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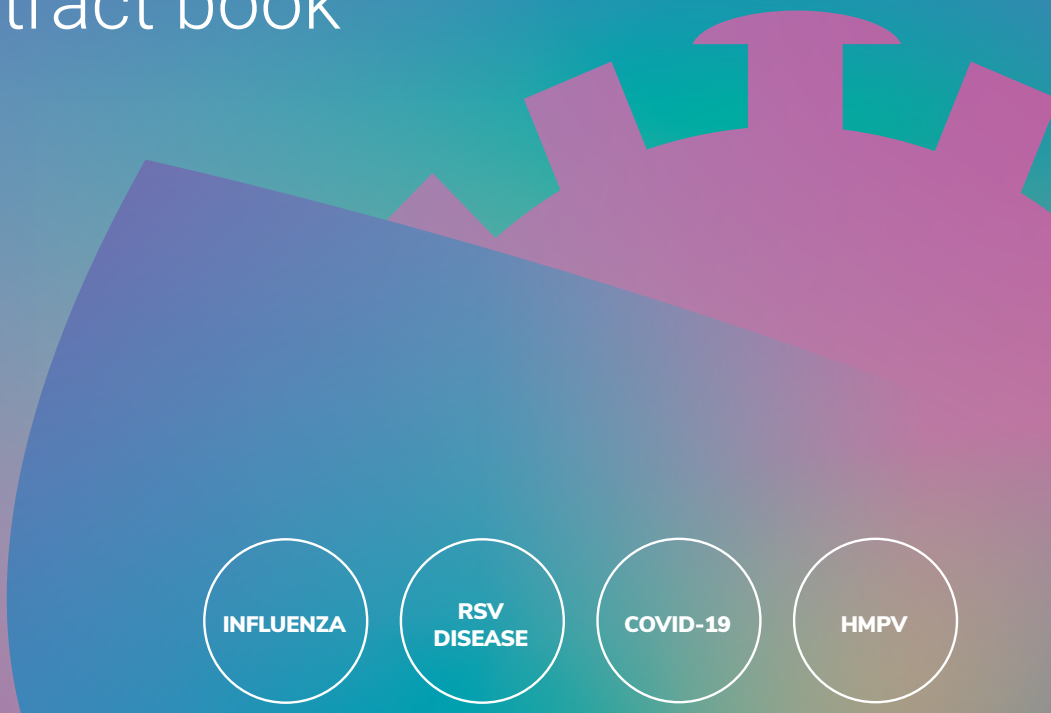

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Abstracts oral sessions overview

INNATE AND ADAPTIVE IMMUNITY

SCS01 • AUDITORIUM 1 - PLENARY HALL • MON 20 OCT 2025 - 11:30 - 13:00

Increasing SARS-CoV-2 IgG4 following repeated mRNA boosters negatively impact antibody functions across Omicron variants and sarbecoviruses ECaS

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Periodic COVID-19 mRNA boosters have been recommended for protection against severe disease and death by SARS-CoV-2, particularly among vulnerable populations. Repeated exposures to mRNA vaccine/booster have been linked to uni-directional IgG subclass switching of spike-specific antibodies towards IgG4 (IgG3→IgG1→IgG2→ωIgG4).

In contrast to IgG3 and IgG1, IgG4 binds poorly to Fc gamma receptors (FcγR) present on NK cells (FcγRIIIa) or phagocytes (FcγRIIa) diminishing Fc-mediated non-neutralising anti-viral activity. However, its impacts across emerging variants and sarbecoviruses remain under explored. Furthermore, IgG4 undergoes Fab-arm exchange in vivo, forming bispecific antibodies which could weaken their binding capabilities to viral variants due to their monovalency. Sequential subclass switching could also favor pre-exisiting immunity biased by ancestral imprinting.

Here, we show that plasma and salivary IgG4 increased substantially following 4 exposures to the mRNA vaccine/booster, in either primary mRNA vaccinees (mRNA cohort; 2 x mRNA vaccine + 2 x mRNA boosters) or primary adenovirus-vector vaccinees (Vaxzevria cohort; 2 x adenoviral-vector vaccine + 4 x mRNA boosters). Elevated class-switched IgG4 negatively correlated with plasma concentrations of IgG1 targeting Omicron spikes trimers (BA.1, XBB.1.5, JN.1), with responses peaking at 4 weeks following their 4th mRNA dose in both cohorts (mRNA: r=-0.53 to -0.66, p<0.01; Vaxzevria: r=-0.65 to -0.71, p<0.01). While ancestral imprinting greatly influenced IgG4 spike responses, particularly against the RBD, IgG4 responses trended similarly to total IgG, resulting in comparable proportions of IgG4 (% IgG4 / total IgG) across Omicron variants.

Omicron spike-specifc plasma antibody engagement with FcγRIIa and FcγRIIIa improved following repeated boosters, however these responses also negatively associated with rising IgG4 levels (BA.1, XBB.1.5, JN.1; mRNA: r=-0.74 to -0.86, p<0.0001; Vaxzevria: r=-0.73 to -0.88, p<0.001). Similar trends were observed in saliva, though IgG4 had a weaker impact. Depletion of IgG4 antibodies increased IgG1 binding against viral variants (+13-16%), highlighting the impact of sequential subclass switching on epitope competition. Similarly, IgG4 depletion also rescued Fc-receptor engagement across Omicron variants, particularly for FcγRIIIa (+11-14% binding; p<0.0001). Spike-specific IgG4 was also cross-reactive to a range of spikes from SARS and SARS-CoV-2 related sarbecoviruses found in bats (RaTG13, RsSHC014, HKU3) and pangolins (GX-P5L, GD-1). Increased proportions of cross-reactive IgG4 negatively associated with FcγRIIa and FcγRIIIa engagement across sarbecoviruses (mRNA: r=-0.54 to -0.93, p<0.01; Vaxzevria: r=-0.63 to -0.94, p<0.01), highlighting the wider impact of class-switched IgG4. Through epitope blocking, we determined that a majority of ancestrally-imprinted RBD IgG4 across both cohorts (mRNA: 61-71%, p<0.0001; Vaxzevria: 43-52%, p<0.0001) targeted the receptor binding motif - the mutational hotspot facilitating binding to the ACE2 receptor. IgG4 depletion revealed that while IgG4 greatly contributed to neutralising ancestral virus, its direct contribution to BA.1 or XBB.1.5 neutralisation were more modest.

Our findings underscore the growing urgency of better understanding IgG4 class-switching following mRNA boosters.

INNATE AND ADAPTIVE IMMUNITY
SCS01 • AUDITORIUM 1 - PLENARY HALL • MON 20 OCT 2025 - 11:30 - 13:00

Impact of Aging on CD8+ T Cell Immunity to Circulating and Pandemic Viruses

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BACKGROUND

Age is a major factor in determining disease duration and outcome during seasonal epidemic and pandemic outbreaks, including influenza and SARS-CoV-2. CD8+ T cells recognize conserved viral proteins, resulting in broad-reactivity across viral strains and subtypes. They provide protection against severe disease outcomes, particularly in the absence of neutralizing antibodies. This makes them an attractive target for novel T cell-based vaccine strategies. However, the mechanisms underlying age-related changes in virus-specific CD8+ T cell immunity and the potential impact of age on T cell-based vaccine effectiveness remains ill defined. We investigate how virus-specific T cell immunity develops across immunologically-distinct phases of human life.

METHODS

The longevity of adults and elderly influenza-specific CD8+ T cells was studied over 7-12 years. CD8+ T-cell responses were analysed directly *ex vivo* by tetramer-associated magnetic enrichment and single-cell multiplex-nested T cell receptor (TCR)-ab RT-PCR for the most prominent human influenza epitope, HLA-A*02:01-M158-66 (A2/M158). Paired TCRαβ-chains were used to track clonotypes over time within individuals.

We examined the impact of age and/or CMV on the generation of *de novo* SARS-CoV-2-specific CD8+ T cell responses in 40 younger (22-40 years) and 37 older (50-66 years) convalescent individuals. Heterotetramer combinatorial coding combined with phenotypic markers allowed in-depth profiling of 35 SARS-CoV-2 epitope-specific CD8+ T cell populations directly *ex vivo*.

RESULTS

We demonstrated that highly functional public (shared) clonotypes dominated the young and adult influenza virus-specific CD8+ T cell repertoires, whereas less functional private (not shared) clonotypes dominated older CD8+ T cell populations. Although both public and private influenza-specific CD8+ T cells are long-lived, they gradually decline with age eventually resulting in the loss of highly-functional public clonotypes.

Conversely, immunosenescence and CMV infections have been speculated to hamper the formation of protective T cell immunity against novel or emerging pathogens due to age and/or CMV related decline in naïve CD8+ T cells. The SARS-CoV-2 pandemic presented a unique opportunity to examine the impact of age and/or CMV on the generation of *de novo* SARS-CoV-2-specific CD8+ T cell responses. We found that immune aging and CMV status did not impact the formation of robust SARS-CoV-2-specific CD8+ T cell responses.

CONCLUSION

Together, our findings suggest that T cell-based influenza vaccines are expected to be more effective in younger individuals, whereas T cell-based vaccines against novel/pandemic viruses are likely to induce robust T cell immunity in both young and older individuals.

INNATE AND ADAPTIVE IMMUNITY

SCS01 • AUDITORIUM 1 - PLENARY HALL • MON 20 OCT 2025 - 11:30 - 13:00

Detection of pre-existing humoral immunity against influenza virus H5N1 clade 2.3.4.4b in unexposed individuals

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The spill-over of Influenza A virus H5N1 clade 2.3.4.4b from cattle to humans highlights the risk of a human H5N1 pandemic. Given the impact of pre-existing immunity on the course and severity of viral infections, we comprehensively assessed the humoral immunity against the H5N1 A/Texas/37/2024 isolate in H5N1-naïve individuals. To this end, we performed complementary binding and neutralization assays on 66 subjects and ranked activities among a panel of 76 influenza A virus isolates. We detected low but distinct cross-neutralizing titers against A/Texas/37/2024 with a 3.9 to 15.6-fold reduction compared to selected H1N1 or H3N2 strains. Moreover, by cloning and evaluating 136 monoclonal antibodies from memory B cells, we identified potent A/Texas/37/2024-neutralizing monoclonal antibodies in five out of six investigated individuals. These antibodies predominantly utilize VH1-69 gene segments, cross-neutralize H1, and compete with antibodies targeting the HA stem. Our findings demonstrate partial pre-existing humoral immunity to A/Texas/37/2024 in H5N1-naïve individuals.

INNATE AND ADAPTIVE IMMUNITY

SCS01 • AUDITORIUM 1 - PLENARY HALL • MON 20 OCT 2025 - 11:30 - 13:00

Systemic and mucosal antibody signatures of protection against sars-cov-2 transmission **ECaS**

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Binding and neutralizing antibodies against the spike (S) protein of SARS-CoV-2 have been associated with a reduced risk of symptomatic disease. However, the precise level of immunity required to prevent infection remains unclear. Here we present a household human cohort study of COVID-19. Fifty-two families were enrolled for a total of 191 subjects: 52 (26.66%) index cases and 139 (73.34%) exposed individuals. Serum and paired nasopharyngeal swabs (NPS) were collected at baseline, and at day 3, 7 and 28. Immunoglobulin subtyping (IgG, IgM, IgA, sIgA) against S1 and S2 domains of the S protein of SARS-CoV-2 and HCoV-OC43 was performed in serum and upper respiratory compartment. Our data showed that 99(71%) of the exposed susceptible contacts were infected while 40 (20.9%) remained negative. Interestingly, negative cases had detectable levels of pre-existing anti -S1 and -S2 SARS-CoV-2 antibodies at baseline in serum. In the systemic compartment, modelling of the protection of S1 vs S2 baseline antibody levels by a binomial logistic regression showed that the probability of disease occurrence decreased with increased anti- SARS-CoV-2 S titers. Besides, serum anti-HCoV-OC43 showed also protective potential protection. However, a multivariate logistic regression adjusted by potential cofounding factors demonstrated that only anti-S1 IgG and anti- HCoV-OC43-S2 IgM correlated with protection from SARS-CoV-2 transmission. More importantly, antibody profiling at the upper respiratory tract showed that all immunoglobulin subtypes quantified against the S protein of SARS-CoV-2 provided protection from transmission, while only HCoV-OC43 IgA and sIgA showed protective potential. Our study offers a detailed immunological profile of individuals exposed to SARS-CoV-2 and provides new insights into antibody correlates of protection. These data may serve as a valuable resource for defining thresholds of protective immunity and guiding the development of next-generation vaccines.

INNATE AND ADAPTIVE IMMUNITY
SCS01 • AUDITORIUM 1 - PLENARY HALL • MON 20 OCT 2025 - 11:30 - 13:00

Obesity modulates tracheal and cardiac responses to influenza A virus infection ECaS

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BACKGROUND

Obesity increases both susceptibility to and severity of influenza A virus (IAV) infection. While IAV targets epithelial cells of the respiratory tract, it is also associated with cardiovascular complications including cardiac pathology. Fundamental molecular mechanisms and the influence of host factors on IAV-induced cardiac pathology remain poorly understood. In this study, we investigate the impact of obesity on IAV-associated cardiac alterations in a large animal model of obesity.

METHODS

Twenty-six castrated male Göttingen minipigs were fed either a standard chow diet or a high-fat, high-fructose, high-cholesterol diet for 103 days. On day 99, animals from each dietary group were inoculated with either H1N2 influenza virus (A/swine/Denmark/12687/2003) or virus-free medium. Viral RNA levels in trachea and the heart were assessed using qPCR. Tissue specific gene expression was studied using RNA-seq and microfluidic qPCR, supplemented by hematoxylin and eosin (H&E) staining to characterize IAV-induced changes in tracheal and cardiac samples from lean and obese animals.

RESULTS

Obesity had minimal impact on gene expression in tracheal tissue but was associated with transcriptional changes and atherosclerosis in the heart. Four days after IAV infection viral RNA levels in the trachea were increased in the obese animals concurrently with 100 more genes being significantly regulated, accompanied by histological evidence of cilia loss and mononuclear cell infiltration in the submucosal layer. The cardiac response of the obese pigs after IAV infection comprised significantly altered gene expression relative to lean pigs with IAV infection, particularly affecting pathways involved in cellular processes involved with stress response and inflammation.

Conclusion: These findings suggest that obesity, as compared to the lean state, is associated with an altered transcriptional response to IAV in both trachea and in the heart. Myocardial gene expression is more affected than tracheal gene expression by obesity alone. Following IAV infection, obese animals showed an altered transcriptional response in both tissues with increases in viral RNA levels, immune cell infiltration, and transcriptional changes in trachea. The heart of obese pigs exhibited pronounced changes in gene expression, particularly in pathways related to stress and inflammation.

INNATE AND ADAPTIVE IMMUNITY
SCS01 • AUDITORIUM 1 - PLENARY HALL • MON 20 OCT 2025 - 11:30 - 13:00

Prior influenza vaccination shapes subsequent vaccine responses in a randomized placebo-controlled trial

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BACKGROUND
Antibody responses to influenza vaccination vary between people and in the same person from one vaccine to the next. Prior vaccination is associated with some of this variation: antibody responses are often lower in repeat vaccinees compared with those not recently vaccinated. It is unclear how much this effect arises from confounders or reflects a true blunting of responses by prior vaccination. These differences might arise in observational studies, for instance, if non-repeat vaccinees have more infections that strengthen future vaccine responses. It is also unclear whether each person responds consistently to different vaccine components or to the same component in different years.

METHODS
We conducted a randomized, placebo-controlled trial of repeated influenza vaccination in healthy adults in Hong Kong. Participants received either a placebo (P) or a recombinant influenza vaccine (V) in each year to establish groups with different vaccination histories (PPPPV, PPPVV, PPVVV, PVVVV, VVVVV). We measured vaccine responses in the first 4 years of the study using hemagglutination inhibition (HAI) and neutralization (FRNT) assays. Non-pharmaceutical interventions against SARS-CoV-2 prevented influenza transmission in Hong Kong and thus eliminated differences in infection rates between groups during the first 3 rounds of annual vaccination. We developed Bayesian hierarchical models to estimate the effects of prior vaccination independently of pre-vaccination titers, accounting for infections once influenza transmission resumed. These models estimate the variance in peak post-vaccination titers attributable to individual and general effects and the impact of vaccination on long-term vaccine responses.

RESULTS
Vaccine-induced antibody boosts were smaller in people with higher pre-vaccination titers, consistent with previously reported “antibody ceiling” effects. However, for the same pre-vaccination titer, each prior vaccination reduced peak post-vaccination titers to some vaccine strains by up to 1.7 log2 units (95% CrI 1.5-2.0), contributing to higher titers in non-repeat compared with repeat vaccinees for up to a year after vaccination. After adjusting for prior vaccination, baseline titers and other covariates, there is weak consistency in a person’s response to different vaccine components and to the same component from one vaccine to the next: Individual effects account for 7% (95% CrI 6-8%) of the total variation in post-vaccination antibody titers, corresponding to 18% (95% CrI 17-20%) of the variation not explained by other factors.

CONCLUSIONS
These results show that prior vaccination decreases post-vaccination antibody titers independently of its effect on pre-vaccination titers. The impacts of prior vaccination are not due to higher infection rates in non-repeat vaccinees. We will use the models to further investigate the consistency of individual responses across years and vaccine components.

PANDEMIC PREPAREDNESS

SCS02 • AUDITORIUM 2 - BREAKOUT • MON 20 OCT 2025 - 11:30 - 13:00

Observed Immunogenicity After Two Doses of an MF59-Adjuvanted Cell Culture–Derived H5N8 Influenza Vaccine (aH5N8c) in Healthy Subjects Aged ≥18 Years

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BACKGROUND

Increasing transmission of avian influenza H5N1 from wildlife and livestock to humans has been observed. This highly pathogenic virus and its genetic reassortants are considered a global threat due to the lack of immunity to H5 viruses in the world population. We assessed the immunogenicity of two doses of an MF59-adjuvanted, cell culture–derived H5N8 A/Astrakhan/3212/2020 influenza vaccine (aH5N8c). This vaccine is part of the Biomedical Advanced Research and Development Authority's pandemic influenza vaccine stockpile. Immunogenicity after a booster dose at 6 months and safety results are reported separately.

METHODS

This Phase 2, randomized, observer-blind, multicenter study enrolled 479 subjects, of whom 239 subjects received two doses of aH5N8c. Participants included 121 aged 18–64 years (60 of whom were poultry workers) and 118 persons aged ≥65 years. Serum samples were collected at baseline (Day 1), Day 22, and Day 43 to assess antibody responses with hemagglutination inhibition (HI) and microneutralization (MN) assays. Primary endpoints included HI and MN responses against H5N8 at Day 43. Analyses included geometric mean titers (GMTs), geometric mean fold increases (GMFIs), seroconversion rates (SCRs), and percentages achieving titers ≥1:40. Subgroup analyses were conducted by age, poultry worker status, prior influenza vaccination, sex, and race.

RESULTS

Table 1: Immunogenicity at Day 43 following two doses of aH5N8c

	Hemagglutination inhibition assay				Microneutralization assay			
Age cohort	GMT (95% CI)	GMFI (95% CI)	SCR, % (95% CI)	HI≥1:40, % (95% CI)	GMT (95% CI)	GMFI (95% CI)	SCR, % (95% CI)	MN≥1:40, % (95% CI)
18–64 years	71.7 (56.7–90.5)	13.7 (10.9–17.4)	75.8 (66.1–83.8)	75.8 (66.1–83.8)	157.6 (113.7–218.4)	19.2 (13.8–26.7)	80.6 (68.6–89.6)	83.9 (72.3–92.0)
≥65 years	64.0 (47.1–87.1)	10.2 (7.4–14.0)	71.0 (61.1–79.6)	73.0 (63.2–81.4)	141.3 (94.0–212.2)	11.1 (7.4–16.6)	70.0 (56.8–81.2)	86.7 (75.4–94.1)

Abbreviations: CI, confidence interval; GMT, geometric mean titer; GMFI, geometric mean fold increase; SCR, Seroconversion Rate; HI, hemagglutination inhibition; MN, microneutralization.

Acknowledgement: This project received federal funds from The Center for the Biomedical Advanced Research and Development Authority (BARDA)

Following the first dose of aH5N8c, an increase in HI titers was observed, with GMFIs of 3.8 in the 18–64 years cohort and 5.6 in the ≥65 years cohort at Day 22. Following the second dose, HI titers continued to rise, reaching GMFIs of 13.7 in younger and 10.2 in older adults at Day 43. SCRs were 75.8% in younger adults and 71.0% in older adults; percentages achieving HI titers ≥1:40 were 75.8% and 73.0%, respectively (Table 1). MN titers as assessed in a subset were generally higher than HI titers, showing robust antibody responses across age cohorts at Day 43. MN GMTs exceeded HI GMTs, with Day 43 MN GMTs of 157.6 in younger adults and 141.3 in older adults. MN SCRs reached 80.6% in younger and 70.0% in older adults (Table 1). No notable differences in immune responses by age, poultry worker status, prior influenza vaccination, sex, or race were observed; baseline titers were uniformly low across subgroups.

CONCLUSIONS

Two doses of aH5N8c three weeks apart elicited strong antibody responses by Day 43 as measured by HI and MN assays. Responses were consistent across age cohorts and subgroups, including poultry workers and those with prior influenza vaccination history. These data further support MF59-adjuvanted cell-based monovalent influenza vaccines as an important, immunogenic public health tool against H5 influenza.

Acknowledgement: This project received federal funds from The Center for the Biomedical Advanced Research and Development Authority (BARDA)

PANDEMIC PREPAREDNESS
SCS02 • AUDITORIUM 2 - BREAKOUT • MON 20 OCT 2025 - 11:30 - 13:00

Immunogenicity of Homologous or Heterologous Booster Vaccinations with MF59-Adjuvanted, Cell Culture–Derived H5N8 or H5N6 Influenza Vaccines in Healthy Subjects Aged ≥18 Years

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BACKGROUND

The highly pathogenic avian influenza H5N1 subtype and its genetic reassortants are considered a global threat due to their ability to infect humans and the lack of immunity to H5 viruses in the human population. We evaluated prime-boost regimens comprising two MF59-adjuvanted, cell culture–derived, H5 vaccines containing either H5N8 A/Astrakhan/3212/2020 (aH5N8c) or H5N6 A/Guangdong/18SF020/2018 (aH5N6c). Both vaccines are part of the Biomedical Advanced Research and Development Authority's pandemic influenza vaccine program. Primary immunogenicity (Days 1–43) and safety results have been presented separately.

METHODS

This Phase 2, randomized, observer-blind study assessed the immunogenicity of aH5N8c booster vaccination administered 6 months after homologous or heterologous priming regimens in 479 adults aged ≥18 years. Participants were randomized to three priming regimens given on Days 1 and 22: Arm A, homologous priming with two doses of aH5N8c (n=239); Arm B, heterologous priming with aH5N8c→aH5N6c (n=120); Arm C, heterologous priming with aH5N6c→aH5N8c (n=120). All participants received aH5N8c booster on Day 202. Hemagglutination inhibition (HI) and microneutralization (MN) antibody responses against H5N8 were assessed at Day 223 (3 weeks post-booster). HI responses were also assessed on Day 209 (7 days post-booster) and Day 382 (6 months post booster). Immunogenicity endpoints included geometric mean titers (GMTs), geometric mean fold increases (GMFIs), seroconversion rates (SCRs), and the percentage of subjects achieving HI/MN titers ≥1:40. Analyses were conducted for the overall population and stratified by age cohort (18–64 years and ≥65 years).

RESULTS

As early as 7 days after aH5N8c booster vaccination, robust antibody responses against H5N8 were observed in all treatment groups, with Day 209/202 HI GMFIs of 4.1, 7.1, and 4.9 in Arms A–C, respectively. HI titers continued to rise, with Day 223/Day 202 HI GMFIs of 5.4, 10.7, and 6.4, in Arms A–C, respectively. The Day 223/202 HI SCRs ranged from 57.7% to 73.5%, and subjects with HI titer ≥1:40 ranged from 82.3% to 86.9%. Day 223/Day 202 MN GMFIs were 8.4, 18.7, and 12.0 in Arms A–C, respectively, with SCRs from 79.6 to 85.9% and MN titers ≥1:40 ranging from 91.4% to 93.8%. HI titers were higher 6 months post-booster (Day 382) than pre-booster values at Day 202 (Table 1). Robust responses were seen in both age groups : Day 223/202 HI SCRs ranged from 67.4% to 85.1% in persons 18–64 years and 47.7% to 62.7% in ≥65 years, while MN SCRs ranged from 85.7% to 96.3% and 71.0% to 83.9% in the younger and older age groups, respectively.

CONCLUSIONS

A single aH5N8c booster dose administered 6 months after priming elicited rapid and robust immune responses across all treatment arms and age cohorts, supporting use of aH5N8c as part of pandemic preparedness planning for H5 influenza viruses.

Acknowledgement: This project received BARDA federal funds

PANDEMIC PREPAREDNESS
SCS02 • AUDITORIUM 2 - BREAKOUT • MON 20 OCT 2025 - 11:30 - 13:00

When can sequence data confirm transmission links in respiratory virus outbreaks? ECaS

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BACKGROUND
Genomic sequencing has become an increasingly important tool for investigations into acute respiratory pathogen outbreaks, particularly for corroborating putative epidemiological transmission events. The fundamental principle underlying the use of genomic sequencing for transmission linkage is that greater genetic similarity between pathogen genomes increases the likelihood of a shared transmission chain. However, unlinked infections can also exhibit highly similar, or even identical, genomes. Commonly used single nucleotide polymorphism (SNP) thresholds only assess whether transmission is plausible given the genetic data in binary terms; they either support or refute transmission. To formally quantify how predictive sequencing data is of a transmission link, one must also account for pathogen prevalence, population-level pathogen genetic diversity, and the strength of the epidemiological signal of transmission. Currently, there is no formal framework to quantify confidence in a transmission link that accounts for these factors. Here, we developed such a formalism and used it to quantify when sequence data do and do not yield sufficient information to confirm or rule out a transmission link for acute respiratory viruses.

METHODS
We quantified sequence data's 'information value' using the concept of likelihood ratios. The likelihood ratio (LR) for a SNP distance quantifies how frequently it is observed in true transmission pairs compared to false transmission pairs. If LR > 1, the SNP distance gives evidence in favor of a link; if LR < 1, it gives evidence to the contrary. Using a mathematical model, we calculated LR and the 'pre-test probability' (that two infections are linked based on the epidemiological link alone) throughout the course of an epidemic. Mathematically, combining the pre-test probability with the likelihood ratio yields the overall probability that two infections are linked. We investigated how the pre-test probability and likelihood ratio depend on evolutionary and epidemiological variables.

RESULTS
We found that the information value of sequence data is highly context-dependent. For example, in a SARS-CoV-2-like epidemic, a genetic distance of 2 SNPs could yield evidence against transmission in the early epidemic, but be evidence in favor of transmission a few weeks later. Similarly, the probability that two infections are linked based on epidemiological signal alone varies by orders of magnitude depending on pathogen prevalence in the population and the strength of the contact link. As a result, the positive predictive value of finding similar viruses for transmission linkage varies extremely strongly by epidemic phase, the nature of the contact link, and genetic similarity. Using our framework, this positive predictive value can be explicitly calculated.

CONCLUSIONS
Accounting for population-level pathogen epidemiological and evolutionary dynamics is essential to ensure that sequence data are accurately interpreted in outbreak investigations. Our framework offers a statistically robust way to quantify confidence in transmission events that accounts for these variables.

PANDEMIC PREPAREDNESS

SCS02 • AUDITORIUM 2 - BREAKOUT • MON 20 OCT 2025 - 11:30 - 13:00

Broad-Spectrum Baseline Immunity Against Highly Pathogenic Avian Influenza A(H5N1) Clade 2.3.4.4b Viruses in Dutch Healthcare Workers: Insights into Both Humoral and Cellular Immune Responses ECaS

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BACKGROUND

Highly pathogenic avian influenza (HPAI) A(H5N1) clade 2.3.4.4b viruses pose a major public health threat. While humans should predominantly be immunologically naïve for A(H5N1) viruses, they are frequently exposed to seasonal influenza viruses by natural infection or are vaccinated against them. Whether immune responses induced by exposure to or vaccination against seasonal influenza viruses have the potential to modulate the course and severity of A(H5N1) infections in humans is unknown. Most studies that have explored the presence of pre-existing population immunity to A(H5N1) viruses have been fragmented, typically investigating either antibody or T-cell cross-immunity, thereby providing an incomplete picture. Here, we employed an immune profiling approach to assess both humoral and cellular immune responses targeting recent A(H5N1) clade 2.3.4.4b viruses in an adult cohort of healthcare workers (HCWs).

METHODS

A(H5N1) baseline population immunity was evaluated in blood samples obtained from a HCW cohort (n=117; 21-67 years) in August or September 2024. HA-binding antibodies were measured by protein microarray (PMA) and functional antibodies targeting HA or NA through hemagglutinin inhibition (HI), neuraminidase inhibition (NI), and antibody-dependent cellular cytotoxicity (ADCC) assays. T-cell responses were measured by an interferon-gamma release assay (IGRA).

RESULTS

HI antibodies targeting recent seasonal influenza viruses were highly prevalent in HCWs (responder rate: 65-95%; geometric mean titer (GMT): 20-90). In contrast, A(H5)-specific HI antibodies were absent in all participants, which correlated well with the lack of binding antibodies to A(H5) HA1 in PMA. Nonetheless, binding antibodies targeting the HA0 of A(H5) clade 2.3.4.4b viruses were found in 62-100% of participants (GMT: 33-333), probably due to HA stalk cross-reactive antibodies that might exert functionality through Fc mechanisms. Indeed, A(H1)- and A(H5) clade 2.3.4.4b-reactive ADCC-mediating antibodies were detected and exhibited a strong correlation. As opposed to HA, high responder frequencies and robust levels of neutralizing antibodies targeting seasonal (responder rate: 96-99%; GMT: 42-291), and avian (responder rate: 96-99%); GMT: 183-201) N1s were observed; the N1 neutralizing antibodies highly correlated. Similarly, T-cell responses directed towards overlapping peptide pools of A(H5) clade 2.3.4.4b or avian N1s were detected in 49-66% and 63-70% of the participants, respectively, and correlated well to responses against seasonal HA or N1 peptide pools.

CONCLUSIONS

These findings demonstrate the abundance of binding antibodies to A(H5) clade 2.3.4.4b that can harbour Fc-effector functions, inhibiting antibodies to avian N1s, and T-cell responses against both in HCWs. The strong correlations between responses against seasonal and avian influenza virus antigens suggest cross-reactive immunity, probably attributed to prior seasonal A(H1N1) exposure. Altogether, this study highlights the presence of partial pre-existing humoral and cellular immunity to A(H5N1) clade 2.3.4.4b viruses in the general population, which might blunt severe disease. This study further underscores the importance of baseline immunity screenings for future pandemic preparedness.

PANDEMIC PREPAREDNESS

SCS02 • AUDITORIUM 2 - BREAKOUT • MON 20 OCT 2025 - 11:30 - 13:00

Evaluating Cross-Protective Antibody Responses to Influenza A(H5N8) Vaccine in High-Risk Groups ECaS

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BACKGROUND

Since 2020, the geographic and host range of highly pathogenic avian influenza (HPAI) clade 2.3.4.4b A(H5Nx) viruses has expanded, with outbreaks among various bird species across Asia, Europe, Africa, the Americas, and even Antarctica. These viruses frequently spill over to mammals, reiterating their zoonotic potential. In 2023, Finland experienced a clade 2.3.4.4b A(H5N1) outbreak, which spread from wild birds to farmed fur animals. Vaccination of high-risk groups, including fur farm and poultry workers, veterinarians, bird ringers, and laboratory personnel exposed to A(H5) HPAI viruses, began in June 2024 using the MF59-adjuvanted inactivated vaccine from Seqirus, based on A(H5N8) A/Astrakhan/3212/2020 virus.

METHODS

This study investigated antibody responses following a two-dose vaccination regimen in 39 subjects. Blood samples were collected before vaccination and three weeks after each dose. Nine participants had previously received two to six doses of A(H5N1) vaccines between 2009 and 2018. We assessed functional antibody responses using microneutralization (MN) and/or haemagglutinin inhibition (HI) assays against the homologous clade 2.3.4.4b A(H5N8) A/Astrakhan/3212/2020 virus, and also clade 1 A(H5N1) A/Vietnam/1203/2004 vaccine virus, clade 2.3.4.4b A(H5N1) A/blue fox/UH/004/2023 and A/Texas/37/2024 viruses isolated during recent outbreaks in fur animals in Finland and cattle in the United States.

RESULTS

Vaccination induced comparable levels of functional antibodies against the homologous vaccine virus and the two other clade 2.3.4.4b viruses. In previously unvaccinated participants, antibody responses against the homologous vaccine virus were significantly elicited after the first dose, with 47% and 73% of participants reaching seroprotection levels based on MN and HI, respectively. After the second dose, antibody levels were boosted, with seroprotection rates of 83% (MN) and 97% (HI). However, vaccination did not induce cross-reactive antibodies against clade 1 A/Vietnam/1203/2004 (MN). In previously unvaccinated participants, a single vaccine dose elicited a significant antibody response against both A/blue fox/UH/004/2023 and A/Texas/37/2024 viruses, with a further increase in antibody levels after the second dose. All previously vaccinated participants achieved seroprotective antibody levels after a single vaccine dose, indicating a robust recall response. These elevated levels persisted following the second dose. A single dose elicited substantial levels of cross-reactive neutralizing antibodies against clade 1 A/Vietnam/1203/2004. Significant increases in antibody levels against both A/blue fox/UH/004/2023 and A/Texas/37/2024 were observed after the first dose in the previously vaccinated participants and remained elevated after the second dose.

CONCLUSIONS

This study demonstrates that the MF59-adjuvanted inactivated A(H5N8) vaccine is expected to confer cross-protection against currently circulating clade 2.3.4.4b H5N1 viruses, but no cross-protection against heterologous clade 1 viruses in previously H5 naïve participants. These findings are significant for occupational health, highlighting the vaccine's potential to protect individuals at increased risk of exposure, by enhancing immune preparedness and reducing vulnerability in high-risk environment.

PANDEMIC PREPAREDNESS
SCS02 • AUDITORIUM 2 - BREAKOUT • MON 20 OCT 2025 - 11:30 - 13:00

OVX836, a Nucleoprotein based vaccine under clinical development, offers protection against Highly Pathogenic Avian Influenza viruses (H5N1 and H7N9) in mouse and ferret models

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BACKGROUND

Influenza A viruses evolution and diffusion pose a significant threat to animal and human health. Zoonotic transmission, particularly from avian strains as H5N1, H7N9, or H9N2, remains a major pandemic risk. While neutralizing antibodies targeting the hemagglutinin (HA) surface protein offer effective protection, HA-based vaccines provide limited coverage against antigenically divergent strains.

Osivax is developing OVX836, a recombinant vaccine targeting the highly conserved nucleoprotein (NP). In humans, OVX836 elicited strong NP-specific T-cells and antibody responses, with a preliminary efficacy signal of protection of 84%.

Here we evaluated the protection provided by OVX836 alone and with SQ adjuvant (a squalene emulsion with QS21 saponin) against H5N1 and H7N9 in pre-infected mice and ferrets. These models reflect human pre-existing NP immunity from repeated seasonal influenza infections.

METHODS

Mice pre-infected with Mouse-Adapted-H3N2-A/Hong-Kong/1/1968, were immunized twice, 21 days apart with either OVX836(30µg), OVX836+SQ(15µg) or a negative control. Twenty-one days after, mice were challenged with 100.6 TCID50 of H5N1-A/Hong-Kong/156/1997 via intranasal instillation. Clinical signs, body weight and survival were monitored daily for 14 days.

Ferrets who were naturally exposed to Influenza and had confirmed haemagglutination-inhibiting antibody response against pH1N1 were immunized twice, 21 days apart with either OVX836(300µg), OVX836+SQ(150µg) or a negative control. NP-specific immune responses were assessed before and after immunizations, by ELISA for anti-NP IgG, and using IFNγ ELISpot for T-cell responses. Twenty-one days after the last immunization, ferrets were challenged with 105 TCID50 of H7N9-A/Anhui/1/2013 via intratracheal instillation. Seven days post-challenge, ferrets were euthanized, then lung examined through gross pathology/histopathology and for viral load (via RT-PCR and TCID50 assays).

RESULTS

Pre-exposure to H3N2-MA virus did not protect mice from H5N1 infection. Indeed, 83% of the mock-immunized mice were euthanized 6 days post-challenge as they lost over 20% of their initial weight. Half of those receiving OVX836 recovered and survived, and 83% of the OVX836+SQ vaccinated mice survived the challenge.

In ferrets, lungs scored for gross-pathology seven days post-H7N9 challenge showed moderate lung damage on average for mock-vaccinated ferrets, ranging from slight to complete. OVX836-vaccinated ferrets had milder damage, from slight to moderate, while OVX836+SQ-vaccinated ferrets exhibited only slight lesions.

OVX836 vaccination boosted NP-specific IgG and T-cell responses. NP-specific IgG titers reached 3.56 Log10 (OVX836) and 3.96 Log10 (OVX836+SQ), with a significant difference. Pre-immunization T-cell responses increased from 72 NP-specific IFNγ SFU/10^6 PBMC to 1372 (OVX836) and 1525 (OVX836+SQ) on average post-vaccination.

CONCLUSION

OVX836, a NP-based vaccine, showed strong efficacy against H5N1 and H7N9 infections, in animals pre-exposed to a seasonal influenza virus. Adding SQ adjuvant allowed antigen dose-sparing and further enhanced protection, improving mouse survival rates and reducing lung damage in ferrets. These promising results highlight OVX836's potential as a broadly protective influenza A vaccine candidate, especially valuable for pandemic preparedness.

FUTURE VACCINATION STRATEGIES - PART 1

SCS03 • AUDITORIUM 1 - PLENARY HALL • MON 20 OCT 2025 - 14:30 - 16:00

A Dendritic Cell-targeting Approach to Deliver a Universal Influenza Vaccine Candidate to the Respiratory Mucosa ECaS

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BACKGROUND

Seasonal influenza vaccines require annual updates due to antigenic drift and waning immunity, highlighting the need for a universal flu vaccine targeting conserved viral epitopes. The extracellular domain of M2(M2e) is a leading universal vaccine antigen candidate, but its weak immunogenicity hinders clinical development. To enhance M2e immunogenicity, we developed a dendritic cell(DC)-targeting vaccine by fusing M2e to a Clec9A-specific monoclonal antibody(Clec9A-M2e). This study evaluates Clec9A-M2e's ability to elicit robust and durable immune responses in young and aged mice, including those with pre-existing flu immunity.

METHODS

- Clec9A-M2e: Three copies of M2e were fused in tandem to the Clec9A monoclonal antibody's heavy chains.

- Immunization: Young(5-6 weeks-old) and aged(10-12 months-old) Balb/c mice received 2µg Clec9A-M2e adjuvanted with 50µg PolyI:C intratracheally in a prime-boost regimen(4-week interval).

- Immune assays: Systemic and mucosal M2e-specific antibody (IgG and IgA) responses were measured via ELISA, with antibody functionality assessed via an ADCC reporter assay. M2e-specific germinal centre(GC) B cells and follicular-helper T cells(TFH) were analyzed by flow cytometry, while antibody secreting cells(ASC) and T cell response were quantified by ELISPOT.

- Challenge experiments: Mice were challenged with lethal H1N1/PR8 at 7 days, 3, 6, and 9 months post-boost(MPB). Protection was evaluated via weight loss monitoring and lung viral titre reduction by plaque assay.

- Pre-existing immunity: Mice were infected with sublethal H1N1/PR8 before receiving a single or double (for aged mice) dose of Clec9A-M2e. M2e-specific immune responses were compared against flu-naïve counterparts.

RESULTS

Clec9A-M2e elicited strong systemic and mucosal M2e-specific responses in young mice, with robust ADCC activity sustained for 6MPB before declining at 9MPB. M2e-specific GC B cells and TFH were also detected up to 6MPB, but not at 9MPB. Consistently, lethal challenge at 7days, 3M, and 6M resulted in full protection, with minimal weight loss and significantly reduced lung viral titers in immunized mice. However, at 9MPB, only partial protection was observed.

In aged mice, Clec9A-M2e induced significant but lower M2e-specific antibody, T cell responses, and ADCC activity, correlating with only 50% protection and moderate lung viral reduction upon challenge

In young mice with pre-existing flu immunity, a single Clec9A-M2e dose boosted M2e-specific antibody titres to levels comparable to prime-boosted flu-naïve mice. Aged mice with pre-existing immunity required two doses of Clec9A-M2e to achieve similar immune responses.

CONCLUSIONS

The Clec9A-targeting strategy enhances M2e immunogenicity, eliciting robust humoral and cellular immunity upon mucosal delivery. The vaccine conferred complete protection in young mice with durable responses sustained for 6 months post-boost. However, aged mice achieved only partial protection with prime-boost immunization. Clec9A-M2e also significantly boosted pre-existing M2e-specific immunity from prior flu infection. These findings support Clec9A-M2e as a promising nasal universal influenza vaccine candidate capable of overcoming M2e's weak immunogenicity.

FUTURE VACCINATION STRATEGIES - PART 1
SCS03 • AUDITORIUM 1 - PLENARY HALL • MON 20 OCT 2025 - 14:30 - 16:00

Development of a next-generation COVID-19 intranasal vaccine based on a multivalent NDV-HXP-S formulation. ECaS

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BACKGROUND
Live-attenuated intranasal vaccines offer great promise to generate mucosal immunity and reduce breakthrough infections after vaccination. Previously, we developed a Newcastle Disease Virus (NDV)-based vaccine expressing the spike (S) protein of SARS-CoV-2, named NDV-HXP-S. This live-attenuated vaccine can be administered intranasally to provide both systemic and mucosal immunity. With the emergence of new SARS-CoV-2 variants of concern, we have developed new NDV-HXP-S variant-based constructs. The aim of this work is to combine these constructs in a multivalent formulation to extend protection against additional SARS-CoV-2 strains.

METHODS:
NDV-HXP-S vaccines covering main SARS-CoV-2 sub lineages were designed and combined in different monovalent and multivalent formulations. To study the replication kinetics of NDV-HXP-S, we developed an amplicon-based Next Generation Sequencing (NGS) technique to quantify the relative percentage of each variant after infection in Vero-E6 cells. Next, we studied NDV-HXP-S mucosal and systemic immunogenicity in the mouse model following a prime-boost intranasal vaccination regimen. Cross-reactivity against a diverse panel of SARS-CoV-2 variants was evaluated using a pseudotyped virus neutralization assay measured from blood serum samples collected four weeks post boost.

RESULTS:
NDV-HXP-S expressing the spike protein of Ancestral, Beta, Gamma, Delta, Omicron BA.1, Omicron BA.5, EG.5, BQ.1.1, XBB.1.5, JN.1 and KP.2 were produced. Replication studies in Vero-E6 cells showed a similar kinetic among all monovalent vaccines. When combined in different multivalent formulations, the same final viral titer as the monovalent vaccines were obtained at 72 hours post infection. However, NGS results showed different relative amounts in a formulation-dependent manner, suggesting competition during co-infection. In vivo, the combination of different variants extended the breadth of immunogenicity against phylogenetically distant SARS-CoV-2 variants of concern. Among all the multivalent formulations tested, a trivalent vaccine composed of Delta, XBB.1.5 and KP.2 NDV-HXP-S induced the highest degree of cross-reactivity.

CONCLUSION:
Overall, these results provide proof of concept for the development of mucosal multivalent NDV-HXP-S vaccines as next generation COVID-19 boosters. Furthermore, the developed methods will help the study of live-attenuated NDV-based vaccine replication and immunogenicity in future clinical trials.

