fli.de, maryna.kuryshko@fli.de, juliane.lang@fli.de

Human infections with U.S. clade 2.3.4.4b H5N1 viruses frequently present with conjunctivitis, suggesting the eye as an important portal of infection. We compared the ocular tropism of U.S. H5N1 (B3.13 and D1.1) and European H5N1/H5N5 viruses in human conjunctival epithelial and corneal endothelial cells. All viruses infected human ocular cells, but the U.S. human H5N1 isolates displayed markedly enhanced replication. European viruses differed substantially in ocular fitness, with one H5N5 isolate exhibiting similarly high replication. Using reverse genetics approach, we identified mutations in PB2, PB1, PA, NA and NS1 that contribute to ocular tropism. Integrated proteomic, cytokine, flow cytometric and imaging analyses revealed distinct host responses induced by human H5N1 B3.13, including differences in innate immune signaling, cytokine production and apoptosis. Following ocular inoculation, U.S. H5N1 viruses established productive infection in ferrets with dissemination to the respiratory tract and brain and efficient direct contact, but not airborne transmission. Viruses recovered from infected ferrets remained genetically stable without reversion of mammalian adaptation-associated signatures. These findings identify viral and host determinants of ocular H5N1 infection and demonstrate that the eye is a functional route of H5N1 infection and direct transmission, with implications for zoonotic risk assessment and infection control.

**Keywords**:

H5N1 clade 2.3.4.4b, Ocular tropism, Zoonotic transmission, Host response, Ferret model

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

Inf26-0054

**Session 3: Viral Pathogenesis / 94**

## The Respiratory Mucus Barrier: Friend or Foe in Zoonotic Influenza Transmission?

**Author:** Maximilian Rohde[1]

**Co-authors:** Fiona Riegel [1]; Cosmin Stefan Butnarasu [2]; Daniel Lauster [3]; Christian Sieben [1]

[1] *Helmholtz Center for Infection Research*

[2] *University of Turin*

[3] *Freie Universität Berlin*

**Corresponding Authors:** maximilian.rohde@helmholtz-hzi.de, christian.sieben@helmholtz-hzi.de

The ongoing spread of a highly pathogenic avian influenza virus (H5N1) in wild birds and poultry has increased global concern regarding the potential for zoonotic and pandemic spread of emerging influenza A viruses (IAV). Before viruses can infect epithelial cells, they must overcome the respiratory mucus barrier. Mucus in the airways can trap particles and promote their removal via

mucociliary clearance. Beyond this physical barrier function, mucins can interact with viral surface proteins, influencing virus mobility, attachment, and infectivity. To study how respiratory mucus influences IAVs, we have collected native respiratory mucus from relevant host species. We have developed standardized biophysical and biochemical methods to quantify the penetration of IAVs through species-specific mucus. This allows us to analyze the penetration behavior and subsequent infection potential of multiple IAV strains. The findings indicate that mucus exerts a differential effect on the penetration efficiency and infectivity of avian- and human-origin influenza strains. These observations suggest that respiratory mucus plays a multifaceted role. Our research revealed that virus-mucus interactions enhance the efficacy of virus infection by potentially protecting against viral degradation. Research into the interaction between respiratory viruses and native mucus samples is crucial to understand how the mucus barrier influences the emergence and transmission of influenza viruses.

**Keywords**:

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-4

**Session 4: Epidemiology and emerging viruses** / **172**

## Keynote: An experimental framework to benchmark MxA resistance in emerging influenza A viruses

**Session 4: Epidemiology and emerging viruses** / **99**

## The 3W Strategy for Avian Influenza Surveillance in Mallards: Water, Wheat & Wait –Optimization through Virus Recovery Methods

**Author:** Joaquin Neumann-Heise[1]

**Co-authors:** Chiara Holzer [2]; Albert Perlas [3]; Lara Urban [4]; Timm Harder [5]; Francesca Isabel Rondi [6]; Sascha Knauf [7]; Anja Globig [1]

[1] *Institute of International Animal Health/One Health, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany*

[2] *Department of Water Microbiology, DVGW-Technologiezentrum Wasser, Karlsruhe, Germany*

[3] *Helmholtz AI, Helmholtz Zentrum München, Neuherberg, Germany; Helmholtz Pioneer Campus, Helmholtz Zentrum München, Neuherberg, Germany*

[4] *Helmholtz AI, Helmholtz Zentrum München, Neuherberg, Germany; Helmholtz Pioneer Campus, Helmholtz Zentrum München, Neuherberg, Germany; School of Life Sciences, Technical University of Munich, Freising, Germany; Institute for Food Safety and Hygiene, University of Zurich, Zürich, Switzerland*

[5] *Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany*

[6] *Scienze Biotecnologiche Veterinarie, Università degli Studi di Milano, Milano, Italy*

[7] *Institute of International Animal Health/One Health, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany; Faculty of Veterinary Medicine, Justus Liebig University, Giessen, Germany*

**Corresponding Author:** joaquin.neumannheise@fli.de

Low Pathogenicity Avian Influenza (LPAI) viruses may serve as precursors and reassortment partners of High Pathogenicity Avian Influenza (HPAI) viruses, highlighting the importance of LPAI surveillance.

We developed a non-invasive "3W"approach, in which mallards (Anas platyrhynchos) are attracted to baited water bins, allowing collection of water samples potentially containing avian influenza viruses (AIV).

Between June 2022 and December 2023, 243 Virocult® swab samples collected from feed-baited bins in the Greifswalder Bodden area (Germany) were analyzed by generic and subtype-specific Influenza A RT-qPCR. Twelve samples (5%) tested positive, yielding subtypes H3N8, H11N9 and H5Nx. However, viral loads were insufficient for sequencing or virus isolation.

To improve sensitivity and increase virus loads for diagnosis, five virus recovery methods were compared experimentally using series of each 40 mL H4N6-spiked ($10^2$, 103, $10^4$ $TCID_{50}$) tap and Bodden water samples, with and without wheat to mimic field sampling conditions. Across all conditions, activated carbon/polyethylene glycol precipitation (ACPEG) and electronegative membrane adsorption (EM) yielded the lowest Ct values, whereas simple swab samples performed worst.

These findings demonstrate the potential of the 3W strategy to non-invasively detect AIV from wild waterfowl in water samples. Enrichment methods, in particular ACPEG and EM, may improve detection sensitivity and facilitate virus characterization.

**Keywords**:

Avian influenza virus; environmental surveillance; mallard (Anas platyrhynchos); virus concentration methods

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

Inf26-0040

**Session 4: Epidemiology and emerging viruses** / **133**

## HPAI passive serological surveillance in wild birds within the OH4Surveillance project

**Author:** Nele Sierens[None]

**Corresponding Author:** nele.sierens@sciensano.be

One Health for Surveillance (OH4Surveillance) is a three-year EU4Health-funded initiative aimed at strengthening zoonotic disease surveillance through a One Health approach. Since 2024, Belgium has participated in a consortium of 11 countries coordinated by Denmark.
This study integrated serosurveillance into the monitoring of highly pathogenic avian influenza (HPAI) by serologically testing wild birds submitted between July 2024 and March 2026 within the EU-mandated routine passive virological surveillance program. The objective was to identify silent viral circulation and past infections. As blood collection is often not feasible in deceased birds, spleen tissue was evaluated as an alternative serological matrix.
Samples were analysed using competitive NP- and H5-ELISAs. ELISA-positive samples were subsequently tested by haemagglutination inhibition (HI) assays.
Of the 128 samples analysed, 69 were ELISA-positive (53.9%), including 30 dual-positive (NP+/H5+),

13 NP-positive only, and 26 H5-positive only. Five ELISA-positive samples lacked sufficient material for HI testing. Among the remaining 64 samples, 31 (48.4%) showed positive H5-specific HI responses.
These findings demonstrate that integrating serosurveillance into routine HPAI surveillance enhances the detection of silent viral circulation and previous infections that would otherwise remain undetected.