Superior protection and prevention of transmission upon influenza infection by adjuvanted mucosal vaccination in the guinea pig influenza model.

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BACKGROUND

During epidemics and pandemics caused by respiratory viruses, vaccines ideally prevent both disease of the infected host, as well as transmission to new hosts. While current intramuscular influenza vaccines generate systemic immune responses, they provide limited protection at mucosal surfaces, reducing their potential to stop influenza virus replication at respiratory mucosa. This emphasizes the role of mucosal immunity in early protection and limiting transmission. Intranasal (IN) vaccination induces both mucosal and systemic immunity, offering advantages over parenteral vaccines.

METHODS

In this study, we assessed IN immunization in guinea pigs using a novel mucosal adjuvant nanoemulsion (NE) combined with IVT, a RIG-I agonist derived from the Sendai virus defective interfering RNA. Four animals per group were vaccinated twice at three-week intervals and challenged with the pandemic H1N1 virus (A/Netherlands/602/2009) four weeks after the final vaccination. The groups included IN administered PBS, IN trimeric recombinant hemagglutinin protein (triHA, A/Michigan/45/2015 H1N1) alone, IN triHA combined with NE/IVT adjuvant (NE/IVT/triHA), as well as intramuscularly (IM) administered triHA adjuvanted with AddaVax (MF59-like adjuvant). Finally, a standard of care control was included: a quadrivalent inactivated influenza vaccine (QIV) given via the IM route twice (prime/boost). Since there is strong interest in rerouting immune responses induced in the periphery by parenteral vaccination to the mucosal sites, we also tested boosting immune responses induced by IM QIV prime with IN NE/IVT/triHA. Finally, we evaluated the impact of pre-existing immunity by including animals previously infected with A/Michigan/45/2015 H1N1 virus. To test the impact of vaccination on prevention of virus transmission in a direct contact model, naive animals were co-housed with vaccinated, infected animals. Virus shedding and transmission, or lack thereof, was confirmed by virus titration in nasal washes, mucosal swabs and seroconversion of sentinel animals.

RESULTS

IN immunization with NE/IVT/triHA mucosal adjuvant elicited humoral responses comparable to IM AddaVax/triHA, and superior nasal mucosal IgA responses relative to all other groups. Boosting QIV vaccination with intranasal NE/IVT/triHA further enhanced serum IgG as well as mucosal IgA compared to QIV prime/boost. Intranasal NE/IVT/triHA vaccination induced the highest cross-neutralizing antibody (nAb) titers against the heterotypic A/Victoria/4897/2022 H1N1 strain. Contrary to QIV vaccination, both IM AddaVax/triHA and IN NE/IVT/triHA immunizations prevented detectable viral shedding post-challenge. Notably, naïve sentinel animals co-housed with IN NE/IVT/triHA-vaccinated guinea pigs were protected from virus transmission, remaining seronegative.

CONCLUSION

Our results demonstrate that the mucosal NE/IVT adjuvant platform, which is given intranasally and induces both robust systemic and mucosal immunity, can protect during direct infection, bolster intramuscular induced vaccine responses and prevent transmission to naïve sentinels.

FUTURE VACCINATION STRATEGIES - PART 1
SCS03 • AUDITORIUM 1 - PLENARY HALL • MON 20 OCT 2025 - 14:30 - 16:00

Broad betacoronavirus immunity and SARS-CoV-2 protection induced by divergent spike-based nucleoside-modified vaccines ECaS

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BACKGROUND
The emergence of zoonotic coronaviruses highlights the need for vaccines that elicit broad and durable protection. Here, we assessed the immunogenicity and cross-protective potential of nucleoside-modified vaccines encoding spike proteins from diverse coronaviruses (CoV) using a murine challenge model.

METHODS
Nineteen lipid nanoparticle (LNP)-formulated spike nucleoside-modified vaccines, each expressing a distinct spike antigen from the four coronavirus subgenera, were evaluated in a homologous prime-boost regimen. Mice were challenged with 5x the 50% lethal doses (LD50) of mouse-adapted severe acute respiratory syndrome coronavirus 2 (MA-10 SARS-CoV-2, USA/WA1/2020). Antibody binding to SARS-CoV-2 spike subdomains—receptor binding domain (RBD), N-terminal domain (NTD) and S2—and cross-binding and neutralizing antibodies to a panel of CoV spikes were assessed.

RESULTS
Sarbecovirus-based vaccines (e.g., SARS-CoV-2, SARS-CoV-1, HKU3-8) conferred full or partial protection after one dose and complete protection after boosting. Vaccines based on MERS-CoV, bat coronavirus GCCDC1, and bovine coronavirus (BCoV) spikes also conferred varying degrees of protection. Sarbecovirus spike vaccinated mice exhibited broad cross-reactive binding antibody responses, particularly within the sarbecovirus subgenus, while reactivity against alpha- and delta-CoV spikes was minimal. MERS-CoV spike vaccination elicited potent cross-reactivity within the merbecovirus subgenus and to a lower magnitude to other beta-CoV spikes in addition to gamma-CoV HKU15 spike. GCCDC1 and BCoV spike induced moderate to high cross-reactivity across the beta-CoVs. Sarbecovirus vaccines induced strong RBD binding and broad S2 reactivity; non-sarbecovirus vaccines showed weaker NTD binding. Cross-neutralizing antibodies to SARS-CoV-2 were primarily detected in SARS-CoV-2 and SARS-CoV-1 vaccine groups. Limited neutralization of SARS-CoV-1, MERS-CoV and 229E pseudoviruses was observed with other CoV spike vaccines.

CONCLUSIONS
These findings support our strategy for a multivalent vaccine for a broad beta-CoV protection and pandemic preparedness.

The Additive Effect of Neuraminidase Inclusion in Influenza Vaccine Formulations using a Self-Amplifying RNA Platform

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Self-amplifying RNA vaccines provide a novel approach to inducing robust, long-lasting immunity1. Neuraminidase (NA) antibodies can inhibit viral release, reducing disease spread and severity, even if hemagglutinin (HA) -based immunity is compromised by antigenic drift. Furthermore, NA is less prone to antigenic variation, making it a more stable vaccine target for broader, more durable influenza protection. Conventional subunit influenza vaccines often contain variable, non-standardized amounts of NA antigen2. By incorporating standardized NA-encoding RNA, sa-mRNA vaccines can elicit potent and broadly protective immune responses.

We evaluated the benefits of incorporating NA into the sa-mRNA vaccine platform under both optimal and suboptimal HA antigen doses. In a prime-boost regimen, ferrets were vaccinated with increasing doses of a single HA antigen (2.5, 0.25, and 0.025 µg) or a combination vaccine expressing both HA and NA proteins, while maintaining a constant NA dose (2.5 µg). Following homologous virus challenge with A/California/04/2009 H1N1 (CAL09), ferrets vaccinated with the NA construct alone exhibited significantly reduced morbidity. Viral loads were reduced in ferrets vaccinated with high-dose HA alone or in combination with NA. However, the added benefit of the NA was most evident when the HA was present at suboptimal doses, as evidenced by a significant reduction in viral loads at days 1 and 5 post-inoculation compared to the HA-only vaccination. Similarly, ferrets receiving the dual-antigen vaccine (HA + NA) exhibited lower viral lung burden at high and medium doses compared to their single antigen counterparts. Serological results further demonstrated that dual-antigen vaccinated ferrets had higher HAI and MN titers at medium and low doses compared to their single HA-antigen counterparts.

In a second experiment, we examined the additional benefits of including NA in cases of HA-vaccine mismatch. Ferrets were vaccinated with heterologous HA sa-mRNA monocistronic constructs containing either a single HA or a dual vaccine HA/NA antigen at a 2.5 µg each, followed by a CAL09 virus challenge. Ferrets that received the single antigen NA vaccine exhibited reduced body weight loss and fever compared to PBS control. Similarly, ferrets vaccinated with the dual HA/NA antigen vaccine - heterologous to the challenge virus - exhibited a shorter duration of virus shedding in nasal washes and tissues.

Overall, our findings highlight the advantages of including NA in influenza vaccine, particularly in the sa-mRNA platform. NA enhances protection by reducing morbidity and viral shedding, especially in case of HA antigenic mismatch or suboptimal HA directed immunity. These findings underscore the potential of neuraminidase as an important component of influenza vaccines, particularly within the framework of self-amplifying RNA technology, where standardized and optimized antigen expression can be precisely controlled.

FUTURE VACCINATION STRATEGIES - PART 1
SCS03 • AUDITORIUM 1 - PLENARY HALL • MON 20 OCT 2025 - 14:30 - 16:00

Evaluation of a broadly protective influenza B virus vaccine based on mosaic hemagglutinin platforms in mice. ECaS

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BACKGROUND

Influenza B viruses cause significant respiratory disease annually, with vaccination being a key strategy to reduce their clinical and socio-economic impact. Recent efforts have focused on developing broadly protective influenza B vaccines. In this context, we explored two vaccine strategies: mRNA-lipid nanoparticles (mRNA-LNPs) and inactivated virus-based vaccines.

METHODS

We first evaluated the immunogenicity of chimeric hemagglutinin and mosaic hemagglutinins using the mRNA-LNP platform. The chimeric constructs were designed by replacing the entire head domain of influenza B hemagglutinins with those from avian influenza virus hemagglutinins. The mosaic hemagglutinins had only the immunodominant epitopes in the heads exchanged. Following the same design, recombinant mosaic influenza B viruses were generated, inactivated, and formulated into vaccines, whose immunogenicity was tested in preclinical mouse models.

RESULTS

Sequential vaccination with mosaic hemagglutinin mRNA-LNPs elicited strong immune responses against conserved head and stalk epitopes. This strategy induced higher levels of cross-reactive antibodies, and antigen-specific CD4+ T cells compared to vaccination with chimeric hemagglutinins, leading to superior protection against the phylogenetically distant B/Lee/1940 virus strain in both passive serum transfer and direct challenge studies. Like the humoral immune responses observed after vaccination with mRNA-LNP, vaccination with mosaic hemagglutinins based on inactivated virus vaccines enhanced cross-reactive immunity against phylogenetically distant influenza B strains that translated in enhanced protection in passive serum transfer and direct challenge studies.

CONCLUSIONS

These preclinical studies underscore the potential of mosaic hemagglutinin-based vaccines and support their progression toward clinical trials for a universal influenza B virus vaccine.

VIRUS AND HOST FACTORS IN PATHOGENESIS - PART 1

SCS04 • AUDITORIUM 2 - BREAKOUT • MON 20 OCT 2025 - 14:30 - 16:00

Avian influenza viruses re-shape the hormonal landscape by inducing pulmonary CYP19A1 expression in golden hamsters

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BACKGROUND

Infection with avian influenza viruses (AIVs) in humans is associated with pneumonia and high mortality. Current outbreaks of H5N1 bird flu in wild birds, poultry, cattle, swine and sporadic infections of humans highlight the urgent need for developing effective countermeasures. Recently, we have identified the CYP19A1 gene (encoding the testosterone-to-estradiol converting aromatase enzyme) as a key driver of estrogen-regulated sex-specific lung inflammation in SARS-CoV-2 infection. In the present study, we established AIV infection in the golden hamster model and shed light on virus-induced changes of the hormonal landscape during the course of infection.

METHODS

We infected male and female golden hamsters intranasally with H5N1 (A/wildgoose/Germany-Nw/AI00581/2024) or H7N9 (A/Anhui/1/2023) AIV (106 pfu). Weight loss, survival and lung function were monitored longitudinally over 21 days. Organs were additionally collected at 3, 6 or 21 days post infection (d p.i.) to determine viral titers in the lung and extrapulmonary organs, to quantify pulmonary immune responses, CYP19A1 and sex hormone receptor (SHR) expression, and to measure circulating sex hormone levels.

RESULTS

Male and female hamsters developed considerable weight loss upon H5N1 AIV infection, while infection with H7N9 presented a rather mild phenotype. Female hamsters initially lost more weight in the early acute phase of infection but recovered slightly faster compared to male hamsters. Lung function was compromised in H5N1-infected male and female hamsters at 6 d p.i. (acute phase), but restored at later time points (recovery phase). H7N9 infection did not affect lung function in female hamsters, in contrast to males. Lungs harvested from H5N1 infected male and female hamsters presented severe tissue inflammation and increased levels of pro-inflammatory cytokines, compared to H7N9 infected lungs. Viral particles were detected in both H5N1 and H7N9 infected lungs at 3 d p.i. but not in any of the extrapulmonary organs (e.g. brain, liver, gonads) assessed. Viral lung titers were slightly elevated in male hamsters compared to females. H5N1 significantly upregulated pulmonary CYP19A1 expression independently of sex, while only a minor increase was observed after H7N9 infection. Induced CYP19A1 was accompanied by changes in the expression of tissue-resident SHRs (estrogen and androgen receptors). Finally, low testosterone and high estradiol levels were observed after both H5N1 and H7N9 infection in male hamsters.

CONCLUSIONS

Recently, we proposed a model wherein SARS-CoV-2 exerts multiple hits on the metabolic HPG axis, ultimately promoting estrogen-mediated lung inflammation. In contrast, changes in the hormonal landscape upon AIV infection seem to stem primarily from the induction of pulmonary CYP19A1 transcription. In the future, we will use our model to develop tailored therapeutic approaches by systematically screening aromatase inhibitors, compounds that interfere with estrogen signaling, and a combination thereof for their potential to mitigate disease in the case of an influenza pandemic.

VIRUS AND HOST FACTORS IN PATHOGENESIS - PART 1
SCS04 • AUDITORIUM 2 - BREAKOUT • MON 20 OCT 2025 - 14:30 - 16:00

Obesity increases the risk for severe COVID 19 – An obese hamster model to study sex-specific differences in the underlying pathophysiology

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BACKGROUND

A major risk factor for developing a severe course of COVID 19 is obesity. We and others have shown that SARS-CoV-2 disseminates from the lung into adipose tissue where it productively replicates. These findings suggest that adipose tissue serves as a yet unrecognized virus reservoir in SARS-CoV-2 infected animals. The overall increased virus load impedes virus clearance and disturbs lipid metabolism. Here, we wanted to establish an obese hamster model for SARS-CoV-2 infection mimicking obesity in humans to study the underlying pathophysiology.

MATERIAL & METHODS

Male and female Syrian hamsters were fed with an obesity inducing diet with high-fat and high-sugar (HFHS) for at least 16 weeks starting directly after weaning. As a control, littermates received a standard chow diet (defined as lean model). For infection experiments, obese hamsters were intranasally inoculated with SARS-CoV-2 or mock-infected with PBS. The animals were weighed and monitored daily for clinical signs of disease until 21 days post-infection (dpi). During the acute and recovery phase of infection, we determined the lung function, viral load and immune response of SARS-CoV-2-infected obese hamsters.

RESULTS

Obese male and female hamsters fed with HFHS diet displayed slightly higher total body weight compared to chow fed animals. Accordingly, we were able to observe significantly increased total organ weight of the lung, kidney, subcutaneous and brown adipose tissues in obese animals. Interestingly, only male hamsters fed with HFHS diet revealed significantly elevated total organ weight for visceral and gonadal adipose tissues. Furthermore, we only detected significantly higher cholesterol and triglyceride levels in the liver of male hamsters fed with HFHS diet but not in females.

SARS-CoV-2 infection in obese hamsters increased disease severity compared to lean animals. Obese hamsters infected with SARS-CoV-2 revealed a prolonged phase of weight loss and impaired recovery compared to infected lean animals. Furthermore, obesity elevated pulmonary viral load and immune response in SARS-CoV-2-infected hamsters.

Moreover, in obese male hamsters SARS-CoV-2 infection results in increased disease severity in comparison to obese females. This is reflected in the fact that infected obese male showed delayed recovery and significantly impaired lung function. Consistently, SARS-CoV-2 infection in obese male hamsters causes increased immune response and viral load in the lungs as well as in the extrapulmonary organs including adipose tissue.

DISCUSSION

In summary, we successfully established an obese hamster model to study SARS-CoV-2 pathophysiology in obesity and found a worsened and prolonged course of disease. This animal model can be utilized to evaluate efficacy of new antiviral intervention strategies in high-risk groups like obesity.

VIRUS AND HOST FACTORS IN PATHOGENESIS - PART 1
SCS04 • AUDITORIUM 2 - BREAKOUT • MON 20 OCT 2025 - 14:30 - 16:00

Functional Balance of HA and NA in Emerging Porcine Influenza A Viruses: Sialoreceptor Dynamics at the Mucosal Interface

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BACKGROUND

A key barrier to cross-species transmission of avian influenza A viruses (IAV) to humans is the shift in hemagglutinin (HA) receptor specificity from avian-type α2-3-linked sialic acids (2-3Sia) to human-type α2-6-linked sialic acids (2-6Sia). Epithelial cells present a mixture of both receptor types, making gradual adaptation through intermediate hosts plausible. Swine express both receptor types and their susceptibility to IAV strains of diverse origin makes them ideal mixing vessels for viral reassortment as witnessed by the genesis of the 2009 H1N1 pandemic.

Current swine IAVs (swIAVs) present a huge genetic diversity that remains underexplored in zoonotic risk assessments. As part of the ICRAD/EPICVIR consortium, this study investigates receptor-specific binding and infection dynamics of six contemporary Belgian swIAV isolates carrying reassorted gene segments of 2009 pandemic and Eurasian-avian origin. We focus on the functional balance between HA and neuraminidase(NA) that, given the distinct sialoglycome profiles, is hypothesized to be a key determinant in overcoming the swine-human species barrier.

In particular, the major role of mucus as a species-specific decoy receptor layer was assessed in detail in view of its relevance to the EPICVIR consortium aim to align molecular virology studies with transmission and pathogenesis studies.

METHODS

HEK293 cell lines expressing defined sialic acid glycotopes were engineered by systematic sialyltransferase knockout/knockin. Glycoproteins produced in these cells were used to measure, in real-time (biolayer interferometry), virus binding and NA-driven virus release allowing to quantify the HA/NA balance. In parallel, infection of the same cells provided the unique opportunity for a direct correlation with infection efficiency. Dilution series of mucus harvested from primary swine or human tracheal cultures were added to each assay to investigate how the different swine IAVs and a comparable human H1N1pdm09 can handle the inhibitory effects of these potential decoy receptors.

RESULTS

SwIAV strains prefered human-type 2-6Sia receptors, though two Eurasian avian-like strains displayed considerable 2-3Sia binding. HA and NA activities as well as their balance varied significantly between and within pandemic and Eurasian avian clades. Mucus only exerted extremely potent inhibition when NA activity was blocked by oseltamivir. Nevertheless, weak-binding strains showed reduced dependence on NA activity for mucus penetration, suggesting that their HA-NA-receptor balance is primarily tuned to the cell surface rather than being essential for escaping from mucus decoy receptors.

CONCLUSIONS

Swine IAVs display diverse HA-NA-receptor interactions with functional cell surface and decoy mucus receptors. Establishment of a HEK293 cell system allows to dissect the sialoglycan repertoire for use in correlative binding and infection studies. Mucus can be analysed in these assays to assess its yet enigmatic role as a host-specific barrier for influenza virus infections. The findings enhance our understanding of swIAV host adaptation and may inform surveillance for strains with zoonotic or pandemic potential.

VIRUS AND HOST FACTORS IN PATHOGENESIS - PART 1
SCS04 • AUDITORIUM 2 - BREAKOUT • MON 20 OCT 2025 - 14:30 - 16:00

Cell Tropism, Replication Dynamics, and effect on neural network activity to Seasonal and Pandemic Influenza A Virus Infection in a hiPSC-Derived Neural Co-Culture Model ECaS

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Influenza A virus (IAV) infection is associated with a wide variety of neurological complications, which vary from a severe encephalitis to mild complications like impaired cognitive functioning. There is substantial evidence that seasonal and pandemic IAVs can enter the central nervous system (CNS) through cranial nerves, but once inside the CNS, the cell tropism, replication efficiency and functional consequences are largely unknown. Therefore, the aim of our study was to investigate the interaction of seasonal and pandemic IAVs with cells of the CNS using a human induced pluripotent stem cell (hiPSC)-derived neural model, consisting of neurons and astrocytes. Analyses included electrophysiology to asses neural functioning using a multi-electrode array (MEA) system.

This hiPSC-derived neural co-culture model was inoculated with pandemic (pH1N1) or a seasonal IAV (H1N1 or H3N2 virus) after which we determined the cell tropism, replication kinetics and electrophysiology. All three viruses infected predominantly neurons, based on immune fluorescent staining for viral proteins. Inoculation of the co-cultures did not result in the production of detectable progeny virus, despite an increase of viral RNA over time. Virus-infected neurons could be detected up to 10 days post infection (dpi). The number of infected neurons remained stable over time, without morphological evidence for cell death or the induction of cleaved caspase-3. Functionally, pH1N1 virus infection of neural cultures resulted in electrophysiological changes, including a reduction in firing rate, burst frequency and network burst frequency in the first days after inoculation. Ongoing experiments are studying the effect of pH1N1 virus infection on long-term potentiation of neural activity.

Altogether, our data show that neurons are susceptible for seasonal and pandemic influenza viruses. Infection seems persistent in neurons and triggers electrophysiological changes. This study shows for the first time, although in vitro, that influenza viruses disrupt neural homeostasis, without efficient virus replication and cell death. These functional changes can contribute to neurological complications during influenza virus infections in the acute and potentially post-acute phase of the infection.

Single-cell profiling of inflammatory lung injury in clade 2.3.4.4b H5N1 influenza infected mice ECas

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INTRODUCTION

The highly pathogenic avian influenza (HPAI) virus H5N1 was first identified in 1996 and subsequently disseminate globally, leading to outbreaks in wild birds and domestic poultry and human infections worldwide. In 2024, an outbreak of clade 2.3.4.4b HPAI H5N1 in bovine herbs happened in USA and spillover into humans, elevating public health risk with further cross-species transmission. It is important to characterize the pathogenesis of clade 2.3.4.4b HPAI H5N1.

METHODS

Mice were intranasally infected with reverse genetically generated clade 2.3.4.4b cattle H5N1 virus. At 3 days post-infection, serum, nasal turbinate and lung tissue were collected for viral titer detection and cytokine profiling. Remaining lung tissue was enzymatically dissociated for single-cell RNA sequencing (scRNA-seq) to delineate host transcriptional responses.

RESULTS

Cattle H5N1 virus caused systemic infection in respiratory organs in mice. Using the scRNA-seq, we analysed the transcriptome of 17 cell types in the lung tissue of mice infected with cattle H5N1 virus or PR8 virus, respectively. Strong antiviral immune response was induced in lung tissue post cattle or PR8 virus infection, but less T cell response in H5N1 infection. Strikingly, cattle H5N1 infection induced extensive neutrophil increase and activated higher NLRP3 inflammasome level in neutrophils. Notably, we found that CXCL10 was highly expressed in neutrophil of H5N1 infected mice and secreted into systemic circulation, suggesting a potential role in neutrophil-mediated immunopathology.

CONCLUSION

Clade 2.3.4.4b cattle H5N1 virus induced extensive inflammatory lung injury. Single-cell RNA sequencing (scRNA-seq) analysis of 17 cell types in infected mouse lungs revealed that the virus triggered a robust antiviral immune response, a marked increase in neutrophil, elevated NLRP3 inflammasome levels within neutrophils, and high expression of CXCL10 highly expression in neutrophils, which was subsequently secreted into circulating system.

A history of obesity increases influenza severity via innate immune training in a canonical NLRP3-dependent manner

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BACKGROUND

Obesity significantly increases the risk of death following an influenza virus infection. Consistent with these clinical observations, we and others have shown that mice with diet-induced obesity develop much more severe influenza than their lean counterparts. Previously, it has been postulateded that this increased severity can be reversed by weight loss, but there is no experimental or observational evidence. Here, a mouse model was employed to study the long-term effects of obesity on anti-viral immunity.

Methods

Four-weeks old C57BL/6 mice were fed a high fat consisting of 43% calories from fat, or a lean diet consisting of 12% calories from fat for 10 weeks. After 10 weeks, mice fed a high-fat diet had a significantly higher total body weight and percentage body fat compared to mice fed the lean diet. Obese mice were then swapped to a lean diet for 10 weeks. Control groups received either high-fat or low-fat diets for 20 weeks. After 20 weeks, mice were infected with influenza A virus (A/Auckland/09(H1N1)) and monitored throughout the infection for changes to body weight and blood oxygen saturation. Following euthanasia, viral titers, and histopathology of the lungs were assessed, as well as systemic cytokine/chemokine responses. ATAC-seq and *ex vivo* stimulation was performed on bone marrow cells from all groups.

RESULTS

After 10 weeks on the lean diet, mice that were previously obese (PO) had a body weight and percentage body fat equivalent to mice that received the lean diet for the entirety of the 20-week treatment period. However, upon infection with influenza virus, PO mice displayed increased viral replication, lung inflammation, body weight loss and pulmonary dysfunction compared to lean mice. Importantly, deficiency in NLRP3 or its canonical pathway components, caspase-1 and gasdermin-D, blocked the long-term effect of murine obesity on susceptibility to severe influenza virus infection. To confirm a role for innate immune training, bone marrow cells from the mice were stimulated *ex vivo*. Bone marrow cells from PO mice showed heightened proinflammatory cytokine responses and changes in chromatin accessability. Bone marrow transfer from PO mice to recipient lean mice led to worsened clinical disease after influenza infection. Treatment of PO mice with an NLRP3 inhibitor, MCC950, partially reduced the severity of influenza virus infection and the *ex vivo* response of bone marrow cells.

CONCLUSIONS

We propose that obesity can have long-term, canonical NLRP3-dependent effects on innate inflammatory cells resulting in impaired anti-viral responses. Understanding the long-term effects that obesity has on anti-viral immunity will help pave the way for development of novel therapeutics to improve the health of the large proportion of the global population who have, or previously have been, obese.

BURDEN OF DISEASE IN ACUTE RESPIRATORY VIRUS INFECTIONS

SPI02 • AUDITORIUM 3 - BREAKOUT • MON 20 OCT 2025 - 14:30 - 16:00

Risk factors associated with severe health outcomes among older adults hospitalized with respiratory syncytial virus (RSV): understanding the pre-vaccine era landscape ECAS

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BACKGROUND

Studies in high-income countries indicate that older adults experience more RSV-related hospitalizations and deaths than younger age groups. Given aging population structures in many countries, updating adult-specific risk factors associated with poor RSV-associated health outcomes is critical. Since 2023, several countries have approved RSV vaccines for older adults, necessitating efforts to identify risk groups to prioritize for vaccination programs. We sought to identify sociodemographic, socioeconomic, and clinical risk factors associated with severe health outcomes among older adults hospitalized with RSV in the pre-vaccine era.

METHODS

We used population-based health administrative data in Ontario, Canada to conduct a retrospective cohort study among adults aged ≥50 years who were hospitalized with community-acquired RSV during 2017-2020. RSV was identified using either Ontario laboratory testing data, which captures >95% of all provincial testing, or validated ICD-10 diagnostic codes. Multivariable modified Poisson regression models, controlling for age, sex, and other potential confounders, were used to assess associations between individual risk factors and each of five outcomes (hospital length of stay [LOS], 30-day mortality, 30-day readmission, ICU admission, and mechanical ventilation [MV]). We compared the direction, magnitude, and 95% confidence intervals of effect estimates to identify common and unique predictors of these outcomes.

RESULTS

Of 3,221 older adults hospitalized with RSV, the mean LOS was 11.2 days (s.d. 23.0); 314 (9.8%) died within 30 days of admission; 343 (10.7%) were readmitted within 30 days post-discharge; 560 (17.4%) were admitted to the ICU; and 212 (6.6%) received MV. Males, individuals with a history of heart failure or chronic kidney disease, and adults at risk of frailty were at greater risk of all five outcomes, even after adjusting for other comorbidities. With each decade of age, the risks of 30-day mortality and LOS progressively increased, whereas the likelihood of ICU and MV admissions decreased, even after adjusting for other factors. The same trend was observed for adults receiving chronic home care services, as well as for those with dementia, active cancer, other immunodeficiencies, and transplant recipients. Finally, rurality was associated with increased risk of ICU admission, whereas the risk was lower for adults residing in neighbourhoods with the highest proportions of racialized and newcomer populations.

CONCLUSIONS

This study highlights the impact of chronic conditions, prevalent in older populations, on the risk of severe RSV outcomes, complementing the growing body of RSV literature in high-income settings. The use of laboratory-confirmed RSV distinguishes our study from most published studies in Canada, which were limited to less reliable clinical assessments. Our findings will contribute towards educating physicians on how to recognize older patients at risk of severe RSV-related outcomes, informing policy on which high-risk subgroups to prioritize for vaccine eligibility, and establishing a baseline for future vaccine effectiveness studies.

SATELLITE SYMPOSIUM ORGANISED BY THE ESWI IDC AND SUPPORTED BY IFPMA AND SANOFI:

WHEN INFECTIONS MEET NCDS: THE BIDIRECTIONAL RELATIONSHIP BETWEEN CARDIOMETABOLIC CONDITIONS AND RESPIRATORY VIRUSES

SAT11 • AUDITORIUM 3 - BREAKOUT • MON 20 OCT 2025 - 16:30 - 17:30

When Glucose Swings Matter: Glycemic Variability and Influenza Outcomes in Critically Ill Patients

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BACKGROUND

In the aftermath of the COVID-19 pandemic, a renewed focus has emerged on the burden of other respiratory viruses, particularly influenza, in intensive care units (ICUs). Influenza continues to cause significant morbidity and mortality, especially among vulnerable populations. Diabetes mellitus is a well-established risk factor for severe influenza outcomes, and growing attention is being paid to the role of glycemic variability (GV)—the fluctuations in blood glucose levels over time—as a potential contributor to poor ICU outcomes. While elevated GV has been linked to increased mortality in general ICU populations, its specific impact on patients with severe influenza remains unexplored. This study aims to assess the association between glycemic variability and clinical outcomes in ICU patients admitted with influenza, thereby providing insight into a potentially modifiable risk factor in this high-risk group.

METHODS

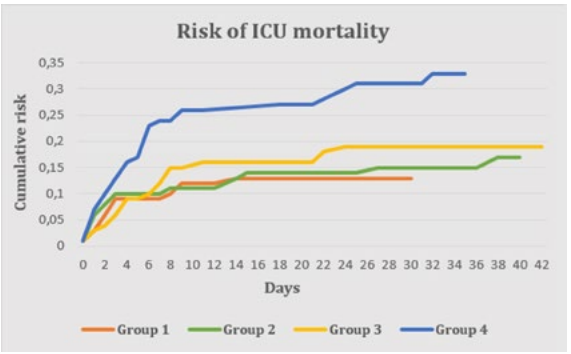
This is a real time data study including influenza patients admitted to 23 ICUs in The Netherlands between November 2023 and March 2024.In this national influenza registry the GV was quantified using the coefficient of variation (CV). The associations between CV and various ICU outcomes were evaluated by using competing risk analysis and Cox-hazard regression.

RESULTS

279 patients were analyzed. ICU mortality increased with rising CV, ranging from 14.5% mortality and a cumulative risk of 13% in the group with low GV, to 32.9% mortality and a cumulative risk of 33% in the group with high GV. In people without diabetes, higher GV was associated with increased risk of ICU mortality, in hospital mortality, treatment with insulin and treatment with corticosteroids. Diabetics did not have a statistically significant increased risk.

CONCLUSION

In conclusion, our study shows that high GV in ICU patients with influenza is significantly associated with increased ICU mortality, particularly among non-diabetic patients. This may be due to a high prevalence of patients with undiagnosed diabetes in groups with high GV.



We also found that elevated GV is associated with the use of insulin therapy, and that insulin use might be associated with worse outcomes. Additionally, our findings highlight a complex interplay between corticosteroid use, GV and ICU mortality. Further research is needed to clarify the exact roles of insulin and corticosteroids in this context. Our study suggests that careful monitoring of GV, potentially through the use of CGM, may contribute to improved outcomes for ICU patients with influenza.

EPIDEMIOLOGY AND SURVEILLANCE

SCS05 • AUDITORIUM 1 - PLENARY HALL • TUE 21 OCT 2025 - 11:00 - 12:30

Age at First Influenza A Virus Infection in Children and Impact of Influenza Vaccination ECaS

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BACKGROUND

Most children are infected by influenza A virus (IAV) by age 5 years. We sought to characterize the age at first IAV infection and to assess the impact of influenza vaccination on this timing.

METHODS:

Children are followed from birth through age 14 years as part of an ongoing community-based prospective cohort study in Managua, Nicaragua. Parents are instructed to bring their children to the study health center at the first sign of illness. Children with medically attended acute respiratory illness are tested for influenza by reverse transcription-polymerase chain reaction (RT-PCR) and blood samples are collected on an annual basis. Blood samples collected at age ≥9 months were tested for IAV nucleoprotein by enzyme-linked immunosorbent assay. Results were combined with data on symptomatic IAV infections detected by RT-PCR to identify the earliest IAV infection. Accelerated failure time models were used to estimate median (interquartile range [IQR]) age at first IAV infection by influenza vaccination status (vaccinated: ≥1 doses vs. unvaccinated: 0 doses) overall and separately by subtype and clinical syndrome (symptomatic [detected by RT-PCR] vs. subclinical [detected by serology]). Event time ratios (ETR) were estimated for vaccination in each model and interval censoring was used for subclinical infections to account for the uncertainty in the timing of the infection. Analyses include data through December 2024.

RESULTS:

A total of 1,530 children born between 2010 and 2024 were analyzed; 50% were female. Most children (80%) had ≥1 IAV infection during study enrollment and 37% were vaccinated prior to censoring. Half of first IAV infections were subclinical and H3N2 was more common than H1N1pdm (44% vs. 20%); however, subtype data was pending for 35% of first infections. Among unvaccinated children, 25% were infected with IAV by age 13.3 months, 50% by age 23.2 months, and 75% by age 36.0 months. Vaccination delayed age at first IAV infection by approximately 5 months (median [IQR]: 28.6 [16.4 to 44.4]; ETR [95% confidence interval (CI)]: 1.24 [1.15 to 1.33]). In stratified analyses, unvaccinated children were older at H1N1pdm infection than at H3N2 infection (median [IQR]: 30.2 [16.7 to 48.3] vs. 23.3 [12.9 to 37.2]), and older at subclinical infection than at symptomatic infection (median [IQR]: 29.9 [18.1 to 44.4] vs. 22.6 [12.2 to 36.9]). Vaccination delayed age at first IAV infection regardless of subtype (ETR [95% CI] for H1N1pdm: 1.89 [1.54 to 2.32]; H3N2: 1.61 [1.42 to 1.83]), and among those with symptomatic infection (ETR [95% CI]: 1.62 [1.43 to 1.84]).

CONCLUSIONS

Half of first IAV infections were subclinical and detected through serology. Among unvaccinated children, approximately half were infected with IAV by their second birthday and three-quarters were infected with IAV by their third birthday. Influenza vaccination delayed age at first IAV infection.

EPIDEMIOLOGY AND SURVEILLANCE
SCS05 • AUDITORIUM 1 - PLENARY HALL • TUE 21 OCT 2025 - 11:00 - 12:30

Association of influenza viral genetic information with disease severity markers in hospitalized patients ECaS

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BACKGROUND
Seasonal influenza causes millions of severe cases worldwide each year. A/H1N1pdm09 has been associated with severe disease among those born after the 1950s, and the reassortant 3C.2a2 clade of A/H3N2 in the 2017-18 season showed higher severity scores. However, the clinical impact of more recently emerged clades remains poorly characterized. The Global Influenza Hospital Surveillance Network (GIHSN) provides standardized clinical and virological data from hospitalized patients with respiratory illness across multiple countries.

METHODS
We analyzed data from hospitalized, laboratory-confirmed influenza patients across 14 countries participating in the GIHSN from August 2022 to October 2023. Disease severity was defined by admission to intensive care unit (ICU) or receipt of oxygen supplementation, and also as two composite definitions: 3-variable definition (at least one of ICU, mechanical ventilation, or in-hospital death), and 4-variable definition (3-variable criteria plus oxygen supplementation). Mixed-effects logistic regression models assessed the associations between influenza subtype and clade with severity, adjusting for age group, sex, comorbidities, influenza vaccination status, antiviral use, and epidemic period (defined by country as ≥5% influenza positivity for two consecutive weeks per WHO FluNet data). Study site was included as a random effect.

RESULTS
A total of 745 influenza-positive patients were included: 263 A/H1N1pdm09, 380 A/H3N2, 102 B/Victoria. The cohort was 52.2% male, with 44.6% aged <5 years, 39.5% aged 5–64 years, and 16.0% aged ≥65 years. Overall, 18.0% had one comorbidity, 15.2% had 2 or more comorbidities, 5.8% received influenza vaccine, and 32.1% received antivirals; 71.3% were enrolled during epidemic periods. Two predominant clades of A/H1N1pdm09 (6B.1A.5a.2a.1 and 6B.1A.5a.1) and three of A/H3N2 (3C.2a1b.2a.2a.1b, 3C.2a1b.2a.2a.3a.1, and 3C.2a1b.2a.2b) were analyzed. A/H1N1pdm09 infection was associated with increased odds of ICU admission (adjusted odds ratios [aOR] 2.7, 95% confidence interval [CI]: 1.1-6.2) and meeting the 3-variable definition (aOR 2.0, 95%CI: 1.0-3.9) compared with A/H3N2. No significant differences in severity markers were observed between the two predominant A/H1N1pdm09 clades. Among A/H3N2, the 3C.2a1b.2a.2b clade showed a non-significant trend toward increased severity using the 4-variables definition compared with the 3C.2a1b.2a.2a.1b clade (aOR 3.2, 95%CI: 0.9-11.1).

CONCLUSIONS
This global analysis highlights the differential impact of influenza subtypes on disease severity in hospitalized patients, and confirms that A/H1N1pdm09 was associated with increased odds of severe outcomes compared with A/H3N2. While differences in severity among A/H3N2 clades were not statistically significant, the observed trend for the 3C.2a1b.2a.2b clade warrants further investigation. Future research should

investigate the role of specific viral mutations in modulating disease severity. These findings reinforce the critical relevance of the GIHSN in providing robust data for global analyses, enhancing our ability to explore emerging patterns and novel insights into influenza. Ongoing genomic surveillance is crucial for understanding and anticipating the clinical impact of emerging influenza variants.

EPIDEMIOLOGY AND SURVEILLANCE
SCS05 • AUDITORIUM 1 - PLENARY HALL • TUE 21 OCT 2025 - 11:00 - 12:30

Population-based Surveillance shows Burden of Hospitalisations and Severe Disease due to hMPV, RSV, and Influenza in Older Adults

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BACKGROUND
Unlike other respiratory viruses, human metapneumovirus (hMPV) lacks extensive surveillance data, leading to gaps in knowledge regarding its burden and seasonal patterns. With potential prophylactics in development, a deeper understanding of hMPV epidemiology is crucial to inform prevention strategies and public health interventions. Here, we systematically identified laboratory-confirmed severe hMPV, respiratory syncytial virus (RSV) and influenza infections and described their severity in hospitalised adults over 60 years of age.

METHODS
A retrospective analysis was conducted using prospectively collected data from the Valencia Hospital Network for the Study of Infectious diseases (VAHNSI), covering 17–46% (depending on the season) of the Valencia Region population (~5 million inhabitants). hMPV, influenza, RSV, parainfluenza, rhino/enterovirus, adenovirus, endemic coronaviruses, and bocavirus were identified using a multiplex RT-PCR from 2014/15 to 2019/20 (during respiratory viruses' surveillance periods only) and in 2022/23 (year-round period) in all patients over 60 years of age admitted with a respiratory diagnosis to the 4-10 hospitals of VAHNSI, including SARS-CoV-2 detection. Hospitalisations due to hMPV, RSV or influenza were described by frequencies and proportions in the overall study period. Severity indicators (ICU admission, mechanical ventilation and in-hospital mortality) were evaluated for those three viruses.

RESULTS
Of 14,593 hospitalisations related to a respiratory infection, 4,851 (33.2%) were viral-positive cases. Influenza, RSV and hMPV were detected in 1,974 (40.7%), 583 (12.0%) and 421 (8.7%) of overall viral-positive cases, respectively. Influenza, RSV and hMPV hospitalisations were admitted to ICU in 32 (1.6%), 9 (1.5%) and 4 (1.0%), used mechanical ventilation in 94 (4.8%), 57 (9.8%) and 25 (5.9%) and had an in-hospital death in 103 (5.2%), 32 (5.5%) and 20 (4.8%) of the cases, respectively.

CONCLUSIONS
Our findings highlight the significant burden of hMPV in older adults, with positivity rates comparable to RSV but lower than influenza. Despite these differences, hMPV-associated mortality was similar to both RSV and influenza, underscoring its clinical relevance. These findings and the post-pandemic shift in viral circulation patterns reinforce the need for enhanced surveillance and targeted prevention strategies, particularly as new prophylactic and treatment options against hMPV are under development.

EPIDEMIOLOGY AND SURVEILLANCE
SCS05 • AUDITORIUM 1 - PLENARY HALL • TUE 21 OCT 2025 - 11:00 - 12:30

Impact of COVID-19 non-pharmaceutical interventions on antibody waning and the resurgence of human respiratory syncytial virus, seasonal coronavirus and influenza virus in a prospective cohort in The Netherlands ECaS

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BACKGROUND

During the COVID-19 pandemic, non-pharmaceutical interventions (NPIs) were introduced to reduce the spread of SARS-CoV-2. This also resulted in a reduction in circulation of several other respiratory pathogens, including seasonal coronaviruses (hCoV), human respiratory syncytial virus (RSV) and influenza virus. This distinctive period offered a unique opportunity to deepen our understanding of waning immunity and exposure to these respiratory pathogens independent from clinical notifications.

METHODS

We measured specific serum IgG antibodies in a nationwide prospective cohort study (PIENTER-Corona) during the COVID-19 pandemic in which serum samples were collected at 3–6-month intervals from 927 participants aged 1–87 years. A multiplex bead-based immunoassay was used for a quantitative assessment of IgG antibodies targeting the four hCoVs, RSV, and multiple subtypes of influenza A and B virus in parallel. Data analysis revealed changes in antibody concentrations of twofold or greater caused by infection, enabling serological differentiation between virus types and subtypes.

RESULTS

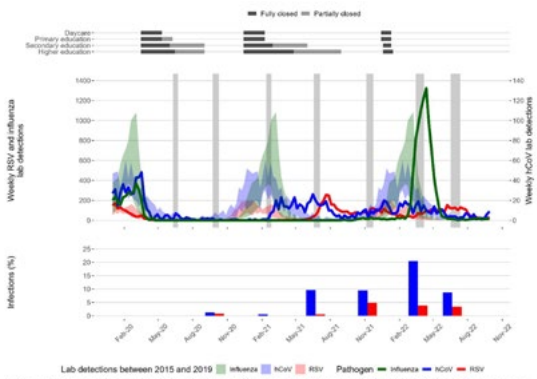

Figure 1 Overview of lab notifications of influenza virus (green), RSV (red) and hCoVs (blue) in The Netherlands. *The shaded wide lines show the minimum and maximum range of weekly lab detections between 2015 and 2019. The colored lines show the number of weekly lab detections between January 2020 and November 2022. The light grey boxes show the sampling periods during the study period. The dark and light grey bars above the graph show the closure of daycare, primary, secondary and higher education during the COVID-19 pandemic. The bottom graph shows the percentage of RSV and hCoV infections in the study population per sampling round. Notifications data is from Virologische Weekstaten.*

Following the relaxation of NPIs, the re-emergence of respiratory pathogens occurred at different time points (Figure 1). On an individual level, we identified changes in antibody concentrations over time that could be attributed to a recent infection with either hCoV, RSV or influenza virus. On a Dutch population level, serological detection of circulation coincided with the increase in notifications of the corresponding viruses, but was quantitatively different from clinical notifications. We detected four times more hCoV infections compared to RSV, while the number of RSV notifications were higher (Figure 1). This discrepancy could be due to a higher number of asymptomatic hCoV infections. During the period of reduced infections due to NPIs, waning of RSV and hCoV antibodies was detected in all age groups, but was highest in children. Children also seem to be the first exposed to hCoV and RSV when notifications returned. In-depth analysis of influenza A and B virus antibodies is still ongoing.

CONCLUSIONS

This nationwide serosurvey enables the monitoring of respiratory virus circulation within the Dutch population, offering a comprehensive view of viral dynamics over time. The unique period of reduced circulation during the COVID-19 pandemic, followed by the resurgence of respiratory viruses, provides valuable insights into immunity and exposure to hCoV, RSV, and influenza virus.

EPIDEMIOLOGY AND SURVEILLANCE
SCS05 • AUDITORIUM 1 - PLENARY HALL • TUE 21 OCT 2025 - 11:00 - 12:30

Epidemiology of hospitalizations in older adults with lab-confirmed hMPV infection: A 14-year time series

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BACKGROUND

The impact of human Metapneumovirus (hMPV) in older adults is not well known. This study analyses the prevalence of hMPV lab-confirmed older adults hospitalizations during 14 consecutive seasons.

METHODS

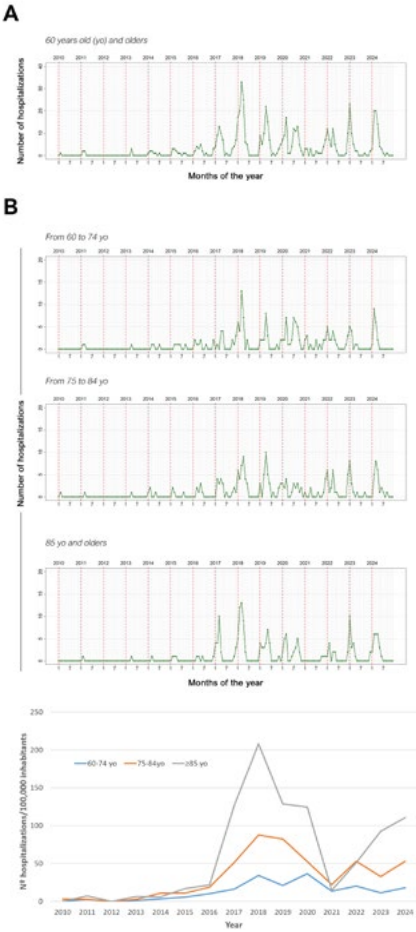
We conducted a retrospective cohort and descriptive study analyzing the prevalence of hMPV laboratory-confirmed hospitalizations in older adults in the two tertiary hospitals of the province of Valladolid (Spain) (Hospital Clínico Universitario de Valladolid and Hospital Universitario Rio Hortega) in the 2010-2024 period. This health area covers approximately a total of 160,000 inhabitants ≥60 years old (yo) patients. Date of diagnosis and hospitalization status were collected from Microbiology databases. A descriptive analysis was performed in patients ≥60 yo, and then data were segregated for 60-74 yo, 75-84 yo and ≥85 yo groups. Prevalence was calculated by age for each year based on population living in this health area.

RESULTS

In the 14 years analyzed, a total of 561 hospitalizations in ≥60 yo with hMPV infection were detected: 184 (32.%) in 65-74 yo, 192 (34.2%) in 75-84 yo and 185 (33.0%) ≥85 yo. In all age groups, a significant increase in hospitalizations was observed since 2017, starting in the month of January, peaking in March and finishing in the month of June (Figure 1A and 1B). Global mean prevalence of hMPV hospitalizations since 2017 onwards was 107.1 hospt/100,000 inhabitants in ≥85 yo, 54.3 hospt/100,000 inhabitants in 75-84 yo and 21.3 hospt/100,000 inhabitants in 60-74 yo people (Figure 2).

CONCLUSION

hMPV hospitalizations in the elderly have an epidemic pattern between January and June, with a clear peak in March. The prevalence of hMPV hospitalizations is especially high in ≥85 yo. The increase of cases after 2017 could be based on the increase of clinical requests of viral diagnosis because of the start of some policies of reduction of antibiotic use in hospitalized patients.



EPIDEMIOLOGY AND SURVEILLANCE
SCS05 • AUDITORIUM 1 - PLENARY HALL • TUE 21 OCT 2025 - 11:00 - 12:30

Epidemiology of human coronaviruses and impact of SARS-CoV-2 vaccination in paediatric patients in Hong Kong ECaS

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BACKGROUND

Human coronaviruses (hCoVs) commonly cause seasonal epidemics of mild, self-limiting upper respiratory tract infections but can lead to severe illness in vulnerable populations, especially young children. Emerging evidence suggests immunological cross-reactivity from exposure to SARS-CoV-2 particularly beta-coronaviruses on endemic hCoVs. However, it remains unclear whether and how the cross-reactivity is protective against infection. This study aimed to investigate the epidemiology of HCoVs among hospitalized paediatric patients in Hong Kong and to assess the potential impact of SARS-CoV-2 vaccination on hCoV-related hospitalizations in the post-pandemic period.

METHODS

Paediatric inpatients up to 17 years of age from two large public hospitals in Hong Kong were tested by PCR for various respiratory viruses SARS-CoV-2 and the four common hCoV subtypes (229E, NL63, OC43, HKU1) between January 2021 and March 2025. Pearson correlation analysis on weekly virus detection proportions was conducted to assess the relationship between activities of SARS-CoV-2 and alpha-HCoVs (229E and NL63) or beta-HCoVs (OC43 and HKU1). A test-negative design was used to evaluate SARS-CoV-2 vaccine effectiveness (VE) in preventing respiratory infections with hCoVs in paediatric inpatients. VE was estimated by a conditional logistic regression model adjusting for sex, age, and prior SARS-CoV-2 infection status, matching on the month of infection.

RESULTS

During the study period, hCoVs activity was extremely low in 2021 and 2022, followed by a resurgence from 2023 onward with several clear epidemics, and an overall positivity rate of 3.1%. Beta hCoVs, particularly OC43, exhibited marked winter peaks, while alpha hCoVs showed a relatively lower but stable circulation. A significant negative correlation ($r = -0.211$, $p = 0.024$) in weekly virus activities was found between SARS-CoV-2 and beta hCoVs, while there was no statistically significant correlation between SARS-CoV-2 and alpha hCoVs. We estimated increasing VE in preventing hCoV-associated hospitalization with an increasing number of COVID-19 vaccine doses received, i.e., 8.2% (95% CI: -16.8% to 27.9%) for partial vaccinated children, 20.9% (95% CI: -4.2% to 40.0%) for completed vaccinated children, and 35.7% (95% CI: -24.5% to 66.8%) for those who were boosted. Point estimates of VE were generally higher against hospitalization from beta hCoVs than alpha hCoVs.

CONCLUSIONS

This study revealed post-pandemic changes in the seasonal patterns of hCoVs among hospitalized paediatric inpatients in Hong Kong, with beta hCoVs showing winter seasonality and an inverse relationship with SARS-CoV-2 activity. SARS-CoV-2 vaccination was associated with a dose-dependent reduction in hCoV-associated hospitalizations in children, suggesting potential cross-protection, especially for beta hCoVs.

MOLECULAR VIROLOGY: VIRUS EVOLUTION, STRAIN SELECTION, STRUCTURE AND REPLICATION

SCS16 • AUDITORIUM 2 - BREAKOUT • TUE 21 OCT 2025 - 11:00 - 12:30

Antigenic drift in the receptor binding site of H1N1 balances antibody evasion and receptor binding

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BACKGROUND

Influenza viruses rapidly evolve to evade host antibodies, a process known as antigenic drift. Antigenic drift occurs dominantly in the hemagglutinin (HA) head domain as antibodies preferentially target more mutable head epitopes. The receptor binding site (RBS) of the head domain is broadly conserved, reflecting its essential role in viral entry by binding to sialic acids (SAs) on host cells. Whether RBS-targeting antibodies, which mimic sialic acid binding, drive antigenic drift has not been investigated.

METHODS

We produced recombinant HAs with single or combinatorial mutations at K133aN, N159K, S186P, and D190A within and nearby the RBS of post-2018 H1N1 viruses to elucidate how these residues impact RBS-monoclonal antibody (mAb) and receptor binding. MAb binding profiles were determined by ELISA and further analyzed by antigenic cartography. Using recombinant HAs, we determined their receptor binding profiles on a Neu5Ac glycan array. To understand antibody evasion mechanisms, we generate structures of two discrete RBS-specific mAbs binding to a pre-2018 H1. Moreover, we generated structures of A/Hawaii/70/2019 and A/Wisconsin/588/2019 with a2,6 SA analog to understand receptor binding.

RESULTS

We identified four mutations (K133aN, N159K, S186P, and D190A) present in two clades of H1N1, with S186P found in both clades, D190A present in clade 5a.1, and K133aN and N159K in clade 5a.2. Notably, K133a, S186 and D190 stabilize SA within the RBS pocket and D190 is a direct SA contact Our data demonstrate that K133aN, N159K, S186P and D190A are sufficient to differentially evade distinct classes of RBS-specific mAbs. This is supported by cryo-EM structures showing the two main classes of RBS-specific mAbs directly binding to K133a, N159, S186 and/or D190. We identified that S186P increases SA binding breadth against diverse core glycans bearing a2,6 SAs, while D190A significantly restricts this profile. In contrast, K133aN and N159K provide an intermediate receptor binding phenotype relative to S186P and D190A.

CONCLUSIONS

These data establish that K133aN, N159K, S186P and D190A are not simply RBS mAb-evading mutations but also have differential consequences for receptor binding. As such, S186P, K133aN, and N159K remain fixed in the circulating H1N1 population, whereas D190A was transient. Additionally, we propose clade 5a.1 was replaced with clade 5a.2 viruses, as the D190A mutation in 5a.1 likely attenuates viral fitness. Together, these data illustrate antigenic drift does occur within the RBS but under the constraints of balancing antibody evasion and receptor binding.

MOLECULAR VIROLOGY: VIRUS EVOLUTION, STRAIN SELECTION, STRUCTURE AND REPLICATION
SCS16 • AUDITORIUM 2 - BREAKOUT • TUE 21 OCT 2025 - 11:00 - 12:30

HA Mutations Driving Immune Escape in Avian Influenza Virus Do Not Enhance Human Receptor Binding

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BACKGROUND

Vaccination against avian influenza viruses (AIVs) reduces disease burden in poultry and the risk of zoonotic transmission. However, vaccination can accelerate antigenic drift in the viral hemagglutinin (HA), raising concerns about viral escape and potential adaptation to humans. Between 1999 and 2002, H7N1 outbreaks in Italy led to prolonged virus circulation in vaccinated poultry. This study investigated the molecular and zoonotic consequences of HA mutations fixed under vaccination pressure.

METHODS

We analyzed HA sequences of H7N1 isolates from 1999 (pre-vaccination) and 2002 (post-vaccination). Using reverse genetics, we generated reassortant viruses containing the 1999 parental backbone and HA genes with or without single or combined mutations observed in 2002 field isolates. Virus replication kinetics were assessed in primary chicken and turkey embryo cells and human cell lines. Receptor-binding profiles were evaluated using solid-phase and glycan-binding assays. Antigenic properties were studied using hemagglutination inhibition assays with chicken, turkey and ferret H7 sera.

RESULTS

Seven amino acid substitutions in the HA1 protein were fixed in viruses isolated post-vaccination. These mutations led to significant antigenic drift, as shown by reduced cross-reactivity with antisera against the 1999 strain. Despite immune escape, the mutations decreased viral binding to avian-type α2,3-linked sialic acid receptors and did not increase binding to human-like α2,6-linked receptors. Virus replication in cell cultures varied depending on the specific mutations, indicating differential effects on fitness. However, none of the mutations conferred enhanced fitness toward human receptors.

CONCLUSIONS

This study demonstrates that vaccination in poultry can select for HA mutations that facilitate immune escape and modulate viral fitness, while maintaining or even reducing avian receptor binding. Crucially, no molecular evidence for increased zoonotic potential was observed. These findings underscore the importance of continuous molecular surveillance of AIVs in vaccinated flocks to monitor antigenic drift and evaluate the risk of interspecies transmission. For pandemic preparedness, this highlights the complexity of balancing vaccine efficacy with evolutionary pressures in enzootic AIV reservoirs.

MOLECULAR VIROLOGY: VIRUS EVOLUTION, STRAIN SELECTION, STRUCTURE AND REPLICATION
SCS16 • AUDITORIUM 2 - BREAKOUT • TUE 21 OCT 2025 - 11:00 - 12:30

Multivalent Colocalization of Influenza Polymerase and Nucleoprotein by ANP32A Reveals the Molecular Basis of Human Adaptation

Aldo Roman CAMACHO ZARCO, Martin BLACKLEDGE

CEA, France

BACKGROUND

Adaptation of avian influenza RNA polymerase (FluPol) to human cells requires mutations on the 627-NLS domains of the PB2 subunit. The E627K adaptive mutation compensates a 33-amino-acid deletion in the acidic intrinsically disordered domain of the host transcription regulator ANP32A, a deletion that restricts FluPol activity in mammalian cells. The function of ANP32A in the replication transcription complex and in particular its role in host restriction remains poorly understood.

METHODS

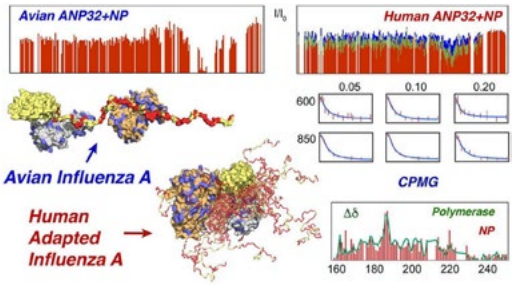
Here we characterize ternary complexes formed between ANP32A, FluPol, and the viral nucleoprotein (NP) through different biohysical techniques and in vitro assays.

RESULTS

We demonstrate that while FluPol and NP can simultaneously bind distinct linear motifs on avian ANP32A, the deletion in the shorter human ANP32A blocks this mode of colocalization. NMR reveals that NP and human-adapted FluPol, containing the E627 K mutation, simultaneously bind the identical extended linear motif on human ANP32A in an electrostatically driven, highly dynamic and multivalent ternary complex.

CONCLUSION

This study reveals a probable molecular mechanism underlying host adaptation, whereby E627K, which enhances the basic surface of the 627 domain, is selected to confer the necessary multivalent properties to allow ANP32A to colocalize NP and FluPol in human cells.



MOLECULAR VIROLOGY: VIRUS EVOLUTION, STRAIN SELECTION, STRUCTURE AND REPLICATION
SCS16 • AUDITORIUM 2 - BREAKOUT • TUE 21 OCT 2025 - 11:00 - 12:30

Molecular basis of 60 years of antigenic evolution of human influenza A(H3N2) virus neuraminidase

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BACKGROUND
Human influenza A viruses escape antibody-mediated immunity through changes in the hemagglutinin (HA) and neuraminidase (NA) glycoproteins but this antigenic evolution has been less well studied for NA compared to HA.

METHODS
Here, the antigenic properties of the NA of more than 300 A(H3N2) viruses isolated since 1957 were quantified with a neuraminidase inhibition enzyme-linked lectin assay (NI-ELLA) and visualized using antigenic cartography. The molecular basis of major antigenic changes was investigated using phylogenetics and recombinant viruses.

RESULTS:
In the ~60 year period investigated, the N2 gene had a somewhat slower rate of evolution compared to H3: 4.46E-3 substitutions/site/year for HA and 3.41E-3 substitutions/site/year for NA. This genetic evolution of N2 was associated with substantial antigenic evolution, where the antigenic drift of N2 neuraminidase was found to be more gradual than described previously for H3 hemagglutinin. In the 3D antigenic map for N2, the viruses appeared less clustered and punctuated than in the H3 map previously investigated. Moreover, we noted that the antigenic changes of neuraminidase and hemagglutinin were discordant, with NA frequently evolving more when HA evolved less and *vice versa.* By reverse genetics, we investigated the individual effect of 104 different amino acid substitutions that occurred over time to conclude that many substitutions had little (but additive) antigenic impact and few had large antigenic impact, again in contrast to previous observations with HA. However, like for HA, the substitutions with highest antigenic impact generally involved large changes in the physicochemical properties of the amino acid residues such as changes in charge, hydropathy index or volume. In the protein NA structure, the amino acid positions associated with antigenic change clustered around the NA active site, at the interface of the NA tetramer and at the lateral side of NA forming a continuous epitope space from one monomer to the next, but some changes even occurred at the basal side of NA.

CONCLUSIONS
We conclude that these data offer opportunities to improve influenza vaccine effectiveness through increased focus on neuraminidase and by empowering molecular surveillance. The discordance between HA and NA drift offers an opportunity for improving vaccine matching; if one component would be mismatched due to ongoing antigenic evolution, the other could still be matched to ensure protection. Ultimately, when the molecular determinants of NA drift are more completely understood, it might be possible to rely on a "sequencing first" NA surveillance approach for vaccine strain selection, as is increasingly done for HA.

MOLECULAR VIROLOGY: VIRUS EVOLUTION, STRAIN SELECTION, STRUCTURE AND REPLICATION
SCS16 • AUDITORIUM 2 - BREAKOUT • TUE 21 OCT 2025 - 11:00 - 12:30

Little evidence for additional mammalian adaptation in bovine-derived H5N1 viruses during replication or transmission in ferrets

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BACKGROUND

In March 2024 an outbreak of H5N1 influenza virus was reported in US dairy cattle and is still ongoing. Viruses of the B3.13 genotype are responsible for almost all these infections. Of ~55 known human B3.13 infections, none were severe and no human-to-human transmission has been detected. However, cattle-derived B3.13 viruses exhibit high pathogenicity in some naturally infected mammals, such as farm cats, and in animal models. Serial passage of B3.13 viruses through cattle, with spillover infections into humans and other mammals, raises pandemic concerns. We therefore characterized within- and between-host evolutionary dynamics of cattle-derived B3.13 H5N1 viruses replicating in, and transmitting between, ferrets.

METHODS

Previously, Eisfeld et al. (Nature 633:426, 2024) characterized two B3.13 H5N1 isolates in ferrets. A/dairy cattle/New Mexico/A240920343-93/2024 (NM93-H5N1), isolated from cow's milk, replicated robustly in ferrets, but did not transmit efficiently by respiratory droplet. A/Texas/37/2024 (huTX37-H5N1), isolated from a human with conjunctivitis, also replicated robustly, killing infected animals, and was transmitted by respiratory droplet in 6 of 24 ferret pairs (Gu et al., Nature 636:711, 2024). Notably, huTX37-H5N1 encodes the mammal-adapting mutation PB2-E627K, while NM93-H5N1 does not. To assess B3.13 virus evolution in ferrets, we examined viral genome sequences in nasal swabs from infected donor and recipient ferrets. We quantified intrahost single nucleotide variants (iSNVs) to identify mutations of interest and used summary statistics to evaluate natural selection during replication and transmission.

RESULTS

Within-host diversity of both viruses remained low in infected ferrets, with most iSNVs remaining below 10% frequency. There was no evidence of selection for additional mammal-adapting mutations, though mutations previously associated with increased fitness in mammals were detected in a few samples at very low frequencies (<10%). In general, nonsynonymous mutations occurred at lower frequencies than synonymous mutations, and synonymous nucleotide diversity (πS) exceeded nonsynonymous diversity (πN), in both viruses across all gene segments. There was no significant change in πN or πS between huTX37-H5N1 donors and recipients, but also few shared iSNVs between transmission pairs.

CONCLUSIONS

Both the cattle-derived NM93-H5N1 and the human isolate huTX37-H5N1 are under weak purifying selection in ferrets, suggesting that both viruses are relatively fit for replication, even though only huTX37-H5N1 possesses the PB2-E627K mutation. We found no evidence for selection on any gene segment during transmission of huTX37-H5N1, in contrast to previous studies, where we observed selection on hemagglutinin (HA) of avian influenza viruses transmitted between ferrets. We speculate that both viruses are relatively fit for replication in mammals; this fitness may combine with the very short infection times in ferrets (animals infected with huTX37-H5N1 died or were euthanized within 6 days of infection) to limit the evolutionary potential of these viruses in many mammalian hosts.

PHARMACEUTICAL AND NON PHARMACEUTICAL INTERVENTION

SCS07 • AUDITORIUM 1 - PLENARY HALL • TUE 21 OCT 2025 - 14:00 - 15:30

Estimated Relative Effectiveness and Public Health Impact of Cell-Based Versus Egg Based Influenza Vaccines During the 2023–2024 Season in the United States

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INTRODUCTION

Egg-adaptive mutations can alter the antigenicity of egg-based influenza vaccines, contributing to reduced effectiveness. Use of cell-based (QIVc) quadrivalent influenza vaccines can improve effectiveness against test-confirmed influenza compared to egg-based (QIVe) vaccines, as demonstrated during the United States (US) 2017–18 to 2019–20 and 2022–23 influenza seasons. Here we estimate the relative vaccine effectiveness (rVE) and potential public health impact of QIVc versus QIVe during the 2023–24 season, across age and other subgroups.

METHODS

rVE was estimated using linked data from electronic health records, medical and pharmacy claims, and laboratory tests in the US. A retrospective test-negative design was applied among individuals aged 6 months–64 years vaccinated with either QIVc or QIVe in the 2023–24 season and tested for influenza within 7 days of an acute respiratory or febrile illness (ARFI). rVE was estimated using doubly robust logistic regression in the full population, pediatric and adult subpopulations, individuals with high-risk conditions and those tested in outpatient settings. Sensitivity analyses adjusted for propensity to be tested and matched on test week. The public health impact was estimated using a compartmental influenza burden averted model.

Figure 1: Adjusted relative vaccine effectiveness of cell-based (QIVc) versus egg-based (QIVe) quadrivalent influenza vaccines against test-confirmed influenza

Analysis	Age	Cases	Controls	Adjusted rVE (95% CI)
Main	6m – 64y	16,678	90,101	19.8% (15.7 – 23.8)
Sensitivity analyses				
Propensity to be tested	6m – 64y	16,678	90,101	20.2% (16.0 – 24.1)
Matched week	6m – 64y	16,678	70,993	20.3% (16.4 – 23.9)
Age Subgroups				
Pediatric	6m – 17y	11,439	49,551	19.6% (13.6 – 25.3)
Infants/Children	6m – 8y	6,837	33,356	17.6% (9.3 – 25.1)
Children/Adolescents	9 – 17y	4,602	16,195	24.3% (15.1 – 32.5)
Adult	18 – 64y	5,239	40,550	18.5% (12.1 – 24.5)
Outpatient Subgroups				
All	6m – 64y	15,701	81,458	19.7% (15.3 – 23.8)
Pediatric	6m – 17y	10,868	45,313	19.5% (13.2 – 25.4)
Adult	18 – 64y	4,833	36,145	19.5% (12.7 – 25.7)
High-Risk Subgroups				
All	6m – 64y	7,761	49,378	14.7% (8.7 – 20.3)
Children/Adolescents/Adults	5 – 64y	6,651	41,351	17.1% (10.9 – 22.9)
Adult	18 – 64y	3,758	30,775	17.1% (9.3 – 24.1)

Table 1: Estimated influenza burden averted by use of QIVc vs QIVe in subjects aged 0–64 years in the United States 2023–24 influenza season[1]

Outcome	Outcomes prevented by QIVe			Outcomes prevented by QIVc			Incremental outcomes prevented by QIVc		
	0 — 17y	18 — 64y	Total (0 — 64y)	0 — 17y	18 — 64y	Total (0 — 64y)	0 — 17y	18 — 64y	Total (0 — 64y)
Symptomatic Cases	4,750,435	2,424,763	7,175,198	5,822,812	3,731,782	9,554,593	1,072,376	1,307,018	2,379,395
Outpatient Visits	2,639,856	932,779	3,572,635	3,245,134	1,456,162	4,701,296	605,278	523,383	1,128,661
Hospitalizations[2]	17,809	16,573	34,382	22,092	27,220	49,312	4,284	10,646	14,930
ICU Visits	2,938	2,735	5,673	3,645	4,491	8,136	707	1,757	2,463
Deaths	203	677	880	253	1,202	1,455	50	525	574

1.Modelled using CDC data on influenza vaccine uptake, influenza incidence, influenza-related healthcare resource use and deaths from the 2023–2024 influenza season.
2.Hospitalizations include both ICU and non-ICU hospital stays.

RESULTS

The final dataset comprised 106,779 vaccinated ARFI patients, including 2,119 (13%) test-positive cases and 14,750 (87%) test-negative controls in the QIVc group and 14,559 (16%) cases and 75,351 (84%) controls in the QIVe group. QIVc was significantly more effective than QIVe in preventing test-confirmed influenza with an estimated rVE of 19.8% (95% CI: 15.7–23.8%) in the full population. Consistent rVE results were observed for sensitivity analyses and all subpopulations (Figure 1). If all vaccinated individuals aged 6 months–64 years in the US received QIVc over QIVe, an additional estimated 2,379,395 symptomatic illnesses would have been prevented, with proportionate reductions in related complications (Table 1).

CONCLUSION

This study demonstrates superior effectiveness of QIVc versus QIVe in preventing test-confirmed influenza in individuals aged 6 months–64 years and across pediatric, adult, high-risk and outpatient subgroups, during the US 2023-24 season.

PHARMACEUTICAL AND NON PHARMACEUTICAL INTERVENTION
SCS07 • AUDITORIUM 1 - PLENARY HALL • TUE 21 OCT 2025 - 14:00 - 15:30

Differential protection of prior infection and repeated vaccination against SARS-CoV-2 infection in Omicron BA.2 to JN.1 predominance: a prospective cohort study ECaS

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BACKGROUND

Prior to the COVID-19 pandemic, the repeated infection of rapidly evolved virus sub-lineages and the practice of repeated vaccination were uncommon, thus had limited evidence. A fundamental assessment of their protection in preventing the risk of infection is important for informing public health strategies, however remained unclear. We aimed to assess the protective effectiveness (PE) of prior infection and repeated vaccination against SARS-CoV-2 Omicron asymptomatic and symptomatic infection in a representative community cohort in Hong Kong during periods of BA.2, BA.4/BA.5, BA.2.75, XBB and JN.1 predominance.

METHODS

In this prospective cohort study, individuals aged 5 or above underwent weekly rapid antigen testing (RAT) with a self-collected pooled nasal and throat swab, regardless of symptom and exposure status during five periods from March 1, 2022 to May 21, 2024, in which the BA.2, BA.4/BA.5, BA.2.75, XBB or JN.1 subvariants were dominant in Hong Kong. Primary outcomes are the hazard of SARS-CoV-2 infection, including asymptomatic and symptomatic infection, and the protective effectiveness (PE) of SARS-CoV-2 prior infection and repeated vaccination. The PE was estimated with a Cox proportional-hazards regression model with time-dependent covariates, allowing for changes in infection and vaccination status over time, after adjustment for demographic factors and pre-existing medical conditions.

RESULTS

In the BA.2, BA.4/BA.5, BA.2.75, XBB and JN.1 predominance, 14% (1729/12020) of participants, 19% (2370/12610), 4% (416/10290), 15% (1541/10212), and 18% (595/3240) respectively tested positive for SARS-CoV-2 infection. Up-to-date vaccination reduced the risk of infection during BA.2 (54%, 95% CI: 49-58) and BA.4/BA.5 (28%, 16-38) predominance, but no statistically significant protection was observed during BA.2.75, XBB and JN.1 predominance. Significant protection against SARS-CoV-2 reinfection was demonstrated for those previously infected, with a PE of 89% (83%-92%), 76% (72-79), 66% (56-74) and 45% (38%-51%) during periods of BA.2, BA.4/BA.5, BA.2.75, and XBB predominance respectively. The PE of prior infection was further boosted with multiple prior infections to 85%, 79%, and 56% for reinfections in the BA.4/BA.5 to XBB periods respectively. Having a recent infection within 6 months (74%, 37-89) or multiple prior infections (34%, 8-53) was protective against reinfection in JN.1 period.

CONCLUSIONS

Our result represented a fundamental assessment of the differential protection of prior infection and repeated vaccination. Of SARS-CoV-2 infections in the community, repeated vaccination provided a significant protection against BA.2 and BA.4/BA.5 infection only, and conferred practically no additional protection in XBB and JN.1 waves, possibly reflecting attenuated effect of repeated vaccination and vaccine escape with subvariant evolution. Prior infection significantly prevented reinfection in BA.2, BA.4/BA.5, BA.2.75, and XBB predominance, with its protection enhanced with recent or repeated infections. Our findings indicated that a recent history of prior infection conferred the most substantial protection against reinfection, including mild and asymptomatic cases in JN.1 predominance.

PHARMACEUTICAL AND NON PHARMACEUTICAL INTERVENTION
SCS07 • AUDITORIUM 1 - PLENARY HALL • TUE 21 OCT 2025 - 14:00 - 15:30

Vaccine effectiveness against medically attended influenza at primary care level in the paediatric population, 2024/25 season, Europe

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BACKGROUND

In Europe, seasonal influenza vaccination is recommended to children with certain medical conditions, and in some countries all children within certain age groups. Influenza vaccines are also available for private purchase. We estimated the vaccine effectiveness (VE) in preventing medically attended, laboratory-confirmed influenza in the paediatric population in primary care in 2024/25 in Europe.

METHODS

We conducted a test-negative case-control study among children (6 months–17 years) in seven European countries, presenting to primary care with acute respiratory infection and swabbed ≤7 days after symptom onset. Cases were RT-PCR positive for influenza; controls were negative. All or a systematic sample of viruses were sequenced in each site. Practitioners collected demographic, clinical and vaccination information through electronic medical records, registry linkage or interview. We used logistic regression to measure the odds ratio of vaccination by comparing cases and test-negative controls, confounder-adjusted for study site, onset date, age, sex, and chronic conditions. The VE was calculated as (1- odds ratio)x100. The VE was estimated among all children against any influenza virus and by (sub)type, and by age group.

RESULTS

We included 1,957 cases and 3,701 controls from weeks 42-2024–12-2025. There were 223 (12%) untyped viruses, 493 (25%) A(H1N1)pdm09, 269 (14%) A(H3N2), 958 (49%) B, and 14 (1%) influenza co-infections. Overall, 14% (534/3,701) of controls were vaccinated. The VE against any influenza virus was 63% (95%CI: 52–71) overall; 51% (95%CI: 31–66), 81% (95%CI: 69–89), 65% (95%CI: 28–84) in children aged 6 months–4 years, 5–11, 12–17 years, respectively. Lowest VE was observed against influenza A(H1N1) pdm09, at 28% (95%CI: -4–51) overall, ranging 0–41% by age group. The majority of sequenced A(H1N1) pdm09 viruses (94%; 144/154) belonged to clade 5a.2a (C.1.9), different from the vaccine strain. The VE against influenza A(H3N2) was 71% (95%CI: 48–85) overall, ranging 47–88% by age group. Of 77 sequenced viruses, 82% (63) were clade 2a.3a.1 (J.2), 12% (9) 2a.3a.1 (J.2.2), 5% (4) 2a.3a.1 (J.2.1), similar to the vaccine strain, and 1% (1) 2a.3a (G.1.3.1). The VE against influenza B was 85% (95%CI: 76–92) overall, ranging 78–91% by age group. Of 116 sequenced B/Victoria viruses, 44% (51) were clade V1A.3a.2 (C.5.1), 29% (34) V1A.3a.2 (C.5.7), 26% (30) V1A.3a.2 (C.5.6), and <1% (1) V1A.3a.2 (C.5), similar to the vaccine strain.

CONCLUSIONS

Overall, vaccination prevented nearly two-thirds of medically attended influenza among vaccinated children at the primary care level during the 2024/25 season in Europe. Protection varied by (sub)type and age group, with highest VE against influenza B, and lowest VE against A(H1N1)pdm09. End-of-season analyses, including clade-specific estimates, are required to better understand these results.

PHARMACEUTICAL AND NON PHARMACEUTICAL INTERVENTION
SCS07 • AUDITORIUM 1 - PLENARY HALL • TUE 21 OCT 2025 - 14:00 - 15:30

Immunogenicity and safety of high dose formulations of mf59-adjuvanted cell-derived influenza vaccine in adults aged 50 years and older: two phase 2 randomised controlled trials

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BACKGROUND

The optimal formulation of an adjuvanted cell-based quadrivalent influenza vaccine (aQIVc) was investigated in dose-finding (V201_01, NCT04782323) and dose-confirmation (V201_07, NCT05501561) studies. Both were phase 2, observer-blind, multicentre trials, randomising participants aged ≥50 years to 7 or 3 formulations of (a)QIVc vs non-adjuvanted QIVc (15 µg hemagglutinin antigen (HA) per strain).

Primary objective: Assess the immunogenicity of different (a)QIVc formulations vs QIVc 28 days post-vaccination.

METHODS

In V201_01, formulations of aQIVc contained 15, 30 or 45µg HA per strain, and 1x, 2x or 3x the dose of MF59 adjuvant included in licenced Fluad quadrivalent®. V201_07 tested formulations that contained 45 µg HA with either 0, 1 or 2x MF59 (abbreviated further as aQIVc[45,1] and aQIVc[45,2]).

RESULTS

In V201_01, 838 of 839 enrolled participants (52.7% aged 50–64 years; 56.6% female; 55.9% White) were exposed. Generally, adjusted geometric mean titer (aGMT) ratios of aQIVc vs QIVc at Day 29 (per-protocol-set [PPS], n=810) increased with antigen and adjuvant dose, but were similar between 2x and 3x adjuvant for all strains.

In V201_07, 1051 of 1056 enrolled participants (52.1% aged 50–64 years; 58.0% female; 83.7% White) were exposed (PPS: n=1022). aGMT ratios for aQIVc[45,2] vs QIVc ranged from 1.27 (95% CI: 1.04;1.55) to 1.86 (1.52;2.27) and were superior (lower 95% CI limit >1.0) for all strains. aQIVc[45,1] was superior to QIVc for some, but not all, strains. aGMT ratios of aQIVc[45,2] over QIVc[45,0] were superior for A/H1N1, A/H3N2 and B/Yamagata and non-inferior for B/Victoria.

Neither study presented safety concerns: in V201_07, rates of solicited adverse events from Day 1 to 7 post-vaccination were highest with aQIVc[45,2] (71.1%) and lowest with QIVc (50.4%); most events were of mild or moderate severity across all groups.

CONCLUSIONS

Multiple dose formulations demonstrated strong immune responses with no safety concerns. aQIVc has been further investigated in a larger study (NCT06015282).

PHARMACEUTICAL AND NON PHARMACEUTICAL INTERVENTION
SCS07 • AUDITORIUM 1 - PLENARY HALL • TUE 21 OCT 2025 - 14:00 - 15:30

High-dose recombinant influenza vaccines induce greater A(H3N2)-reactive antibodies and overcome attenuation associated with repeated vaccination compared to standard-dose egg- or cell-based vaccines: results from a RCT in healthy younger adults

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BACKGROUND

When grown in eggs, influenza viruses can acquire mutations within the haemagglutinin that enable better growth in eggs but alter their antigenicity and reduce vaccine effectiveness. Immunogenicity and effectiveness may further be compromised by repeated vaccination, which can focus the antibody response and limit its breadth. Both these problems occur more commonly for A(H3N2) viruses. Cell-grown and recombinant-haemagglutinin vaccines may avoid egg-acquired adaptations, but it is unclear whether they also overcome attenuation associated with repeated vaccination.

METHODS

We conducted a randomized immunogenicity trial (ClinicalTrials.gov: NCT05479370) to assess whether high-dose recombinant quadrivalent influenza vaccines (QIV-R) were more immunogenic than standard-dose egg-grown (QIV-E) and cell-grown (QIV-C) vaccines. We recruited healthy adults aged 21-49 years into 2 strata based on the number of vaccines received in the prior 5 years: infrequent (≤1) and frequent (≥3). Participants within each stratum were randomised 1:1:1 to receive QIV-R, QIV-C or QIV-E. Sera collected before and 14-21d post-vaccination were tested in haemagglutination inhibition assay against representative vaccine viruses grown in both eggs and cells. Post-vaccination geometric mean titre (GMT), seropositivity, geometric mean fold-rise and seroconversion against vaccine antigens were compared, adjusted for pre-vaccination titre and vaccination history.

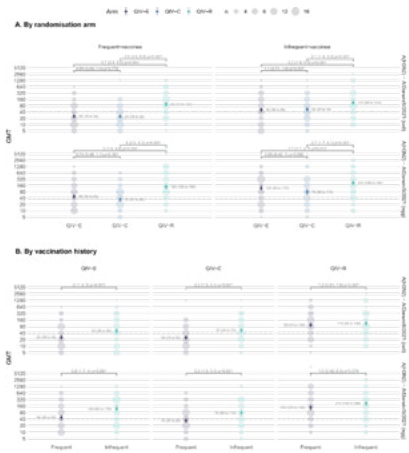

Figure: Post-vaccination geometric mean titres (GMT) by A. Randomisation arm and B. Vaccination history against cell and egg-grown A(H3N2) vaccine antigens. Solid diamonds with error bars show the GMT, adjusted for pre-vaccination titre, vaccination history, and the interaction of vaccination history and randomisation arm. Adjacent text provides the estimates. Bubbles show the raw data (unadjusted titres) for each randomisation arm, with size indicative of the number of observations with that titre. The dashed line at 40 indicates the standard threshold for seropositivity. Text at the top of each facet with brackets shows the linear contrast comparing randomisation groups; i.e. the ratio of post-vaccination GMTs with p-values for the t-test comparing means.

RESULTS

359 participants (150 male, 209 female) were enrolled and provided pre- and post-vaccination blood samples. Adjusted GMTs against cell-grown A(H3N2) were significantly higher for the group who received QIV-R (GMT=100, 95%CI 84-120) than QIV-C (GMT=35; 95%CI 29-43; p<0.001) or QIV-E (GMT=36, 95%CI 29-43; p<0.001). While pre-vaccination GMTs were higher among frequent vaccinees irrespective of randomisation arm, post-vaccination GMTs were roughly 2-fold higher among infrequent compared to frequent vaccinees who received QIV-E or QIV-C and were comparable for the group receiving QIV-R (GMT=110, 95%CI: 84-150 v GMT=93, 95%CI 70-120). Improvements were also observed for A(H1N1)pdm09 and B/Victoria. Influenza infections were detected in 5/120 QIV-E, 4/120 QIV-C and 1/119 QIV-R vaccinees.

INTERPRETATION

Accumulated evidence, including the results of this study, suggest that high-dose QIV-R is more effective and immunogenic than standard-dose QIV-E or QIV-C. These data also suggest that QIV-R provides a better antibody boost in repeat vaccinees. For frequently vaccinated groups, such as healthcare workers, QIV-R may be preferable to overcome negative interference from annual vaccination. Further work is needed to ascertain whether these gains are also realised in older adults and target groups for vaccination, and how they translates to vaccine effectiveness.

MATHEMATICAL MODELLING AND PROJECTIONS, INCLUDING LIMITATIONS

SCS08 • AUDITORIUM 2 - BREAKOUT • TUE 21 OCT 2025 - 14:00 - 15:30

A Framework for Measuring Population Immunity Against Influenza Using Individual Antibody Titers ECas

Weijia XIONG (1), Xiaotong HUANG (1), Tran Thi Nhu THAO (2), Nguyen Thi Le THANH (2), Liping PENG (1), Ranawaka A. P. M. PERERA (1), Vicky J. FANG (1), Bingyi YANG (1), Sook-San WONG (3), Nancy H.L. LEUNG (1), Shenna G SULLIVAN (4), Dennis K. M. IP (1), Malik PEIRIS (1,5), Ted M. ROSS (6,7,8,9), Maciej F. BONI (2,10), Benjamin J. COWLING (1,11), Tim K. TSANG (1)

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BACKGROUND

Measuring population immunity is crucial for epidemic control and prevention of infectious diseases. While correlates of protection have been identified at the individual level for some pathogens, methods to translate these into population-level immunity metrics remain underdeveloped. We developed and validated a framework to construct population immunity estimators derived from individual serological measurements.

METHODS

This study developed a framework to assess whether large data sets of individual immune measurements can be translated into and validated as population immunity metrics. Using influenza virus and HAI titers as our test case, we assessed four estimators: (1) Geometric Mean titer (GMT); (2) the proportion of non-naïve individuals; (3) the proportion of immune individuals, defined as the weighted average of protection levels across the number of individuals in different HAI titer levels; (4) the relative reduction in reproductive number defined as the proportional decrease in disease transmissibility due to population immunity (i.e., $1 - Re/R_0$), reflecting the extent to which existing immunity reduces the transmissibility of influenza viruses. The first 3 estimators were calculated separately by age groups (children, adults and elderly), and then weighted by the age distribution of the respective study population based on local census data13. The fourth one was already age-weighted in the estimation process. Lower values indicate lower population immunity.

We validated these estimators by assessing their ability to predict dominant influenza subtypes and their correlation with population-level incidence data. Then, we conducted SEIR model simulations to evaluate factors affecting their reliability (Figure 1). Finally, we conducted simulation studies to determine optimal sample sizes required for accurate population immunity estimation, The framework was applied to data from longitudinal cohorts in Hong Kong, Vietnam, and the USA.

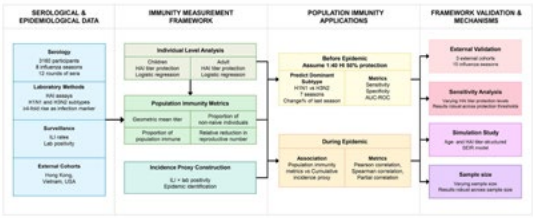
RESULTS

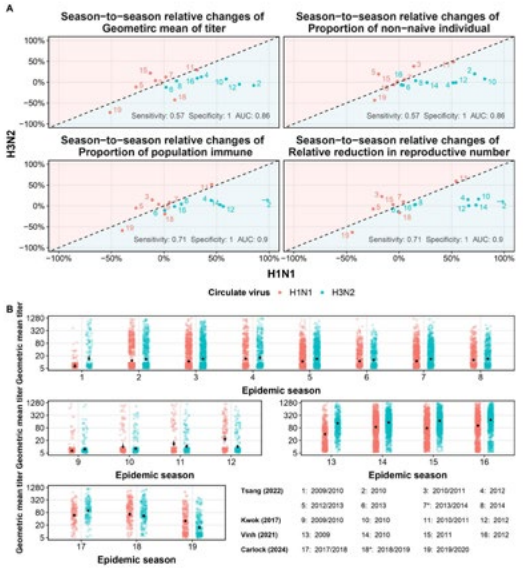
Using influenza as a model system, we analyzed 68,158 serum samples across 4 studies covering 19 epidemics in 2009-2020, establishing four hemagglutination-inhibiting (HAI) antibody titer-based estimators: geometric mean titer, proportion of non-naïve individuals, proportion of population immune, and relative reduction in reproductive number. We found that subtype-specific relative changes of these estimators from previous seasons predicted predominant subtypes in upcoming seasons with up to 80% sensitivity and 100% specificity. In a longitudinal cohort spanning eight influenza seasons with serum collection during epidemics, we found significant negative correlations between each estimator and subsequent

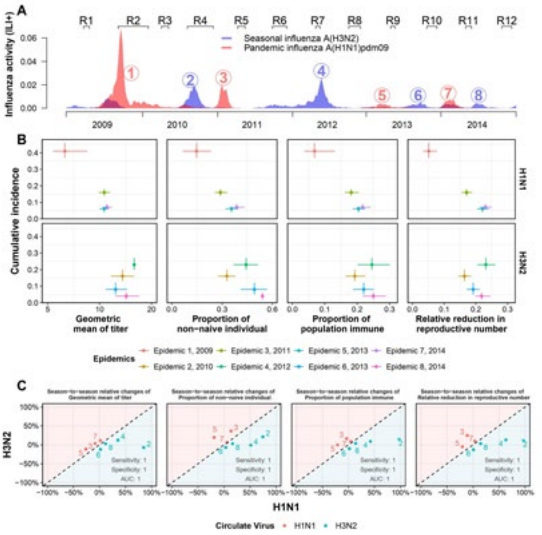
cumulative incidence for H1N1 viruses, but weaker correlations for H3N2 viruses. These relationships remained consistent regardless of the specific protection threshold assigned to different antibody titer levels, provided individual-level protection was at least moderate. Simulation studies revealed that lower effective reproductive numbers and higher antibody waning rates diminished the correlation strength.