**Keywords**:

**Professional status of the speaker**:

Graduate student

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26- 33

**Session 4: Epidemiology and emerging viruses / 158**

# Childhood Immune Imprinting and Seasonal Influenza-Driven Boosting of Cross-Reactive Avian Influenza A Antibody Landscapes in Adults: Evidence from a German Population-Based Longitudinal Cohort (2020-2024)

**Author:** Yaw Awuku-Larbi[1]

**Co-authors:** Manuela Harries [1]; Brit Häcker [1]; Jessica Krepel [1]; Carolina Klett-Tammen [1]; Alex Dulovic [2]; Veronika Jäger [3]; Wencke Stracke [3]; André Karch [3]; RESPINOW Study Consortium ; Berit Lange [1]

[1] *Helmholtz Centre for Infections Research*

[2] *Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen*

[3] *Institute of Epidemiology and Social Medicine, University of Münster*

**Corresponding Authors:** jessica.krepel@helmholtz-hzi.de, andre.karch@ukmuenster.de, berit.lange@helmholtz-hzi.de, brit.haecker@helmholtz-hzi.de, veronika.jaeger@ukmuenster.de, manuela.harries@helmholtz-hzi.de, alex.dulovic@nmi.de, yaw.awuku-larbi@helmholtz-hzi.de, wenckemarie.stracke@ukmuenster.de, carolina.klett-tammen@helmholtz-hzi.de

Avian A(H5N1) in birds/poultry is a pandemic threat, but how imprinting and seasonal immunity shape antibody recognition of avian subtypes is unclear. We assessed whether birth-cohort imprinting was associated with H5N1/H7N9 binding antibodies and whether H1N1/H3N2 infections boosted antibodies within matched HA groups.
We analysed 7,232 sera from 2,871 adults in the population-based MuSPAD cohort (born 1927–2001), sampled in 2020–2024. Binding antibodies to group 1 antigens (H5N1, three H1N1 strains) and group 2 (H7N9, three H3N2 strains) were measured by Luminex. Birth-year imprinting probabilities were estimated from German influenza circulation data. Infection was defined by ≥2 log2 titre rise. Age-adjusted mixed models assessed the H5N1–H7N9 contrast and HA-group-specific boosting.
Higher group 1 imprinting probability was associated with a higher H5N1–H7N9 contrast (β=0.82 log2 BAU; 1.76-fold increase; p<0.001), independent of age, and remained stable over time (ICC=0.85). H1N1 infection increased group 1 titres 3.4-fold versus 1.2-fold for group 2; H3N2 showed the reciprocal pattern (group 2: 3.2-fold; group 1: 1.1-fold; interaction p<0.001). Vaccination increased both groups similarly (~2.3-fold).
Birth-cohort imprinting shapes avian influenza binding-antibody landscapes, while seasonal infection induces HA-group-specific boosting. These patterns may support age-stratified pre-pandemic risk assessment and interpretation of vaccination-induced cross-reactive responses.

**Keywords**:

Influenza A virus; Antibodies, Viral; Immunologic Memory; Seroepidemiologic Studies; Vaccination; Cross reactions

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-81

**Session 5: Virus host cell interaction II / 173**

## Keynote: New insights into infectivity and immunopathology of zoonotic H5Nx viruses in humans

**Keywords**:

**Professional status of the speaker**:

**Junior scientist status**:

**Registration ID**:

**Session 5: Virus host cell interaction II / 90**

## Beyond H5N1: Influenza A virus infection in bovine udder organoids

**Authors:** Prerna Arora[1]; Henrik Fritsch[1]

**Co-authors:** Miaomiao Yan [1]; Inga Grotha [1]; Christiane Pfarrer [2]; Paul Becher [1]

[1] *Institute of Virology, University of Veterinary Medicine Hannover, Hannover, Germany*

[2] *Institute for Anatomy, University of Veterinary Medicine Hannover, Hannover, Germany*

**Corresponding Authors:** henrik.fritsch@tiho-hannover.de, miaomiao.yan1@outlook.com, prerna.arora@tiho-hannover.de, christiane.pfarrer@tiho-hannover.de, paul.becher@tiho-hannover.de, inga.grotha@tiho-hannover.de

Influenza A viruses (IAVs) have recently expanded their host range to cattle, raising critical questions about tissue tropism, replication capacity, and consequences for dairy production and zoonotic risk. The detection of highly pathogenic avian influenza H5N1 in bovine milk highlights the mammary gland as a previously underappreciated site of infection and viral shedding.

To study mammary-specific influenza biology, we established bovine mammary gland organoids (MGOs) derived from primary epithelial cells and maintained in chemically defined media. This scalable ex vivo model recapitulates key features of the bovine udder, including luminal–basal polarity, lineage-specific cytokeratin expression, and lactation-associated differentiation, enabling physiologically relevant infection studies.

Using this system, we compared infection dynamics of avian, swine, and human IAVs. Viruses from diverse host origins productively infected MGOs, largely independent of culture conditions. Mammalian-adapted H1N1 strains replicated with efficiencies comparable to bovine H5N1, whereas low pathogenic avian H9N2 showed restricted replication, indicating subtype-specific constraints in mammary tropism.

Together, this work establishes bovine mammary organoids as a platform to investigate influenza virus–host interactions in the udder and provides insight into determinants of mammary infection with implications for animal health, dairy safety, and zoonotic risk assessment.

**Keywords**:

Influenza A virus, H5N1, bovine mammary gland, organoids, udder, lactation

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-0020

**Session 5: Virus host cell interaction II / 96**

# BTN3A3 resistance of emerging influenza A viruses is shaped by Mx1 of mammalian intermediate host species.

**Author:** Jamina Molsen[1]

**Co-authors:** Katharina Schmitz [1]; Martin Schwemmle [1]; Peter Reuther [1]; Kevin Ciminski [1]

[1] *Institute of Virology, Medical Center University of Freiburg, Freiburg, Germany*

**Corresponding Authors:** martin.schwemmle@uniklinik-freiburg.de, kevin.ciminski@uniklinik-freiburg.de, jamina.molsen@uniklinik-freiburg.de, peter.reuther@uniklinik-freiburg.de, katharina.schmitz@uniklinik-freiburg.de

Zoonotic infections with influenza A viruses (IAVs) of animal-origin are typically self-limiting but can occasionally give rise to pandemics provided human host barriers are overcome. The recently identified human BTN3A3 (butyrophilin subfamily 3 member A3) is a potent restriction factor for zoonotic IAVs. Human-adapted IAVs overcome BTN3A3 restriction through adaptive residues 52 (N, H, Q) or 313 (Y, V) in the viral nucleoprotein (NP). Notably, these residues are associated with resistance to myxovirus resistance 1 (Mx1) proteins from human, swine, and bats. We thus hypothesized that BTN3A3 resistance might emerge as a result of Mx1-mediated selection pressure in intermediate hosts expressing antivirally active Mx1. To test this, we generated reassortant viruses carrying NP of different mammalian-derived IAVs and assessed viral replication in BTN3A3-expressing cells. Combining comparative mutagenesis with polymerase reconstitution assays, we found that NP residues conferring resistance to bat or swine Mx1 proteins also confer BTN3A3 resistance. Because bats and swine lack antivirally active BTN3A3, these adaptive residues emerged under Mx1-mediated selection pressure. Our findings demonstrate that resistance to the human restriction factor BTN3A3 can evolve in Mx1-competent intermediate hosts, providing new insight into the zoonotic potential of animal IAVs and the role of BTN3A3 in limiting zoonotic transmission.