CONCLUSIONS

Our framework provides a systematic approach for evaluating population immunity estimators that could inform preparedness efforts for seasonal epidemics and potentially extend to other infectious diseases.







MATHEMATICAL MODELLING AND PROJECTIONS, INCLUDING LIMITATIONS
SCS08 • AUDITORIUM 2 - BREAKOUT • TUE 21 OCT 2025 - 14:00 - 15:30

serojump: A Bayesian tool for inferring infection timing and antibody kinetics from longitudinal serological data

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INTRODUCTION

Understanding the dynamics of acute infectious diseases at both individual and population levels is essential for effective public health preparedness and response. Serological assays, which measure biomarkers of humoral immunity, offer valuable insight into immune responses following infection or vaccination. However, traditional serological analysis methods—such as binary seropositivity and fixed seroconversion thresholds—often rely on heuristics that overlook individual variability in antibody kinetics and timing of infection. This can lead to biased estimates of infection burden and immune responses.

METHODS

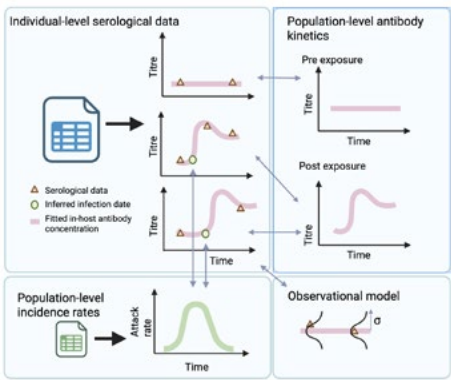
To overcome these limitations, we developed serojump, a novel probabilistic modeling framework and accompanying software package. Serojump leverages individual-level serological time series data to jointly infer infection status, estimate infection timing, and characterize post-exposure antibody kinetics. We validated the method using both simulated data and real-world SARS-CoV-2 serological datasets from The Gambia. In simulation studies, serojump accurately recovered individual infection status, population-level antibody trajectories, and relationships between biomarkers and protection, even under observational noise.

RESULTS

When benchmarked against conventional serological heuristics, serojump demonstrated superior sensitivity in identifying infections and greater precision in estimating infection timing. Applied to longitudinal SARS-CoV-2 data collected during the Delta wave in The Gambia, serojump uncovered missed infections with sub-threshold antibody rises and detailed immune responses across multiple biomarkers following vaccination and infection. These findings highlight key advantages over static threshold-based approaches.

DISCUSSION

Serojump provides a pathogen-agnostic and flexible framework for extracting richer insights from serological datasets. By accommodating individual variation in antibody responses, it enables more accurate estimation of infection dynamics, immune boosting, and potential correlates of protection. As an open-source tool, serojump supports a wide range of study designs and infectious disease applications, offering a valuable resource for researchers and public health practitioners.



MATHEMATICAL MODELLING AND PROJECTIONS, INCLUDING LIMITATIONS
SCS08 • AUDITORIUM 2 - BREAKOUT • TUE 21 OCT 2025 - 14:00 - 15:30

Integrating Serology and PCR to Infer RSV Infection Dynamics and Correlates of Protection in a Community Cohort in The Gambia

David HODGSON (1,5), Laura GATCOMBE (2), Tom COLEMAN (2), Sheikh JARJU (3), Rhys WENLOCK (3), Ben LINDSEY (4), Madikoi DANSO (3), Natalie BARRATT (4), Irina GROUNEVA (4), Marie GOMES (3), Ya-Jankey JANGE (3), Dawda JOBE (3), Beate KAMPMANN (1), Ashley OTTER (2), Adam KUCHARSKI (5), **Thushan DE SILVA** (3,4)

1: Center for Global Health, Charite, Berlin; 2: UK Health Security Agency, UK; 3: Vaccines and Immunity Theme, MRC Unit The Gambia at LSHTM, The Gambia; 4: The Florey Institute of Infection, University of Sheffield, UK; 5: Centre for Mathematical Modelling of Infectious Disease, LSHTM, UK

INTRODUCTION

Respiratory Syncytial Virus (RSV) is a leading cause of respiratory illness worldwide, particularly affecting young children and older adults. Gaining insight into the dynamics of RSV infection and immunity is critical for guiding vaccine development and public health planning. This study analyses antibody responses to RSV using data from a household cohort in The Gambia, combining longitudinal serological measurements with PCR-confirmed symptomatic and asymptomatic infections to assess antibody kinetics and correlates of protection.

METHODS

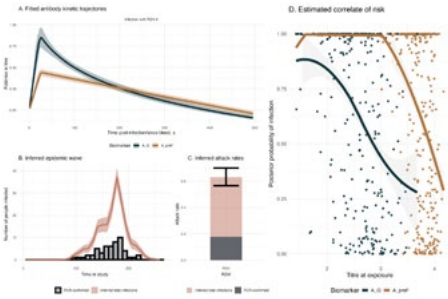
We applied serojump, a Bayesian framework that jointly models antibody kinetics and infection risk. The analysis focused on key RSV biomarkers—preF, postF, G and M protein IgG antibodies in 349 individuals — measured across three timepoints over 52 weeks. The model incorporated both PCR-confirmed infections detected through weekly sampling and cases inferred from serology to provide a comprehensive view of RSV transmission.

RESULTS

By accounting for changes in preF, postF, G, and M antibodies over time, we estimated an overall attack rate of 62% (95% CrI: 56–70), compared to just 19% detected by PCR alone. 82% of PCR-confirmed infections were asymptomatic. Infants under five years had the highest attack rate (70%, 95% CrI: 70–86), followed by older children (65%, 95% CrI: 48–66) and adults (57%, 95% CrI: 48–66). PreF and G antibodies showed distinct kinetics, with peak responses occurring around 8 days post-infection. The peak fold-rise in preF was 2.8 (95% CrI: 2.5–3.0). Higher pre-infection antibody levels were associated with a lower risk of infection, and preF emerged as a stronger correlate of protection than other biomarkers.

DISCUSSION

We demonstrate a high annual attack rate for RSV in The Gambia, leading to frequent boosting of RSV-specific IgG, predominantly from asymptomatic infections. These findings underscore the value of incorporating serologically inferred infections into RSV surveillance to capture the true burden of infection. While preF antibodies are already established as correlates of protection (CoP) against RSV disease, our findings suggest they may also serve as CoP against largely asymptomatic infection—an important distinction for understanding transmission and immunity. This will be further strengthened by ongoing analysis of paired RSV-specific mucosal IgA. The serojump framework offers a robust tool for future seroepidemiological studies of RSV and other respiratory viruses, with significant implications for understanding population-level immunity.



MATHEMATICAL MODELLING AND PROJECTIONS, INCLUDING LIMITATIONS
SCS08 • AUDITORIUM 2 - BREAKOUT • TUE 21 OCT 2025 - 14:00 - 15:30

Joint reconstruction of influenza A(H1N1) and A(H3N2) antibody dynamics to estimate the risk of influenza virus infection

Tim TSANG (1), Ranawaka PERERA (1,2), Vicky FANG (1), Dennis IP (1), Malik PEIRIS (1,2), Gabriel LEUNG (1,3), Benjamin COWLING (1,3), Simon CAUCHEMEZ (4)

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BACKGROUND

In typical influenza A seasons, co-circulation of influenza A(H1N1) and A(H3N2) virus are common, despite one subtype being dominant. In the context of cross-reactivity, joint consideration of hemagglutination-inhibiting (HAI) titers of both subtypes may improve the identification of infections.

METHODS

We develop a novel Bayesian model to jointly estimate the antibody titer dynamics of H1N1 and H3N2 to identify infections, allowing for boosting and waning, measurement errors, and cross-reactivity between them. We apply this approach to a large cohort of 2367 individuals followed for up to 6 years in Hong Kong.

RESULTS

On average, HAI titers increased 25-fold and 12-fold for children and adults after H1N1 infections, respectively. For H3N2, the average rise was 18-fold for children and 10-fold for adults. HAI titers then declined by 19% and 27% annually after H1N1 infections in children and adults, respectively, and by 11% and 12% after H3N2 infections. In eight epidemics, the infection risks for adults with HAI titers < 10 were 1.9%-25% for dominating strains and 0.2-4.3% for non-dominating strains. For children, risks were 4.3%-50% and 0.2-10% for dominating and non-dominating strains, respectively. Investigating 11,325 person-epidemics across eight epidemics, we probabilistically identified 3,174 infections (95% CrI: 3,106-3,242), of which 275 (95% CrI: 250-300) were co-infected by H1N1 and H3N2. Among 2,002 observed cases with a 4-fold or greater rise, we estimated 65% (95% CrI: 63%-66%) were caused by homologous infections and 7.1% (95% CrI: 6.4%-7.8%) by heterologous infections. Among 766 observed cases with a 2-fold rise, we estimated 35% (95% CrI: 33%-36%) and 16% (95% CrI: 15%-17%) resulted from homologous and heterologous infections, respectively.

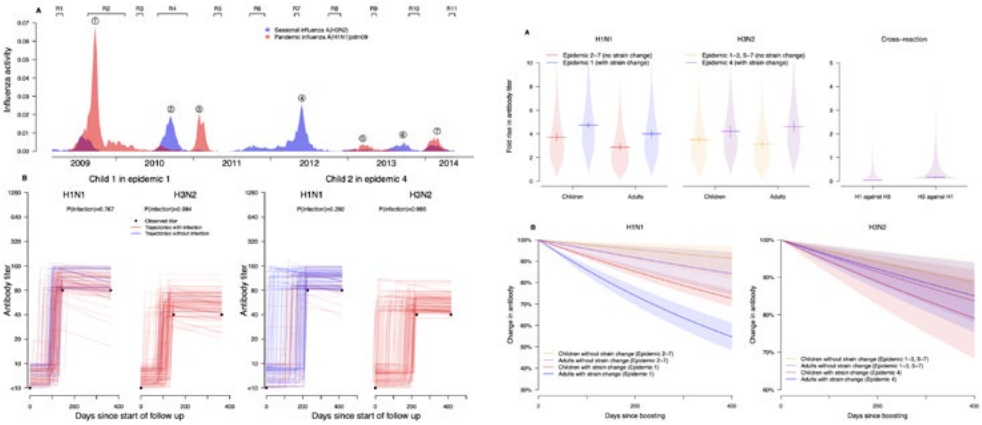
CONCLUSIONS

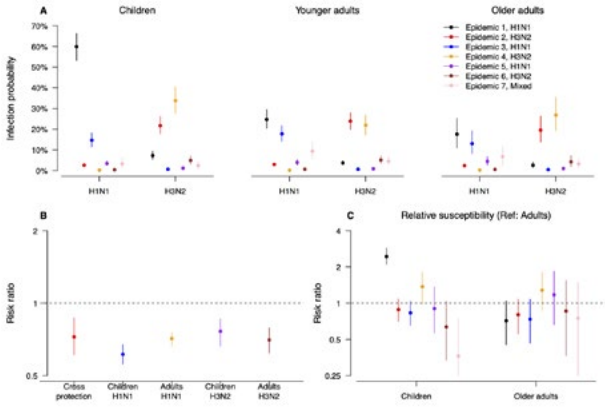
Our inferential framework clarifies the contributions of co-infections, and cross-reactivity to characterize individual infection risk.

Figure 1. Timelines of study and illustration of individual HAI titer trajectories reconstruction. Panel A. Timelines of our study, rounds of blood sample collection, and local influenza activity based on surveillance data. Panel B: Illustration of HAI titer trajectories, infection status, infection time, and pre-epidemic HAI titer in two example individuals.

Figure 2. Estimated distribution of individual boosting and waning. Panel A: Estimated boosting distribution in HAI titer after infection by subtype, and the distribution of cross-boosting due to infections of different subtypes. Panel B. The waning after boosting in HAI titer from infection by subtype.

Figure 3. Infection probability and its determinant among seven epidemics. Panel A. Estimates of infection probabilities for children, younger adults, and older adults in the six epidemics during our study period. Panel B. The protection associated with a 2-fold increase in HAI titers for children and adults, and cross-protection from infection of different subtypes in the same epidemics. Panel C. The model estimates of age-relative susceptibility for seven epidemics.





MATHEMATICAL MODELLING AND PROJECTIONS, INCLUDING LIMITATIONS
SCS08 • AUDITORIUM 2 - BREAKOUT • TUE 21 OCT 2025 - 14:00 - 15:30

Investigating the predictive power of machine learning algorithms for antigenic novelty of influenza H3 viruses ECas

Lucy GREENWOOD, Ricardo AGUAS
University of Oxford, United Kingdom

BACKGROUND
Seasonal influenza results in hundreds of thousands of deaths every year across the world, despite improvements in vaccine development. The current vaccine selection and production pipeline is cumbersome, resulting in long lags from novel strain detection to protection via vaccination. This contributes to a lack of population immunity against emerging antigenically novel strains thus enabling their fixation through fast selective sweeps. This lag is due in large part to the need to empirically establish the level of cross immunity between strains through haemagglutination inhibition (HI) assays.

We hypothesise that machine learning algorithms trained on existing HI data and routinely collected sequence data can establish a highly predictive genotype to phenotype (GP) map. Used in real-time, such methods would allow for early detection of antigenically relevant novel strains that would warrant a vaccine update.

METHODS
Using existing publicly available HI and amino acid sequence data, machine learning models have been developed to predict antigenic distance between pairs of influenza strains. These models leverage features derived from the amino acid sequences in combination with physicochemical properties such as hydrophobicity, charge, polarity, and beta-sheet propensity. Input features include both raw differences in these properties between aligned residues and more advanced encodings that incorporate structural context, such as residue surface accessibility and structural proximity to other residues. To reduce overfitting and prioritize potentially biologically meaningful signals, residue positions consistently ranked with high feature importance across random forest runs for each physicochemical property were extracted. This filtering prioritizes structurally exposed and immunologically relevant positions while excluding buried or invariant sites less likely to drive antigenic evolution, contributing to model interpretability.

RESULTS
We provide comparative predictive performance of several GP maps, demonstrating that higher levels of accuracy are generally seen when considering structural elements of the proteins compared to models that only consider the raw physiochemical differences at each position. We demonstrate the validity of the proposed use of GP maps for early detection of strains of interest for vaccine update.

CONCLUSIONS
Once such a GP map is established, and refined with additional recent data, it can be used both to inform flu vaccine updates and explore the fundamental limits of the genetic and antigenic evolution of influenza. Such methods can be expanded to explore all possible antigenically relevant evolutionary trajectories to investigate the existence of broadly protective strains that could be included in a shift mitigating vaccine.

MATHEMATICAL MODELLING AND PROJECTIONS, INCLUDING LIMITATIONS
SCS08 • AUDITORIUM 2 - BREAKOUT • TUE 21 OCT 2025 - 14:00 - 15:30

Comparative Pre-symptomatic Transmission Potential of Influenza A and SARS-CoV-2 in Households ECas

Chengyao ZHANG (1), Vicky J. FANG (1), Kwok-Hung CHAN (2), Gabriel M. LEUNG (1,3), Dennis K. M. IP (1), J. S. Malik PEIRIS (1,4), Benjamin J. COWLING (1,3), Tim K. TSANG (1,3)

1: WHO Collaborating Centre for Infectious Disease Epidemiology and Control, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region, China.; 2: Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region, China.; 3: Laboratory of Data Discovery for Health Limited, Hong Kong Science and Technology Park, New Territories, Hong Kong.; 4: HKU-Pasteur Research Pole, The University of Hong Kong, Hong Kong Special Administrative Region, China.

BACKGROUND
Symptom-based strategies, including case isolation and contact quarantine, are widely adopted to control epidemics of respiratory infectious diseases, while the effectiveness is influenced by the pre-symptomatic transmission. Previous studies revealed that influenza A and SARS-CoV-2 infections could occur prior to the onset of clinical illness, and both viruses were found to have viral shedding before symptoms. However, it is challenging to distinguish between pre-symptomatic and symptomatic infections, as infection times are usually unobserved.

METHODS:
Our study introduces a new framework to estimate the proportion of pre-symptomatic transmission while considering characteristics affecting transmission heterogeneity. We conducted case-ascertained household studies in Hong Kong, analyzing 493 households affected by influenza A between 2008 and 2017, and 100 households impacted by SARS-CoV-2 in December 2022. An individual-based hazard model was used to describe transmission dynamics and quantify effects of various factors associated with individual susceptibility and infectiousness. Our model estimated the distribution of infectiousness profile which represented the probability of transmission occurring at each time point relative to symptom onset, and depended on the length of incubation, enabling the calculation of the proportion of pre-symptomatic transmission.

RESULTS:
We estimated that the transmission of influenza A started 1 day before symptom onset, and the mean of infectiousness profile was 0.49 (95% credible interval (CrI): 0.20, 0.79) days after symptom onset. Meanwhile, the infectiousness profile of SARS-CoV-2 began 6 days prior to illness, with a mean equal to 3.73 (95% CrI: 2.02, 6.08) days before the presence of symptoms. Therefore, 3.70% (95% CrI: 2.58%, 5.21%) of influenza A infections and 57.04% (95% CrI: 43.50%, 69.17%) of SARS-CoV-2 transmission occurred before symptom onset. Besides, we found younger age was associated with increased influenza A susceptibility and infectiousness, while higher viral load and fever symptoms also indicated an increased infectiousness for influenza A. Our model was assessed to be adequate and with no systematic bias. We also observed that our findings remained robust regardless of whether the infectiousness profiles were assumed to follow Poisson or Gamma distributions.

CONCLUSIONS
We revealed that compared to influenza A, SARS-CoV-2 had a significantly higher potential of pre-symptomatic transmission leading to a substantial amount of infections occurring during the incubation period. Therefore, traditional symptom-based measures were not sufficient to control SARS-CoV-2 outbreaks, and additional social distancing policies are necessary. Our study provides a reliable framework to estimate the proportion of pre-symptomatic infections, which can be applied to other pathogens in future works.

IMPLEMENTATION OF ADULT AND RISK GROUPS NATIONAL IMMUNISATION PROGRAMMES

SPI04 • AUDITORIUM 3 - BREAKOUT • TUE 21 OCT 2025 - 14:00 - 15:30

Effectiveness of Maternal Influenza Vaccination in Preventing Influenza Infection in Infants Aged ≤ 6 Months in Korea

Yoonsun YOON (1), Ji Young PARK (2), Young June CHOE (3), Yun-Kyung KIM (2)

1: Korea University Guro Hospital, Korea, Republic of (South Korea); 2: Korea University Ansan Hospital, Korea, Republic of (South Korea); 3: Korea University Anam Hospital, Korea, Republic of (South Korea)

BACKGROUND

Infants under 6 months of age are at increased risk of severe influenza but are ineligible for direct vaccination. Maternal influenza vaccination during pregnancy is recommended to provide passive protection to young infants. Although Korea has offered free influenza vaccination to pregnant women since October 2019, evidence on vaccine effectiveness (VE) in infants is limited. This study aimed to evaluate the effectiveness of maternal influenza vaccination in preventing laboratory-confirmed influenza in infants aged ≤ 6 months in Korea.

METHODS

We conducted a test-negative case-control study during the 2023–2024 and 2024–2025 influenza seasons at three tertiary hospitals in Korea. Infants aged ≤6 months hospitalized with influenza-like illness (ILI) who underwent rapid antigen or PCR testing were enrolled. Cases were defined as influenza-positive, and controls as influenza-negative. Maternal vaccination status and gestational timing were obtained through medical records and interviews. VE was calculated using the formula: VE = (1 – odds ratio) × 100.

RESULTS

Among 327 enrolled infants, 47.4% were born to vaccinated mothers. Overall VE against laboratory-confirmed influenza was 55.6%. VE by season was 30.2% in 2023–2024 and 71.4% in 2024–2025. Influenza infection occurred in 3.2% of infants of vaccinated mothers and 7.0% of those of unvaccinated mothers (OR = 0.444). When restricted to appropriately timed vaccination (after 13 gestational weeks and excluding those within 2 weeks of delivery), VE was 34.9%. VE in infants under 3 months was 55.4%. VE against influenza A was 39.6%, and all three cases of influenza B occurred in infants of unvaccinated mothers.

CONCLUSION

Maternal influenza vaccination significantly reduced influenza risk in infants, particularly with timely vaccination. These findings support maternal immunization as an essential public health strategy to protect young infants.

Preliminary observation of impact of Maternal RSV Vaccination on Infant Hospitalizations in Mendoza, Argentina: A Comparative Study of the 2023 and 2024 Seasons

Juan Manuel FERNANDEZ MUÑOZ (1), Abigail MORETA (1), Anahé Belén PACHECO (1), Iris Soledad AGUILAR (2), María Marcela ROSALES (2), Carlos ESPUL (1)

1: Ministry of Health of Mendoza, Argentine Republic; 2: Departamento Provincial de Inmunizaciones

BACKGROUND

Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract infections and hospitalization in infants worldwide. In Argentina, seasonal RSV epidemics significantly burden the healthcare system, particularly among infants under six months of age. In 2023, the Ministry of Health of Argentina incorporated an RSV vaccine into the National Immunisation Programme, targeting pregnant women between 32 and 36 weeks of gestation with a single dose of a bivalent RSVpreF vaccine. The province of Mendoza implemented this strategy in early 2024, achieving over 81% coverage among pregnant women. This study aims to evaluate the impact of the maternal RSV vaccination campaign on the burden of RSV in infants during their first six months of life, by comparing epidemiological indicators between the 2023 (pre-vaccine) and 2024 (post-vaccine) seasons.

METHODS

This retrospective analysis included all confirmed RSV-positive cases reported to the national surveillance system (SISA) and the provincial epidemiological bulletins during the 2023 and 2024 seasons in Mendoza, Argentina. We focused on the pediatric population, particularly on infants under one year. We extracted data on RSV positivity, hospital admissions, ICU admissions, and virus subtype (A or B). Implementing maternal RSV vaccination in 2024 allowed for a comparative assessment of disease severity indicators between the pre- and post-vaccination periods.

RESULTS

The total number of RSV-positive cases was comparable between the two seasons: 1004 in 2023 and 1014 in 2024. In 2023, 600 cases occurred in children under one year of age, including 41 infants under one month. In 2024, there were 537 cases in infants under one year, with only 16 in the first month of life. However, a higher number of hospitalizations (732 vs. 571) and ICU admissions (110 vs. 70) were reported in 2024 compared to 2023. Regarding viral subtypes, RSV-A predominated in 2023, while RSV-B was more prevalent in 2024.

CONCLUSION

Despite high maternal vaccine coverage in Mendoza in 2024, no reduction in hospitalization or ICU admissions among RSV-positive infants was observed during the first post-vaccination season. Although a decrease in cases under one month may suggest early passive protection, the overall severity indicators were unexpectedly higher. The shift in circulating RSV subtype between seasons may also influence clinical outcomes. These preliminary findings highlight the need for further analyses to understand vaccine impact in real-world settings, accounting for virological, clinical, and immunological variables.

FUTURE VACCINATION STRATEGIES - PART 2

SCS09 • AUDITORIUM 1 - PLENARY HALL • WED 22 OCT 2025 - 11:00 - 12:30

A Novel Antigenically Central HA mRNA Vaccine Confers Humoral and Cellular Immune Responses and Protects Ferrets Against Challenge With a Heterologous A(H5) Clade 2.3.4.4b Highly Pathogenic Avian Influenza Virus ECaS

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BACKGROUND

The recent increased incidence of A(H5) highly pathogenic avian influenza virus (HPAIV) infections in mammals raises concerns about a novel pandemic. Pandemic preparedness is impeded by the continuous antigenic evolution of the A(H5) hemagglutinin (HA). In the pursuit to develop a subtype-wide A(H5) vaccine, we previously successfully employed antigenic cartography to rationally design an antigenically central A/Anhui/1/2005 HA vaccine antigen (AC-Anhui). A split-inactivated vaccine based on AC-Anhui HA elicited antibody responses that broadly cover the A(H5) antigenic space. Here, we aimed to compare the immunogenicity and protection afforded by an mRNA vaccine based on AC-Anhui with one on the widely stockpiled vaccine clade 2.3.4.4b A/Astrakhan/3212/2020 (Astrakhan) against the recent clade 2.3.4.4b A(H5) A/Texas/37/2024, using ferrets as a preclinical model.

METHODS

Six ferrets per group were intramuscularly vaccinated in homologous or heterologous prime-boost regimens with mRNA vaccines containing the AC-Anhui HA and/or Astrakhan HA, or with PBS, with a one-month interval between prime and boost. Blood samples were collected on days 0, 28, and 42 after prime to assess A(H5)-specific T-cell responses through IFN-g enzyme-linked immunosorbent spot (ELISpot), followed by T-cell phenotyping. On day 0, 28, 54, and 63 after prime, blood was collected to assess antibody responses by hemagglutination inhibition (HI) assays. Fifty-nine days after prime, animals were challenged intranasally and intratracheally with 103 TCID50/mL of HPAIV A/Texas/37/2024. Four-days post-inoculation, ferrets were euthanized and tissues (e.g. respiratory tissues (nasal turbinates, trachea and lung), lymphoid tissues (tracheobronchial lymph node and spleen), jejunum, and cerebrum) were collected for virological and histopathological analyses.

RESULTS

Following two immunizations, HI antibodies and H5-specific CD8+ T-cell responses against the vaccine antigens were detected, demonstrating the immunogenicity of A(H5) mRNA vaccines in ferrets. Contrary to PBS vaccinated animals, mRNA vaccinated animals were protected against clinical presentation, severe disease, virus replication in the lower respiratory tract, and extra-respiratory viral dissemination upon challenge, regardless of the vaccination regimen. Differences observed between PBS and every mRNA vaccinated group were significantly different for all measured parameters during the challenge experiment (e.g. bodyweight, body temperature, virus titers in throat and nose swabs, and tissues). Within the mRNA vaccinated groups, protection conferred by the AC-Anhui homologous and heterologous vaccine regimens was very comparable to non-inferior to that conferred by the antigenically closer Astrakhan homologous. When testing post-challenge sera (day 63) against a total of 78 antigenically similar and distinct A(H5) antigens, ferrets vaccinated with AC-Anhui homologous showed the highest and the broadest HI antibody responses. In contrast, antibody profiles revealed that Astrakhan mRNA vaccination resulted in the lowest and narrowest HI antibody responses.

CONCLUSIONS

These results highlight the immunogenicity and protection capacity of A(H5) mRNA vaccines in ferrets and the promise of the AC-Anhui antigen, showcasing its relevance for (pre)pandemic preparedness.

Breast milk-derived secretory IgA provides broad protection against influenza A virus ECas

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BACKGROUND
Passive immunization strategies could play a critical role in bridging the gap between the onset of a pandemic or outbreak and the widespread implementation of specific vaccines. They can also provide protection against routine infections, especially in those who are immunocompromised. Current passive immunization approaches rely on intravenously administered plasma or monoclonal antibodies, which pose challenges in terms accessibility and scalability. Novel passive immunization strategies are therefore needed.

Mucosally-administered antibodies offer an alternative to traditional intravenous delivery, particularly in the context of respiratory pathogens. However, large-scale production of respiratory mucosal antibodies is challenging and costly. Breast milk presents an attractive alternative source of mucosal antibodies, as it is readily accessible and rich in secretory IgA. We therefore investigated the antiviral properties of breast milk-derived secretory IgA.

METHODS
Breast milk was collected from 120 lactating individuals, with a heterogenous vaccination and infection history. Secretory IgA (sIgA) was purified from breast milk and pooled together. ELISAs were used to determine binding activity against a panel of respiratory viruses. Neutralization potency of breast milk-derived IgA against the same panel was also tested. Challenge experiments were conducted using BALB/c and antibody deficient (Jh-/-) mice, with animals receiving breast milk-derived IgA intranasally prior to infection with influenza A virus (IAV).

RESULTS
Breast milk-derived IgA demonstrated broad antiviral activity against representative strains of IAV, respiratory syncytial virus (RSV), and adenovirus. Specifically, breast milk-derived IgA was able to bind and neutralize both group 1 and group 2 IAV, as determined by ELISA and microneutralization assays. Additionally, it contained broadly neutralizing antibodies against IAV, neuraminidase-inhibiting antibodies, and could antibodies capable of eliciting Fc-dependent effector mechanisms. Interestingly, breast milk-derived IgA also contained antibodies capable of recognizing the emerging highly-pathogenic H5N1 2.3.4.4b clade.

In vivo, intranasal administration of breast milk-derived IgA protected both immunocompetent and immunocompromised mice from morbidity and mortality after IAV infection. Treated mice exhibited reduced tissue damage and dampened inflammatory responses, further supporting the therapeutic potential of breast milk-derived IgA administered to the respiratory mucosa.

CONCLUSIONS
More innovative passive immunization strategies are needed to protect vulnerable populations at the onset of a pandemic. We demonstrated that breast milk-derived IgA has broad anti-IAV activity *in vitro* and can protect against morbidity and mortality in both immunocompetent and immunocompromised mouse models. Our findings highlight breast-milk derived IgA as a promising alternative passive immunization strategy for respiratory infections.

FUTURE VACCINATION STRATEGIES - PART 2
SCS09 • AUDITORIUM 1 - PLENARY HALL • WED 22 OCT 2025 - 11:00 - 12:30

Hemagglutination inhibition antibody titer as correlate of protection against influenza virus infection: a systematic review and meta-analysis

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BACKGROUND

Hemagglutination inhibition (HAI) antibody titer is used as a vaccine correlate of protection (CoP) to predict vaccine efficacy and accelerate approval of seasonal inactivated influenza vaccines. A post-vaccination serum HAI titer of 40 was estimated to be associated with a 50% reduction in risk of influenza virus infection (i.e. 50% protection), although estimates from subsequent studies differed. We performed a systematic review and meta-analysis on the association of HAI titer and influenza infection risk.

METHODS

We identified studies in humans (observational studies, vaccine trials and human challenge studies) published before 26 June 2024 using search terms (influenza) AND ('haemagglutin*' or 'hemagglutin*' or 'HAI' or 'HI' or 'tit*') AND ('protect*'). We included studies reported ≥5 infections and either (i) numbers of infected individuals for two or more pre-infection HAI titer levels, or (ii) estimate and 95% confidence interval (CI) of protection level associated with a HAI titer of 40. For (i), we estimated the protection level associated with an HAI titer of 40 as (1-RR)x100%, where RR is the risk ratio of infection risk at an HAI titer of 40 compared to at a titer <10. We classified studies by whether the virus strain in vaccination, HAI assay and infection were the same. We evaluated heterogeneity with the *I2* statistic, and derived pooled estimate of protection using Mantel-Haenszel fixed-effect model where appropriate.

RESULTS

We identified 31,815 titles, screened 513 full-texts and included 53 studies. Reported protection level estimates or estimates calculated from studies using serologic definition of infection, or from challenge studies, were highly heterogeneous. Using infection data from studies with virologic-defined infection, in studies where (vaccine strain,) HAI tested strain and infecting strain were the same, we estimated a pooled RR of 0.53 (95% CI 0.47, 0.59) associated with an HAI titer of 40 in studies with recent vaccination, translating to a protection level of 46% (95% CI 41%, 51%); and a pooled RR of 0.53 (95% CI 0.41, 0.69) in studies without reported vaccination in the past 6 months. We estimated HAI titers of 46, 813 and 6993 were associated with 50%, 80% and 90% protection respectively. Separately, we estimated a pooled RR of 0.81 (95% CI 0.75, 0.87) in studies where HAI tested strain was different from infecting strain.

CONCLUSIONS

Community-acquired infection with virologic definition of infection allowed more consistent estimates of protection level associated with an HAI titer against influenza virus infection. Minimal studies were available to assess differences in homologous protection between ages, or to assess cross-protection from a mismatched vaccine against infection by a drifted virus mediated through HAI antibodies targeting the drifted virus. Higher HAI titer associated with more than 50% protection may be considered for accelerated vaccine approval especially for vulnerable populations.

Pooled Long-Term Safety Analysis of a Phase 1/2/3 Randomized, Observer-Blind, Controlled Study of the Self-Amplifying mRNA COVID-19 Vaccine ARCT-154 in Adults

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BACKGROUND

Self-amplifying (sa-) mRNA technology allows for use of lower vaccine doses due to host cells making copies of vaccine mRNA. sa-mRNA COVID-19 vaccines have been shown to be well-tolerated and immunogenic. The sa-mRNA vaccine ARCT-154 encodes the S-protein of the Wuhan-Hu-1 virus variant D614G. ARCT-154 has proven to be immunogenic and efficacious with a good safety profile up to 92 days post-vaccination in a large multi-phase study (Hồ *et al*. Nat Commun. 2024;15:4081). Here, we present the pooled long-term safety analysis.

METHODS

Recruitment of participants involved 16 clinical centers in Vietnam (NCT05012943). Enrolled participants were healthy adults ≥18 – <60 years (Phase 1) and adults ≥18 years in Phases 2 and 3, including those at high risk of severe COVID infection. They were randomized 3:1 (Phases 1, 2, 3a) or 1:1 (Phase 3b) to receive two doses of either ARCT-154 or placebo (sterile saline) 28 days apart. To ensure all participants in Phases 1, 2 and 3a/b received immunization against COVID-19 there was a switchover at Day 92 when placebo recipients from all phases received ARCT-154 as two doses 28 days apart. Participants from Phase 2 and 3a who received ARCT-154 as the initial vaccination were re-randomized to receive a third dose of ARCT-154 or placebo. Participants from Phases 1 and 3b who received ARCT-154 as the initial vaccination received placebo as two doses 28 days apart. Safety data were collected in Phases 1/2/3a/3b from the initial vaccination up to Day 394 (end of study). Safety data included adverse events (AEs) within 28 days after each vaccination across all phases, serious AEs (SAEs) and medically-attended AEs (MAAEs). All AEs were coded using MedDRa system organ class and preferred terms.

RESULTS

The safety analysis population comprised 16,396 participants, all of whom received at least one dose of ARCT-154 at Days 1 and 29 or at switchover (Days 92 and 120). Between switchover and Day 394, across all phases, SAEs occurred in less than 2.5% of ARCT-154 recipients, and one vaccine-related SAE was reported. MAAEs between switchover and Day 394 were experienced by approximately 34% of ARCT-154 participants in Phases 1/2/3a and 20% in Phase 3b. Less than 0.5% of MAAEs were considered to be vaccine-related. Both SAEs and MAAEs were reported at a similar frequency in the placebo and ARCT-154 groups. The number of deaths between switchover and Day 394 for ARCT-154 was low (0.2%; n=12) and similar in the placebo group.

CONCLUSIONS

ARCT-154 was well-tolerated in all phases of the study and no safety concerns were identified. Reported AEs were generally transient, mild to moderate in severity, and mostly unrelated to the vaccine. Its long-term safety profile supports use of ARCT-154 in annual vaccination campaigns at a population level.

FUTURE VACCINATION STRATEGIES - PART 2
SCS09 • AUDITORIUM 1 - PLENARY HALL • WED 22 OCT 2025 - 11:00 - 12:30

Decades of Evidence Revisited: A Systematic Review on Anti-Neuraminidase Antibody Responses and Influenza-related Outcomes

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BACKGROUND

Antibody responses to haemagglutinin (HA) are widely accepted as an indicator of protection against influenza, while anti-neuraminidase (NA) responses have not been similarly recognized. However, studies suggest that NA-specific antibodies may reduce illness, viral shedding and infection severity. In this work, we aimed to better understand the clinical evidence linking anti-NA responses to influenza protection.

METHODS

We conducted a systematic literature review to identify and qualitatively synthesize historical peer-reviewed evidence on characteristics and value of anti-NA immune responses against influenza-related outcomes. The review followed the Cochrane Handbook for Systematic Reviews of Interventions, with searches in Embase, MEDLINE, pre-print databases and grey literature to capture evidence not included in these databases. We grouped each publication by the observed strength of relationship between pre-existing anti-NA responses and outcomes as defined by the authors. Examples of groups include: "statistically insignificant" meaning no statistically significant association; "significant association" meaning ≥1 statistically significant but not necessarily independent association; "independent association/predictive relationship" meaning significant association after adjusting for anti-HA titres.

RESULTS

The final selection comprised 36 studies published between 1972 and 2024, including prospective cohort (16), human-challenge (12), randomized control trials (6), case-control (1), and retrospective cohort (1) studies. Pre-existing immunity was categorized as hybrid immunity (22) (a combination of natural exposure and vaccination), vaccinated vs. unvaccinated comparators (8), naturally acquired influenza only (4), and vaccination only (2). Endpoints included in underlying studies: laboratory-confirmed infection (28), symptom occurrence/severity (14), symptom duration (6), viral shedding (11), severe influenza complications (1), and viral transmission (1).

Associations were found between higher pre-existing anti-NA responses and reduced risk or severity of influenza-related outcomes, sometimes independent of anti-HA responses. However, the type and strength of these relationships varied, ranging from statistically insignificant to significant, with some studies finding an independent/predictive relationship. The most substantial body of literature focused on laboratory-confirmed infection (n=28), where most studies documented at least a significant association (n=20), and some studies found an independent predictive relationship (n=10) between anti-NA response and clinical outcomes. These relationships persisted irrespective of heterogeneity in study design, patient characteristics, and antibodies assays used.

CONCLUSIONS

Despite heterogeneity in study design, origin of pre-existing anti-NA response, and clinical settings between studies, there is strong evidence suggesting an association between pre-existing anti-NA immunity and protection against influenza outcomes.

These findings imply that NA antibodies from natural infection or immunization could play a role alongside HA antibodies in reducing the clinical burden of influenza. Improved understanding of the characteristics and magnitude of anti-NA responses can elucidate mechanisms of action of existing influenza vaccines and contribute to the design of next-generation vaccines.

FUTURE VACCINATION STRATEGIES - PART 2
SCS09 • AUDITORIUM 1 - PLENARY HALL • WED 22 OCT 2025 - 11:00 - 12:30

Immunogenicity and Reactogenicity of an mRNA-Based Seasonal Influenza and SARS-CoV-2 Multicomponent Vaccine (mRNA-1083) in Adults ≥65 Years With Comorbidities

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BACKGROUND

Infectious disease burden in older adults underscores the need for effective vaccine strategies; mRNA vaccines have emerged as a promising platform due to their ability to induce robust immune responses and favorable safety profiles, demonstrating effectiveness across various populations, including older adults. In a phase 3 study, mRNA-1083, an investigational multicomponent mRNA vaccine targeting seasonal influenza and SARS-CoV-2, met noninferiority criteria and induced higher immune responses than recommended standard-of-care COVID-19 and influenza vaccines against SARS-CoV-2 and the 3 clinically-relevant influenza strains in adults aged ≥65 years, with an acceptable safety and tolerability profile. Here, we present immunogenicity and reactogenicity data in adults aged ≥65 years stratified by age (65-74 and ≥75 years) and comorbidity (cardiorespiratory and endocrine-metabolic conditions) subgroups.

METHODS

This phase 3, randomized, observer-blind, active-controlled study (NCT06097273) randomly assigned (1:1) adults in 2 age cohort substudies (Cohort A: ≥65 years; Cohort B: 50-64 years) to receive 1 dose of mRNA-1083 (and saline placebo) or co-administered influenza (Fluarix [Cohort B] or Fluzone HD [Cohort A]) and COVID-19 (Spikevax) vaccines. Immunogenicity endpoints included geometric mean antibody levels measured by hemagglutination inhibition assay for influenza and pseudovirus neutralization assay for SARS-CoV-2; geometric mean fold rises from baseline were also assessed. Participant pre-existing medical conditions were reviewed to determine comorbidity subgroups of cardiorespiratory and endocrine-metabolic conditions.

RESULTS

Overall, 4037 participants were randomized in Cohort A to receive mRNA-1083 (n=2025) or Fluzone HD+Spikevax (n=2012). Among vaccinated participants, 3184 were aged 65-74 years and 831 were ≥75 years; 887 had cardiorespiratory and 2202 had endocrine-metabolic comorbidities. Immune responses elicited by mRNA-1083 were generally comparable between the 65-74 and ≥75-year age groups, as well as among individuals with cardiorespiratory and endocrine-metabolic comorbidities compared with the overall study population. mRNA-1083 was generally well-tolerated, with most solicited local and systemic adverse reactions reported as grade 1 or 2 across both age groups and in participants with comorbidities. The reactogenicity profile was comparable in participants aged ≥75 years relative to those aged 65-74 years as well as in participants with cardiorespiratory and endocrine-metabolic comorbidities compared with the overall study population.

CONCLUSIONS

Immune responses elicited by mRNA-1083 were generally similar between adults aged 65-74 and ≥75 years, and between participants with or without cardiorespiratory and endocrine-metabolic comorbidities. mRNA-1083 was generally well-tolerated with an acceptable reactogenicity profile across these subgroups. These findings collectively support the suitability of mRNA-1083 for use in older adults, including those with significant underlying comorbidities.

HUMAN CHALLENGE STUDIES

SCS11 • AUDITORIUM 2 - BREAKOUT • WED 22 OCT 2025 - 11:00 - 12:30

Disease characteristics and immunological profiles obtained from a newly developed hMPV human challenge model

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BACKGORUND

Human metapneumovirus (hMPV) is a leading cause of respiratory infections, especially in young children, the elderly, and immunocompromised individuals. The human challenge model (HCM) has played a major role in advancing vaccines and antiviral drug development. To date, there has been no hMPV HCM model with the appropriate disease characteristics to enable product efficacy testing. Here we describe the development of a hMPV human challenge model.

METHODS

A hMPV A2 strain, isolated from a clinical sample in October 2022, underwent extensive *in vitro* characterization prior to GMP production. A human challenge study was conducted with healthy adult participants experimentally inoculated intranasally with approx. 5 log FFU/mL hMPV A2. Participants were monitored 24/7 within a quarantine unit for 12 days post viral inoculation. Subjects were assessed by symptom diary cards, cold perception questionnaires, NPS for viral load, ECG, bloods and spirometry for safety as well as serum and PMBC collection for immunological profiling. Participants returned for a final Day 28 follow up visit.

RESULTS

All participants safely completed the quarantine with no SAEs or AEs of concern. Symptoms indicative of upper respiratory tract (URT) infection, peaking on day 4, including sneezing, runny nose, and nasal congestion were observed. Perceived colds started by Day 3 and lasted for a median duration of 7 days. qPCR and infectious viral assays showed high infection rates and strong viral load curves in inoculated participants. Serological neutralisation titres were obtained for each participant pre and post challenge (day 28) to assess the role of pre-existing immunity against the challenge strain of hMPV as well as providing insighted on cross protection from other pneumoviruses.

CONCLUSION

A new A2 hMPV human challenge model was demonstrated to be safe and well tolerated, generating robust symptoms and viral load providing a strong platform for the assessment of new vaccines and antiviral treatments.