**Keywords**:

BTN3A3, influenza A virus, Mx1, zoonosis, host barriers

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

**21**

**Session 5: Virus host cell interaction II / 146**

# Host Membrane Lipid Composition Regulates Influenza A Virus Attachment and Membrane Fusion

**Author:** Steinar Mannsverk[1]

**Co-authors:** Ana Giraldo Villamil [1]; Peter Kasson [2]

[1] *Uppsala University*

[2] *Uppsala University, Georgia institute of Technology*

**Corresponding Authors:** steinar.mannsverk@icm.uu.se, kasson@gmail.com, ana.villamil@icm.uu.se

Influenza A virus (IAV) initiates infection by binding sialylated glycans on the host cell surface, yet the membrane factors regulating this interaction remain incompletely understood. Here, we investigated how the host membrane lipid composition regulates IAV attachment and fusion using single-virus microscopy in chemically defined membrane systems, complemented by biophysical measurements.

We found that sphingomyelin markedly enhanced IAV attachment to GD1a glycosphingolipid receptors in a cholesterol-dependent manner, identifying a cooperative role for these lipids in promoting high-avidity viral binding. Our data support a model in which sphingomyelin and cholesterol drive nanoscale liquid-liquid phase separation, locally concentrating viral receptors and thereby enhancing multivalent virus-receptor interactions. Interestingly, the same membrane composition delayed viral fusion, suggesting that the lipid environment established during attachment also influences subsequent membrane fusion. Consistent with these findings, cholesterol depletion significantly reduced IAV binding to A549 cells.

Together, our results identify host membrane lipid composition as a key regulator of influenza virus entry and suggest that nanoscale membrane organization enhances viral attachment while modulating downstream fusion. These findings provide new insight into how host membrane architecture contributes to influenza infection and may represent a target for antiviral intervention.

**Keywords**:

viral attachment, membrane fusion, biophysics, single-virus microscopy

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26- 96

**Session 6: Antivirals & vaccines** / **174**

# Keynote: Antivirals against influenza viruses: where do we stand and where do we go?

**Session 6: Antivirals & vaccines** / **136**

# Fragment-based drug discovery against the Influenza virus nucleoprotein

**Author:** Amina Qadir[1]

**Co-authors:** Ervin Fodor [1]; Guido Paesen [1]; Jonathan Grimes [1]; Lisa Dollhopf [1]; Paul Brennan [1]; Pooja Gupta [1]; Thomas Bowden [1]; Annette Vondelft [1]

[1] *University of Oxford*

**Corresponding Authors:** guido.paesen@strubi.ox.ac.uk, pooja.gupta@strubi.ox.ac.uk, paul.brennan@cmd.ox.ac.uk, thomas.bowden@strubi.ox.ac.uk, jonathan.grimes@strubi.ox.ac.uk, lisa.dollhopf@cmd.ox.ac.uk, ervin.fodor@path.ox.ac.uk, path1397@ox.ac.uk, annette.vondelft@cmd.ox.ac.uk

The influenza A virus nucleoprotein (NP) is an essential and highly conserved component of the viral ribonucleoprotein (vRNP) complex, where it mediates viral RNA encapsidation and coordinates multiple steps of the viral replication cycle. Despite its essential role, NP remains unexplored as a therapeutic target.

To identify chemical starting points for structure-based inhibitor design, we performed a crystallographic fragment screen using the XChem platform at Diamond Light Source. A library of 690 fragments was screened against the monomeric NP mutant, and diffraction datasets were analysed using the PanDDA2 pipeline. We identified 53 fragment hits occupying 12 distinct binding sites on NP, demonstrating that the protein contains multiple ligandable pockets. The most prominent site corresponded to the NP oligomerization interface, with 21 fragments binding within the tail-loop pocket that mediates NP-NP interactions during vRNP assembly. Several fragments clustered around residue E339, a residue previously shown to be critical for NP oligomerization, supporting the functional relevance of this site.

Current efforts are directed towards biophysical validation of the identified fragment hits and their optimisation using structure-guided medicinal chemistry. Overall, this ongoing work represents a significant step toward the development of inhibitors that disrupt
viral ribonucleoprotein assembly.

**Keywords**:

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-80

**Session 6: Antivirals & vaccines** / **92**

# How to control HPAIV in Poultry? Exploring Vaccine Acceptance of Poultry Farmers as a Key Element in the One Health context

**Authors:** Sarah Heynen[1]; Andreas Münkel[1]; Tristan Winkelmann[2]; Bettina Schneider[2]; Amely Campe[2]; Silke Rautenschlein[1]

[1] *University of Veterinary Medicine Hannover, Clinic for Poultry*

[2] *University of Veterinary Medicine Hannover, Institute of Biometry, Epidemiology and Information Processing*

**Corresponding Author:** sarah.heynen@tiho-hannover.de

Zoonotic highly pathogenic avian influenza viruses (HPAIV) are endemic in Europe and a threat to poultry but also mammalian health. Control strategies relied on culling of infected birds. The Delegated Regulation (EU) 2023/361 enables now the European Union member states to decide for vaccination. However, the impact of a vaccination campaign depends on the successful implementation at farm level.
Using a mixed-methods approach, this study investigated HPAIV-vaccination acceptance of poultry farmers (PF). A Knowledge, Attitude and Practice online survey was conducted (n=98; 27 with outbreak experience) and revealed knowledge gaps and feasibility concerns: over 50% of PF were unaware of key requirements going along with HPAIV-vaccination, and less than half considered them feasible. While 75% supported emergency and 41% preventive vaccination, acceptance increased with outbreak experience.
Narrative interviews allowed a deeper insight into the structural barriers, including gaps in knowledge transfer, mismatches between regulation and farm realities, and tensions in trust toward authorities. The farmer's acceptance of a vaccination approach was conceptualized as a socially embedded decision process framework. Integration of the PF perspectives in the decision-making process is critical for vaccination acceptance, which subsequently allows reduction in HPAIV field pressure by reduced shedding rates of vaccinated birds and transmission dynamics within the One Health context.

**Keywords**:

bird flu, Europe, KAP, vaccine acceptance, survey

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-12

**Session 6: Antivirals & vaccines / 110**

# Improving Immune Responses In Avian IAV H5 Immunization By Sustained Antigen Release Technology

**Authors:** Alina Iakubovskaia[1]; Arja Pasternack[1]; Emilia Peltoniemi[2]; Ilkka Julkunen[2]; Laura Kakkola[2]; Mika Jokinen[3]; Milla Runsala[3]; Olli Ritvos[1]; Pekka Kolehmainen[2]; Sophie Winter[2]

[1] *University of Helsinki*

[2] *University of Turku*

[3] *DelSiTech*

**Corresponding Author:** sjwint@utu.fi

Recent global outbreaks of highly pathogenic avian influenza A(H5N1) highlight the need for vaccines that can prevent human transmission. Seqirus's zoonotic influenza A(H5N8) vaccine (approved in 2024) protects against clade 2.3.4.4b H5N1 viruses but requires a booster, which may reduce compliance. Prolonged antigen exposure can strengthen humoral immunity; therefore, sustained antigen release using DelSiTech Silica Matrix™ Technology may improve vaccine performance.

This study compared humoral responses in mice after extended (silica-formulated), escalating (seven doses; 0.2%–63.3%), or bolus subunit immunization. C57BL/6 mice received subcutaneous recombinant H5 hemagglutinin (~3.75 µg) with/without Addavax adjuvant. Encapsulated H5 microparticles were embedded in silica hydrogel for sustained release. Serum H5-specific IgG, IgM, IgA, and H1/H3 cross-reactivity were measured at weeks 3 and 6 by ELISA.

Escalating dosing induced IgG but was lower than silica groups, which produced strong IgG at week 3 with only minor decline by week 6. Adjuvant was needed for strong IgG in all groups except silica. Weak IgM appeared only in escalating-dose groups; IgA was not detected. Limited H1/H3 cross-reactivity occurred only in the escalating dose + adjuvant group.

Overall, single-dose silica formulation generated superior IgG compared with bolus vaccination.

**Keywords**:

Avian influenza, H5, hemagglutinin, sustained antigen release, silica technology

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-55

**Session 7: Virus Replication and Innate Immunity** / **175**

# Keynote

**Session 7: Virus Replication and Innate Immunity** / **117**

# 90 kDa ribosomal S6 Kinase 1 promotes endosomal acidification during the early Influenza A virus infection phase

**Author:** Nicole Oberberg[None]

**Co-authors:** Darisuren Anhlan ; Franziska Rodner ; Yvonne Boergeling ; Ursula Rescher ; Stephan Ludwig ; André Schreiber

**Corresponding Author:** nicole.oberberg@uni-muenster.de

Influenza A viruses (IAV) activate MAPK/ERK in a biphasic manner to promote viral replication. The family of 90 kDa ribosomal S6 kinases (RSK) belongs to the downstream effectors of MAPK/ERK and is involved in cell growth, proliferation and differentiation. We have previously shown, that the RSK1 isoform mediates the nuclear release of newly produced vRNPs via direct phosphorylation of

the viral nucleoprotein during late IAV replication. Within this study, we aimed to identify a so far unknown contribution of RSK1 during the early IAV replication by promoting endosomal acidification and thereby facilitating the cytoplasmic release of viral genomes.
Inhibition of RSK1-3 isoforms with the specific inhibitor BI-D1870 as well as siRNA-mediated knockdown of RSK1 resulted in a decreased internalization of VSV-pseudotyped H1N1 viruses, human IAVs and also HPAIVs. Furthermore, endosomal acidification was reduced upon the missing RSK1 function. Super resolution microscopy (STED) and proximity ligation assays showed that RSK1 interacts with late endosomes. We found colocalization of RSK1 with the v-ATPase, which was confirmed by co-immunoprecipitation of RSK1 with the v-ATPase subunit V1E1. Fusion assays of viral and endosomal membranes revealed that RSK1 manipulation reduced the fusion efficiency between those membranes.
In summary, this work demonstrates for the first time that RSK1 is misused by human IAVs and also HPAIVs to promote the release of viral genomes into the cytoplasm.

**Keywords**:

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26- 63

**Session 7: Virus Replication and Innate Immunity / 114**

# Understanding the selection process of adaptive mutations 271A, 590S and 591R in the PB2 polymerase during the emergence of the triple-reassortant H3N2 swine influenza virus

**Author:** Margot SARRAT[1]

**Co-authors:** Florence Mompart [1]; Thomas Peyret [1]; Fatima Sikt [1]; Baby Naznin [1]; Christelle Camus [1]; Romain Volmer [1]

[1] *INRAE ENVT*

**Corresponding Authors:** baby.naznin@envt.fr, christelle.camus@envt.fr, fatima.sikht@envt.fr, margot.sarrat@envt.fr, florence.mompart@envt.fr, romain.volmer@envt.fr, thomas.peyret@envt.fr

In 1998, an H3N2 swine influenza virus emerged following reassortment between avian, human, and swine viruses, acquiring avian-derived PB2 and PA segments. PB2 subsequently adapted to pigs through the acquisition of the triple mutation 271A, 590S, and 591R. Although the key role of the viral polymerase complex in the adaptation of avian influenza A viruses to mammals is well established, and these three mutations were conserved in H1N1pdm09, the contribution of this combination of mutations and the context in which it was selected remain poorly understood. Using a loss-of-function approach, we compared the replication of four variants of the 1998 H3N2 triple-reassortant swine virus carrying different combinations of avian- and swine-derived PB2 and PA segments *in vitro* in MDCK and NPTr cells and *ex vivo* in swine precision-cut lung slices (PCLS), using replication kinetics and serial passage experiments. We found that PB2 mutations 271A, 590S, and 591R conferred only a moderate selective advantage when associated with a swine-derived PA, but a markedly greater advantage in the presence of an avian-derived PA. Our study highlights the importance of the viral genetic background in shaping the phenotypic effects of adaptive mutations and emphasizes the need to consider interactions between viral genes when assessing influenza emergence and pandemic risk. These findings suggest that the PB2 triple mutation may have been selected in swine before PA adapted to its new host.

**Keywords**:

adaptative mutations, interactions, selection, emergence

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-61

**Session 7: Virus Replication and Innate Immunity / 121**

# Immunopathology of H5N1 highly pathogenic avian influenza viruses in layer chickens.

**Author:** Samuel Kindylides[1]

**Co-authors:** Bénédicte Lambrecht [1]; Fiona Ingrao [1]; Lionel Tafforeau [2]; Mieke Steensels [1]

[1] *Sciensano*

[2] *University of Mons*

**Corresponding Authors:** mieke.steensels@sciensano.be, lionel.tafforeau@umons.ac.be, samuel.kindylides@sciensano.be, fiona.ingrao@sciensano.be, benedicte.lambrecht@sciensano.be

Since 2021, clade 2.3.4.4b H5N1 highly pathogenic avian influenza viruses (HPAIVs) have become predominant worldwide, displaying increased virulence and dissemination in poultry and waterfowl. Within the framework of the WiLiMan-ID project, our goal is to gain insight into the mechanisms shaping host-pathogen interactions and contributing to the enhanced virulence of recent HPAIVs. To this end, we demonstrated that three strains, a clade 2.3.2.1c virus from 2013 and two clade 2.3.4.4b isolates (genotypes AB and BB), displayed different immunopathological and transmission profiles in layer chickens. To identify the viral and host factors involved in the virulence and spread of these viruses, an *ex vivo* infection model based on chicken monocyte-derived dendritic cells was developed. As key antigen-presenting cells, dendritic cells bridge innate and adaptive immunity, contributing to inflammatory responses through pathogen sensing and cytokine production. The clade 2.3.4.4b viruses showed significantly increased replication in this model when compared to clade 2.3.2.1c, historically of lower virulence in waterfowl. Additionally, RT-qPCR analysis revealed distinct immune gene expression profiles induced by these three HPAIV strains, including strong differences in key antiviral interferon-stimulated genes. These findings represent an important first step towards identifying new molecular determinants that may contribute to the increased virulence of recent H5N1 HPAIVs.