HUMAN CHALLENGE STUDIES
SCS11 • AUDITORIUM 2 - BREAKOUT • WED 22 OCT 2025 - 11:00 - 12:30

Evolution of Human Metapneumovirus (HMPV) over the last 20 years and superior isolation of HMPV from clinical samples in organoid-derived bronchial cell cultures to isolation in monolayer cell line cultures

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BACKGROUND
Human Metapneumovirus (HMPV) is a globally prevalent respiratory virus that, together with RSV and Influenza, ranks among the top three viral causes of respiratory illnesses (RI) in children, immune compromised and the (frail) elderly. Similar to RSV, two major genetic lineages (A and B) of HMPV are circulating worldwide and continue to evolve into new lineages. Traditional isolation of HMPV in standard monolayer cell lines is not always successful. Recently, it was shown that, upon inoculation of human organoid-derived bronchial (ODB) cultures, HMPV primarily targeted ciliated cells. These observations lead to the hypothesis that isolation of virus from clinical specimen in this ODB model could be more successful than in standard monolayer cultures.

As RSV vaccination programs roll out and interventions for HMPV emerge, accurate assessment of the genetic variation and evolution of HMPV, along with identifying optimal culture conditions, is crucial for understanding its true impact and the development of antiviral therapies that effectively target all genotypes. Here, we studied HMPV evolution between 2005 and 2021 and evaluated human ODB cultures for efficient isolation of HMPV from clinical samples.

METHODS
We analyzed 130 whole HMPV genome sequences obtained from samples collected from individuals hospitalized with RI, and partial fusion and attachment protein gene sequences from samples collected from patients visiting general practitioners, between 2005 and 2021. In addition, the efficiency of HMPV isolation from 36 clinical samples was compared between ODB cultures and Vero-118 cells.

RESULTS:
Phylogenetic analyses demonstrated that the viruses clustered in the 4 main genetic lineages: A1, A2, B1, and B2. In 2003, the A2 lineage split into A2.1 and A2.2 and in 2008, the A2.2 lineage split into lineages A2.2.1 and A2.2.2. Since 2015 the A2.2.2 lineage is primarily defined by viruses with a duplication (either 111 nt or 180 nt) in the G gene. A gradual evolution over time was only clear for lineage A2.2.2 and not so much for the other lineages. No differences were observed in dominant lineages between patients being hospitalized and those consulting general practitioners.

Evaluation of ODB culture and Vero cells for the isolation of HMPV showed that the isolation efficiency of serotype A viruses was comparable in both cultures, while isolation of serotype B viruses was profoundly more efficient in the ODB cultures than in Vero-118 cells.

CONCLUSION:
This study showed the emergence and potential extinction of genetic HMPV lineages throughout recent decades. Based on these findings, we propose criteria for the designation of new genetic lineages to support a more systematic classification of HMPV. Additionally, the results indicate that primary epithelial cultures represents a superior method for isolating HMPV from clinical specimens. These models may also aid in identifying more relevant targets to preventing HMPV infections.

HUMAN CHALLENGE STUDIES
SCS11 • AUDITORIUM 2 - BREAKOUT • WED 22 OCT 2025 - 11:00 - 12:30

A Study of the Efficacy, Safety and Immunogenicity of mRNA vaccines in an Influenza B Challenge Model in Healthy Adults

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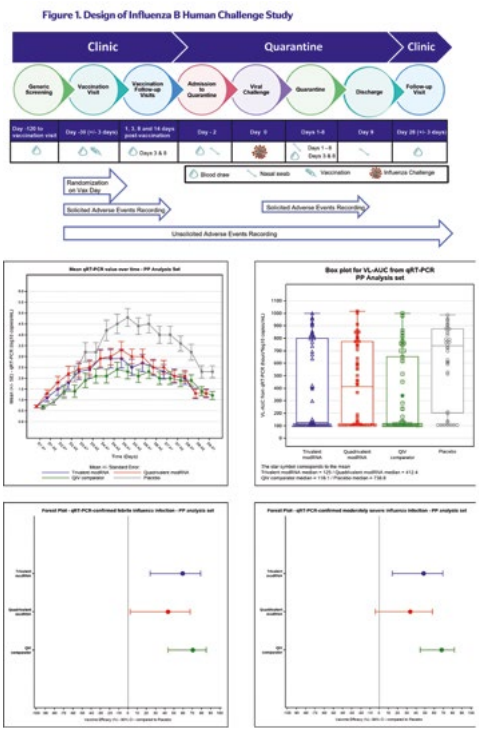
BACKGROUND

Influenza remains a major cause of morbidity and mortality globally. Modified nucleoside messenger RNA (mRNA) vaccines induce robust humoral and cellular immune responses and allow for a close match to circulating strains, offering the potential to improve vaccine effectiveness. A Phase 2a controlled human infection model (CHIM) was used to evaluate the efficacy of mRNA vaccines against challenge with a B/Victoria lineage virus. As seasonal incidence of influenza B is often variable, the CHIM model offers an opportunity to supplement data on vaccine efficacy from clinical studies.



METHODS

Healthy adults 18-55 years of age were randomized to receive 1 of 3 active vaccines (optimized trivalent mRNA [tIRV], quadrivalent mRNA [qIRV], licensed quadrivalent influenza vaccine [QIV] comparator) or placebo, and inoculated approximately 30 days later with a B/Victoria lineage influenza virus that is antigenically similar to the vaccine strain. Post-inoculation, participants were monitored in a quarantine unit, where nasopharyngeal swabs were collected twice daily for quantitative RT-PCR and viral culture (Figure 1). Pre- and post-vaccination HA-specific antibody titers were measured using a hemagglutination inhibition assay (HAI) and a focus reduction neutralization assay (FRNA). T cell immunity was quantified using intracellular cytokine staining. Following vaccination, solicited adverse events were captured for a minimum of 7 days. Post-inoculation symptoms were collected on diary cards daily.

RESULTS

A total of 200 vaccinated participants underwent viral challenge. All active vaccine arms demonstrated statistically significant reductions versus placebo for primary efficacy endpoints, including viral shedding and symptomatic illness (Figure 2). All active vaccine arms elicited HA-specific antibody responses and CD4+ T cell responses; only the mRNA vaccine arms elicited CD8+ T cell responses. Vaccination was generally safe and well-tolerated, with no serious adverse events, deaths or adverse events leading to discontinuation reported.

CONCLUSIONS

This is the first known study to demonstrate efficacy of seasonal influenza mRNA vaccines against influenza B in a CHIM study. Vaccination with tIRV and qIRV mRNA vaccines, as well as the licensed QIV vaccine, induced humoral and cell-mediated immune responses and protected from subsequent challenge with influenza B virus by significantly reducing the viral load, incidence of infection, and symptomatic disease.

HUMAN CHALLENGE STUDIES
SCS11 • AUDITORIUM 2 - BREAKOUT • WED 22 OCT 2025 - 11:00 - 12:30

Development of a Severe Acute Respiratory Syndrome – Coronavirus 2 (SARS-CoV-2) Omicron BA5 human challenge model (HCM) for the assessment of new vaccine and anti-viral therapies in seropositive subjects

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BACKGROUND

hVIVO successfully conducted the world's first human challenge trial (HCT) for Severe Acute Respiratory Syndrome – Coronavirus 2 (SARS-CoV-2) using a wild-type virus SARS-CoV-2/human/GBR/484861/2020 in seronegative subjects. The continued evolution of SARS-CoV-2 and resulting emergence of new viral variants and the roll out of first-generation vaccines requires continual monitoring of vaccine efficacy and updates to new treatments. Here we describe the development of a new SARS-CoV-2 Omicron BA5 human challenge model.

METHODS

A SARS-CoV-2 Omicron BA5 [(BA.1.1.529 lineage)] strain, isolated from a clinical sample in 2022, underwent extensive *in vitro* characterization prior to GMP production. A human challenge study was conducted to characterise the new strain in 18-40 yrs, healthy, serosuitable adult volunteers experimentally inoculated intranasally using a dose escalation/de-escalation design to ensure safety and establish appropriate endpoints for future investigational medicinal product (IMP) studies. Participants were monitored 24/7 within a quarantine unit for up to 14 days post viral inoculation. Subjects were assessed by symptom diary cards, ECG, bloods and spirometry for safety. Mid-turbinate swabs (MTS) and throat swabs (TS) for viral load (VL) were collected twice daily and VL assayed by both qPCR and viral culture. Immunological samples including serum and peripheral blood mononuclear cells (PBMC)s were collected for immunological assessments. Participants returned for follow up visits on Day 28, 90 and 180.

RESULTS

Inoculation with all virus doses was safe and well tolerated with no significant adverse events (SAE)s or AEs of concern, and no stopping criteria were met. Across the doses assessed, increased inoculation titres associated with increased infection and symptomatic infection rates. qPCR and infectious viral assays showed robust infection (50% to 100%) and moderate to severe symptomatic infection rates (17% to 67%). Onset of viral shedding was rapid, with high titres being observed within a day or two of inoculation. Virus was detected via both MTS and TS sampling, with higher more consistent VL profiles from MTS. Symptoms were primarily upper respiratory in nature, supported by systemic symptoms and cough.

CONCLUSIONS

We report the first SARS-CoV-2 Omicron BA5 human challenge study results in non-naïve (seropositive), previously vaccinated, adults. Robust, safe and tolerable infections were associated with symptomatic disease. These results provide a strong foundation for evaluating vaccine and treatment efficacy in the Omicron human challenge model.

HUMAN CHALLENGE STUDIES
SCS11 • AUDITORIUM 2 - BREAKOUT • WED 22 OCT 2025 - 11:00 - 12:30

Establishing a Controlled Human Infection Model with a contemporary Respiratory Syncytial Virus B challenge agent in healthy volunteers

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BACKGROUND

Controlled human infection model (CHIM) studies (or human challenge studies) are increasingly used for evaluating the efficacy of novel vaccines or therapeutic compounds. Respiratory Syncytial Virus (RSV) challenge studies to date have been performed almost exclusively using the GMP-manufactured A/Memphis/37b as a challenge agent, a strain first isolated in 2001. There is an increasing need for additional RSV challenge agents as the A/Memphis/37b strain exhibits decreasing similarity to currently circulating RSV strains, due to continual viral evolution. Moreover, the impact of RSV B strains on infections and hospitalizations has been increasingly recognized in recent years. The Inno4Vac consortium is a public-private partnership, funded by the IMI2/EU/EFPIA Joint Undertaking. As part of Inno4Vac, we aim to establish a controlled human infection model (CHIM), using an RSV B strain, isolated from a patient in 2022, that was characterized and selected to generate a Master Virus Bank (MVB) under GMP-conditions. In this study, we have evaluated the safety and infectivity of this RSV B strain in healthy volunteers.

METHODS

This is an open label, outpatient, first-in-human CHIM trial performed at the Centre for Human Drug Research (CHDR) in Leiden, The Netherlands. Healthy volunteers (age 18-55) are screened for eligibility and inoculated intranasally with RSV B, with a target infection rate (viral shedding on ≥2 consecutive days) of 70% or higher. Depending on interim symptoms, safety, and viral shedding, the starting dose of 1 x 104 TCID50 may be increased between cohorts. Participants pay daily visits for safety assessments and nasal sampling for virology; self-reported symptom questionnaires are completed daily via electronic diary for two weeks.

RESULTS

Thus far, 10 of the planned 30 participants have been inoculated, with a starting dose of 1 x 104 TCID50. No safety issues have emerged; inoculation of the remaining participants is planned from August to September 2025. The final results for all participants, including safety, symptomatology and viral shedding are expected in October 2025.

CONCLUSIONS

We are developing a novel RSV B CHIM using a GMP-manufactured strain, possibly suitable for studies to evaluate the efficacy of novel vaccines and compounds against RSV infection. The study design facilitates selection of an appropriate virus dose level in a safe manner. In addition, the CHIM allows for extensive assessment of RSV disease and immunological responses. This model could enable a contemporary RSV B challenge strain to be added to the available challenge agent repertoire. Moreover, the outpatient setting of the CHIM decreases the burden for study participants and lowers operational burden and costs.

ACKNOWLEDGEMENTS

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CLINICAL MANIFESTATIONS, BURDEN OF DISEASE AND MANAGEMENT

SCS10 • AUDITORIUM 3 - BREAKOUT • WED 22 OCT 2025 - 11:00 - 12:30

Burden of Influenza Hospitalisation (Including With Recent COVID-19) Among Individuals With Immunocompromising Conditions and Other Comorbidities: An Exploratory Analysis Using the INFORM Study Cohort

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BACKGROUND

Using routine healthcare data from the INFORM (INvestigation oF cOvid-19 Risk among iMmunocompromised populations) study (ISRCTN53375662), we estimated the burden of influenza hospitalisation (including with recent COVID-19) in England among subgroups of individuals (aged ≥12 years) with immunocompromising conditions (ICs) or other comorbidities (e.g., cystic fibrosis; CF).

METHODS

We performed an exploratory analysis using the INFORM study cohort comprising de-identified, routinely collected data from a 25% sample of the English population (Evans et al. *Lancet Reg Health*, 2023;35:100747). Influenza hospitalisation was defined as ≥1-night stay with an influenza International Classification of Diseases (ICD)-10 code recorded in any position in Hospital Episode Statistics, and was classified as 'with recent SARS-CoV-2 infection' if a COVID-19 episode (identified using diagnosis codes or positive test results) starting within the prior 6 months was recorded. Analysis focused on individuals aged ≥12 years between July 2022 and June 2023. Individuals were grouped according to predefined IC or comorbidity based on diagnoses and/or treatments during the 5-year baseline period using primary and secondary care data. Crude incidence rates were calculated and age- and sex-adjusted incidence rate ratios (aIRRs) (with 95% confidence intervals; CIs) were estimated using a Poisson model with offset for person-time at-risk.

RESULTS

In total, 12,075,920 individuals aged ≥12 years were included in the analysis. Mean (standard deviation; SD) age was 45.3 (20.6) years, with 20.5% aged ≥65 years; 50.2% were female; and 3.9% had any IC. Between July 2022 and June 2023, the overall rate of influenza hospitalisation was 10.51 (95% CI 10.45, 10.57) per 10,000 person-years, with 13.1% (n=1635; N=12,505) of individuals hospitalised for/with influenza having evidence of recent SARS-CoV-2 infection. **Table 1** shows the absolute and relative risks of influenza hospitalisation for any IC and for 10 selected ICs and other comorbidities. The risk of hospitalisation was >2-times higher in individuals with any IC than those without (aIRR, 2.25 [95% CI 2.14, 2.37]). Stem cell transplant (SCT) recipients and those with CF had the highest relative risks for influenza hospitalisation (aIRR, 32.41 [95% CI 24.26, 43.29] and 35.04 [95% CI 26.86, 45.70] respectively, versus individuals without SCT or CF).

CONCLUSIONS

Individuals with IC and comorbidities (who tend to be older adults) experience higher rates of influenza hospitalisation than individuals without these conditions. Additionally, recent SARS-CoV-2 infection is more prevalent in the IC and high-risk subgroups with the highest risks of severe influenza (CF, SCT and haematological malignancies).

Table 1. Absolute and relative risk of hospitalisation for/with influenza in individuals aged ≥12 years in the INFORM cohort between July 2022 and June 2023

IC or Comorbidity Subgroup	Size of Subgroup (n, % of Total Population)	Hospitalisations in Subgroup (n, % of Total Hospitalisations)	Proportion of Influenza Hospitalisations With Prior SARS-CoV-2	Crude IR of Influenza Hospitalisation per 10,000 PY (95% CI)	Adjusted IRR for Influenza Hospitalisation (95% CI)[a]
Any IC condition	475,900, 3.9%	2060, 16.3%	18.9%	45.01 (44.72, 45.30)	2.25 (2.14, 2.37)
Haematological malignancies (active treatment in ≤6 months prior)	6785, 0.07%	160, 1.3%	28.1%	198.05 (195.87, 200.23)	9.19 (7.84, 10.78)
Organ transplant (≤5 years prior)	7375, 0.06%	85, 0.7%	23.5%	116.41 (114.1, 118.72)	11.26 (9.05, 14.02)
Stem cell transplants (≤2 years prior)	1565, 0.01%	50, 0.4%	30.0%	319.15 (314.1, 324.2)	32.41 (24.26, 43.29)
Secondary immunodeficiency with active treatment with non-corticosteroid immunosuppressive or immunomodulatory therapy(ies)	11,285, 0.09%	80, 0.6%	25.0%	74.07 (72.2, 75.94)	4.18 (3.35, 5.23)
Cystic fibrosis	2210, 0.02%	55, 0.4%	27.3%	260.10 (255.91, 264.29)	35.04 (26.86, 45.70)
Pulmonary hypertension	17,780, 0.1%	250, 2.0%	20.0%	198.05 (195.87, 200.23)	4.61 (4.05, 5.25)
Chronic liver disease	128,090, 1.1%	650, 5.1%	17.7%	52.37 (51.81, 52.93)	3.87 (3.57, 4.19)
Multiple sclerosis	16,520, 0.1%	75, 0.6%	20.0%	47.52 (45.98, 49.06)	3.72 (2.96, 4.68)
Chronic heart disease	498,685, 4.1%	3355, 26.6%	18.0%	70.71 (70.43, 70.99)	3.42 (3.27, 3.58)
Chronic lung disease (COPD, asthma, bronchiectasis)	1,660,590, 13.8%	5610, 44.4%	13.9%	34.25 (34.1, 34.4)	3.86 (3.73, 4.01)

[a]Compares incidence in individuals with the specified condition with incidence in individuals without the specified condition.
CI, confidence interval; COPD, chronic obstructive pulmonary disease; IC, immunocompromising condition; IR, incidence rate; IRR, incidence rate ratio; PY, person-years.

CLINICAL MANIFESTATIONS, BURDEN OF DISEASE AND MANAGEMENT
SCS10 • AUDITORIUM 3 - BREAKOUT • WED 22 OCT 2025 - 11:00 - 12:30

Is clinical outcome pathogen related? Characteristics and Outcomes of ICU Patients with Severe Acute Respiratory Infections: Focusing on Respiratory Syncytial Virus, Human Metapneumovirus, Influenza virus, and Parainfluenza virus ECaS

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INTRODUCTION

Viral severe acute respiratory infections (SARI) are a major cause of intensive care unit (ICU) admission, with a significant burden and mortality. Comparative clinical data of patients admitted to the ICU with virus infections other than SARS-CoV-2 or influenza virus (IV) infection are limited. Therefore, this study investigates patient characteristics, clinical outcomes, and ventilation parameters of ICU patients admitted with SARI caused by Respiratory syncytial virus (RSV), Human metapneumovirus (HMPV), IV, or Parainfluenza virus (PIV).

METHODS

A retrospective cohort study was conducted of patients with SARI admitted to the ICU of the Spaarne Gasthuis, a Dutch secondary teaching hospital, between 2017 and 2023.

RESULTS

277 patients were included, with RSV (n=51), HMPV (n=40), IV (n=142), and PIV (n=44) infections respectively. Pre-existing hematological malignancies were more common in RSV patients. No significant differences were found in length of hospital stay or ventilation parameters across the respective virus groups. Median duration of ICU stay was four days (IQR 2-7). Bacterial co-infections, pulmonary infiltrates and a higher ROX-index were more common in patients with noninvasive ventilation (NIV) failure. Hospital mortality rates were not different between the groups; RSV (25.5%), HMPV (15%), IV (24.6%), and PIV (20.5%).

CONCLUSION

This study analyzed ICU patients with SARI caused by HMPV, RSV, IV, or PIV, revealing four key findings: high ICU, hospital and 1 year mortality rates with no differences and similar mechanical ventilation parameters between the groups, risk factors for NIV failure linked to prolonged ventilation, and co-morbidities associated with severe disease.

CLINICAL MANIFESTATIONS, BURDEN OF DISEASE AND MANAGEMENT
SCS10 • AUDITORIUM 3 - BREAKOUT • WED 22 OCT 2025 - 11:00 - 12:30

Hospital Burden of Respiratory Syncytial Virus (RSV) in Adults Aged 18 to 60 Years in France: A Nationwide Analysis (2018–2023)

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1: Moderna; 2: Steve Consultants; 3: CHU Saint Etienne

BACKGROUND

Respiratory syncytial virus (RSV) is well known for its impact on infants and older adults, but its burden in younger adults remains underexplored. Emerging evidence suggests that adults aged 18 to 60 years, particularly those with comorbidities, may require significant hospital care, including intensive care unit (ICU) admission. Robust real-world data are needed to support targeted prevention and policy planning. Our objective is to describe the hospital burden associated with RSV-related hospitalizations in adults aged 18 to 60 years in France, with a focus on patients with cardio-respiratory comorbities as those are known to be the most at risk for severe disease due to RSV.

METHODS

We conducted a retrospective observational study using the French national hospital discharge database (PMSI). All hospitalizations between July 1, 2018, and December 31, 2023, for patients aged [18-60[with an ICD-10 diagnosis of RSV-related disease (J12.1, J20.5, J21.0, B97.4), recorded as primary, related, or associated diagnosis, were included. Outcomes included ICU admission, length of stay (LoS), in-hospital mortality, and hospitalization costs. Analyses were stratified by comorbidities of interest which are cardio-respiratory diseases based on previous hospital ICD-10 code diagnoses (DP/DR/DAS) over a 1-year history before the hospitalization for RSV.

RESULTS

Among the 4,789 RSV-related hospitalizations identified in adults aged 18–60 years from 2018 to 2023, 27.6% (n=1,321) involved patients with at least one cardio-respiratory comorbidity. Across the study period, the ICU admission rate in the overall adult population ranged from 16.5% to 22.5%, and from 17.9% to 26.7% among those with cardio-respiratory comorbidities. The mean length of stay (LoS) reached up to 12.4 days (SD: 17.6) in the overall group, and 15.0 days (SD: 17.0) in the subgroup with comorbidities. The proportion of patients requiring mechanical ventilation ranged from 15.0% to 21.1% overall, and from 16.5% to 25.3% among those with cardio-respiratory comorbidities. The in-hospital mortality rate was 3.0% in the general adult population and 4.5% among patients with cardio-respiratory comorbidities. Finally, the mean cost per hospitalization ranged from €6,309 to €8,829 in the overall population, and from €7,402 to €11,192 in patients with cardio-respiratory comorbidities.

CONCLUSION

The associated burden of RSV related hospitalization among adults aged 18-60 years is substantial and cardio-respiratory comorbidities are associated with greater risk of severe outcomes including longer length of stays and in-hospital deaths. These findings support the need for enhanced recognition and prevention strategies in this population.

CLINICAL MANIFESTATIONS, BURDEN OF DISEASE AND MANAGEMENT
SCS10 • AUDITORIUM 3 - BREAKOUT • WED 22 OCT 2025 - 11:00 - 12:30

Clinical outcomes among older adults hospitalized with respiratory syncytial virus and influenza infection – a retrospective study from Israel

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BACKGROUND

Respiratory syncytial virus (RSV) is increasingly recognized as a cause of severe respiratory illness and mortality among older adults. Limited information is available in Israel regarding clinical outcomes of RSV, particularly in comparison to influenza. This study compared clinical outcomes associated with RSV and influenza infections among hospitalized older adults.

METHODS

A retrospective, single-center analysis was conducted of initial hospital admissions among adults aged ≥60 years who tested positive for RSV or influenza via standard-of-care RT-PCR from nasopharyngeal samples between 2016-2023. Coinfections of RSV and influenza were excluded. Clinical characteristics and outcomes were obtained from electronic medical records. The primary outcome was a composite of severe endpoints: 30-day all-cause mortality, intensive care unit (ICU) admission, mechanical ventilation, or vasopressor support during hospitalization. Secondary outcomes included 90-day all-cause mortality, all-cause re-hospitalization within 90 days, tachyarrhythmia and cardiovascular events at 30 days. Missing data was handled using multiple imputation by chained equations. An inverse-probability treatment weighting (IPTW) using the propensity score was used to adjust for potential confounding. Covariate balance was assessed using standardized mean differences (SMD), with SMD ≤0.1 indicating adequate balance. Weighted and unweighted relative risks (RRs) were estimated using a log binomial model to compare clinical outcomes between the RSV and influenza cohorts.

RESULTS

The analysis included 817 RSV and 2,113 influenza patients. While unweighted SMDs indicated baseline imbalances, IPTW improved covariate balance. Residual imbalance was noted for Charlson score (SMD=0.11), chronic cerebrovascular disease (SMD=0.14), and chronic rheumatic and connective tissue disease (SMD = 0.13). In unweighted RR models, RSV infection was associated with an increased risk for the composite primary outcome (RR 1.21, 95% CI 1.05-1.38) and 90-day all-cause mortality (RR 1.29, 95% CI 1.11-1.48) compared to influenza. No significant difference was observed for 90-day rehospitalization (RR 1.07, 95% CI 0.93-1.23) (Table 1). In weighted RR models, the differences between the groups were attenuated: the estimates of the composite primary severe outcome (RR 1.01, 95% CI 0.85-1.21), 90-day all-cause mortality (RR 1.05, 95% CI 0.87-1.25) and re-hospitalization within 90 days (RR 1.04, 95% CI 0.86-1.26) were similar between RSV and influenza patients (Table 2). Among RSV infected older adults, cardiovascular complications, including tachyarrhythmia and ischemic events were common (146/817, 17.9%, and 81/817, 9.9%, respectively).

CONCLUSIONS

Among hospitalized older adults, RSV infection is associated with clinical outcomes comparable to those of influenza, including 30 and 90-day all-cause mortality, risk of ICU admission, mechanical ventilation, vasopressor support, and rehospitalization within 90 days. This data provides support for the implementation of RSV vaccination programs among older adults to reduce RSV-related severe clinical outcomes.

Table 1: Characteristics of RSV and influenza patients before and after inverse probability of treatment weighting (IPTW)

	Pre-IPTW			Post-IPTW		
	Influenza (n=2,113)	RSV (n=817)	SMD	Influenza	RSV	SMD
Demographics:						
Gender:						
Female (n, %)	976 (46.2)	435 (53.2)	**0.14**	1346.8 (47.6)	1381.8 (45.8)	0.04
Male (n, %)	1137 (53.8)	382 (46.8)		1484.4 (52.4)	1637.1 (54.2)	
Age (median [IQR]) years	77.5 (9.6)	78.9 (9.7)	**0.15**	77.7 (9.5)	78.0 (9.9)	0.03
RSV cycle threshold (Ct) (mean (SD))	27.2 (6.1)	28.5 (5.9)	**0.22**	27.6 (6.2)	27.8 (6.2)	0.03
Comorbidities:						
BMI (mean (SD))	27.2 (5.3)	27.1 (5.8)	0.02	27.0 (5.2)	27.2 (5.6)	0.03
BMI ≥30 (n, %)	568 (26.9)	190 (23.3)	0.08	708.9 (25.0)	639.1 (21.2)	0.09
BMI <18.5 (n, %)	56 (2.7)	22 (2.7)	<0.01	67.8 (2.4)	63.3 (2.1)	0.02
Current and prior smoking (n, %)	537 (25.4)	187 (22.9)	0.06	677.5 (23.9)	683.0 (22.6)	0.03
Prior hospitalization (n, %)	336 (15.9)	182 (22.3)	**0.16**	472.1 (16.7)	613.5 (20.3)	0.09
Charlson score (median [IQR])	5.8 (2.5)	6.2 (2.5)	**0.17**	5.8 (2.5)	6.0 (2.5)	**0.11**
Aids or HIV (n, %)	2 (0.1)	1 (0.1)	0.01	2.1 (0.1)	1.1 (0.0)	0.02
Cerebrovascular (n, %)	453 (21.4)	202 (24.7)	0.08	617.0 (21.8)	845.8 (28.0)	**0.14**
Chronic pulmonary (n, %)	600 (28.4)	262 (32.1)	0.08	817.3 (28.9)	860.8 (28.5)	0.01
Congestive heart failure (n, %)	505 (23.9)	244 (29.9)	**0.13**	683.6 (24.1)	791.0 (26.2)	0.05
Dementia (n, %)	161 (7.6)	74 (9.1)	0.05	223.1 (7.9)	287.1 (9.5)	0.06
Diabetes (n, %)	702 (33.2)	270 (33.0)	<0.01	918.5 (32.4)	1040.0 (34.4)	0.04
Hemiplegia or paraplegia (n, %)	72 (3.4)	34 (4.2)	0.04	100.0 (3.5)	115.2 (3.8)	0.02
Liver disease (n, %)	72 (3.4)	34 (4.2)	0.04	96.7 (3.4)	117.6 (3.9)	0.03
Malignancy (n, %)	488 (23.1)	208 (25.5)	0.06	640.5 (22.6)	743.5 (24.6)	0.05
Myocardial infarction (n, %)	312 (14.8)	122 (14.9)	<0.01	397.9 (14.1)	447.6 (14.8)	0.02
Peptic ulcer disease (n, %)	83 (3.9)	39 (4.8)	0.04	120.1 (4.2)	135.4 (4.5)	0.01
Vascular disease (n, %)	154 (7.3)	53 (6.5)	0.03	195.1 (6.9)	229.2 (7.6)	0.03
Renal disease (n, %)	397 (18.8)	201 (24.6)	**0.14**	519.3 (18.3)	657.3 (21.8)	0.09
Rheumatic and connective tissue disease (n, %)	74 (3.5)	21 (2.6)	0.05	93.4 (3.3)	184.7 (6.1)	**0.13**
Leukemia, lymphoma, metastatic malignancy (n, %)	191 (9.0)	82 (10.0)	0.03	256.2 (9.0)	192.7 (6.4)	0.1
Immunosuppression (n, %)	156 (7.4)	154 (18.8)	**0.34**	255.4 (9.0)	262.9 (8.7)	0.01
HSCT (n, %)	49 (2.3)	27 (3.3)	0.06	69.6 (2.5)	52.7 (1.7)	0.05

IPTW - inverse probability of treatment weighting; SMD - standardized mean differences

Table 1: Characteristics of RSV and influenza patients before and after inverse probability of treatment weighting (IPTW) (cont.)

	Pre-IPTW			Post-IPTW		
	Influenza (n=2,113)	RSV (n=817)	SMD	Influenza	RSV	SMD
Clinical characteristics:						
Fever ≥38 (n, %)	1078 (51.0)	291 (35.6)	**0.31**	1337.0 (47.2)	1553.6 (51.5)	0.08
Temperature <36 (n, %)	104 (4.9)	46 (5.6)	0.03	165.3 (5.8)	131.7 (4.4)	0.07
Cough (n, %)	212 (10.0)	633 (77.5)	**1.85**	726.9 (25.7)	826.9 (27.4)	0.04
Chest pain (n, %)	97 (4.6)	110 (13.5)	**0.31**	170.1 (6.0)	167.5 (5.5)	0.02
Dyspnea (n, %)	855 (40.5)	589 (72.1)	**0.67**	1311.7 (46.3)	1336.7 (44.3)	0.04
Maximal pulse rate (median [IQR])	105.4 (25.2)	107.3 (25.3)	0.07	105.8 (25.5)	106.4 (24.5)	0.02
Minimum saturation at collection (median [IQR])	87.1 (9.1)	86.3 (9.7)	0.08	87.0 (9.3)	87.1 (8.6)	0.02
Minimum systolic blood pressure (mean (SD))	105.1 (20.8)	105.8 (22.6)	0.04	105.3 (21.1)	106.4 (23.8)	0.05
Maximal lymphocytes (median [IQR])	1.3 (5.1)	1.6 (8.7)	0.04	1.4 (5.6)	1.3 (5.9)	<0.01
Maximal C-reactive protein (median [IQR])	89.3 (80.6)	90.2 (86.3)	0.01	89.3 (80.8)	93.1 (85.0)	0.05
Maximal creatinine (median [IQR])	1.2 (1.0)	1.4 (1.2)	0.1	1.2 (1.1)	1.3 (1.1)	0.04
Minimum albumin (mean (SD))	3.4 (0.5)	3.4 (0.6)	0.07	3.4 (0.5)	3.3 (0.5)	0.08
Positive respiratory culture (n, %)	16 (0.8)	8 (1.0)	0.02	21.3 (0.8)	35.8 (1.2)	0.04

IPTW - inverse probability of treatment weighting; SMD - standardized mean differences

Table 2: Estimating the relative risk of outcomes between RSV and influenza using a log-binomial model.

Cohort	Unweighted RR (95% CI)	Weighted RR (95% CI)
Composite primary outcome (ICU admission, mechanical ventilation, vasopressor support & 30-day all-cause mortality		
RSV+	1.21 (1.05, 1.38)	1.01 (0.85, 1.21)
Influenza+	Reference	Reference
90-day all-cause mortality		
RSV+	1.29 (1.11, 1.48)	1.05 (0.87, 1.25)
Influenza+	Reference	Reference
Re-hospitalization within 90 days due to any reason		
RSV+	1.07 (0.93, 1.23)	1.04 (0.86, 1.26)
Influenza+	Reference	Reference

CLINICAL MANIFESTATIONS, BURDEN OF DISEASE AND MANAGEMENT
SCS10 • AUDITORIUM 3 - BREAKOUT • WED 22 OCT 2025 - 11:00 - 12:30

The Effect of Revaccination and Reinfection on Symptom Severity and Prevalence in a Post Covid-19 Condition Cohort

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BACKGROUND

The possible effect of antigenic exposures on Post-Covid-19 Condition (PCC, or long COVID), remains debated. This observational cohort study aims to assess the effect of COVID-19 vaccine booster doses (revaccination) and reinfection on the severity of four and prevalence of 13 self-reported PCC-associated symptoms.

METHODS

Participants with self-attributed PCC recruited in the period of May 2021 to June 2024 via longcovid.rivm.nl received a baseline questionnaire and a follow-up every 3 months for up to 24 months. Participants aged ≥18 years with symptoms persisting >3 months attributed to SARS-CoV-2 were included (n=4665).

The effects of revaccination (second, third, fourth dose) and reinfection (second, third infection) on PCC-associated symptom severity and prevalence were analyzed independently, comparing within (0-3 months before versus 3-6 months after revaccination/reinfection), and between groups (revaccinated/reinfected individuals versus non-revaccinated/non-reinfected individuals, after the event), using Benjamini-Hochberg corrected permutation tests.

Participants with revaccination/reinfection were 1:1 iteratively matched to non-revaccinated/non-reinfected individuals by pre-event vaccinations/infections, and, for prevalence analysis, symptom presence/absence. Propensity scores were adjusted for time since infection, questionnaire submission date, sex, age class, education level, number of comorbidities, and, for severity analysis, initial severity score.

PRELIMINARY RESULTS

The prevalence of 5 out of 13 symptoms stayed stable across all analyses. Following second-dose revaccination, severity of cognitive failure and bodily pain were significantly higher in non-revaccinated individuals versus revaccinated individuals, with a significant decrease in revaccinated individuals. Fatigue and dyspnea significantly decreased in severity in both revaccinated- and non-revaccinated individuals. While loss of smell prevalence showed no significant changes within both revaccination/non-revaccination groups, non-revaccinated groups reported higher average prevalence than revaccinated.

Severity of fatigue decreased in both reinfected and non-reinfected individuals, but significantly only in non-reinfected individuals. Prevalence of memory problems and PEM increased in the non-reinfected group, resulting in higher prevalence than among reinfected groups, in which it remained stable.

CONCLUSIONS

Findings indicate positive effects of a second vaccination dose in self-attributed PCC patients on cognitive functioning and bodily pain severity, and prevalence of loss of smell, whereas additional doses showed no significant differences. Moreover, non-reinfected groups showed increased prevalence of PEM and memory problems, while reinfected groups did not.

CLINICAL MANIFESTATIONS, BURDEN OF DISEASE AND MANAGEMENT
SCS10 • AUDITORIUM 3 - BREAKOUT • WED 22 OCT 2025 - 11:00 - 12:30

RSV in Patients with Hematological Malignancies: A Multi-centre Retrospective Cohort Study ECAS

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BACKGROUND

While respiratory syncytial virus (RSV) is well established as a cause of severe respiratory illness in pediatric populations, its burden in adult risk groups remains undercharacterized. In contrast to influenza and SARS-CoV-2, data on RSV in patients with hematological malignancies are scarce. Emerging studies indicate these patients may face heightened risks, yet cohort-based clinical data remain limited. With the recent approval of two RSV vaccines—nirsevimab and RSVpreF (Abrysvo)—a better understanding of the disease burden in this vulnerable population is urgently needed to inform vaccination strategies and clinical management.

METHODS

We conducted a retrospective cohort study across three institutions: Spaarne Gasthuis Teaching Hospital (Netherlands), Erasmus MC (Netherlands), and the cellular and immunotherapy centre at wits donald gordon (South Africa). Respiratory samples from adult patients testing positive for RSV between 2017 and 2025 were identified from institutional databases. Inclusion criteria encompassed patients with underlying hematological malignancies, including those who had received stem cell transplantation. Clinical parameters, outcomes, and cost-related data were extracted and analyzed.

RESULTS

A total of 70 patients with RSV infection were identified: approximately 60 were post-stem cell transplant. Among these, we observed high ICU admission rate, with a 1-year mortality of around 38% and prolonged median hospital stays (14 days [IQR 9–22]). RSV-related complications included respiratory failure (requiring mechanical ventilation) and secondary bacterial infections. Testing and treatment approaches were highly variable across centers. A preliminary cost analysis revealed substantial financial impact during episodes of RSV-related hospitalization in stem cell recipients; detailed cost breakdowns will be included upon final analysis.

CONCLUSIONS

This multi-centre retrospective study provides the first comparative clinical cohort data on RSV infections in patients with hematological malignancies across African and European settings. The findings underscore a significant clinical burden, with high rates of ICU admission, extended hospitalization, and notable mortality. The variability in diagnostic and therapeutic strategies highlights an urgent need for standardized care pathways. These results emphasize the importance of prioritizing this group for RSV prevention measures, including targeted vaccination strategies and improved clinical protocols.

ANTIVIRAL AND IMMUNE THERAPY

SCS12 • AUDITORIUM 1 - PLENARY HALL • WED 22 OCT 2025 - 14:00 - 15:30

Effectiveness of the approved antiviral drugs for mild/moderate infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Hong Kong

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BACKGROUND

The effectiveness of antiviral drugs against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Hong Kong remains mostly underexplored, with a limited evaluation of the performance of the approved antiviral drugs for mild/moderate SARS-CoV-2 infection against clinically relevant outcomes. In view of the gaps in knowledge, we sought to assess the effectiveness of the two approved antiviral drug regimens (300mg nirmatrelvir with 100mg ritonavir; and 800mg molnupiravir, both administered orally twice daily over 5 days) for treatment of mild/moderate SARS-CoV-2 infection in Hong Kong.

METHODS

We conducted a cohort study of 18-64-year-olds, with laboratory-confirmed SARS-CoV-2 infected from April 2022 to January 2023 in Hong Kong. We compared those treated and not treated with the antiviral drugs. The outcomes were disease progression (development or worsening of symptoms), SARS-CoV-2-related mortality, all-cause mortality, and intensive care unit (ICU) admission among those hospitalised following treatment. We summarised SARS-CoV-2 infections and the study participants' characteristics by treatment status and antiviral drug type. Using a conditional logistic regression model with a 5-strata propensity score-matched analysis and adjusting for potential confounders identified from the literature, we conducted stratified analysis for 18-44-year-olds and 45-64-year-olds by symptom status at treatment commencement (asymptomatic and symptomatic) and expressed results as adjusted odds ratios (ORs) with associated 95% confidence intervals (CIs).

RESULTS

We included 206,418 persons, of which 47% were asymptomatic and 53% were symptomatic. Compared with no treatment, nirmatrelvir/ritonavir and molnupiravir were both associated with significantly decreased odds of disease progression in both 18-44-year-olds and 45-64-year-olds; OR: 0.20 (95% CI 0.09-0.42) and 0.14 (0.10-0.20), respectively for 18-44-year-olds, and 0.10 (0.04-0.26) and 0.19 (0.14-0.25), respectively for 45-64-year-olds. While both drugs had no association with the other outcomes in 18-44-year-olds except for a significantly decreased odds of ICU admission with molnupiravir, there was an associated significantly decreased odds of the outcomes in 45-64-year-olds except for all-cause mortality with molnupiravir. We made similar observations in asymptomatic persons, and for disease progression in the symptomatic 45-64-year-olds. Irrespective of symptom status in 45-64-year-olds, compared with molnupiravir, nirmatrelvir/ritonavir was associated with significantly decreased odds of disease progression, SARS-CoV-2-related mortality, and all-cause mortality; OR: 0.39 (0.22-0.70), 0.20 (0.10-0.38), and 0.33 (0.21-0.53), respectively.

CONCLUSIONS

The approved antiviral drugs for non-severe SARS-CoV-2 infection were effective against clinically relevant outcomes in 18-64-year-olds in Hong Kong; however, mostly in the 45-64-year-olds. Nirmatrelvir/ritonavir was superior to molnupiravir in 45-64-year-olds, but not in 18-44-year-olds although the assessment for 18-44-year-olds was limited by sample size. Nevertheless, our findings may suggest an age-based policy for use of these drugs for non-severe SARS-CoV-2 infection.

ANTIVIRAL AND IMMUNE THERAPY

SCS12 • AUDITORIUM 1 - PLENARY HALL • WED 22 OCT 2025 - 14:00 - 15:30

Impact of Antiviral Timing and Vaccination on Viral Shedding in Hospitalized Older Adults with COVID-19 During Omicron Predominance

ECaS

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BACKGROUND

The two oral antivirals for COVID-19, molnupiravir and nirmatrelvir-ritonavir, have been shown to reduce disease severity, but their effects on virological outcomes remains less well characterized.

METHODS

We analyzed viral burden, measured by RT-PCR cycle threshold (Ct) values, in elderly patients hospitalized with symptomatic COVID-19 in Hong Kong public hospitals from May 23, 2022, to January 23, 2023, a period dominated by Omicron sublineages. We examined the effect of antiviral therapy on viral shedding duration, with a focus on timing of treatment initiation, and explored whether the observed treatment effects were affected by patients' immunity background as defined by their COVID-19 vaccination status.

RESULTS

Among 3,759 patients with symptomatic COVID-19 included, 1,318 received molnupiravir and 1,613 received nirmatrelvir-ritonavir. Nirmatrelvir-ritonavir, especially when initiated early, was associated with significantly reduced viral shedding duration compared to untreated patients (mean difference: -2.4 days; 95% CI: -2.7, -2.2), whereas the effect of molnupiravir was more modest (-0.6 days; 95% CI: -0.9, -0.3). The virological benefit of nirmatrelvir-ritonavir appeared to be most pronounced in unvaccinated individuals (-4.9 days; 95% CI: -6.4, -3.5).

CONCLUSIONS

Early treatment with nirmatrelvir-ritonavir not only improves clinical outcomes in hospitalized COVID-19 patients, but also provides significant benefit of reducing viral burden, which may help lower transmission risk. These findings emphasize the importance of prompt antiviral therapy and suggest that considering both vaccination status and antiviral use can further protect high-risk groups and help control the spread and severity of COVID-19 in future outbreaks.

ANTIVIRAL AND IMMUNE THERAPY
SCS12 • AUDITORIUM 1 - PLENARY HALL • WED 22 OCT 2025 - 14:00 - 15:30

Analysis of potential drug combinations against influenza by specifically targeting the viral polymerase ECaS

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INTRODUCTION
Influenza A viruses (IAV) remain a significant global health threat, causing seasonal epidemics and pandemics. The growing emergence of drug-resistant IAV strains necessitates the exploration of new antiviral strategies. Targeting the RNA-dependent RNA polymerase (RdRp), an essential component of viral replication, represents a promising approach to overcome existent antiviral resistance.