**Keywords**:

H5N1, HPAIV, dendritic cells, innate immune response, interferon, chicken

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-7

**171**

## Poster Prize and Closing Remarks

**151**

## Loss of hemagglutination ability by H3N2 influenza A virus, subclade K

**Author:** Ruonan Liang[1]

**Co-authors:** Pascal Lexmond [2]; Oliver Grant [1]; Mark Pronk [2]; Roland J. Pieters [1]; Ron AM Fouchier [2]; Geert-Jan Boons [1]; Björn Koel [2]; Robert de. Vries [1]

[1] *Utrecht University*

[2] *Erasmus University Medical Center*

**Corresponding Author:** r.liang@uu.nl

Seasonal human H3N2 influenza viruses, subclade K (J.2.4.1), have been the predominant influenza A viruses in the Northern hemisphere influenza season of 2025/2026. Since 2024, the vaccine virus A/Darwin/6/21 has emerged into different antigenic variants. To assess antigenicity, the hemagglutination inhibition (HI) assay is the most widely used, and a loss of binding to turkey erythrocytes could significantly hamper this process. Antigenic changes are frequently caused by amino acid substitutions near the hemagglutinin (HA) receptor-binding pocket, which can also affect receptor binding properties, such as hemagglutination. In this study, we explored how substitutions in or around the HA receptor-binding site affect binding to glycans at the molecular level. We employed ELISA, glycan array, flow cytometry, hemagglutination assays, and tissue staining. Substitutions at positions 140, 192, and 223 establish a new HA background of the viruses that emerged in 2024. Computational analysis of HA in complex with elongated glycan reveals that mutation F192 forms a CH-Pi to stabilize the binding. Based on this background, substitutions in antigenic sites A and B within subclade K viruses exhibit a binding preference to elongated glycans, which are not displayed on turkey erythrocytes. Conversely, our previously established glyco-remodeled erythrocytes are efficiently bound by these subclade K H3N2 viruses and could support influenza surveillance and vaccine development.

**Keywords**:

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26- 101

**118**

## Influenza A virus and its reliance on host cell metabolism

**Author:** Chiara R. Bojarzyn[1]

**Co-authors:** Jens Kleinehr [1]; Michael Schöfbänker [1]; Katharina Daniel [2]; Nadine Bieß [3]; Matthias Behrens [3]; Hans-Ulrich Humpf [3]; Stephan Ludwig [1]; Eike R. Hrincius [1]

[1] *Institute of Virology Muenster*

[2] *Institute of Virology Cologne*

[3] *Institute of Food Chemistry Muenster*

**Corresponding Author:** c_boja03@uni-muenster.de

Influenza A viruses (IAVs) rely heavily on host cell metabolism for productive replication. Several studies have demonstrated that IAVs actively modulate host cellular processes. It has already been shown that suppression of glucose metabolism inhibits viral replication. Interestingly, inhibition of glycolysis disrupts IAV replication by impairing viral polymerase function resulting in reduced synthesis of viral genomic RNA. This raises the question of whether this is a glycolysis-specific effect or a general consequence of metabolic inhibition. There is limited knowledge about how IAV polymerase regulation interacts with metabolic pathways. Here, we demonstrated that inhibition of the main metabolic pathways glutaminolysis, oxidative phosphorylation (OXPHOS), fatty acid synthesis (FAS), and pentose phosphate pathway led to a reduction of viral titers. Moreover, inhibition of glutaminolysis, FAS, and OXPHOS led to an imbalance in the cellular glycolysis and respiration networks, with an extended period of viral transcription and a sharp decline in replication. Furthermore, we investigated effects of the tricarboxylic acid cycle intermediate oxaloacetate on viral replication and showed that it nearly fully reversed the glycolysis inhibition mediated reduction on viral titers. Our results suggest that the regulation of IAV replication is closely linked to the cellular metabolic state, showing the potential of modulating metabolism as a host-targeted antiviral strategy.

**Keywords**:

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-26

**153**

# Rational design of A/H1N1pdm09 improves LAIV dual-species fitness and in vivo efficacy

**Author:** shaun cooper[1]

**Co-authors:** David Burbidge [1]; Jack Hirst [1]; Katherine Scott [1]; Oliver Dibben [1]

[1] *AstraZeneca*

**Corresponding Authors:** shaun.cooper@astrazeneca.com, oliver.dibben@astrazeneca.com, jack.hirst@astrazeneca.com, katherineanne.scott@astrazeneca.com, david.burbidge@astrazeneca.com

Live attenuated influenza vaccine (LAIV) requires efficient replication in eggs (manufacture) and the upper respiratory tract (administration). In H1N1, unfavourable egg adaptations can compromise dual-species fitness. A strategy targeting HA residues 125, 127, 222 and 223 was effective, but later limited by genetic instability and antigenic mismatch.
To identify more robust solutions, we evaluated broader HA adaptations linked to dual-species replication. A deep scanning library of 2,304 HA variants from a recent H1N1 strain was generated,

rescued and serially egg passaged, with NGS used to identify predominant genotypes. Selected variants were characterised for fitness, stability, antigenicity, receptor binding, and efficacy tested in a ferret challenge model.
The dominant genotype (LIB) contained adaptations at 7 of 8 targeted residues and increased replication 8-fold in eggs and 400-fold in human nasal cells versus parental wild-type LAIV. Iterative refinement identified receptor-binding motif 187E, 222Q, 223L and 225A (EQLA) which was applied to strains from 2015-2023, conferring enhanced dual-species replication via increased human-like receptor binding. LIB and EQLA LAIV were genetically stable and antigenically matched to parental wild type.
In vivo, LIB- and EQLA LAIVs protected against wild-type challenge and induced >300-fold higher neutralising antibodies to wild-type virus. These findings support a rational design approach for development of effective H1N1 LAIV.