OBJECTIVE
This study aims to target the viral RNA polymerase by employing a 'double-hit' strategy to inhibit viral replication. It focuses on evaluating the antiviral potential of combining polymerase inhibitors (Favipiravir, Baloxavir) with Ivermectin, a nuclear transport inhibitor. The objective was to first assess the individual effects of these drugs and then analyze their combined efficacy, addressing challenges posed by resistance and enhancing therapeutic outcomes.

METHODS
Two approaches were employed to evaluate the antiviral activity of the drugs, both individually and in combination. A minigenome assay designed to measure RdRp activity using reconstituted H1N1 polymerase and an infection assay with a clinical H3N2 isolate. Drug efficacy was assessed by determining the IC50 and analyzing drug interactions through combination studies, supported by statistical modeling to interpret the results comprehensively.

RESULTS
Favipiravir and Baloxavir effectively inhibited RdRp activity and viral replication, with Baloxavir showing higher potency. In the minigenome assay, IC50 values were 71.38 µM for Favipiravir, 0.0102 µM for Baloxavir, and 7.414 µM for Ivermectin. In the infection assay, IC50 values were 4.149 µM for Favipiravir, 0.00053 µM for Baloxavir, and 4.496 µM for Ivermectin. However, combination treatment of Baloxavir and Ivermectin showed antagonistic effects, as synergy scores confirmed: -33.18 (ZIP) and -44.71 (Loewe) in the minigenome assay, with similar trends observed in the infection assay.

CONCLUSION AND OUTLOOK
This study investigated the combination of Baloxavir and Ivermectin against IAV, revealing antagonistic effects with no additional benefit over individual therapies. These findings underline the challenges of antiviral combination strategies. Future research should validate results with recent IAV isolates as well as highly pathogenic strains of IAV, such as H5N1 or H7N9, explore human lung cell lines, and consider alternative combinations like Favipiravir with Ivermectin. Advancing these efforts will aid in developing effective antiviral strategies and improving public health responses to IAV infections.

ANTIVIRAL AND IMMUNE THERAPY
SCS12 • AUDITORIUM 1 - PLENARY HALL • WED 22 OCT 2025 - 14:00 - 15:30

Treatment with pictilisib-loaded lipid nanoparticles reduces influenza A virus infection in vitro and ex vivo ECaS

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BACKGROUND

Influenza A viruses (IAVs) remain a significant global health concern, causing seasonal outbreaks, pandemics, and zoonotic transmissions with high morbidity and mortality, particularly among children, the elderly, and immunocompromised individuals. Despite annual vaccination being the primary preventive strategy, its limited coverage and inability to protect against emerging subtypes necessitate the development of novel antiviral interventions. Current Food and Drug Administration (FDA)- and European Medicines Agency (EMA)-approved anti-IAV drugs face resistance challenges, emphasizing the need for host-directed therapies that target cellular factors supporting viral replication. Phosphatidylinositol 3-kinases (PI3Ks), particularly class I, play a key role in cellular processes exploited by IAVs, making them attractive targets for antiviral development. Among potential candidates, pictilisib, a PI3K inhibitor, has demonstrated antiviral properties, though its adverse effects in clinical trials remains a critical limitation.

METHODS

To address this, pictilisib was encapsulated within lipid nanoparticles (LNPs) as a drug carrier system. *In vitro* experiments using lung epithelial cells were conducted to assess cytotoxicity via cell count and lactate dehydrogenase (LDH) assays. Non-toxic concentrations were selected to evaluate the impact of both free and LNP-encapsulated pictilisib on viral replication efficiency, using plaque assays and Western blot analyses. Cytokine and chemokine expression profiles were examined through flow cytometry to characterize immune responses to the treatment. To further investigate the efficacy of LNP-based drug delivery in a more complex biological system, offering a closer representation of *in vivo* conditions, *ex vivo* mouse lung slice infections were performed. Here, plaque assays assessed viral suppression in infected tissue sections, while histological staining provided insights into viral spread within the lung after treatment.

RESULTS

LNP-encapsulated pictilisib exhibited antiviral and anti-inflammatory effects comparable to free pictilisib *in vitro*. In addition, the establishment of an *ex vivo* drug testing system allowed a more precise evaluation of therapeutic efficacy. *Ex vivo* analyses demonstrated improved viral suppression compared to treatment with free pictilisib, underscoring the benefits of nanoparticle-based drug delivery in host-directed therapy.

CONCLUSION

This study highlights the potential of integrating PI3K inhibition with advanced nanocarrier technology to improve IAV treatment efficacy. Crucially, the host-directed therapy component mitigates drug resistance, offering a promising avenue for further exploration in combating infectious diseases.

ANTIVIRAL AND IMMUNE THERAPY
SCS12 • AUDITORIUM 1 - PLENARY HALL • WED 22 OCT 2025 - 14:00 - 15:30

Comparative assessment of in vivo antiviral activity of anti-influenza drugs (AD ASTRA)

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BACKGROUND

Influenza infects many millions each year, with considerable associated morbidity and mortality, and is the most likely causative agent of a future pandemic. Despite this, there is a paucity of evidence that available antivirals are clinically beneficial and uncertainty regarding their relative *in vivo* antiviral efficacy. There is an urgent need to assess the relative antiviral efficacies of anti-influenza. The pharmacodynamic measure of acceleration of the rate of viral clearance has been used successfully to evaluate and compare treatments for COVID-191,2. The advantage of this approach is that it requires small sample sizes to accurately compare clinical antiviral activity, and can deliver results quickly. This approach has not been trialed in influenza.

METHODS

AD ASTRA is a platform study evaluating antiviral efficacy of available therapeutics in early symptomatic influenza infection (symptom onset < 4 days) in previously healthy patients aged 18-60 years. The patients are randomized to one of the treatment arms (favipiravir, oseltamivir, or baloxavir) or a no study drug arm. The density of influenza virus genomes in the throat is determined from a validated qPCR assessment of daily serial oropharyngeal swabs taken in duplicate on days 0–7 and 14. The treatment effect is defined as a proportional change in the population mean clearance slope relative to the no study drug. The success/ futility thresholds for stopping have been set at 20%.

RESULTS

The first unblinded interim analysis was conducted after the first 50 patients were enrolled (44 patients with evaluable data: favipiravir, n=14; oseltamivir, n=14; no study drug, n=16). The results suggest that this methodology can determine relative *in vivo* antiviral efficacy. The data of the estimated viral clearance half-lives and antiviral efficacies of favipiravir and oseltamivir are shown in Figure 1. Depending on decisions by the DSMB to further unblind, based on pre-defined success/ futility criteria, further data may be presented later.

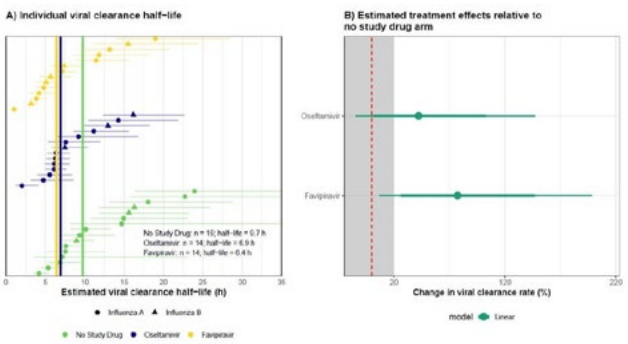

Figure 1. A) Estimated viral clearance half-lives B) Estimated antiviral efficacies relative to no study drug

CONCLUSIONS

This methodology is able to quickly and accurately demonstrate the *in vivo* antiviral activity of anti-influenza with small numbers enrolled, as was the case with COVID-19. This method can be used to answer urgent questions about comparative *in vivo* efficacy of anti-influenza for both seasonal and pandemic influenza.

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ANTIVIRAL AND IMMUNE THERAPY
SCS12 • AUDITORIUM 1 - PLENARY HALL • WED 22 OCT 2025 - 14:00 - 15:30

Structural characterization and preclinical development of a heavy chain-only antibody that protects against group 1 and group 2 influenza A viruses

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BACKGROUND
While human monoclonal antibodies capable of neutralizing both group 1 and group 2 influenza A viruses by targeting the hemagglutinin (HA) stem have been reported, a broadly neutralizing heavy chain-only antibody with similar activity has yet to be identified. Heavy chain-only antibodies naturally occur in camelid species, such as llamas. These heavy chain-only antibodies are comprised of a single variable domain, which typically is very stable and can be easily formatted. Our aim was to isolate single-domain antibodies (sdAbs) that can neutralize both group 1 and group 2 influenza A viruses.

METHODS:
A llama was immunized with H1, H3, H5, and H7 HA immunogens to generate a phage display library, which was used for bio-panning. Selected sdAbs were genetically fused to a human IgG1 Fc domain and expressed in transiently transfected HEK293S cells. The resulting sdAb-hIgG1 Fc constructs were evaluated in a microneutralization assay against H1N1 (PR8 and H1N1pdm 2009), H3N2 (X31), H5N1 (NIBRG-14), H7N7 (SC35M), and H2N2 (A/Singapore/1/57) viruses. The sdAb-hIgG1 Fc constructs were also administered intraperitoneally or intranasally to mice, which were subsequently challenged with a potentially lethal dose of the aforementioned viruses. Cryogenic electron microscopy was used to determine the structure of a selected sdAb in complex with H3 HA.

RESULTS:
We identified a sdAb, named 1CX29, that can neutralize H1N1, H2N2, H5N1, and H7N7 influenza viruses with IC50s ranging from 50 to 100 nM. 1CX29-hIgG1 Fc fusions neutralized these viruses, as well as H1N1 and H3N2 viruses sampled from hospitalized influenza patients (2024-2025 season), with IC50s ranging from 10 to 20 nM. Furthermore, 1CX29-hIgG1 Fc administered at a dose of 5 mg/kg by intraperitoneal injection, protected mice against an otherwise lethal challenge with H1N1pdm 2009, NIBRG-14 (H5N1), X31 (H3N2), and SC35M (H7N7) viruses. Intranasal administration of 1CX29-Fc fusion constructs at a dose of 50 µg/kg protected mice against challenge with the aforementioned viruses and against the historical pandemic H2N2 strain A/Singapore/1/57.

Cryo-EM-based structural analysis of 1CX29 in complex with H3 HA revealed that sdAb 1CX29 binds to a conserved alpha helix in the stem region of HA. This structural insight in the epitope-paratope interface guided the design of a humanized 1CX29 derivative, which, as a fusion with hIgG1 Fc, had very favorable developability characteristics.

CONCLUSIONS:
The broad influenza A virus-neutralizing 1CX29-based heavy chain-only antibody described here, can be developed in a candidate biologic to protect against seasonal and pandemic influenza.

VIRUS EVOLUTION AND STRAIN SELECTION

SCS13 • AUDITORIUM 2 - BREAKOUT • WED 22 OCT 2025 - 14:00 - 15:30

High-throughput neutralization measurements correlate strongly with evolutionary success of human influenza strains ECaS

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BACKGROUND

Human influenza viruses rapidly acquire mutations in their hemagglutinin (HA) protein that erode neutralization by antibodies from prior exposures. New HA variants with reduced neutralization are generally the most evolutionarily successful, and repeatedly replace the current dominant variant(s) in a process known as antigenic drift. As a result, people are reinfected roughly every five years and vaccines are updated annually to attempt to match the currently dominant influenza strains.

METHODS

To rapidly profile human immunity to influenza, we use a sequencing-based assay to measure neutralization titers for 78 recent H3N2 HA strains against a large set of children and adult sera, measuring ~10,000 total titers. These measurements quantify the heterogeneity of neutralizing antibody immunity to influenza across different members of the population.

RESULTS

There is substantial person-to-person heterogeneity in the titers against different viral strains, both within and across age cohorts. We also show that the growth rates of H3N2 strains in the human population in 2023 are highly correlated with the fraction of sera with low titers against each strain. Notably, strain growth rates are less correlated with neutralization titers against pools of human sera, demonstrating the importance of population heterogeneity in shaping viral evolution.

CONCLUSIONS

Overall, our results suggest that high-throughput neutralization measurements of human sera against many different viral strains can help explain the evolution of human influenza. We are now looking towards repeating this approach to measure circulating antigenic diversity of both pdmH1N1 and H3N2 variants on a timeline that would help inform influenza vaccine-strain selection.

VIRUS EVOLUTION AND STRAIN SELECTION
SCS13 • AUDITORIUM 2 - BREAKOUT • WED 22 OCT 2025 - 14:00 - 15:30

Antigenic mapping of influenza A viruses using deep learning-based prediction of hemagglutination inhibition titers

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BACKGROUND

Seasonal influenza remains a significant public health challenge through unpredictable antigenic drift, where accumulated mutations enable immune evasion and necessitate vaccine updates. Comprehensive antigenic characterization has been hindered by the absence of real-world A(H1N1)pdm antigenic mapping and post-2012 A(H3N2) maps due to insufficient pairwise hemagglutination inhibition (HAI) titrations.

METHODS

We compiled 64,301 A(H3N2) titers (94 reference, 4,125 test viruses) and 65,505 A(H1N1)pdm titers (42 reference, 4,747 test viruses) collected from 2012–2022. An end-to-end transformer was trained to predict HAI titers directly from paired HA1 amino-acid (AA) sequences, capturing non-linear interactions and generalising to unseen strains. Performance was evaluated with 10-fold cross-validation and season-to-season hold-outs. Model predictions filled the incomplete virus–serum matrices, after which multidimensional scaling generated antigenic maps from (i) the original incomplete and (ii) the completed matrices. We used Gaussian mixture models to determine antigenic clusters, and Gradient-weighted Class Activation Mapping (Grad-CAM) to identify substitutions linked to cluster transitions.

RESULTS

Across cross-validation folds out model showed mean absolute error below one log-unit (comparable to laboratory variability) and placed 98 % of predictions within a four-fold range for both subtypes. Rolling nowcasts retained 86–98 % (A(H3N2)) and 90–99 % (A(H1N1)pdm) four-fold accuracy, even after the post-2020 drop in A(H1N1)pdm cross-reactivity.

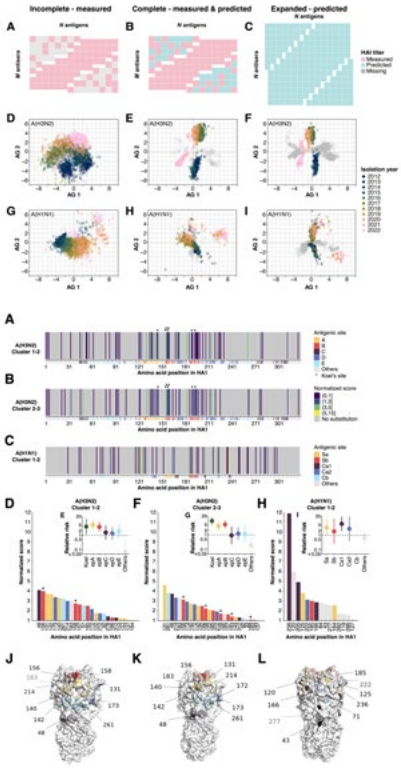
We found that antigenic maps built from incomplete, measured HAI titers showed little clustering patterns, but imputing missing off-diagonal titers with model predictions revealed clear subtype-specific clusters while preserving strong map-to-titer correlations (filled: r = 0.84/0.94; expanded: r = 0.91/0.97; all $p < 0.01$).

Our antigenic mapping identified three A(H3N2) clusters between 2012-2022 with transitions occurring approximately every 6 years. Notably, genetically diverse co-circulating subclades 3C.2a and 3C.3a (2015-2020) formed a single antigenic cluster. A(H1N1) formed three less temporally distinct clusters, with a novel cluster emerging of clade 5a.2 post-COVID-19. For both subtypes, pairwise antigenic distances among viruses isolated in the same year were bimodally distributed during transition years, whereas years dominated by a single cluster showed a unimodal pattern.

Using interpretable Grad-CAM analysis, we identified AA substitutions linked to antigenic cluster transitions that align with previous laboratory findings. Key A(H3N2) sites primarily occurred within established antigenic sites, especially in antigenic site A (Relative risk (RR): 5.1, 95% confidence interval (CI), 2.5-10.2) and B (3.7, 1.7-8.2) compared to a random site. A(H1N1)pdm showed fewer key mutations, with some outside recognized antigenic regions.

CONCLUSIONS

Our deep learning approach accelerates antigenic characterization in surveillance at global scale and can be transferred to other variable pathogens, providing an actionable bridge between genomic sequencing and vaccine strain selection.


Incomplete - measured
Complete - measured & predicted
Expanded - predicted
N antigens
HAI titer
Measured
Predicted
Missing
A(H3N2)
A(H1N1)
AG 1
AG 2
AG 3
Isolation year
Amino acid position in HA1
Antigenic site
Others
Normalized score
No substitution
Relative risk

Site specific predictability of amino acid substitutions of seasonal influenza viruses ECaS

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INTRODUCTION

Influenza vaccines are the primary public health tool to reduce influenza disease burden. Accurate forECaSting of seasonal influenza virus evolution is essential for vaccine strain match but the predictability of influenza virus evolution remains challenging. Fixation or loss of mutations depend on many factors, including their intrinsic fitness benefit, interference effects arising from competing variants, and stochastic epidemiological dynamics. However, given that haemagglutinin (HA) epitope sites experience strong immune selection pressure, predicting HA epitope evolution could suffice for vaccine design. Here, we investigate the predictability of A/H3N2 influenza virus evolution at the site specific amino acid (AA) level.

METHODS

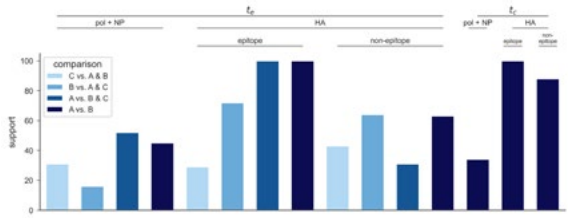
We downloaded all influenza A/H3N2 HA, PB2, PB1, PA, and NP sequences collected between 2000 and 2019 from human hosts from the GISAID EpiFlu database. Using a hidden Markov model, we classified AA substitutions into three groups based on their frequency trajectory: fixed (Group A), widely circulating but never fixed (Group B), and transient (Group C). For each AA substitution, we estimated the time of first detectable global frequency (te) and the time of established circulation (tc). We performed 100 independent down-samples of sequences and reconstructed maximum likelihood phylogenies to estimate the Local Branching Index (LBI, a proxy measure for mutation fitness benefit) distribution of each AA substitution at te and tc. We then used Mann-Whitney U tests to investigate if the LBI distributions of fixating substitutions (Group A) differ significantly from non-fixating groups (Group B and C).

RESULTS

At te, the LBI distributions of HA epitope AA substitutions that eventually fixed (Group A) were consistently significantly distinct ($p < 0.05$) in all down-sampled sets from those that did not (Group B and C) (Figure 1), suggesting that the evolutionary fate of HA epitope AA substitutions could be predictable upon reaching detectable global frequencies. In contrast, this was only observed in 44% of down-sampled sets for HA non-epitope AA substitutions at te, which only increased to 88% at tc, suggesting that HA non-epitope AA substitutions likely experience stronger interference effects. For AA substitutions in the polymerase complex and nucleoprotein, we found low down-sample support at both te (16–54%) and tc (34%), indicating the predictability of non-HA AA substitutions is far more limited.

CONCLUSION

Our results suggest that HA epitope substitutions that fix eventually may be predictable upon reaching detectable global frequencies. However, the predictability of non-epitope and non-HA substitutions is limited due to interference and stochastic effects.



VIRUS EVOLUTION AND STRAIN SELECTION
SCS13 • AUDITORIUM 2 - BREAKOUT • WED 22 OCT 2025 - 14:00 - 15:30

Informal settlements and COVID-19: viral phylodynamic, respiratory microbiome, and public health implications.

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Respiratory infections, particularly COVID-19, have been extensively studied globally, but data from Latin American informal settlements remain limited. In Buenos Aires city, these areas exhibit population densities up to eight times higher than urban neighborhoods, along with inadequate housing, limited social distancing, and reduced access to clean water and sewage systems. Such conditions may accelerate viral spread and alter the nasopharyngeal microbiome, which influences immune responses and pathogen colonization.

We analyzed SARS-CoV-2-positive patients from informal settlements and urban areas during the first pandemic wave (2020). Viral complete genomes and bacterial 16S rRNA (V4 region) were sequenced (Illumina).

Three dominant SARS-CoV-2 lineages circulated during the first wave in Argentina: B.1.499, N.3, and N.5. Phylogeographic analysis estimated that B.1.499 and N.3 originated almost simultaneously in different informal settlements within Buenos Aires city. The origin of the B.1.499 lineage was inferred to be in "Barrio 31" (PP = 0.9) around March 5, 2020 (95% HPD: February 17–March 15), while the N.3 lineage was inferred to have originated in "Barrio 1-11-14" (PP = 0.99) around March 19, 2020 (95% HPD: February 29–April 4). Each settlement showed local dominance of a single lineage, with limited co-circulation, despite their proximity and overlapping timelines. This aligned with a peak in reported COVID-19 cases within these areas (up to 100 times higher than in other neighborhoods) nearly one month before broader community transmission in Buenos Aires. Several significant viral migration events from settlements to other city neighborhoods and Argentinean provinces were found, especially B.1.499 (Bayes factors>3 and PP>0.8). Demographic reconstruction showed limited spread and early lineage diversification plateau for N.3 by mid-2020, while B.1.499 diversified until October 2020. Both lineages had limited international spread: B.1.499 was sporadically detected in North America, Europe, and Japan; N.3 remained mostly within Argentina. Lineage N.5 emerged later (April 8, 2020; 95% HPD: March 23–April 22) in Buenos Aires Province (PP = 0.99), expanding post-peak and diversifying through October 2020. It became predominant by late 2020 but was replaced by Gamma and Lambda in early 2021. Though exported abroad, no sustained transmission chains were observed.

Microbiome analysis showed greater bacterial richness in informal settlement residents and revealed significant differences in bacterial diversity between city and settlement residents. For alpha diversity, indices showed higher bacterial richness and evenness in residents of informal settlements (Shannon: $p = 4.45\times10^{-7}$; Simpson: $p = 1.64\times10^{-5}$). For beta diversity, Bray-Curtis dissimilarity revealed distinct microbial compositions between the two groups ($p = 0.001$).

These results highlight how informal settlements acted as early epicenters for lineage emergence and spread. Combined with microbiome disparities, the findings underscore the impact of structural inequities on COVID-19 dynamics and call for targeted interventions in vulnerable populations.

VIRUS EVOLUTION AND STRAIN SELECTION
SCS13 • AUDITORIUM 2 - BREAKOUT • WED 22 OCT 2025 - 14:00 - 15:30

Understanding the evolution of Influenza A/H3N2 polymerase complex

ECaS

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Seasonal influenza virus leads to annual epidemics characterized by respiratory infections that could potentially cause severe illness in high-risk groups. Influenza virus has a low fidelity RNA polymerase complex with poor proof-reading which results in a high viral mutation rate that allow the virus to rapidly evolve. Since its introduction in 1968, influenza A/H3N2 viruses have continued to circulate in the human population. However, the molecular basis underlying the evolution of the polymerase complex remain largely unknown. In this study, we aim to characterise and study the molecular evolution of H3N2 influenza polymerase gene segments (PB2, PB1 and PA).

We reconstructed the likely historical order of substitutions in the polymerase complex of H3N2 spanning the years 1968-2024. We found more than 100 individual non-synonymous amino acid mutations that have fixed over the last 50+ years across all three polymerase proteins. We performed in vitro luciferase assay to measure changes in RNA polymerase replication activity owing to stepwise accumulation of amino acid substitutions since 1968. The early stages of the virus's adaptation to the human host were marked by a 5-fold increase in replicative fitness from 1968 to 2008. However, from 2008 onwards, the replication activity plateaued and these stagnation periods suggest that other phenotypic changes could be driving the evolution of influenza polymerase in the later years. To investigate what other likely factors could be underlying polymerase evolution, we will perform other relevant analyses such as molecular dynamics simulations to characterize protein stability and protein-to-protein interaction dynamics. We will also investigate if host innate immunity drives polymerase evolution by additionally using interferon response as a read-out on the minigenome assay.

THREATS FROM THE ANIMAL WORLD

SCS14 PLE • AUDITORIUM 1 - PLENARY HALL • WED 22 OCT 2025 - 16:00 - 17:30

Genomic insights into the broadening host range of clade 2.3.4.4b H5 avian influenza viruses

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Istituto Zooprofilattico Sperimentale delle Venezie, Italy

BACKGROUND

Since late 2020, the HPAI epizootic driven by H5Nx clade 2.3.4.4b viruses has reached such a wide geographic dissemination to be considered a true panzootic. This clade has demonstrated an extraordinary adaptability, spreading across continents, infecting over 500 avian species and more than 80 mammalian species, and causing sustained mammal-to-mammal transmission as well as several human cases. This study aims to evaluate the impact of the diversifying evolution of clade 2.3.4.4b viruses on the host tropism, geographical spread and zoonotic potential.

METHODS

We performed in-depth analyses of 5807 full genomes of clade 2.3.4.4b H5Nx collected in Europe between October 2020 and March 2025. Genotypes were identified using Genin2 (https://github.com/izsvenezie-virology/genin2) and further validated through phylogenetic analyses using IQTREE v2.2.6. Fast Unconstrained Bayesian AppRoximation available in the HyPhy 2.5.60(MP) was used to identify sites under positive selection. Molecular markers associated with increased zoonotic potential were identified using FluMut (https://github.com/izsvenezie-virology/FluMut). The impact of different gene compositions on host and geographic spread was estimated on two genotype-specific datasets using BEAST v1.10.4.

RESULTS

Our findings highlight the extraordinary genetic plasticity of clade 2.3.4.4b, which in the last four years has resulted in over 90 distinct genotypes emerging through multiple reassortment in Europe. Genetic diversity was higher in wild birds, particularly Anseriformes, and varied across five successive waves, peaking in epidemiological year 2021-2022. Despite the diversity, the success of this clade appears linked to the stable maintenance of three key gene segments: HA, M, and N1. This core genetic stability coupled with the flexible reassortment of other segments has enabled the virus to form host-adapted genome constellations and expand into new ecological niches. Notably, genotypes adapted to Charadriiformes, such as EA-2022-BB, have been associated with spillovers into fur farms, likely facilitated by seabirds' behaviour at the interfaces with the production system. Conversely, Anseriformes-associated genotypes have shown broader host promiscuity and have driven most outbreaks in domestic birds.

To date, clade 2.3.4.4b retains avian-type receptor specificity, although our results showed that mammalian-adaptive markers may rapidly be acquired following virus replication in mammalian species.

CONCLUSIONS

The genetic plasticity of clade 2.3.4.4b viruses enables unprecedented host range and geographic expansion, increasing the opportunities to enter new ecological niches and to evolve under the influence of both intrinsic and extrinsic factors determined by the novel host populations it encounters. Despite retaining an avian-type receptor-binding profile, the virus not only managed to infect multiple mammalian hosts, but was also able to occasionally sustain onward transmission and acquire adaptive mutations in these species. In this context, strengthened surveillance efforts are essential to promptly identify the emergence of new variants which may represent an increased threat to endangered species or human health.

THREATS FROM THE ANIMAL WORLD
SCS14 PLE • AUDITORIUM 1 - PLENARY HALL • WED 22 OCT 2025 - 16:00 - 17:30

Emergence of a Novel Reassortant Clade 2.3.2.1e Avian Influenza A/H5N1 Virus Associated with Human Cases in Cambodia

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BACKGROUND

Highly pathogenic avian influenza (HPAI) H5N1 viruses have circulated in Cambodian poultry since 2004, periodically causing zoonotic infections. After nearly a decade without human cases, Cambodia experienced a resurgence of human H5N1 cases between February 2023 and March 2025, with 19 confirmed infections, primarily caused by a novel reassortant clade 2.3.2.1e (formerly known as clade 2.3.2.1c) avian influenza A/H5N1 virus raising significant public health concerns. This study investigates the factors driving this resurgence, focusing on viral evolution, genomic epidemiology, and public health implications.

METHODS

Samples were collected from “One Health” outbreak investigations associated with human cases and ongoing active pathogen surveillance programs at live bird markets (LBMs) across multiple provinces in Cambodia. Samples were processed and tested using qRT-PCR, virus isolation, and whole genome sequencing using the Oxford Nanopore GridION followed by genomic epidemiology, in depth phylogenetic and reassortment analysis. Epidemiological linkages were assessed based on case investigations.

RESULTS

Initial cases in February 2023 were linked to a long-circulating clade 2.3.2.1e (former clade 2.3.2.1c) virus, consistent with prior outbreaks in Cambodian poultry. However, the surge in cases from October 2023 onwards was associated with a novel reassortant A/H5N1 genotype. This virus emerged through two successive reassortment events, whereby the regionally endemic clade 2.3.2.1e acquired genes from clade 2.3.4.4b and low pathogenic avian influenza viruses introduced by migratory birds. The resulting genotype spread across Cambodia and Vietnam, replacing endemic strains. Genomic signatures indicate multiple adaptive mutations, including PB2 E627K, associated with enhanced polymerase activity, virulence, and replication capacity in avian and mammalian hosts. Additional markers, such as HA T156A and PB1 H99Y, were identified, suggesting enhanced binding affinity to mammalian receptors.

CONCLUSIONS

The emergence and rapid spread of a novel reassortant A/H5N1 virus in Cambodia underscores the ongoing risk of zoonotic spillover in Southeast Asia, exacerbated by high-density poultry farming, migratory bird interactions, and transboundary poultry trade. The resurgence in human cases and detection of mammalian-adaptive mutations in these viruses underscore the need for active surveillance, public health vigilance, and coordinated “One Health” approaches to mitigate further spillovers as the genetic landscape of HPAI is rapidly shifting across endemic regions.

THREATS FROM THE ANIMAL WORLD
SCS14 PLE • AUDITORIUM 1 - PLENARY HALL • WED 22 OCT 2025 - 16:00 - 17:30

Increased attachment to and replication of recent avian H5N1 influenza A viruses from clade 2.3.4.4b in human airway epithelial cells ECaS

Lisa BAUER, Lonneke LEIJTEN, Matteo IERVOLINO, Varun CHOPRA, Laura VAN DIJK, Mark POWER, Monique SPRONKEN, Willemijn RIJNINK, Mathis FUNK, Rory DE VRIES, Mathilde RICHARD, Thijs KUIKEN, Debby VAN RIEL
Erasmus MC, Netherlands, The

BACKGROUND
Highly pathogenic avian influenza (HPAI) H5 viruses of the A/Goose/Guangdong/1/1996 (GsGd) lineage pose significant global risks to wildlife, domestic animals, and humans. Recent cross-species transmission events to mammals, including humans, highlight this. Important determinants for cross-species and intra-species transmission in mammals include the ability to attach to and replicate in respiratory epithelial cells. Although these factors have been investigated for HPAI H5N1 viruses in the past, limited studies are available for currently circulating strains from clade 2.3.4.4b.

METHODS
We assessed the attachment *in situ*, as well as the replication kinetics and innate immune responses *in vitro* of three recent H5N1 clade 2.3.4.4b viruses—including bovine and avian isolates—and compared it to a well-characterized 2005 HPAI H5N1 clade 2.1.3.2 virus and a seasonal H3N2 virus. We compared the pattern of virus attachment to human respiratory tract by virus histochemistry. Additionally, we studied the replication kinetics, as well as innate immune responses in human nasal respiratory epithelium and tracheo-bronchial epithelium cultures *in vitro*. Lastly, we investigated the polymerase complex activity using a minigenome assay.

RESULTS
A sequence alignment of the surface glycoprotein HA revealed that the recent clade 2.3.4.4b viruses differed five amino acids in or surrounding the receptor binding domain compared to the older clade 2.1.3.2 H5N1 virus. Overall, clade 2.3.4.4b viruses attached more abundantly to the human upper and lower respiratory tract than clade 2.1.3.2 H5N1 virus. In nasal respiratory epithelium cultures, clade 2.3.4.4b viruses replicated efficiently. In tracheo-bronchial epithelium cultures, one clade 2.3.4.4.b replicated to higher titers than the clade 2.1.3.2 H5N1 virus, almost as efficiently as H3N2 virus. This increased replication was not associated with an increased polymerase activity of clade 2.3.4.4b H5N1 virus. The efficient replication of clade 2.3.4.4b H5N1 virus induced a robust innate immune response almost comparable to H3N2 virus.

CONCLUSIONS
The pattern of virus attachment and replication efficiency of clade 2.3.4.4b H5N1 viruses resembled that of H3N2 virus more closely than a clade 2.1.3.2 H5N1 virus. This could contribute to an increased risk for both human infection and virus adaptation to humans.

THREATS FROM THE ANIMAL WORLD
SCS14 PLE • AUDITORIUM 1 - PLENARY HALL • WED 22 OCT 2025 - 16:00 - 17:30

Bovine Myxovirus Resistance Protein 1 mediates antiviral activity against human and avian influenza A viruses

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BACKGROUND
Highly pathogenic avian influenza (HPAI) H5N1 viruses from clade 2.3.4.4b have recently been associated with infections in an unprecedent number of mammalian species, including dairy cattle. This virus has now been detected in cattle in multiple farms and greater than 65 human infections have also been reported. Little is known regarding the nature of interferon inducible genes (ISGs) in cattle, including their ability mediate antiviral activity against either human or avian influenza A viruses (IAV). Here, we characterised the induction and antiviral activity of bovine Myxovirus Resistance (Mx) proteins 1 and 2 - orthologs of well-characterised human ISGs known to selectively restrict avian influenza viruses.

METHODS:
Firstly, induction of bovine (b)Mx1 and bMx2 in differentiated primary mammary epithelial cells (primary site of H5N1 infection) was compared to tracheal cells following *in vitro* infection with HPAI H5N1 by qPCR. Secondly, we used lentivirus transduction to generate MDBK (immortalised bovine kidney cells) cells with inducible overexpression of bMx1 and bMx2 to assess their antiviral activity. We further generated cell lines with mutations in bMx1 to test whether altered intracellular localisation affected antiviral function. Finally, we utilised reverse genetics viruses in a loss-of-function experiment to determine if mutations in the viral nucleoprotein (NP) that are known to reduce sensitivity to human Mx1 (hMx1), also reduced sensitivity to bMx1.

RESULTS:
We show that both bovine (b)Mx1 and bMx2 were induced to higher levels in primary dairy mammary epithelial cells compared to tracheal cells. Inducible overexpression of bMx1, but not bMx2, inhibited replication of different human and avian IAVs, but not influenza B viruses. Moreover, bMx1 inhibited the replication of a several HPAI H5N1 viruses from clade 2.3.4.4b, suggesting that viruses from this clade have not yet acquired resistance to bMx1-mediated antiviral defenses. Mutations that changed intracellular localisation of bMx1 also abrogated its antiviral activity. Furthermore, using reverse genetics we showed that mutations in viral nucleoprotein (NP) associated with reduced sensitivity to hMx1, also reduced sensitivity to bMx1. This highlights that selection pressures to evade bMx1 restriction may also make the viruses less sensitive to hMx1, increasing their pandemic potential.

CONCLUSION:
This study provides key insights into the antiviral activity of bovine Mx proteins, demonstrating that clade 2.3.4.4b HPAI H5N1 viruses remain susceptible to bMx1 restriction. Our results also suggest that evolutionary pressures to evade bMx1 restriction can also select for variants with reduced sensitivity to hMx1, which will significantly increase the pandemic risk potential of this virus. These results underscore the importance of characterising ISGs in animals at the animal–human interface, particularly given the continued circulation of HPAI H5N1s in mammals such as cattle that are in close contact with humans.

VIRUS STRUCTURE AND REPLICATION

SCS06 • AUDITORIUM 1 - PLENARY HALL • THU 23 OCT 2025 - 11:00 - 12:30

Proteomic analysis of the subcellular reorganization during Influenza A infection in human cells ECas

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BACKGROUND

Influenza A virus (IAV) remains a persistent global health threat, infecting approximately 10% of the world's population annually. Beyond seasonal epidemics, IAV can drive unpredictable pandemics with high morbidity and mortality. Despite decades of research, many biological processes underlying how the virus hijacks host systems and reorganizes cellular architecture remain poorly understood. Viral replication is deeply intertwined with subcellular dynamics, as viruses must localize their proteins to appropriate compartments to access host machinery. Mass spectrometry-based proteomics, especially in spatial contexts, has emerged as a powerful tool to study these interactions. By using omics approaches, we can map disease progression through multidimensional datasets and capture temporal and spatial host responses.

METHODS

Here, we use high-resolution spatial proteomics to systematically characterize protein localization changes during IAV infection, providing novel insights into virus-induced cellular remodeling. We infected human lung epithelial A549 cells with the influenza A/WSN/33 virus and collected samples at multiple time points. To enrich for diverse subcellular compartments, a 9-step differential centrifugation protocol was applied, generating 10 fractions per replicate. Tandem mass spectrometry using data-independent acquisition (DIA) enabled comprehensive protein quantification. Following normalization and data integration, we analyzed changes in both protein abundance and subcellular localization.

RESULTS

Our dataset includes over 7,800 uniquely identified proteins, of which approximately 30% show changes in abundance during infection. We observed widespread protein relocalization events, revealing that many host responses are driven by spatial reorganization rather than expression level shifts.

CONCLUSIONS

Our study highlights how IAV exploits host compartmentalization to advance its replication and evade defenses. The findings underscore the importance of spatial proteomics in virology and cellular biology, offering a valuable resource for further investigations. Ultimately, characterizing temporal-spatial changes during infection enhances our understanding of viral pathogenesis and opens new avenues for therapeutic intervention targeting subcellular processes.

VIRUS STRUCTURE AND REPLICATION
SCS06 • AUDITORIUM 1 - PLENARY HALL • THU 23 OCT 2025 - 11:00 - 12:30

Influenza NS1-Mediated N(6)-Methyladenosine Modification Controls NS mRNA Splicing

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INTRODUCTION

The investigation into how NS1 regulates its mRNA splicing has spanned decades and yielded diverse insights. N6-methyladenosine (m6A) modification is a well-known RNA modification that has been found in influenza virus (IAV) RNAs since 1976. This modification regulates viral RNA processes, but its role in NS segment splicing remains unclear. This study investigates how NS1 exploits m6A modification and the m6A reader YTHDC1 to control splicing of its own NS mRNA.

METHODS

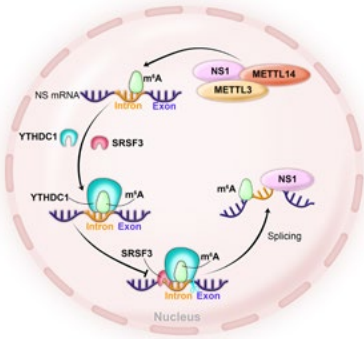
The viruses utilized in this study were generated through reverse genetics. To investigate the interaction between RNA and protein, an RNA-immunoprecipitation assay was employed. The splicing activity was evaluated through RT-qPCR analysis using RNA extracted from cells infected with the indicated viruses.

RESULTS

Our investigation confirmed that NS1 protein negatively regulates NS segment splicing. To address the role of m6A, infection with an m6A-deficient mutant virus (WSN/A385C) increased spliced NEP mRNA levels compared to wild-type, confirming m6A's role in splicing inhibition. The m6A reader YTHDC1 binds modified NS RNA and blocks the splicing factor SRSF3 from accessing the mRNA; knockout of YTHDC1 restored SRSF3 binding and elevated spliced NEP levels, demonstrating YTHDC1's role in repressing NS mRNA splicing. This regulatory mechanism—where NS1 promotes m6A addition, enabling YTHDC1 to inhibit the interaction between SRSF3 and NS mRNA and reduce splicing efficiency—is conserved across human and non-human IAV strains, highlighting the evolutionary importance of the m6A site at position A385 in balancing NS1 (unspliced) and NEP (spliced) mRNA production for optimal viral replication.

CONCLUSIONS

Our findings provided direct evidence that NS1 protein promotes m6A modification on the NS segment, recruiting YTHDC1 to block SRSF3-mediated splicing. This ensures a well-balanced production of unspliced NS1 mRNA for viral replication. The conserved A385 m6A site and YTHDC1-SRSF3 interplay represent novel targets for antiviral strategies aimed at disrupting viral RNA processing. This study reveals a sophisticated mechanism by which IAV hijacks host epitranscriptomic machinery to balance viral gene expression.



VIRUS STRUCTURE AND REPLICATION
SCS06 • AUDITORIUM 1 - PLENARY HALL • THU 23 OCT 2025 - 11:00 - 12:30

Reassortment dynamics between clade 2.3.2.1 and 2.3.4.4b A(H5) influenza viruses ECas

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The segmented nature of the influenza virus genome allows for the generation of novel reassortant progeny when a single cell is infected by more than one influenza virus. Since 2020, the ongoing A(H5) panzootic has led to the introduction of clade 2.3.4.4b A(H5) to multiple countries, including those with ongoing endemic circulation of diverse non-2.3.4.4b clade A(H5) viruses.

Since 2014, Bangladesh and Cambodia have experienced endemic circulation of 2.3.2.1a and 2.3.2.1e clade A(H5) viruses, respectively. A(H5) viruses of clade 2.3.4.4b were detected in poultry in Cambodia in 2021, and Bangladesh in 2023. Following initial introduction, clade 2.3.4.4b viruses continued to co-circulate with endemic clade viruses in both countries. Since 2023, Cambodia has reported multiple detections of novel A(H5) 2.3.2.1e x 2.3.4.4b reassortants in humans and this new genotype has replaced previously circulating 2.3.2.1e and 2.3.4.4b viruses in poultry. Despite an imported human case of A(H5) infection caused by a 2.3.2.1a x 2.3.4.4b reassortant in Australia, the genotype replacement observed in Cambodia has not been reported in Bangladesh, nor has ongoing surveillance in-country reported the Australian genotype.

The reasons for the different reassortment behaviours between 2.3.2.1a and 2.3.2.1e viruses remain unclear. Here, we aimed to describe the reassortment dynamics of 2.3.2.1a and 2.3.2.1e viruses when co-infected with a 2.3.4.4b virus in an *in vitro* avian model and characterise progeny viruses of a known parental lineage.

A representative virus of each clade 2.3.2.1a, 2.3.2.1e, and 2.3.4.4b was plaque-purified, and quantified by plaque assay. Growth kinetics of parental viruses were assessed in embryonic fibroblast cells derived from embryonated chicken (CEF) and duck (DEF) eggs to establish relative fitness prior to reassortment. The representative 2.3.4.4b will be co-infected with either 2.3.2.1a or 2.3.2.1e in CEF and DEF cells and passaged in the respective cell type. For each passage, supernatant will be plaque-purified, with plaques sequenced to determine the genotype composition. Major genotypes will be isolated and replication kinetics characterised in normal human bronchial epithelial (NHBE), CEF, and DEF cells to determine relative replication kinetics.

We have previously presented preliminary data suggesting differential replication kinetics of the pre- and post-reassortment wildtype viruses from human infections in Cambodia using the NHBE model. By simulating natural reassortment events using known parental viruses, we hope to understand whether reassortment differs between 2.3.2.1a and 2.3.2.1e viruses in an *in vitro* model avian system, and the impact this may have on pandemic risk. Understanding the dynamics of reassortment upon co-infection may uncover functional constraints that help predict these events where viruses co-circulate.

VIRUS STRUCTURE AND REPLICATION
SCS06 • AUDITORIUM 1 - PLENARY HALL • THU 23 OCT 2025 - 11:00 - 12:30

Human respirovirus infection driven by heteromultivalent binding of sialoglycotopes

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Human respiroviruses (RV) within the *Paramyxoviridae* bind to sialoglycan receptors to infect the upper respiratory tract (URT). α2-6-linked sialic acids (2-6Sia) are much more prevalent than 2-3Sia at this location. In agreement herewith, adaptation from 2-3Sia binding to 2-6Sia binding is deemed crucial for zoonotic influenza A viruses (IAVs) to become human IAVs, although we recently showed that a that a strict 2-6Sia specificity is not an absolute requirement. In contrast, human RVs appear to prefer binding to 2-3Sias, which apparently does not prohibit their efficient transmission. In view of this discrepancy, we studied RV receptor-binding and entry requirements using cell-based glycan arrays and biolayer interferometry binding assays. Our results indicate that RVs are strictly dependent on 2-3Sias for binding and entry. Cells only displaying 2-6Sia were not infected at all. Human RV infection was mostly restricted to sialylated Galβ1-4GlcNAc termini, a feature shared with human IAVs. Binding and infection of these RVs was much less efficient via sialylated Galβ1-3GalNAc found on core I O-glycans, in contrast to avian IAV and Newcastle disease virus. Similar as previously shown for IAVs, also for RVs heteromultivalent interactions were observed, in which low affinity receptors contribute to the interaction of viruses with a receptor-coated surface or to infection efficiency when present in addition to the preferred receptor. Thus, while human RVs were strictly dependent on 2-3Sia for entry, non-preferred 2-6Sia nevertheless contributed to infection. These results show that receptor specificity of RVs is less dichotomous as generally assumed and may provide an explanation for the ability of 2-3Sia-specific RVs to infect the 2-6Sia-rich URT.

VIRUS STRUCTURE AND REPLICATION
SCS06 • AUDITORIUM 1 - PLENARY HALL • THU 23 OCT 2025 - 11:00 - 12:30

RSV Polymerase L Recruits Rab11a to Facilitate Ribonucleoprotein Transport

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BACKGROUND

Respiratory syncytial virus (RSV) is an enveloped, negative-sense, single-stranded RNA virus. Viral ribonucleoproteins (vRNPs), which are composed of genomic RNA that is encapsidated by the nucleoprotein N and associated with the polymerase complex (comprising polymerase L, P and M2-1), support the synthesis of viral RNA. This process occurs in cytoplasmic viral factories, leading to the production of new vRNPs. Our previous research showed that vRNPs are then transported along microtubules by co-opting the Rab11a-marked recycling endosome pathway to reach the plasma membrane for virion assembly. Immunoprecipitation assays in RSV-infected cells revealed an interaction between Rab11a and vRNPs. This study aims to elucidate the mechanisms by which RSV RNPs are recruited to Rab11a endosomes.

METHODS

To analyze interactions between Rab11a and RNP components, we employed a combination of immunoprecipitation assays, GST pull-down experiments with purified proteins, and immunofluorescence colocalization assays in cells expressing the various partners. We determined the specific form of Rab11a (GDP- or GTP-bound) and the domains involved in these interactions, as well as the L domains, using Rab11a mutants and L fragments with the same approaches. Additionally, biolayer interferometry was used to confirm direct interactions and measure the affinity between partners. To this end, the L-P complexes were immobilized on Ni-NTA sensors via a His6 tag, then exposed to a range of Rab11a concentrations (50-3200 nM) in the presence or absence of GTP.

RESULTS

We first identified the viral polymerase L as the vRNP component interacting with Rab11a. Rab11a-HA specifically indeed co-immunoprecipitated specifically with L, but not with other RNP components, when transiently expressed. Furthermore, Rab11a colocalized with RNPs components only in the presence of L. GST pull-down experiments confirmed a direct interaction between GST-Rab11a in its active form and purified L. Our biolayer interferometry data supported these results by showing a specific and particularly stable interaction between L and Rab11a in the presence of GTP with an affinity of approximately 0.5 µM. Our data also showed L-Rab11a interaction is abolished by mutations in the switch I region of Rab11a, which is known to interact with other Rab11a partners. Additionally, experiments using truncated L constructs revealed that the two C-terminal domains of L (MTAse domain and CTD domain) are both necessary and sufficient for the interaction with Rab11a.

CONCLUSIONS

Taken together, our results suggest that RSV RNP transport from viral factories to the budding sites relies on a direct interaction between the C terminal part of the viral polymerase L and the Switch I region of GTP-bound Rab11a. Further localization of the precise Rab11a binding site on the viral polymerase may provide a novel target for the development of antiviral drugs against influenza.

VIRUS STRUCTURE AND REPLICATION
SCS06 • AUDITORIUM 1 - PLENARY HALL • THU 23 OCT 2025 - 11:00 - 12:30

Transient RNA structure-driven insertions in H5 hemagglutinin required for highly pathogenic avian influenza virus genesis are not dependent on polymerase origin.

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BACKGROUND

Highly pathogenic avian influenza viruses (HPAIVs) pose a serious threat to both animal and human health. These viruses emerge in poultry from low pathogenic avian influenza viruses (LPAIVs) after acquiring mutations that enable systemic replication, causing near 100% mortality rates. The key genetic change underlying HPAIV genesis is the conversion from a monobasic cleavage site in the hemagglutinin (HA) gene into a multi-basic cleavage site (MBCS), which is restricted to viruses of the H5 and H7 subtypes. Our group recently showed that trapping of the viral RNA-dependent RNA-polymerase (RdRp) on a purine-rich sequence by transient RNA structures promotes insertions in H5 HA required for MBCS formation1. Our initial work was conducted using the RdRp of A/Indonesia/5/2005. Here, we assessed the robustness of the trapped polymerase model across polymerases of varying host origin (avian or mammalian), influenza virus subtype (H5 or H7), and pathogenicity (HPAIV and LPAIV).

METHODS

Insertion types and frequencies at the H5 HA cleavage site were assessed using a virus-free influenza replication system previously developed in our group. This system involves using a panel of engineered HA RNA templates differing in cleavage site sequence and predicted RNA structures, designed based on *in silico* predictions of transient RNA structure folding models. These templates were replicated in HEK-293T cells using reconstituted influenza virus RdRp (PB2, PB1, PA and NP). Insertions were reliably detected by circular sequencing. Insertion frequencies were corrected for background insertions by human polymerase I upon initial plasmid transcription. RdRps were sourced from seven influenza viruses: two human (H3N2 and pH1N1), two H5 (LPAIV H5N2 and HPAIV H5N1, with and without PB2-E627K mammalian adaptation), and two H7 viruses (LPAIV H7N9 and HPAIV H7N7).

RESULTS

Insertion patterns across HA RNA mutants and mutant phenotypes were consistent across all RdRp backgrounds. No insertions were observed in the consensus LPAIV RETR cleavage site. Substitution to the purine-rich cleavage site sequence RKKR led to a clear increase in insertion frequencies. Stabilization of the predicted transient cRNA structure, previously shown to promote insertions at the H5 cleavage site, markedly increased insertion frequencies, while disruption of this structure sharply reduced them. Although overall insertion frequencies were lower when avian, non-mammalian-adapted polymerases were used, likely due to reduced activity in mammalian cells, the qualitative pattern across HA mutants remained unchanged.

CONCLUSIONS:

These findings support and extend our group's previous work, confirming that both RNA sequence at the HA cleavage site and transient RNA structures formed by flanking sequences are the key drivers of insertional MBCS acquisition in H5 HA, independent of the RdRp origin. These findings refine the mechanistic understanding behind HPAIV genesis and have implications for monitoring viruses at risk of gaining systemic infectivity.

Funk et al., bioRxiv 2024. doi: https://doi.org/10.1101/2024.01.11.574333

VIRUS AND HOST FACTORS IN PATHOGENESIS - PART 2

SCS17 • AUDITORIUM 2 - BREAKOUT • THU 23 OCT 2025 - 11:00 - 12:30

Respiratory Syncytial Virus G Protein Enhances Viral Dissemination Through TLR2-NFκB-Mediated NLRP3 Priming and Pyroptotic Cell Death

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Respiratory syncytial virus (RSV) remains a leading cause of severe lower respiratory tract infections worldwide. While infants and immunocompromised individuals were considered to have the highest morbidity and mortality risk, recent epidemiological studies have also demonstrated the major impact of RSV infection on the health of older adults. This disease burden stems from both direct viral cytopathogenicity, and a dysregulated immune response characterized by excessive IL-6 and IL-8 production. Also, secondary complications such as superinfections and cardiovascular events have an impact. While recently approved vaccines and monoclonal antibodies mark major progress in intervention strategies, there remains an urgent need to elucidate molecular mechanisms driving RSV pathogenesis, to develop targeted therapeutic strategies for high-risk populations.

The RSV attachment glycoprotein G mediates virus binding through a CX3C-like chemokine motif located within the central conserved domain. In addition, RSV G protein also exists in a secreted form (sG) which possesses immunomodulatory properties. In the present study, we expressed recombinant RSV-A sG and performed pulldown experiments to show that it binds to TLR2, resulting in the triggering of NFκB activity and the secretion of IL-6, IL-8, VEGF, and CCL2. In an *in vitro* model, sG pretreatment enhanced RSV titers in the supernatants of airway epithelial cultures, thus demonstrating a novel role for sG in enhancing viral replication which is distinct from its canonical receptor-binding activity.

We have identified a two-phase inflammasome activation process: sG-TLR2-MyD88-NFκB signaling primes uninfected cells through NLRP3 upregulation and ROS accumulation, a prerequisite for inflammasome complex assembly, while subsequent RSV infection provides the second signal for robust caspase-1 activation and pyroptosis. This sequential activation helps explain how sG promotes viral spread in airway epithelia by preconditioning neighboring cells for inflammasome-driven lysis and facilitating viral egress. These findings shed additional light on the role of sG in RSV pathogenesis, beyond immune evasion. sG coordinates both inflammatory cytokine production and a pyroptotic exit strategy for progeny virus. This dual mechanism may at least in part explain clinical observations of prolonged viral shedding alongside hyperinflammation in severe RSV cases.

It is attractive to consider that sG-driven pyroptosis priming has implications for the development of therapeutic intervention. Targeting the sG-TLR2 interface could simultaneously reduce inflammatory damage and viral spread, addressing two key drivers of RSV pathogenesis. While further validation is needed, for instance in advanced human lung models, these results provide a rationale for evaluating CX3C motif-targeted vaccination strategies to prevent viral attachment and sG-mediated priming. Separately, NLRP3 inhibitor usage during active infection may well mitigate pyroptosis-driven tissue damage in high-risk populations.

VIRUS AND HOST FACTORS IN PATHOGENESIS - PART 2
SCS17 • AUDITORIUM 2 - BREAKOUT • THU 23 OCT 2025 - 11:00 - 12:30

Reverse Zoonosis Shapes Transmission and Virulence of Recent H5N1 Clade 2.3.4.4b in Diverse Bird Species

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BACKGROUND
Highly pathogenic avian influenza viruses (HPAIVs) of the H5 subtype, particularly clade 2.3.4.4b H5N1, continue to evolve and pose a major threat to both animal and human health. While sporadic human infections have been reported, the recent detection of H5N1 in dairy cattle in the United States underscores the virus's capacity to cross species barriers. The zoonotic and epizootic potential of mammal-adapted viruses reintroduced into bird populations remains poorly understood. We aimed to assess whether bovine- and human-derived H5N1 viruses differ in their fitness, virulence, and transmissibility in avian hosts and to identify associated molecular signatures.

METHODS
We compared two closely related H5N1 strains isolated in the USA in 2024: one from an infected dairy cow and one from a human patient. Using reverse genetics and standard virological techniques, we assessed replication kinetics, clinical outcomes, and transmission efficiency in chickens, turkeys, and mallards. In vitro replication was tested in primary avian cells. Polymerase activity and host innate immune responses were evaluated using minigenome assays. Neuraminidase (NA) activity and thermostability were measured by MUNANA and HA assays. Viral genomic segments were analyzed for adaptive mutations.

RESULTS
The bovine-adapted virus displayed reduced virulence and transmission in chickens and to a lesser extent in turkeys. In contrast, the human-derived virus caused higher mortality, faster replication, and efficient transmission in both species. Mallards remained asymptomatic but shed virus at high titers. In vitro, both viruses replicated similarly in avian cells, but the human-derived strain showed significantly higher polymerase activity, driven by changes in PB2. It also elicited stronger interferon responses in chicken cells. A single NA mutation in the human virus increased sialidase activity, thermal stability, and rapid elution from chicken erythrocytes, likely enhancing viral fitness. These differences, despite close genetic relatedness, indicate rapid adaptation at critical molecular sites.

CONCLUSIONS
Our findings reveal that mammalian adaptation of H5N1 in cattle may reduce viral fitness in avian hosts, whereas the human-derived strain maintains high virulence and transmissibility in birds. This suggests that human infections may serve as a source of avian-adapted virus variants, creating a bidirectional zoonotic risk. Silent shedding in mallards further highlights their role as reservoirs. These data emphasize the need for integrated molecular surveillance at the avian–mammalian interface to detect and mitigate cross-species transmission events. Understanding viral evolution in both directions, mammal-to-bird and bird-to-mammal, is crucial for pandemic preparedness.

VIRUS AND HOST FACTORS IN PATHOGENESIS - PART 2
SCS17 • AUDITORIUM 2 - BREAKOUT • THU 23 OCT 2025 - 11:00 - 12:30

Autoantibodies against type I IFNs in patients with zoonotic H7N9 infection

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The factors contributing to the species barrier that prevents human infections with avian influenza A viruses (IAV) are not fully understood. Recently, we performed a whole-genome sequencing study suggesting a deficiency in the interferon (IFN)-regulated antiviral factor MxA increased the susceptibility to H7N9 in 6.5% of patients. However, the cause of increased susceptibility in most patients remains unclear. We hypothesize that autoantibodies (auto-Abs) neutralizing type I IFNs may weaken the species barrier. Such auto-Abs are known to increase the risk of severe disease in infections with viruses including seasonal IAV, SARS-CoV-2, and West Nile virus.

Using a multiplex bead-based assay, we screened for auto-Abs binding to IFNα2, IFNβ1b or IFNω in 199 H7N9 patients and healthy Chinese individuals, including 269 poultry workers and 262 close contacts. Neutralizing capacity was assessed with a luciferase reporter assay.

We detected auto-Abs neutralizing at least one IFN at low concentrations in 18.1% of H7N9 patients. 17.1% neutralized even high IFN levels. In contrast, such auto-Abs were present in only ~1% of healthy controls, consistent with published general population data. Only 1.5% of patients had auto-Abs against IFNβ1b, while most neutralized IFNα2 or both IFNα2 and ω. As seen in other studies, the prevalence was higher in older individuals. One third of auto-Ab-positive patients were >70 yr old. Notably, the proportion of women testing positive for these auto-Abs (24.5%) exceeded that of men (15.8%). Auto-Ab-positive sera also blocked IFN-induced antiviral responses in vitro.

Our findings suggest that auto-Abs neutralizing type I IFNs may increase susceptibility to zoonotic IAV infections and further highlight the role of the type I IFN system in the species barrier. Similar mechanisms could also enhance the vulnerability to H5N1. Determining type I IFN-neutralizing auto-Ab levels may help identify individuals at higher risk for zoonotic IAV infections.

Assessing the pathogenicity of contemporary bovine and human clade 2.3.4.4b HPAI H5N1 viruses using human organoid systems representing lung and brain

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BACKGROUND

The unprecedented transmission of contemporary clade 2.3.4.4b highly pathogenic avian influenza (HPAI) H5N1 viruses between mammals in the US has raised concerns about the risk of spillover into the human population. Historically, HPAI H5N1 virus infection in humans resulted in severe respiratory disease, as well as neurological complications. While human infections with clade 2.3.4.4b contemporary HPAI H5N1 viruses have largely resulted in mild disease, the potential pathogenicity of these viruses in the respiratory tract and central nervous system is poorly understood.

METHODS

Human lung organoids (hLOs), human cerebral organoids (hCOs), and human nasal epithelium were infected with contemporary or historical HPAI H5N1 virus isolates to evaluate virus replication, cell death, and host responses.

hLOs consisted of adult stem cell-derived Type II alveolar cells (AT2s) that, under air-liquid interface, can be differentiated into Type I alveolar cells (AT1s). hCOs are derived from human induced pluripotent stem cells, contain neurons and astrocytes, and form a 3D environment that loosely mimics interactions with neighboring cells in an organism.

RESULTS

Upon inoculation with contemporary clade 2.3.4.4b and historical HPAI H5N1 isolates the replication efficiently, induction of cell death, and innate response differed. While virus replication of historical isolate A/Vietnam/1203/04 was more efficient in AT2s, the contemporary A/Texas/37/2024 replicated more efficiently in AT1s. In AT2s, reduced innate immune responses were detected following infection with contemporary compared to historical isolates. However, no such differences were observed in AT1s. Higher levels of virus replication were observed for contemporary than historical HPAI H5N1 viruses in human nasal epithelium.

In hCOs, similar levels of virus replication were observed for all HPAI H5N1 viruses; no replication was observed upon inoculation with a seasonal H1N1 virus. scRNAseq analysis showed that in HPAI H5N1 virus-inoculated organoids, viral reads were the main driver of variation in our dataset, with up to 90% of reads being viral in infected cells. Neuronal cells were the predominant infected cell type, with some involvement of astrocytes. By contrast, an innate immune response was detected primarily in non-infected or lowly-infected cells, and not until 3-days post-inoculation.

CONCLUSIONS

The reduced virus replication and innate immune responses in hLOs infected with contemporary clade 2.3.4.4b HPAI H5N1 viruses circulating in dairy cattle suggest that these viruses are less pathogenic to humans than older HPAI H5N1 viruses. hCO data indicate that the contemporary HPAI H5N1 viruses have retained neurotropism, but suggest that other factors, potentially inflammatory responses in non-neural tissues, contribute to the development of neurological complications following infection in humans. Together, our data highlight the value of human organoid cultures to assess the pathogenicity of emerging influenza A viruses.

This work is supported by the Intramural Research Program of NIAID, NIH.

Knockdown of Irf9 within vagal sensory ganglia exacerbates disease severity during pulmonary influenza A viral infection ECaS

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BACKGROUND

Interferon regulatory factor 9 (IRF9) is a critical transcription factor for type I and II interferon responses during viral infection and thus plays an important role in antiviral responses. We recently showed that during respiratory infection with Influenza A virus (IAV), *Irf9* gene expression is significantly upregulated in vagal sensory neurons – innervating the respiratory tract. These neurons – essential for monitoring and regulating the environment within the airways and lungs – adopt an antiviral inflammatory phenotype during respiratory viral infection with IAV, characterised by antiviral related transcriptional changes. Although IRF9 is well-known in immune cell signalling, its role in sensory neurons in the peripheral nervous system during viral infection remains poorly understood. Moreover, these signalling pathways may present a novel and underexplored therapeutic target in respiratory viral disease.

METHODS

In a murine model of IAV respiratory infection (male, C57B6/J mice, 12-weeks; Auckland/1/09 H1N1 strain), the role of IAV-induced vagal ganglia *Irf9* expression was assessed by knocking down ganglia expression. Vagal knockdown of *Irf9* was obtained by targeted delivery of an adeno-associated viral vector encoding *Irf9* short hairpin RNA (AAV-IRF9shRNA). Disease severity was measured using whole body plethysmography, body weight and clinical scoring. Additionally, pathogenesis pathways were assessed through proteomics of the vagal ganglia, lung histology, viral titre and cytokine concentration.

RESULTS

IAV-infected AAV-IRF9shRNA animals exhibited significantly greater weight loss (76.8±1.1%; 84.3±1.0%, *p=0.0032*) accompanied with more severe clinical symptoms (26.8±0.8; 12±0.8, *p<0.0001*) compared to IAV infected controls receiving a blank vector. Additionally, AAV-IRF9shRNA animals demonstrated increases in airflow obstruction (291.7±18.8%; 196.6±24.6%, change from baseline 6 days post infection, *p=0.0071*) – measured as midexpiratory flow. Despite these indications of more laboured breath, tidal volume and minute volume were reduced to baseline levels 6 days post infection in AAV-IRF9shRNA animals. Furthermore, there were no differences in pulmonary pathology measured between groups. Proteomic analysis of the vagal ganglia revealed downregulation of pathways associated with (innate) immune responses - specifically to antigen presentation – and programmed cell death in AAV-IRF9shRNA animals compared to their controls.

CONCLUSION

These findings reveal that vagal sensory neurons play a multifaced role in the host response to IAV infection, that is not yet understood. While Irf9 knockdown in these neurons exacerbated symptomatology, this occurred independently of disease pathology highlighting a nuanced neuroimmune interaction. Importantly, these results underscore the complexity of targeting vagal sensory neurons as a therapeutic approach, as these interventions may inadvertently affect respiratory function. Further investigation is warranted to have a deeper understanding of the mechanisms to which vagal sensory neurons contribute disease severity and to assess the feasibility of neuromodulatory strategies in respiratory viral disease.

Genome-wide CRISPR/Cas9 screen identifies host factors potentially modulating influenza A virus polymerase fidelity

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The evolution of H5 and H7 influenza A viruses (IAV) from low pathogenic to high pathogenic viruses has significant implications for both animal and human health. This first step in this transition involves the acquisition of a polybasic cleavage site at the viral hemagglutinin through sequence insertions during transcription. Therefore, the fidelity of the IAV polymerase complex, composed of PB2, PB1 and PA subunits, is likely to contribute to this gain-of-function process. Numerous host factors are known to interact with the IAV polymerase but it is not known if any are involved in controlling its error rate. Here, we performed a genome-wide CRISPR/Cas9 screen to identify host factors that affect IAV polymerase fidelity. To create an IAV polymerase-specific screen that could be run at BSL2, we used a transfection-based minireplicon system that used a reporter segment consisting of a slippage-prone sequence derived from the HA cleavage site of A/whistling swan/Shimane/499/83 ahead of an out-of-frame GFP gene, driven by a polycistronic plasmid expressing PB2, PA and NP and a separate PB1 plasmid. We sorted the GFP-positive cells into 3 populations (low, medium and high). From three replicate screens, we identified around 1500 genes across the three populations that significantly affected GFP expression, both positively and negatively, compared to the unsorted controls. We found that the loss of many cellular genes repressed GFP expression by the IAV polymerase, but majority of these were involved in translational processes and thus likely reflected inhibition of overall IAV and/or cellular gene expression (the latter required to launch the minireplicon system). Conversely, the loss of many cellular genes upregulated GF expression, but some of these were interferon-associated genes and thus again, the increased GFP expression could be due to greater overall RNP activity. However, we were able to identify several genes with a possible connection to IAV polymerase fidelity, particularly certain transcription-related genes responsible for chromatin remodelling and mRNA decay. Some of these gene products had also been shown to modulate IAV replication, supporting their functional importance. Experiments are underway to test these candidate genes in more detail. By understanding the molecular mechanisms driving the IAV polymerase fidelity, this will be useful for future risk assessment and pandemic preparedness.

LATE BREAKERS: NOVEL AND OUTSTANDING DISCOVERIES

SCS15 PLE • AUDITORIUM 1 - PLENARY HALL • THU 23 OCT 2025 - 14:00 - 15:40

mRNA-1010, an mRNA-Based Influenza Vaccine, is Safe and Efficacious in Adults Aged ≥50 Years, Including Individuals at High Risk for Severe Disease

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BACKGROUND

mRNA-1010, a novel mRNA-based trivalent influenza vaccine targeting vaccine-matched influenza A and B strains, previously demonstrated superior immunogenicity compared to licensed standard-dose (SD) and high-dose comparators.1 We present safety and relative vaccine efficacy (rVE) from the end of influenza season analysis of the pivotal phase 3 trial comparing mRNA-1010 to SD influenza vaccination in adults ≥50 years and in prespecified high-risk subgroups.

METHODS

This phase 3, randomized, double-blind, active-controlled study in adults ≥50 years (NCT06602024) enrolled participants from 11 countries throughout the Northern Hemisphere. Participants were randomized 1:1 to receive a single dose of trivalent mRNA-1010 37.5 µg (12.5 µg hemagglutinin mRNA per strain) or SD comparator (Fluarix 45 µg or Fluarix Tetra, Influsplit Tetra, or Alpharix Tetra 60 µg). The primary efficacy endpoint was rVE in preventing the first episode of RT-PCR–confirmed protocol-defined influenza-like illness (ILI) caused by influenza A or B strains beginning at least 14 days after study vaccination through the end of the influenza season. Prespecified high-risk subgroups included participants aged ≥65 years and those with high-risk medical conditions and/or obesity at baseline.

RESULTS

In the 2024-2025 influenza season, 40,703 participants received a study vaccine (mRNA-1010, n=20,350; SD comparator, n=20,353). The median age was 64 years (range 50-97 years); 56.9% were female; 82.6% were White, 13.2% Black; and 10.4% Hispanic/Latino. Nearly half of participants were ≥65 years (n=19,464). Median duration of follow up was 181 days (1-227 days). Solicited adverse reactions (SARs) within 7 days were more frequently reported in the mRNA-1010 than SD comparator group; most reactions were mild or moderate, transient, and self-limiting. Incidence and severity of local and systemic SARs was lower in the older age group in both treatment arms. mRNA-1010 demonstrated an rVE of 26.6% (95% CI, 16.7%-35.4%) against RT-PCR–confirmed protocol-defined ILI compared to the SD comparator, meeting prespecified superiority criteria (lower bound of the 95% CI >9.1%; 1-sided p=0.0005); the observed rVE was consistent in subgroups of participants ≥65 years (27.4%; 95% CI, 12.1%-40.0%) and those with underlying high-risk comorbidities (22.3%; 95% CI, 8.0%-34.3%) or obesity (27.5%; 95% CI, 10.6%-41.3%) (Figure 1).

Figure 1: rVE Against RT-PCR-Confirmed Protocol-Defined ILI By Any Influenza A or B Strain Overall and By High-Risk Subgroup (Per Protocol Immunogenicity Subgroup)

ILI		Numbers of Events: mRNA-1010 (N = 20,179)	Numbers of Events: Licensed Influenza Vaccines (N = 20,174)	Relative Vaccine Efficacy (95% CI)
Overall		411/20179	557/20124	26.6% (16.7, 35.4)
Age	≥ 65 Years	182/9,637	250/9,623	27.4% (12.1, 40)
	65-74 Years	138/7,307	191/7,289	28.0% (10.4, 42.2)
	≥ 75 Years	44/2,330	59/2,334	25.3% (10.4, 49.5)
Risk Factors	≥ 1 Condition	241/11,465	309/11,457	22.3% (8.0, 34.3)
BMI	≥ 30 kg/m²	153/7,958	208/7,892	27.5% (10.6, 41.3)

-20 -10 0 10 20 30 40 50 60

Vaccine Efficacy, % (95% CIs)

rVE is defined as 100 × (1 - hazard ratio (mRNA-1010 vs. the Active Comparator)). The rVE and the CI are based on a stratified Cox proportional hazards model with the study vaccination group as a fixed effect, adjusting by the randomized stratification factors. Comorbidities include cardiac disease, chronic respiratory disease, diabetes, advanced liver disease, advanced renal disease, neurologic disease, obesity, and autoimmune disease.

CONCLUSIONS

mRNA-1010 demonstrated superiority over SD comparator aligned with other enhanced influenza vaccines in the prevention of RT-PCR–confirmed protocol-defined influenza disease in adults ≥50 years. The high observed rVE was consistent across high-risk subgroups, including those aged ≥65 years and those with high-risk comorbidities at baseline.

Reference 1. Soens M, et al. Vaccine. 2025;50:126847.doi:10.1016/j.vaccine.2025.12684

LATE BREAKERS: NOVEL AND OUTSTANDING DISCOVERIES
SCS15 PLE • AUDITORIUM 1 - PLENARY HALL • THU 23 OCT 2025 - 14:00 - 15:40

A real-life study revealed the presence of more than 10% nirsevimab escape mutants in RSV-B breakthrough infections.

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BACKGROUND

Nirsevimab, a long-acting monoclonal antibody targeting the RSV fusion (F) protein, has been widely used since 2023 in several countries, including France. While RSV-A strains in breakthrough infections have remained susceptible, RSV-B strains with resistance-associated substitutions (RAS) at the nirsevimab-binding site have been detected in 4 out of the 56 RSV-B breakthrough infections reported across various studies. To fully characterize the molecular aspects, frequency and risk factors of these resistant strains, we conducted a large observational real-life study of RSV viral breakthrough.

METHODS

During the 2024–25 RSV season, we conducted a multicentre, national, observational study in infants under one year of age with RSV infection, either (i) nirsevimab-treated breakthrough infection or (ii) nirsevimab-untreated infection. Full-length RSV genome sequencing was performed, and changes in the F protein (site Ø) relative to reference sequences were analysed. The impact of amino acid substitutions at site Ø was assessed using a fusion inhibition assay, and the susceptibility of RSV isolates from treated and untreated patients to nirsevimab was assessed using a microneutralisation assay.

RESULTS

A total of 858 RSV-positive infants under 12 months were enrolled in the study and underwent whole genome sequencing. Of these, 439 were nirsevimab-naïve (181 RSV-A and 234 RSV-B), while 419 experienced breakthrough infections after receiving nirsevimab (195 RSV-A and 184 RSV-B). High-quality sequences were obtained for 794 samples, enabling detailed analysis. No resistance-associated substitutions (RAS) were found in the nirsevimab-naïve group. In the breakthrough group, two cases of RSV-A (1%) exhibited a novel F:K209E mutation that conferred nirsevimab resistance. Among the RSV-B breakthrough cases, nine infants had known RAS, one of which almost one year after nirsevimab administration; 14 had novel amino acid changes which conferred intermediate to high-level resistance in phenotypic assays. Overall, 23 (12.5%) of the RSV-B breakthrough infections harboured viruses with intermediate to high-level nirsevimab resistance.Preliminary statistical analysis indicated that prematurity, older age at infection, and a longer interval between nirsevimab administration and RSV detection are associated with the emergence of resistance.

CONCLUSION

These results highlight the complex molecular basis of emerging resistance patterns and confirm that, while resistance selection is rare in RSV-A infections, it occurs more frequently in RSV-B infections. The detection of a highly resistant RSV-B variant almost a year after nirsevimab administration underscores the risk of late resistance selection in situations of low antibody pressure. These findings emphasise the importance of sustained molecular surveillance during the second RSV season for infants who received nirsevimab in their first year.

LATE BREAKERS: NOVEL AND OUTSTANDING DISCOVERIES
SCS15 PLE • AUDITORIUM 1 - PLENARY HALL • THU 23 OCT 2025 - 14:00 - 15:40

Adjuvanted inactivated zoonotic influenza A(H5N8) vaccination induces antibody and T-cell responses to emerging HPAI clade 2.3.4.4b A(H5N1) viruses in healthcare workers

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BACKGROUND

Recent outbreaks of highly pathogenic avian influenza (HPAI) viruses of the A(H5) hemagglutinin (HA) subtype, particularly those belonging to clade 2.3.4.4b and clade 2.3.2.1e, underscore their pandemic threat. For individuals at risk of occupational exposure, a MF59-adjuvanted inactivated A(H5N8) vaccine based on clade 2.3.4.4b strain A/Astrakhan/3212/2020 produced by Seqirus is available. However, data on functional antibody and T-cell responses induced by this vaccine remain limited. This study evaluated the breadth and magnitude of immune responses induced by this vaccine, and compared those in naïve HCW and HCW previously vaccinated with A(H5) influenza vaccines.

METHODS

The zoonotic influenza vaccine Seqirus was procured in the Netherlands and offered to 39 HCW at risk of occupational exposure (laboratory employees). Its immunogenicity was evaluated at baseline, and on days 7 and 28 after each of two vaccine doses (given at a 28-day interval). Of those 39 HCW, 6 had been previously vaccinated with antigenically distinct zoonotic influenza vaccines, others were considered naïve. HA-binding antibodies were measured via multiplex protein microarray (PMA), and functional antibodies were quantified by hemagglutinin inhibition (HI), neuraminidase inhibition (NI), and antibody-dependent cellular cytotoxicity (ADCC) assays. T-cell responses were detected by interferon-gamma release assays (IGRA) and activation-induced marker (AIM) assays.

RESULTS

HI antibodies targeting clade 2.3.4.4b A(H5) viruses were effectively induced in all naïve participants. These included antibodies targeting the vaccine A/Astrakhan/3212/2020 virus, but also cross-reactive antibodies with HI activity against the recent bovine-derived A/Texas/37/2024 virus. Next, post-boost sera were tested against 80 antigenically diverse A(H5) influenza viruses from our A(H5) antigenic map (Kok et al., https://doi.org/10.1101/2024.08.06.606696), revealing HI reactivity against almost all tested viruses in previously vaccinated participants, in contrast to clade 2.3.4.4b-restricted HI response in naïve participants. NI antibody responses to the N8 vaccine component were effectively induced. Moderate A(H5)-specific ADCC responses were detected at baseline, which increased considerably by day 28 after second vaccination. Strong T-cell responses targeting multiple H5 antigens (clades 2.1.3.2, 2.3.4, and 2.3.4.4b) and N8 were induced. Notably, the strongest T-cell response induction was against internal viral proteins (M1 and NP), which were already present at baseline and therefore probably cross-reactive.

CONCLUSION

Our findings show strong induction of HI antibodies targeting clade 2.3.4.4b A(H5) viruses by the zoonotic influenza vaccine Seqirus in naïve individuals. In addition to HI antibodies, the induction of functional NI and ADCC antibodies targeting vaccine antigens was detected. Importantly, all participants displayed detectable T-cell responses post vaccination. While naïve individuals developed robust yet restricted HI responses to clade 2.3.4.4b, previously vaccinated individuals showed broad HI reactivity across all genetic A(H5) clades. These results demonstrate the immunogenicity of the MF59-adjuvanted zoonotic influenza vaccination and highlight the potential of heterologous prime-boost vaccination strategies to induce broad A(H5) immunity.

LATE BREAKERS: NOVEL AND OUTSTANDING DISCOVERIES
SCS15 PLE • AUDITORIUM 1 - PLENARY HALL • THU 23 OCT 2025 - 14:00 - 15:40

Translational efficacy of CD388, a novel Drug Fc-Conjugate (DFC), in mouse influenza infection models: application to prevention efficacy in the recently completed Ph2b NAVIGATE clinical study

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BACKGROUND

Cidara Therapeutics recently announced positive Phase 2b results for CD388, a stable antiviral drug-Fc conjugate aimed at universal influenza prevention. CD388 combines a neuraminidase inhibitor with a human IgG1 Fc fragment, extending half-life. Nonclinical studies showed effectiveness against various influenza A and B strains in mice. This presentation discusses the translation of nonclinical efficacy to clinical outcomes.

METHODS

Mouse studies used 6–8-week-old female BALB/c mice, challenged intranasally with a lethal dose of influenza A or B. Mice (n=5 per group) received a single CD388 dose either 2 h after (treatment) or 7 days before (prophylaxis) viral challenge.

The Phase 2b clinical trial was a randomized, double-blind, placebo-controlled study in healthy adults (18-64 years). Participants received placebo or CD388 (150, 300, or 450 mg). Plasma concentrations were measured during suspected breakthrough infections confirmed by RT-PCR. Exposure-response (ER) modeling was performed using logistic regression to relate PCR positivity to CD388 concentrations.

RESULTS

Both treatment and prophylaxis in mice demonstrated potent CD388 activity against 12 seasonal and multiple pandemic (pdm09) influenza isolates, including oseltamivir-resistant neuraminidase variants (H275Y). Across all subtypes, plasma concentrations ≥1 µg/mL at time of infection conferred complete protection and established the minimum target concentration for efficacy in humans.

In Ph2b, CD388 met the primary endpoint, demonstrating statistically significant prevention efficacy (PE) for each of three dose groups in individuals who received a single dose of CD388 at the beginning of the flu season and were evaluated for laboratory and clinically confirmed influenza over 24 weeks. All secondary efficacy endpoints were also met. A single dose of 150, 300, or 450 mg conferred PEs of 58%, 61%, and 76%, respectively, from symptomatic influenza over 24 weeks versus placebo. Plasma concentration analysis at breakthrough infection showed that lower concentrations correlated with higher infection rates. ER modeling revealed a clear concentration-response relationship, with low PCR positivity at 1 µg/mL, indicating strong concordance with preclinical findings.

CONCLUSION

Clinical data demonstrate strong alignment between efficacious exposures in preclinical mouse influenza models and prevention efficacy observed in a recent Phase 2b trial.

LATE BREAKERS: NOVEL AND OUTSTANDING DISCOVERIES
SCS15 PLE • AUDITORIUM 1 - PLENARY HALL • THU 23 OCT 2025 - 14:00 - 15:40

Effectiveness of HD-IIV against laboratory-confirmed influenza infection and patient-centered outcomes in the community: a DANFLU-2 sub-study

ECaS

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BACKGROUND

Disease specific patient reported outcomes (PROs) are rarely included in real-world influenza vaccine effectiveness studies, limiting patient-centered insights on disease outcomes. In DANFLU-2, a pragmatic randomized controlled trial, we addressed this gap by evaluating the impact of high-dose versus standard-dose inactivated influenza vaccine (HD-IIV; SD-IIV) on symptomatic, laboratory-confirmed influenza (LCI) and PROs using novel methods in a sub-group of participants.

METHODS

DANFLU-2 is an open-label, active-controlled, individually randomized trial conducted in Denmark across three influenza seasons (2022/23, 2023/24, and 2024/25). In the 2024/25 season, 152,288 adults aged ≥65 years were randomized to receive HD-IIV or SD-IIV. This sub-study aimed to enroll 10,000 participants prior to randomization on a first-come basis. Participants with influenza-like illness (ILI) were instructed to self-collect nasal and oropharyngeal swabs at home, post the samples to a centralized laboratory for PCR testing, and to digitally complete the Respiratory Infection Intensity and Impact Questionnaire (RiiQ) for 7-14 days post-symptom onset. The RiiQ assesses five domains with 6-7 items in each, rated on a four-point scale (none to severe) (*Figure 1*). We calculated relative vaccine effectiveness (rVE) as 1 minus relative risk of LCI. Sensitivity analysis used a test-negative design approach. Vaccination arm was linked to PCR results and RiiQ responses after May 31, 2025.

RESULTS

A total of 10,140 participants were enrolled into this sub-study. 1,273 swabs underwent testing against an extended virological respiratory PCR panel. RNAseP positivity was 97.8% confirming the self-collected swabs were of good quality. Positivity rates for samples are detailed in *Table 1*. Relative vaccine effectiveness of HD-IIV vs SD-IIV against symptomatic LCI in the community was 42.2% (95% CI: 16.4–60.0). Sensitivity analyses yielded consistent findings with an rVE of 44.2% (95% CI: 17.6–62.1). Of the 121 LCI events, 97 (80.2%) were linked to a completed RiiQ. Participants who received HD-IIV reported significantly lower odds of experiencing moderate or severe respiratory symptoms (OR 0.57; 95% CI:0.38 – 0.85), systemic symptoms (OR 0.59; 95% CI:0.39 – 0.91), and impact on daily activities (OR 0.59; 95% CI:0.36 – 0.96), based on at least one moderate or severe item per domain for at least one day (*Table 2*). Results for respiratory and systemic symptoms remained significant when the analysis was expanded to a minimum of two moderate or severe items per domain for at least two consecutive days.

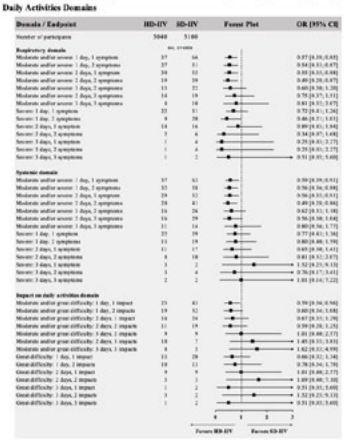
CONCLUSION

This study adds a novel and valuable dimension to influenza vaccine research. These findings demonstrate a reduction of symptomatic LCI in patients receiving HD-IIV over SD-IIV, as well as a lessening of symptom severity and impact on daily life. Importantly, the high quality of the self-collected swabs and good compliance with RiiQ support the feasibility and reliability of using decentralized tools in the older adult population.

Table 1: Characteristics and virus PCR positivity from all submitted self-collected swabs

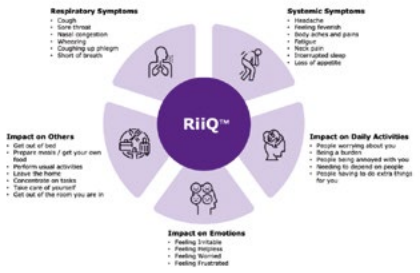
	HD-IIV	SD-IIV	Total
Participants – no. (%)	5,040 (49.7)	5,100 (50.3)	10,140 (100)
Age – yr (SD)	73.3 ± 6.1	73.2 ± 6.3	73.2 ± 6.2
Female sex – no. (%)	2,550 (50.1)	2,572 (50.4)	5,102 (50.3)
Total swabs – no. (%)	627 (49.3)	646 (50.7)	1273 (100)
RNAseP – no. (%)	610 (97.3)	635 (98.3)	1,245 (97.8)
Virus Positivity of Total Swabs			
Influenza A – no. (%)	43 (6.9)	76 (11.8)	119 (9.3)
Influenza A (H1N1 pdm09) – no. (%)	19 (3.0)	33 (5.1)	52 (4.1)
Influenza A (H3N2) – no. (%)	24 (3.8)	43 (6.7)	67 (5.3)
Influenza B – no. (%)	1 (0.2)	1 (0.2)	2 (0.2)
Adenovirus – no. (%)	1 (0.2)	2 (0.3)	3 (0.2)
Corona 229E – no. (%)	3 (0.5)	4 (0.6)	7 (0.5)
Corona HKU1 – no. (%)	7 (1.1)	14 (2.2)	21 (1.6)
Corona NL63 – no. (%)	33 (5.2)	25 (3.9)	58 (4.6)
Corona OC43 – no. (%)	50 (8.0)	34 (5.3)	84 (6.6)
Human metapneumovirus – no. (%)	20 (3.2)	21 (3.3)	41 (3.2)
Parainfluenza 1 – no. (%)	5 (0.8)	9 (1.4)	14 (1.1)
Parainfluenza 2 – no. (%)	6 (1.0)	6 (0.9)	12 (0.9)
Parainfluenza 3 – no. (%)	7 (1.1)	12 (1.9)	19 (1.5)
Parainfluenza 4 – no. (%)	2 (0.3)	2 (0.3)	4 (0.3)
RSV A – no. (%)	8 (1.3)	12 (1.9)	20 (1.6)
RSV B – no. (%)	29 (4.6)	13 (2.0)	42 (3.3)
Entero/Rhinovirus – no. (%)	88 (14.0)	102 (15.8)	190 (14.9)
SARS-CoV 2 – no. (%)	64 (10.2)	70 (10.8)	134 (10.5)

Table 2: RiiQ Results – Impact of HD-IIV vs SD-IIV on Respiratory, Systemic and Impact on Daily Activities Domains



Odds ratios were estimated based on the minimum number of participants meeting various endpoint definitions, progressively stricter in terms of severity/impact, cumulation of them (up to three distinct items), and duration (up to three consecutive days).

Figure 1: Overview of Respiratory Infection Intensity and Impact Questionnaire (RiiQ) Domains



The RiiQ contains five domains, each one with individual items that assess specific symptoms or impacts. Participants respond to each item using a four-point severity scale: none, mild, moderate, or severe.