**Keywords**:

pdm09H1N1, vaccine efficacy, dual species replication, vaccine optimisation, LAIV

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-103

**89**

# Test Abstract #1

**Authors:** Inga Regling[None]; Smith[None]

**Co-author:** Miller

**Corresponding Author:** inga.regling@orgalution.de

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**Keywords**:

Keyword 1, 2, 3

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-1

**95**

## The cellular AP-1 cFos regulates influenza A virus replication

**Authors:** Antoine Gerodez[1]; François Dufrasne[2]; Olivier Denis[3]; Mieke Steensels[1]; Bénédicte Lambrecht[1]; Lionel Tafforeau[4]; Caroline Demeret[5]; Cyril Barbezange[2]

[1] *Avian Virology and Aquatic Diseases, Sciensano, Belgium*

[2] *National Influenza Center, Sciensano, Belgium*

[3] *Immune Response Service, Sciensano, Belgium*

[4] *Cell Biology laboratory, University of Mons, Belgium*

[5] *Interactomics, RNA and Immunity laboratory, Institut Pasteur, France*

**Corresponding Author:** antoine.gerodez@sciensano.be

Influenza A virus (IAV) infection induces activating protein-1 (AP-1) transcription factors as part of the host antiviral response. In our study, we identified cFos as the most strongly upregulated AP-1 factor during IAV infection and, unexpectedly, uncovered a proviral role for this transcription factor. Fluorescence microscopy and functional analyses demonstrated that cFos promotes IAV infection through its nuclear transcriptional activity rather than its cytoplasmic function in lipid synthesis. Using siRNA-mediated knockdown, we further showed that cFos enhances cell survival and suppresses interferon-β expression during infection, thereby creating conditions favorable for viral propagation. In addition, minigenome assays and strand-specific RT-qPCR analyses suggested a potential contribution of cFos to IAV transcriptional activity. Together, these findings identify cFos as a key host factor exploited by IAV to optimize replication through modulation of innate immunity, apoptosis, and viral gene expression. More broadly, our work highlights that transcription factors may support influenza virus replication, providing new insights into host–virus interactions.

**Keywords**:

Influenza A; activating protein 1; AP-1; cFos; innate immunity; apoptosis; viral transcription

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26- 32

**98**

## A bovine H5N1 virus efficiently replicates in differentiated human nasal epithelial cells at the temperature of the upper airways

**Author:** Étori Aguiar Moreira[1]

**Co-authors:** Samuel Constant [2]; Charlène Constant [2]; Lisa Butticaz [1]; Michele Wyler [1]; Teodora David [3]; Peter M. Grin [1]; Charaf Benarafa [1]; Volker Thiel [3]; Marco P. Alves [3]

[1] *Institute of Virology and Immunology (IVI)*

[2] *Epithelix Sàrl*

[3] *University of Bern*

**Corresponding Authors:** peter.grin@ivi.admin.ch, lisa.butticaz@ivi.admin.ch, teodora.david@unibe.ch, charlene.constant@epithelix
michele.wyler@ivi.admin.ch, gert.zimmer@ivi.admin.ch, etori.aguiarmoreira@ivi.admin.ch, volker.thiel@unibe.ch,
marco.alves@unibe.ch, samuel.constant@epithelix.com, charaf.benarafa@ivi.admin.ch

Highly pathogenic avian influenza (H5N1) viruses of clade 2.3.4.4b have caused significant losses in bird populations worldwide and repeatedly infected mammals, including humans, without sustained human to human transmission. Here we show that an H5N1 virus (H5N1-Tex/24) isolated from bovine milk in Texas in 2024 replicates just as efficiently in differentiated human nasal epithelial cells as a pandemic H1N1 virus strain from 2009 (H1N1), at both 37 ℃ and 33 ℃. The adaptive mutations PB2 M631L and PA K497R promoted replication at 33 ℃ but had no effect on replication at 37 ℃. An H5N1 virus (H5N1-BE/22) isolated from a pelican in 2022, which lacked these mutations, replicated efficiently at 37 ℃ but poorly at 33 ℃, and this limitation was not overcome by the introduction of the PB2 M631L and PA K497R mutations. The differentiated nasal epithelial cell cultures expressed receptors for both human and avian influenza viruses. Accordingly, no HA mutations associated with altered receptor specificity were detected. H5N1-Tex/24 was able to effectively suppress the production of interferon-lambda, yet remained sensitive to the antiviral effects of this cytokine. These findings suggest that H5N1-Tex/24 possesses intrinsic traits supporting efficient replication in differentiated human upper airway cell cultures.

**Keywords**:

avian influenza; zoonosis; pandemic preparedness; virus transmission; species barrier; receptor usage; tropism; adaptation; innate immunity

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-39

**129**

## Oreoch-1 Inhibits Early Influenza A Infection Across Strains

**Author:** Bianca Maria Nastri[1]

**Co-authors:** Annalisa Chianese [1]; Carla Zannella [1]; Anna De Filippis [1]; Stephan Ludwig [2]; André Schreiber [2]; Massimiliano Galdiero [1]

[1] *Department of Woman, Child and General and Specialized Surgery, University of Campania "Luigi Vanvitelli", Naples, 80138, Italy.*

[2] *Institute of Virology (IVM), Westfaelische Wilhelms Universitaet, Muenster, Nordrhein-Westfalen, 48149, Germany*

**Corresponding Author:** biancamaria.nastri@unicampania.it

Introduction Antimicrobial peptides are small components of the innate immune system with broad-spectrum antimicrobial activity and immunomodulatory properties. Oreochromicins, isolated from

the gills of Nile tilapia (Oreochromis niloticus), have previously demonstrated antiviral activities in vitro. In this study, we investigated for the first time the antiviral activity of Oreoch-1 against influenza A viruses (IAV), including laboratory-adapted and highly pathogenic H5N1 strains.
Materials and Methods Cytotoxicity was evaluated using the MTT assay and antiviral activity was assessed by plaque reduction assays. The direct interaction between Oreoch-1 and viral particles was investigated, and EC50 and EC90 values were determined for human and highly pathogenic avian strains. Time-of-addition experiments using a pseudotyped VSV expressing influenza hemagglutinin and neuraminidase were performed to confirm the stage of viral inhibition. Early replication analyses and Western blotting further characterized the effect on initial infection events.
Results Oreoch-1 exhibited strong inhibitory activity against all tested influenza A strains. The peptide acted primarily during the early stages of infection by directly interacting with viral particles, preventing efficient viral entry.
Conclusions Oreoch-1 demonstrates broad antiviral activity supporting its potential as a promising antiviral against emerging influenza viruses.

**Keywords**:

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-74

**Opening lecture** / **168**

# Opening talk: Martin Beer (FLI)

**113**

# Progress toward a deep mutational scanning of the H9 HA to better understand H9N2 avian influenza evolution

**Author:** Alexandre RIBEIRO[1]

**Co-authors:** Romain Volmer [1]; Mariette Ducatez [1]; Maxime Fusade-Boyer [1]

[1] *IHAP, UMR1225, Université de Toulouse, INRAe, ENVT, Toulouse, France*

**Corresponding Authors:** alexandre.ribeiro@envt.fr, romain.volmer@envt.fr, maxime.fusade-boyer@envt.fr, mariette.ducatez@envt.fr

Low-pathogenic H9N2 avian influenza is endemic in poultry in many regions of the world, causing significant economic loss and representing a persistent zoonotic threat with pandemic potential as recognized by the WHO. To better understand how each residue of the H9 HA tolerates mutations, we aim to perform a deep mutational scanning (DMS) of its entire coding sequence: this represents a total of 10 659 possible mutations.
We have successfully generated a plasmid mutant library with extensive mutational diversity along the H9 gene, that will serve as template for the generation of the mutant virus library. For biosafety consideration, we use a pseudotyped lentiviral system with adapted and optimized conditions to yield high viral H9N2 pseudovirus titers, notably the use of modified 293T as target cells. Finally, a crucial step for DMS experiments is to ensure the genotype-phenotype link when generating the

mutant pseudovirus library. Using a previously established two-step protocol to ensure this link, we have successfully rescued mutant H9N2 pseudovirus from cells integrated with lentiviral genomes containing our H9 mutant genes.
These results establish the foundation for subsequent mutations fitness mapping of relevant phenotypic characteristics of the H9 HA such as receptor affinity or antibody selective pressure. Ultimately, DMS data will inform phenotypic assessment of newly collected H9 HA sequences.

**Keywords**:

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-62

**120**

# The overlooked role of mucus as a species-specific infection barrier

**Author:** Mengying Liu[1]

**Co-authors:** Albert van Dijk [1]; Kristien Van Reeth [2]; Henrik Clausen [3]; Cornelis A. M. de Haan [1]; Erik de Vries [1]

[1] *Utrecht University*

[2] *Ghent University*

[3] *University of Copenhagen*

**Corresponding Authors:** c.a.m.dehaan@uu.nl, kristien.vanreeth@ugent.be, e.devries@uu.nl, a.vandijk1@uu.nl, hclau@sund.ku.dk, m.liu3@uu.nl

Contemporary swine influenza A viruses (IAV-S) of the Eurasian-avian lineage (EAav) present a pandemic threat by being antigenically largely distinct from the IAV-S that caused the 2009 pandemic (Pdm09). Cross-species transmission only rarely occurs and the role of respiratory mucus as a decoy receptor-loaden species barrier is understudied. IAV infection requires binding to specific sialic acids (Sia) receptors at the epithelial cell surface that likely differ from the decoy receptors present on respiratory mucins. Sia binding to the viral hemagglutinin (HA) needs to be compensated by sialidase activity of the viral neuraminidase (NA) to promote traversal of the mucus and to remain motile on the cell surface.
Penetration of mucus overlaying primary swine and human tracheal cultures was absolutely dependent on NA activity while efficient infection also required NA activity on cell surface receptors. Importantly, large differences exist in decoy effects between swine and human mucus, likely caused by marked differences between their sialoglycomes. Glycoengineered HEK293 cells expressing defined sialylated glycotopes were used to quantify virus binding and NA-mediated release and correlated to infectivity of HEK293 cells and primary cells. Swine and human tracheal mucus was added to dissect species-specific inhibition patterns. We hypothesize that the balance between these parameters is, more than the individual parameters, determining infection efficiency.

**Keywords**:

Swine influenza A virus (swIAV), Mucus glycome, Sialic acid receptors, Hemagglutinin–neuraminidase balance (HA–NA balance), host adaptation, cross-species transmission,
Glycoengineering, Zoonotic potential

**Professional status of the speaker**:

Postdoc

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-51

**123**

# Host immunity and viral genotype modulate HPAIV feather replication and shedding

**Author:** Dylan Andrieux[1]

**Co-authors:** Laura Lebouteiller [1]; Manuela Crispo [1]; Jean-Luc Guérin [1]; Nicolas Gaide [1]; Sébastien Soubies [1]

[1] *Univ Toulouse, ENVT, INRAe, UMR 1225 IHAP*

**Corresponding Authors:** manuela.crispo@envt.fr, jean-luc.guerin@envt.fr, nicolas.gaide@envt.fr, dylan.andrieux90@gmail.com, sebastien.soubies@envt.fr, laura.lebouteiller@envt.fr

H5Nx highly pathogenic avian influenza viruses (HPAIV) represent a major threat to animal and public health. In birds, HPAIV shedding mainly occurs via respiratory and digestive routes. These viruses also replicate in vascularized immature feathers, thus representing an additional shedding route. Understanding virus-feather interactions is crucial to limit viral spread and adapt biosafety. Our study aims to characterize feather infection dynamics by several HPAIV, and assess the impact of immunity on viral replication in feathers.
An in ovo model highlighted early feather infection events by Gs/Gd viruses with evidence of sequential infection from feather endothelium to pulp, and epithelium. To assess feather susceptibility independently of systemic dissemination, hatched duck-derived feather explants were infected. All HPAIV and low pathogenicity avian influenza viruses tested were able to infect explants. Upon in vivo challenge, HPAIV replication in naïve animal feathers resulted in necrosis and epithelial debris while replication was blocked in feathers after anti-H5 vaccination of ducks. Interestingly, endothelial infection was more readily detected in ovo than in vivo.
Collectively, our data support a model in which HPAIV infect immature feathers through viremia, leading to epithelial infection and likely contributing to environmental shedding. While many AIV are able to replicate in feather, virus-dependent viremia and host immune status may modulate access to feathers.

**Keywords**:

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-49

**164**

# FLUPREP: A National Early-Warning System for Emerging Influenza Threats

**Authors:** Peter Reuther[1]; Hardin Bolte[1]; Andreas Hocke[2]; Stefan Hippenstiel[2]; Katharina Katz[3]; Merle Böhmer[3]; Thorsten Wolff[4]; Linda Brunotte[5]; Stephan Ludwig[5]; Donata Hoffmann[6]; Tim Harder[6]; Martin Beer[6]; Martin Schwemmle[1]

[1] *University Medical Center Freiburg –Institute of Virology*

[2] *Charité –University Medicine Berlin*

[3] *Bavarian Health and Food Safety Authority (LGL)*

[4] *Robert-Koch Institute (RKI)*

[5] *University Medical Center Münster –Institute of Virology*

[6] *Friedrich-Loeffler Institute (FLI)*

**Corresponding Authors:** martin.schwemmle@uniklinik-freiburg.de, peter.reuther@uniklinik-freiburg.de

Influenza A viruses frequently breach species barriers, posing a persistent pandemic threat. Mitigating this threat requires systems capable of detecting high-risk strains at the animal–human interface and implementing targeted countermeasures before human spillover occurs. Although international frameworks like TIPRA or IRAT routinely assess IAV risks, data gaps and lengthy consensus-building can hamper national preparedness. Hence, Germany needs its own rapid early warning system for emerging influenza threats.
To fill this gap, we established FLUPREP, a national One Health consortium funded by the BMFTR. Uniting six core partners—RKI, Charité Berlin, FLI, University of Münster, University Medical Center Freiburg, and LGL—FLUPREP seeks to proactively characterize IAVs emerging at the human–animal interface to generate comprehensive risk profiles that drive evidence-based pandemic preparedness. Central to FLUPREP is an integrated experimental pipeline that evaluates pandemic potential by quantifying key risk factors, such as human-type receptor specificity, airborne transmissibility, and resistance to diverse immune barriers. The measured data are then converted into a pandemic risk score calibrated against contemporary and historical IAVs. Graded risk score thresholds will act as actionable triggers, enabling local, state, and federal authorities to scale response measures from routine surveillance to emergency protocols, securing Germany's future pandemic resilience.

**Keywords**:

**Professional status of the speaker**:

Senior Scientist

**Junior scientist status**:

No, I am not a junior scientist.

**Registration ID**:

INF26-31

**141**

# Detection of antibodies against influenza virus in Benin livestock

**Authors:** Baby Naznin[1]; Fatima-Zohra Sikht[1]

**Co-authors:** Arthur Kocher [1]; Ashley Webb [2]; Fidélia Djegui Djegui [3]; Laura Lebouteiller [1]; Mariette Ducatez [1]; Maxime Fusade-Boyer [1]; Pamela McKenzie ; Rachidatou Adjin [3]; Richard Webby [2]; Sébastien Soubies [1]

[1] *Univ Toulouse, ENVT, INRAE*

[2] *St jude Children's Research Hospital, Memphis, TN, USA*

[3] *LADISERO, Parakou, Benin*

**Corresponding Authors:** baby.naznin@envt.fr, fatima.sikht@envt.fr, laura.lebouteiller@envt.fr, maxime.fusade-boyer@envt.fr, arthur.kocher@univ-tlse3.fr, richard.webby@stjude.org, ashley.webb@stjude.org, sebastien.soubies@envt.fr, djegui_fidelia@yahoo.fr, mariette.ducatez@envt.fr, pamela.mckenzie@stjude.org

Since the first outbreak in March 2024, the Highly Pathogenic Avian Influenza Virus (HPAIV) H5N1, clade 2.3.4.4b, has infected both poultry and dairy farms in multiple states of the United States (US). Norway and United Kingdom have also reported H5 virus in small ruminants having found seropositive sheep in 2023 and 2025, respectively. The Netherlands reported one seropositive cow in a farm in January, 2026. Clade The virus has recently been detected for the first time in Australian wild birds. Very limited surveillance for animal influenza is carried out in Africa despite the fact that clade 2.3.4.4b viruses have been repeatedly detected in wild and domestic birds in the last few years. Our study aimed to assess whether livestock in the vicinity of HPAIV H5N1 outbreaks in poultry in Benin (West Africa) have been exposed to influenza virus. Ten cattle, 1 donkey and 2 swine sera were positive for anti-AIV NP antibodies (IDVet multispecies ELISA kit). However, seroneutralisation assays against A/mallard duck/France/24-1065_pp37/2024(H5N1) (European DI genotype) were negative, suggesting absence of anti-H5 neutralizing antibodies. Hemagglutination inhibition assays are ongoing to identify the HA subtype specificity of the identified antibodies. The seropositivity in ELISA tests highlights the importance of surveying livestock susceptibility to AIV in regions outside of the US. More work is warranted to understand the putative role of these hosts in virus evolution and spread.

**Keywords**:

detection, livestock, neutralizing antibodies, surveillance

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-92

**143**

# Impact of H5 vaccination in ducks on influenza virus evolution

**Author:** Mohamed El Sayes[1]

**Co-authors:** Alice Michel [1]; Charlotte Foret-Lucas [1]; John Franks [2]; Richard Webby [2]; Siham Fellahi [3]; Mariette Ducatez [1]

[1] *INRAE, ENVT, IHAP, Toulouse University, Toulouse 31300, France*

[2] *Department of Host-Microbe Interactions, St. Jude Children's Research Hospital, Memphis, TN 38105, USA*

[3] *Department of Veterinary Pathology and Public Health, Institut Agronomique et Vétérinaire Hassan II, B.P. 6202, Rabat, Morocco*

**Corresponding Authors:** john.franks@stjude.org, richard.webby@stjude.org, fellahisiham2015@gmail.com, mohamed.elsayes@envt.fr, charlotte.foret@envt.fr, mariette.ducatez@envt.fr, alice.michel@envt.fr

High pathogenicity avian influenza (HPAI) H5Nx viruses of clade 2.3.4.4b have become spread globally in the last few years. In early 2024, the USA reported the first outbreak of HPAI H5N1 clade

2.3.4.4b genotype B3.13 in dairy cattle. In France, HPAI H5N1 of clade 2.3.4.4b (genotype DI) caused numerous outbreaks in wild and domestic birds. Since 2022, around 32 million birds have been culled across France due to detection of H5N1 virus. This led to a drastic change in EU regulations including the implementation of vaccination programs in domestic ducks in France that started in autumn 2023.
Based on the hypothesis that vaccine-induced immunity pressure can lead to emergence of escape mutant viruses, this study aimed to understand the impact of vaccination on virus evolution. We constructed an attenuated reverse genetics (6+2) HA "delta"(HA with a LP cleavage site) genotype B3.13 virus on a PR8 backbone. This RG virus was passaged twice in embryonated eggs from vaccinated ducks. Across 4 replicates, NGS was performed to assess viral genetic diversity. No hemagglutinin mutation was observed, but a neuraminidase N42D mutation was consistently detected.
Additional passages in eggs from vaccinated ducks will be conducted using RG H5 (6+2) viruses representing different genotypes from different countries. Overall, we will assess the possible emergence of antigenically drifted H5N1 viruses and study their putative impact in the context of vaccination failure and phenotypic changes

**Keywords**:

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-93

**134**

# Impact of respiratory microbiota disruption on mucin glycosylation during influenza D virus-associated polymicrobial infection

**Author:** Thomas Peyret[None]

**Co-authors:** Baptiste Six [1]; Emmanuel Maes [1]; Fatima-Zohra Sikht [2]; Gilles Meyer [2]; Guénaëlle Derolez [2]; Louise Debusschere [1]; Marie Corbin [3]; Mariette Ducatez [2]; Maverick Monie–Ibanes [2]; Nao Yamakawa [1]; Núria Mach [2]

[1] *PAGés - PLBS*

[2] *IHAP unit - INRAE / ENVT*

[3] *University of Glasgow*

**Corresponding Authors:** m.corbin.1@research.gla.ac.uk, louise.debusschere@univ-lille.fr, guenaelle.derolez@envt.fr, fatima.sikht@envt.fr, thomas.peyret@envt.fr, nuria.mach@envt.fr, gilles.meyer@envt.fr, maverick.monie-ibanes@envt.fr, baptiste.six@univ-lille.fr, nao.yamakawa@univ-lille.fr, mariette.ducatez@envt.fr, emmanuel.maes@univ-lille.fr

Bovine respiratory disease (BRD) results from complex interactions between the host, the respiratory microbiota, and multiple respiratory pathogens, including influenza D virus. Mucins, the major structural components of respiratory mucus, constitute the first line of defense against inhaled pathogens. They carry O-glycans whose terminal sialic acid residues mediate interactions between influenza viruses and their host. However, the spatiotemporal changes in mucin glycosylation during respiratory infection remain poorly characterized.

The aim of the study is to evaluate the impact of respiratory microbiota disruption on mucin glycosylation remodeling during polymicrobial respiratory infection. To address this question, an in vivo calf model was established in which animals, treated or not with antibiotics were challenged with a combination of influenza D virus, bovine coronavirus and Mycoplasma bovis. Nasal swabs

and bronchoalveolar lavages samples were collected over time to monitor changes in O-glycans and sialic acids profiles along the respiratory tract using glycomic approaches.

Preliminary analyses of non-infected animals showed that antibiotic treatment reshapes the global O-glycan profile, with coordinated changes across multiple glycan structures. Analyses of infected animals are ongoing and will determine how microbiota disruption influences respiratory mucin glycosylation during influenza D virus-associated polymicrobial infection.

**Keywords**:

Glycans, sialic acids, glycomic, mucins, influenza D virus, polymicrobial infection, microbiota disruption

**Professional status of the speaker**:

PhD student

**Junior scientist status**:

Yes, I am a junior scientist.

**Registration ID**:

INF26-86