LATE BREAKERS: NOVEL AND OUTSTANDING DISCOVERIES
SCS15 PLE • AUDITORIUM 1 - PLENARY HALL • THU 23 OCT 2025 - 14:00 - 15:40

Induction of strong innate immune response in human alveolar epithelium is correlated with pathogenicity of avian influenza viruses

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BACKGROUND

Human infections with avian influenza A viruses (AIVs), including H5N1 and H7N9 subtypes, historically resulted in severe disease marked by respiratory distress, cytokine storm, and mortality in some cases. Currently, clade 2.3.4.4b H5N1 AIVs are widely distributed in wild and domestic species in North America, and 70 human cases have been reported in the United States since 2024. In contrast to previous outbreaks, human infections with clade 2.3.4.4b H5N1 AIVs have largely resulted in mild disease with conjunctivitis as a primary symptom. However, several severe cases requiring hospitalization have been reported. Notably, the majority of severe cases were associated with infection by genotype D1.1 H5N1 viruses, as opposed to the co-circulating B3.13 genotype found in most human cases. Due to limited case numbers and confounding epidemiological factors, potential differences in pathogenicity between D1.1 and B3.13 genotype H5N1 AIVs, and what host or viral factors contribute to these differences, are not understood.

METHODS

We utilized human lung organoids (hLOs) and hLO-derived air-liquid interface cultures to compare virus replication, cell death, and host responses to AIVs in primary human type 1 and type 2 alveolar epithelial cells. We compared two contemporary clade 2.3.4.4b genotype B3.13 viruses (A/Texas/37/2024, A/bovine/Ohio/B24OSU-342/2024), two clade 2.3.4.4b genotype D1.1 viruses (A/Wyoming/01/2025, A/Nevada/10/2025), and two historical AIVs (A/Vietnam/1203/2004 H5N1, A/Anhui/1/2013 H7N9) isolated from fatal human cases.

RESULTS

In type 2 alveolar epithelial cells, D1.1 genotype viruses and A/Vietnam/1203/2004 replicated to similar titers, while B3.13 genotype viruses were slightly attenuated. Activation of innate immune responses, indicated by up-regulation of interferon-stimulated genes (IFITM3, MX1, ISG15, ISG20, RIG1, OAS1, IFIT1) and pro-inflammatory cytokines (IFNb, TNFa, IL-6) at the RNA level, differed between viruses. Isolates obtained from severe or fatal cases (A/Wyoming/01/2025, A/Vietnam/1203/2004, A/Anhui/1/2013) induced significantly stronger innate immune responses compared to viruses isolated from mild cases. Distinct patterns of virus replication and innate immune activation were observed in type 1 alveolar cells, where clade 2.3.4.4b genotype B3.13 replicated to higher titers, but still did not trigger up-regulation of interferon-stimulated genes or pro-inflammatory cytokines.

CONCLUSIONS

These data suggest that induction of strong innate immune responses in human alveolar epithelium may contribute to pathogenicity of AIVs, and highlight the potential applications of human organoid systems for risk assessment of emerging viruses.

This research was supported by the Intramural Research Program of the National Institutes of Health (NIH).

Abstracts poster sessions overview

104

Development and Evaluation of a Novel mRNA Vaccine Against Influenza Virus: Hemagglutinin Expression in Lipid Nanoparticles

Aida ABBASI

ReNAp company, Iran, Islamic Republic of

Combating Influenza Variants: In Vitro Assessment of a Multivalent mRNA Vaccine

Abstract

Objectives: Influenza viruses present a persistent global health threat, demanding innovative vaccine approaches that offer broad and adaptable protection against seasonal epidemics and potential pandemics. This study aimed to: (1) Develop a multivalent mRNA vaccine encoding hemagglutinin (HA) proteins from multiple influenza strains, formulated within lipid nanoparticles (LNPs); (2) Evaluate the in vitro expression of HA proteins from this multivalent mRNA vaccine; and (3) Conduct quality control tests to ensure the formulation's integrity and functionality. We chose a mRNA platform due to its advantages for rapid development and scalability, vital for pandemic preparedness.

Methods: A multivalent mRNA vaccine was designed and synthesized to express HA proteins from several influenza strains. The mRNA was then formulated into lipid nanoparticles (LNPs) for enhanced delivery and stability. In vitro expression of HA proteins was assessed using Western blot assays. Quality control tests were performed on the LNP-formulated mRNA vaccine.

Results: The Western blot and ELISA analyses confirmed successful in vitro expression of HA proteins from the multivalent mRNA vaccine. The quality control tests demonstrated that the LNP formulation exhibited acceptable particle size, high encapsulation efficiency of mRNA, and maintained the integrity of the mRNA cargo.

Conclusion: This study demonstrates the successful development and in vitro expression of a multivalent mRNA-LNP vaccine encoding HA proteins from multiple influenza strains. These findings suggest that this mRNA-based vaccine holds promise as a next-generation influenza vaccine capable of inducing a broad immune response. Further research will focus on in vivo evaluation of the vaccine's efficacy and immunogenicity. The adaptability of the mRNA platform allows for quick modification to address emerging viral variants, making it a valuable tool in the fight against future pandemics.

105

Evaluation of Reverse Genetics-Based H5N8 (Clade 2.3.4.4b) Vaccine with Different Adjuvants and Routes for H5N1 Protection in Chickens

Kairat TABYNOV (1,2,3), Aidana KUANYSHBEK (1,4), Leila YELCHIBAYEVA (1), Kuantay ZHARMAMBET (1), Zauresh ZHUMADILOVA (2), Gleb FOMIN (2), Nikolai PETROVSKY (5), Olaitan C SHEKONI (6), Gourapura J RENUKARADHYA (6), Kaissar TABYNOV (1,2,3)

1: International Center for Vaccinology, Kazakh National Agrarian Research University, Almaty, Kazakhstan;2: Central Reference Laboratory, M. Aikimbayev National Scientific Center for Especially Dangerous Infections, Almaty, Kazakhstan;3: T&TvaX LLC, Almaty, Kazakhstan;4: National Collection of Deposited Strains, Almaty Branch of National Reference Veterinary Center, Almaty, Kazakhstan;5: Vaxine Pty Ltd, 11 Walkley Avenue, Warradale, SA, 5046, Australia;6: Center for Food Animal Health, Department of Animal Sciences, College of Food Agricultural and Environmental Sciences, The Ohio State University (OSU), Wooster, OH, 44691, USA

Background: Highly pathogenic avian influenza (HPAI) H5Nx viruses pose a significant threat to poultry health globally, with recent outbreaks of H5N1 and H5N8 causing substantial economic and ecological damage. Vaccination remains a cornerstone for controlling HPAI, but the efficacy of vaccines depends on the choice of adjuvant and delivery route. This study evaluated the safety, immunogenicity, and efficacy of a reverse genetics-based DIVA-compatible inactivated H5N8 vaccine (IDCDC-RG71A) formulated with different adjuvants and administered via subcutaneous (SC) or intranasal (IN) routes in chickens.

Methods: The study was conducted in a Biosafety Level 3 (BSL-3) facility using influenza serum antibody-negative (SAN) chickens. Vaccine formulations were prepared with various adjuvants, including Montanide ISA 78 VG, ISA 71 R VG, GEL P PR (Seppic, France), and mannose-conjugated chitosan nanoparticles, and administered via subcutaneous (SC) or intranasal (IN) routes. Safety was assessed by monitoring clinical symptoms and growth dynamics over 35 days. Immunogenicity was evaluated by measuring hemagglutination inhibition (HI) antibody titers, specific IgA and IgY levels, and $CD4^+$ and $CD8^+$ T cell proliferation. Efficacy was tested by challenging vaccinated chickens with a virulent H5N1 strain (A/mute swan/Mangystau/1-S24R-2/2024) and monitoring survival rates, viral shedding, and histopathological changes in lung and liver tissues.

Results: SC-administered vaccines, particularly those with Montanide ISA 78 VG and ISA 71 R VG adjuvants, induced robust HI antibody responses (100% seropositivity) and provided 100% protection against H5N1 challenge. These vaccines also significantly reduced viral shedding and prevented lung and liver lesions. In contrast, IN formulations, even after two doses, failed to induce high HI titers and provided no or minimal protection (0-40% survival). The ISA-78-SC formulation showed superior immunogenicity, with significantly higher ($p<0.05$) $CD4^+$ T cell proliferation compared to other groups. Mucosal IgA responses were detected in IN-administered groups but did not correlate with protection.

Conclusions: This study highlights the importance of adjuvant selection and delivery route in maximizing HPAI vaccine efficacy. SC-administered H5N8 vaccines with Montanide adjuvants demonstrated superior protection against H5N1 infection, reducing viral shedding and preventing tissue damage. These findings support the development of potent, DIVA-compatible vaccines for regions affected by endemic HPAI, offering significant implications for poultry health and biosecurity.

106

Risk of nosocomial respiratory syncytial virus versus influenza among adult patients in acute care hospitals

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1: HUS Helsinki University Hospital, Finland;2: Finnish Institute for Health and Welfare;3: Finnish Vaccine Research

Introduction While nosocomial influenza is common, the risk of transmission in acute care remains unclear in the absence of systematic surveillance. Even less is known about the risk of nosocomial respiratory syncytial virus (RSV) among immunocompetent adults.We compared the proportions and incidences of nosocomial cases caused by these two similar respiratory infections among hospitalized adults. Methods A retrospective study was conducted at two tertiary care hospitals in Southern Finland. Data on all hospitalized adult patients with a positive RSV or influenza test during 2016–22 were used to detect all nosocomial and community-acquired RSV and influenza cases. The proportion of nosocomial cases of all hospitalized cases was calculated. The incidences of nosocomial cases per 1000 bed-days were calculated by season and ward type for the five seasons before the COVID-19 pandemic. Results Nosocomial RSV and influenza occurred in 2.8% and 8.1% of all hospitalized adult patients with a laboratory-confirmed infection. Over five seasons, 2016–20, the total incidences of nosocomial RSV and influenza cases per 1000 bed-days were 0.027 (95% confidence interval: 0.013, 0.050) and 0.32 (0.27, 0.39). Nosocomial RSV infections were especially poorly recorded with a virus-specific ICD-10 diagnosis code listed for only 16.7% of RSV and 59.8% of nosocomial influenza patients. Conclusion Despite preventive measures, the incidence of nosocomial influenza was more than tenfold, and the proportion of nosocomial cases was almost threefold compared with RSV among hospitalized adults in acute care. Nosocomial infections were poorly documented in medical records. Prevention and surveillance of both nosocomial influenza and RSV should be improved also among immunocompetent adult patients.

107

ECaS

Three years of the Adult Immunization Board: evolution, key accomplishments, and future directions

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1: Department of Health Sciences, University of Florence, Italy; 2: Centre for the Evaluation of Vaccination (CEV), University of Antwerp, Antwerp, Belgium

Background: The Adult Immunization Board (AIB) is an international, and multidisciplinary group of experts in the field of adult immunization which was established in 2022 to create a collaboration platform to address critical gaps in adult vaccination strategies across European countries. Its mission is to provide evidence-based guidance on fundamental technical and strategic issues, while investigating the progress of adult immunization programs at European and (sub)national levels.

Methods: The AIB operates by organizing two yearly meetings: a technical meeting to discuss a specific technical aspect with subject-matter experts, and a country meeting with local stakeholders to explore and discuss local practices and foster collaboration and coordinated action. These meetings bring together academics, public health experts, healthcare professionals, representatives of the civil society/advocacy groups and policymakers to identify barriers, share best practices, and generate actionable outputs. Insights are disseminated through peer-reviewed publications, detailed reports, and digital platforms. AIB members attend international congresses and conferences to disseminate our results.

Results: Since 2022, the AIB has conducted one kick-off meeting and four major meetings (another one is planned): two technical meetings, and two country meetings in Italy and Finland. The technical meeting at Antwerp 2023 dealt with strategies for the estimation of the vaccine-preventable disease burden, such as data gaps for certain diseases and risk groups; the 2024 meeting in Prague explored strategies for integrating vaccines into National Immunization Programs, identifying barriers like behavioral and operational challenges, and proposing enhanced coordination across Europe. The country meetings addressed local immunization systems: the 2023 meeting in Florence, Italy, emphasized regional inequalities, strategies to reduce vaccine hesitancy, and life-course vaccination integration. The Finland meeting particularly underscored the role of robust electronic registries in improving vaccination program monitoring. These efforts created new connections (>300 people involved), sparked collaboration and catalyzed systemic change; the meetings are documented in detailed reports and peer-reviewed publications based on each meeting findings. Other key AIB deliverables in progress include the Vaccines for Adults Tracker, which monitors vaccines authorized and used in Europe, a European textbook on adult vaccination, which aims to address knowledge and practice gaps in (future) healthcare professionals dealing with vaccination, and a decalogue for policy action to overcome barriers to adult immunization in Europe. The AIB has also participated in over 50 congresses and conferences, including ECCMID, EUPHA, and ESWI, presenting findings and developing and promoting collaborations and coordinated action.

Conclusions: The achievements of the AIB over the past three years demonstrate its critical role in improving adult immunization and collaboration in Europe, promoting lifelong vaccination. Future priorities include continuing its work, expanding its reach and accelerate efforts to implement and optimize adult immunization programs at the European, national and sub-national level.

110

Viral etiologies of lower respiratory tract infections in children < 5 years of age in Addis Ababa, Ethiopia: a prospective case–control study

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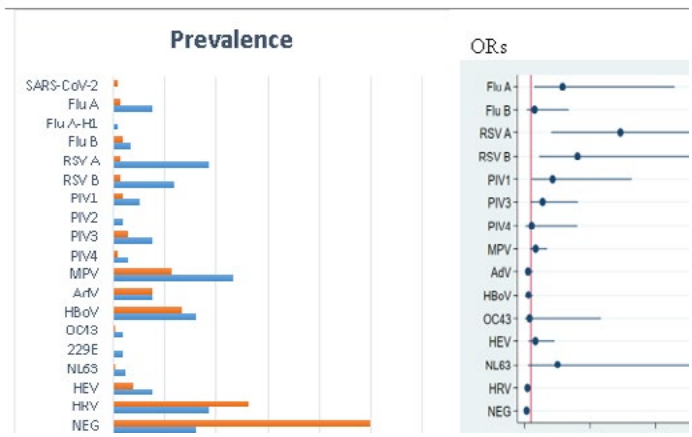
1: Armaour Hansen Research Institute, Ethiopia;2: Department of Biomedical Sciences, College of Natural and Computational Sciences, Addis Ababa University, Addis Ababa, Ethiopia

BackgroundLower respiratory tract infections (LRTIs) are a major cause of morbidity and mortality in children worldwide and disproportionally affect Sub-Saharan Africa. Despite the heaviest burden of LRIs in Ethiopia, to date, no published studies have reported a comprehensive viral etiology of LRTIs among children in Ethiopia. The objective of this study was to determine and estimate the etiological contribution of respiratory viruses to LRTIs in < 5 years children in Ethiopia.

MethodsA prospective case–control study was conducted from September 2019 to May 2022 in two major governmental hospitals, St. Paul Hospital Millennium Medical College and ALERT Hospital in Addis Ababa, Ethiopia. Nasopharyngeal/oropharyngeal samples and socio-demographic and clinical information were collected from children under 5 years. A one-step Multiplex real-time PCR (Allplex™ Respiratory Panel Assays 1–3) was done to detect respiratory viruses. STATA software version 17 was used for the data analysis. We computed the odds ratio (OR), the attributable fraction among exposed (AFE) and the population attributable fraction (PAF) to measure the association of the detected viruses with LRTIs.

ResultsOverall, 210 LRTIs cases and 210 non-LRTI controls were included in the study. The likelihood of detecting one or more viruses from NP/OP was higher among cases than controls (83.8% vs. 50.3%, p = 0.004). The multivariate logistic regression showed a significantly higher detection rate for RSV A (OR: 14.6, 95% CI 4.1–52.3), RSV B (OR: 8.1, 95% CI 2.3–29.1), influenza A virus (OR: 5.8, 95% CI 1.5–22.9), and PIV 1 (OR: 4.3, 95% CI 1.1–16.4), among cases when compared with controls. The overall AFE and PAF for RSV A were (93.2% and 17.3%), RSV B (87.7% and 10.4%) and Influenza A virus (82.8% and 6.3%), respectively. The mean CT values were significantly lower for only RSV B detected in the case groups as compared with the mean CT values of RSV B detected in the control group (p = 0.01).

ConclusionsRSV, Influenza A and PIV 1 viruses were significantly associated with LRTIs in < 5 years children in Addis Ababa, Ethiopia. Therefore, we underscore the importance of developing prevention strategies for these viruses in Ethiopia and support the importance of developing and introducing an effective vaccine against these viruses.



115

Korea 2023-2024 Influenza and Respiratory Viruses Laboratory Surveillance Report

SangHee WOO, Nam-Joo LEE, Jaehee LEE, Jee Eun RHEE, Eun-Jin KIM

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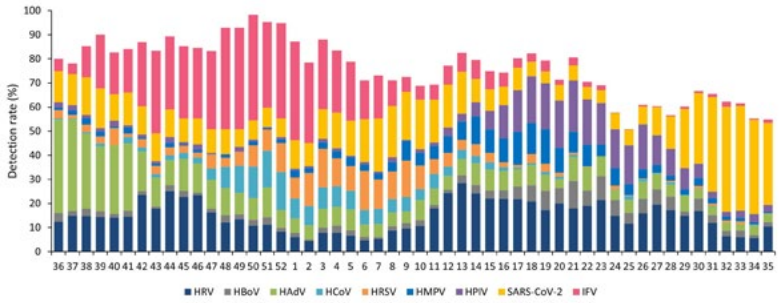
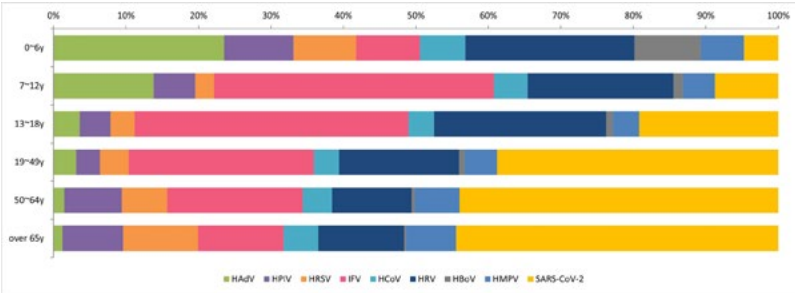
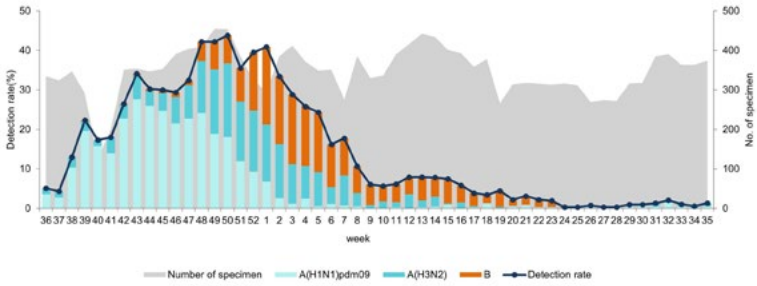
During the 2023-2024 season from 36 weeks in 2023 to 35 weeks in 2024, real-time RT-PCR were performed on 18,040 respiratory specimens to analyze the detection status of the causative pathogen and the characteristics of the virus. As a result, influenza viruses were detected 15.1% of the total specimens, with subtype distributions as follows: A(H1N1)pdm09 (41.7%), A(H3N2) (29.3%) and B type (29.1%). As a result of analyzing the genotype, it was confirmed that a phylogenetic group similar to the vaccine strains, and antiviral resistance mutations were not detected. The antigen of isolated influenza virus had effective neutralizing activities for the vaccine strains and had lack of drug resistance to treatments (Oseltamivir, Zanamivir, and Peramivir, Baloxavir). For respiratory viruses, SARS-CoV-2 was the most frequently detected (15.1%), followed by rhinovirus (14.9%), adenovirus (9.9%), parainfluenzavirus (5.6%), respiratory syncytial virus (5.0%), human metapneumovirus (4.2%), human coronavirus (3.8%), and bocavirus (3.2%). Our division will conduct continuous surveillance of the epidemiological trends of respiratory viruses, including influenza, and ensure the timely provision of data for public health interventions and vaccine strain selection.






Detection rate(%)
No. of specimen
week
Number of specimen
A(H1N1)pdm09
A(H3N2)
B
Detection rate

118

High circulation of avian influenza H9N2 subtype in live bird markets: a new emerging threat in Senegal

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1: Institut Pasteur De Dakar, Senegal;2: Département de Biologie Animale, Faculté des Sciences et Techniques, Université Cheikh Anta DIOP, Dakar, Sénégal

Background

Avian influenza virus (AIV) of the H9N2 subtype has gained increasing attention in recent years due to its widespread circulation in poultry populations and sporadic zoonotic transmission to humans. In Senegal, only one human case of AIV H9N2 infection has been reported so far, despite ongoing influenza sentinel surveillance since 1996. However, until now, this surveillance was only focused on humans and the country has never experienced documented H9N2 infection in poultry even though unusual poultry outbreaks associated with mortalities are occasionally reported. So here, we present results of an active avian influenza surveillance effort focusing on live-bird markets (LBM).

Methods

The Senegalese National Influenza Center initiated in December 2023 an active influenza surveillance in two LBM in Dakar. Each week, fresh feces, water troughs, carcass washing water and cloacal swabs from birds are collected in each market. Samples are examined by RT-PCR for the presence of, among others, AIV H9, H7 and H5 subtypes, which are then characterized further by next-generation sequencing.

Results

From December 2023 to October 2024, 499 samples were tested, and AIV was detected in 58.3% of them. Among these, A/H9N2 was the only subtype detected in both markets, with a detection rate of 47.7% (82/172) in Thiaroye and 35.3% (42/119) in Tilene, resulting in an overall positivity rate of 42.6% (124/291). Genome sequencing of 22 A/H9N2 isolates, including 11 poultry drinking water samples, 7 carcass wash water samples, 3 fecal samples, and 1 cloacal swab, yielded 7 complete and 15 partial genomic sequences. Phylogenetic analyses of the resulting sequences showed that the A/H9N2 isolates obtained in this study formed a monophyletic cluster and were closely related to the Senegalese human strain (A/Senegal/0243/2019) identified through the national influenza sentinel surveillance program. These strains were also closely related to the A/H9N2 viruses of the G1 lineage circulating in neighboring countries, suggesting cross-border transmission. The A/H9N2 strains carried the low pathogenicity RSSR/GLF motif at the HA cleavage site and possessed several key amino acid mutations, including HA-I155T and HA-Q226L, which are associated with human host adaptation, PB2-T105V, PB2-A661T, and PB2-A588V, which are linked to the human-to-human transmission and increased polymerase activity, NS2-T14M, NS2-M100I, NS1-I106M, NS1-V222M, NS1-E223A, NS1-I226V, NS1-E227G, and NS1-P228S, which are known to alter virulence (increased or reduced) in humans or mice, and M2-S31N, which promotes drug resistance. Seven potential N-glycosylation sites were predicted in the HA protein and six in the NA protein. The selection pressure analysis revealed that the A/H9N2 isolates were primarily under neutral evolution or purifying selection pressure.

Conclusion

Overall, our findings highlight the potential for cross-species transmission of Senegalese A/H9N2 viruses, emphasizing the need for sustained monitoring of these viruses in both animal and human populations.

122

Influence of Clinical Characteristics on the Detection Probability of Respiratory Pathogens in Children with Acute Lower Respiratory Infections in Addis Ababa, Ethiopia

Fiseha Wadilo WADA

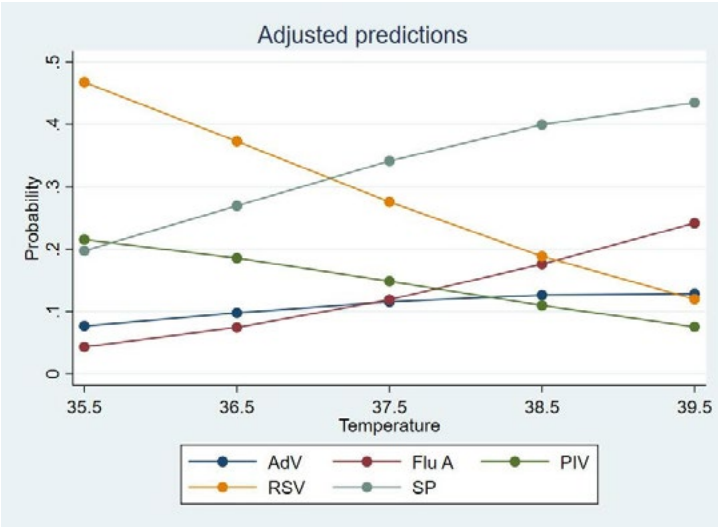
Armaour Hansen Research Institute, Ethiopia

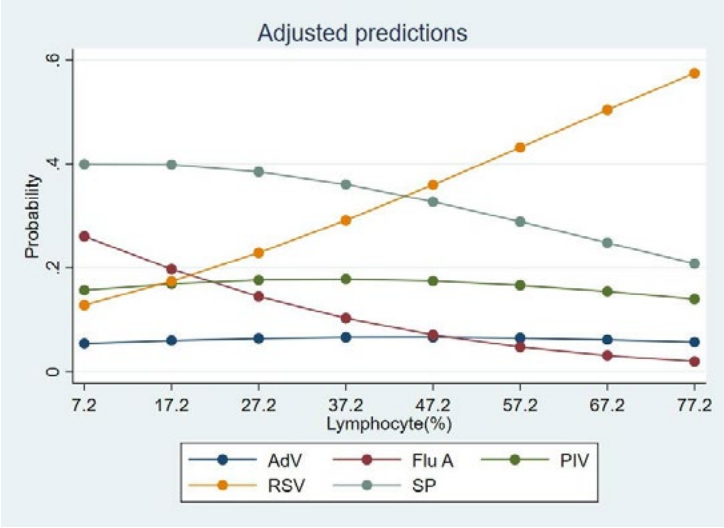
Background: To investigate how the clinical characteristics of children with acute lower respiratory infections (ALRIs) impact the probability of detecting specific respiratory pathogens.

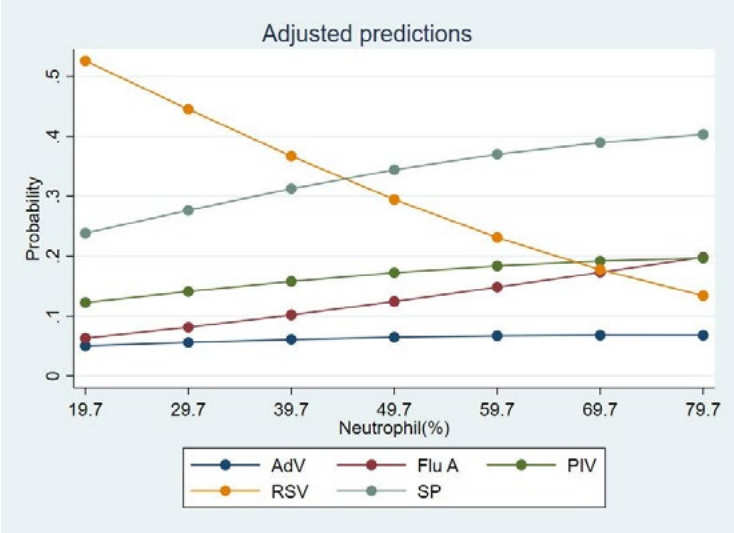
Methods: Nasopharyngeal /oropharyngeal samples, socio-demographic and clinical information were collected from children under 5 years in Addis Ababa, Ethiopia. A one-step Multiplex real-time PCR (Allplex™ Respiratory Panel Assays 1-4) was used to detect respiratory pathogens. STATA software version 17 was used for the data analysis.

Results: The probability of detecting RSV in a child with a temperature of 35.5°C is 0.47, while it drops to 0.12 at 39.5°C. On the contrary, the probability of detecting **S. pneumoniae** in a child with a temperature of 35.5°C is 0.20, while at 39.5°C it rises to 0.44. On the other hand, as the percentage of lymphocytes in the blood increases from 7.2% to 77.2%, the likelihood of detecting RSV also increases from 0.13 to 0.57, while the likelihood of detecting **S. pneumoniae** decreases from 0.34 to 0.21. Conversely, as the percentage of neutrophils in the blood increases from 19.7% to 79.7%, the likelihood of detecting RSV decreases from 0.53 to 0.13, while the likelihood of detecting **S. pneumoniae** increases from 0.24 to 0.40.

Conclusions: In conclusion**,** the clinical characteristics of the child may help predict respiratory pathogens linked to ALRIs, but incorporating biomarker tests could enhance the accuracy of identifying these pathogens. Further research is needed to understand and determine interaction mechanisms between respiratory pathogens and their clinical implications.




Adjusted predictions
Probability
0
.2
.4
.6
7.2
17.2
27.2
37.2
47.2
57.2
67.2
77.2
Lymphocyte(%)
AdV
Flu A
PIV
RSV
SP


Adjusted predictions
Probability
0
.1
.2
.3
.4
.5
19.7
29.7
39.7
49.7
59.7
69.7
79.7
Neutrophil(%)
AdV
Flu A
PIV
RSV
SP

128

ECaS

Integrative Multi-Omics Analysis Reveals Microbiota Alterations and Clinical Indicators Predictive of Pulmonary Fibrosis Progression Following SARS-CoV-2 Infection

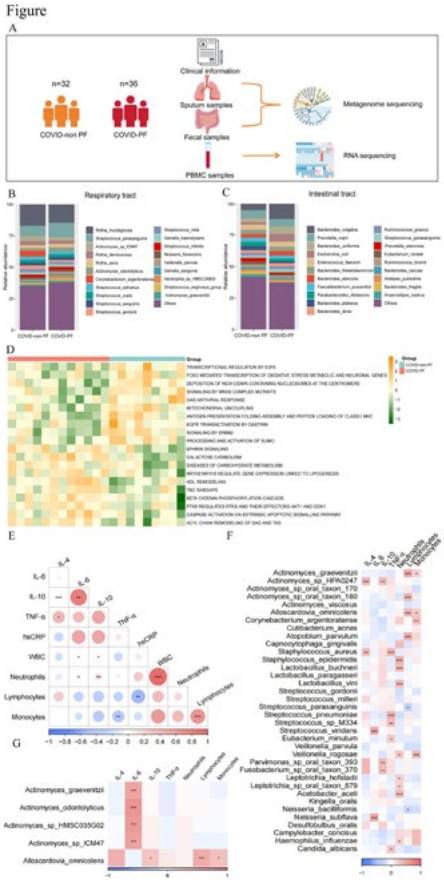
Chang LIU, Jiaqi BAO, Jili NI, Shufa ZHENG, Yu CHEN

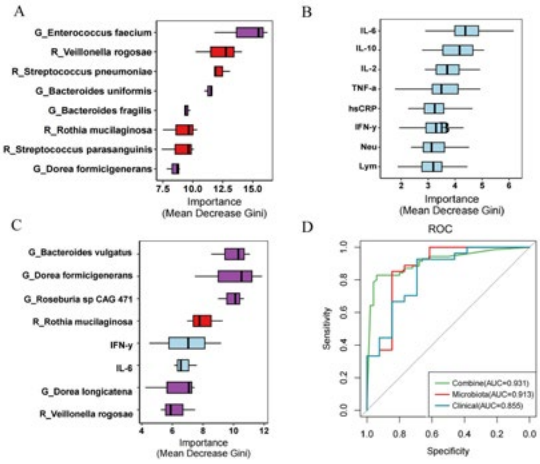
The First Affiliated Hospital, Zhejiang University School of Medicine, China, People's Republic of

Background: Pulmonary fibrosis (PF) following SARS-CoV-2 infection is a severe, often irreversible condition that leads to respiratory dysfunction. Despite growing concerns about PF, research on its impact on the respiratory and intestinal microbiota remains limited. This study employs a multi-omics approach to investigate alterations in both microbiota profiles and clinical indicators in patients with PF following SARS-CoV-2 infection. The aim is to develop a predictive model for the onset and progression of PF, incorporating risk stratification to enable early targeted therapeutic interventions and facilitate timely clinical decision-making, ultimately mitigating irreversible parenchymal remodeling and improving long-term respiratory outcomes.Methods: In this study, 68 patients with confirmed SARS-CoV-2 infection were categorized into two subgroups: those with pulmonary fibrosis (COVID-PF) and those without (COVID-non-PF). To investigate microbiota alterations associated with PF, metagenomic sequencing was performed on sputum and fecal samples from COVID-non PF and COVID-PF patients. Additionally, peripheral blood mononuclear cells (PBMCs) underwent transcriptome sequencing, and gene expression profiles were analyzed using Gene Set Enrichment Analysis (GSEA) to identify PF-related pathway alterations. Furthermore, Spearman's correlation analysis was employed to examine the relationship between clinical parameters and microbial genus abundance in both groups. A random forest classifier was developed to predict PF risk based on integrated respiratory-intestinal microbiota profiles as well as clinical indicators.Results: Our findings demonstrate distinct differences in the respiratory and intestinal microbiota between COVID-PF and COVID-non PF patients. In the respiratory tract, COVID-PF patients showed a reduced relative abundance of Rothia mucilaginosa, while Actinomyces odontolyticus and Veillonella parvula were elevated. In the intestinal tract, the relative abundance of Parabacteroides distasonis and Anaerostipes hadrus was significantly lower, while Bacteroides vulgatus exhibited an increasing trend. Transcriptomic analysis of PBMCs further identified immunomodulation-related pathways associated with PF development. Moreover, strong correlations were found between fibrosis-related cytokines (IL-4, IL-6, IL-10) and respiratory microbiota composition, highlighting their potential role in the progression of PF. A machine learning model integrating microbiota profiles and clinical indicators provided robust predictions for early PF risk stratification and targeted management of fibrosis following SARS-CoV-2 infection.

Conclusions: This study demonstrates that SARS-CoV-2-associated PF have a significant impact on the composition of the respiratory and intestinal microbiota. Changes in both microbiomes, along with clinical indicators, can predict the progression and prognosis of PF. These findings offer new insights into disease mechanisms and suggest viable options for early detection and personalized treatment strategies for PF in SARS-CoV-2-infected patients.

Figure


A
n=32
n=36
COVID-non PF
COVID-PF
Clinical information
Sputum samples
Fecal samples
PBMC samples
Metagenome sequencing
RNA sequencing
B
Respiratory tract
C
Intestinal tract
D
E
F
G


A
G_Enterococcus faecium
R_Veillonella rogosae
R_Streptococcus pneumoniae
G_Bacteroides uniformis
G_Bacteroides fragilis
R_Rothia mucilaginosa
R_Streptococcus parasanguinis
G_Dorea formicigenerans
Importance
(Mean Decrease Gini)
B
IL-6
IL-10
IL-2
TNF-a
hsCRP
IFN-y
Neu
Lym
Importance
(Mean Decrease Gini)
C
G_Bacteroides vulgatus
G_Dorea formicigenerans
G_Roseburia sp CAG 471
R_Rothia mucilaginosa
IFN-y
IL-6
G_Dorea longicatena
R_Veillonella rogosae
Importance
(Mean Decrease Gini)
D
ROC
Sensitivity
Specificity
Combine(AUC=0.931)
Microbiota(AUC=0.913)
Clinical(AUC=0.855)

129

Rational design of live attenuated influenza vaccine viruses for optimised dual-species replication

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Background: Live attenuated influenza vaccine (LAIV) is manufactured in embryonated hens' eggs and delivered intranasally, with vaccine virus replication in the upper respiratory tract driving mucosal and systemic immune responses. Consequently, efficient replication in both egg and human systems is critical for high yield and high vaccine effectiveness (VE). Egg-adaptation of A/H1N1pdm09 influenza virus haemagglutinin (HA) proteins has been implicated in limiting the replication of some LAIV candidate vaccine viruses (CVVs) in primary human nasal epithelial cells (hNEC). Subsequently, an approach to optimisation of the A/H1N1pdm09 HA protein was shown to restore hNEC replication while maintaining yield in eggs. However, the ongoing evolution of A/H1N1pdm09 viruses, and their propensity for egg-adaptation, highlights the need for a more flexible, rational design-based approach for achieving reliable dual-species LAIV virus replication.

Methods: Initially, egg-adaptation of the 220-loop was confirmed to interfere with the targeted dual-species phenotype of A/H1N1pdm09 LAIV strains. A deep-mutational scanning (DMS) approach combining egg and human models was then used to screen thousands of sequences of the 220-loop alone, or in combination with other loci across the HA protein, identifying a range of novel sequence motifs able to support dual-species replication.

Results: The impact of these novel motifs on HA antigenicity was also explored, with some sequence combinations able to maintain antigenic match to parental viruses despite significant sequence differences. In parallel, it was found that a A/H1N1pdm09 CVV with optimised replication but reduced antigenic match was able to provide superior protection in vivo than its antigenically matched parent. This raises the possibility that optimisation of dual-species replication could even be prioritised over antigenic match for optimal A/H1N1pdm09 LAIV VE.

Conclusions: This work demonstrates the potential to apply rational design approaches to the optimisation of A/H1Npdm09 LAIV CVVs, to maintain high VE alongside robust vaccine supply.

132

ECaS

Multiplex Assay for Measuring IgG Antibodies to Avian Influenza A(H5N1) Clade 2.3.4.4b and Seasonal Influenza A

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Background. Highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b caused widespread mortality in wild birds worldwide and localized outbreaks in mammals in 2023. In 2024, infections spread extensively to dairy farms across the United States, with several human cases also being reported. Given the threat of a larger epidemic, we need efficient serological tools to assess immune responses induced by currently available vaccines against H5N1 viruses and to support serosurveillance of potential human infections. **Methods.** We developed an in-house antibody assay to evaluate immune responses to the new A(H5N8) Zoonotic Influenza vaccine (Seqirus). This in-house fluorescent multiplex immunoassay (FMIA) measures serum IgG against A(H5N1) clade 2.3.4.4b haemagglutinin (HA) and multiple HA1 and HA3 antigens from recent seasonal influenza A vaccine strains simultaneously in a single analysis. We assessed the assay's repeatability and reproducibility and tested for cross reactivity between all antigens. Variation was assessed by calculating coefficient of variation (CV), for which <20% was used as limit for good performance. After this validation we analysed responses of 39 subjects from samples collected before vaccination and three weeks post 1st and 2nd dose. Nine subjects had been vaccinated against avian influenza also during 2009–2018. The IgG concentrations (FMIA U/ml) were compared to A(H5N8) A/Astrakhan/3212/2020 microneutralisation titers. **Results.** Antibodies binding to all six influenza antigens could be measured in the same analysis without notable cross-reactions (CV<11%). The assay produced repeatable results within (CV<6%) and between test days (CV<14%) as well as between laboratory workers (CV<14%). H5-specific IgG levels increased in all subjects following vaccination. Following 1st dose, H5-IgG concentrations increased 3-fold in those previously unvaccinated for avian influenza and 20-fold in those previously vaccinated. Antibody levels after 2nd dose increased 10- and 17-fold from baseline in previously unvaccinated and vaccinated, respectively. The H5 IgG concentrations and microneutralisation test titers against A/Astrakhan/3212/2020 vaccine virus had strong correlation (Pearson correlation 0.88, **P**<0.001). **Conclusions.** With FMIA, antigenically similar proteins can be compiled into a single multiplex assay with low assay cross-reactivity. The ability to flexibly incorporate new antigens into an existing assay enables rapid responses to potential pandemic threats posed by emerging influenza viruses and allows for the evaluation of antibody responses induced by new vaccines.

133

FLU-v, a broad-spectrum influenza vaccine, induces broadly reactive cellular responses and NK cell activating antibody responses.

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FLU-v is a broad-spectrum influenza vaccine composed of four short synthetic peptides that originate from M1, M2, NP-A and NP-B influenza proteins. The regions covered by the peptides are highly conserved within influenza A and B strains. FLU-v has demonstrated acceptable safety and long-lasting immunity in phase I and phase II clinical trials. In an H1N1 human challenge, administration of a single dose of adjuvanted FLU-v induced reduction of mild to moderate disease and reduction in influenza symptoms. Vaccination with FLU-v induces activation of CD4+ and CD8+ T-cells, measured by multiparametric flow analysis, and broadly cross-reactive cellular responses, as measured by IFN-γ and Granzyme-B ELISpot after incubation of PBMCs from vaccinated subjects with a panel of different inactivated influenza A strains (H1N1, H3N2, H5N1, H7N9) and a B strain (Yamagata lineage).

Despite FLU-v being designed as a T-cell vaccine, vaccination also induced a 23 -fold increase in IgG titres 42 days post-vaccination in the adjuvanted FLU-v group and, on day 180 post-vaccination, the titres remained 12-fold higher than at pre-vaccination. IgG subclass analysis demonstrated that vaccination induced IgG1 and IgG3 antibodies which are the main activators of antibody dependent cellular cytotoxicity (ADCC), effected primarily by NK cells. Due to location of the FLU-v epitopes in internal influenza antigens, FLU-v specific antibodies do not have neutralisation potential but their binding to the surface of influenza-infected cells could trigger NK cytotoxicity and thus, provide protection against influenza disease progression. The NK cell line GFP-CD16 (176V) NK-92 was used to evaluate NK cell activation, defined as cells expressing the degranulation marker CD107a. A significant increase in the number of NK-92 cells expressing CD107a was observed after incubation with serum from day 42 post-vaccination compared to pre-vaccination serum, indicating the potential for anti-FLU-v antibodies to activate NK cells. In addition, a stronger correlation between FLU-v specific IgG, and NK cell activation was detected compared to correlations with the individual IgG1 and IgG3 subclasses, suggesting that both subclasses contribute towards NK cell activation.

In conclusion, a single dose of adjuvanted FLU-v induces cellular responses that are broadly cross-reactive, in addition to long-lasting IgG antibodies that can activate NK cell effector functions. Both modes of action could provide broad protection against influenza by impairing disease progression.

134

Immunogenicity and efficacy evaluation of a novel pan-coronavirus vaccine, UNICOR-v.

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UNICOR-v is a pre-pandemic pan-coronavirus vaccine composed of twelve short synthetic peptides whose conserved sequences are found in alpha, beta, gamma and delta-coronaviruses belonging to the **Orthocoronavirinae** subfamily. To design the vaccine, all available protein sequences were aligned to identify areas of high conservation. These areas were then analysed in our proprietary epitope prediction algorithm to identify reactive epitopes for different human HLAs. Conserved regions with high density of predicted epitopes were identified from only two proteins, the RNA-dependent RNA-polymerase (Nsp12) and the membrane (M) protein.

These regions were manufactured as synthetic peptides, emulsified with Montanide ISA-51 adjuvant (Seppic) and injected subcutaneously in mice and rabbits. The results showed that antigens P1, P2, P3 and P9 and P11 were consistently the most reactive in all studies. No differences were found in the T cell responses if the 12 peptides were given as three separate injections, or as a single injection, or if animals received a prime or prime & boost two weeks apart. However, for antibody responses, only peptides P1, P2 and P3 induced peptide-binding antibody titers in mice and those were stronger if the peptides P1-P3 were administered as a separate injection rather than all peptides P1-P12 pooled together in a single injection. Additionally, prime and boost vaccinations were required to achieve a high titer antibody response. In rabbits, the mix of the 12 peptides was evaluated as prime & boost compared to vehicle only placebo. The vaccine induced high titer antibody responses to all twelve peptides compared to placebo.

Efficacy of UNICOR-v was evaluated in mouse models for MERS-CoV (hDPP4 transgenic mice), SARS-CoV-1 (BALBc mice), and SARS-CoV-2 (C57BL6 and BALBc mice). Different doses of UNICOR-v were evaluated delivered subcutaneously as prime & boost 2 weeks apart in hDPP4 transgenic mice. As expected from a vaccine that does not induce neutralising antibodies, little effect was observed on day 2 post-infection in either of the challenge models. However, the 10 and 20nmol doses of UNICOR-v were the most effective at reducing clinical disease, viremia and lung histopathology on day 4 post-inoculation. UNICOR-v also showed to increase survival of infected animals when they were monitored for up to 10 days post-infection.

UNICOR-v is a promising vaccine to be used as pre-pandemic and should be evaluated in combination with current Covid-19 vaccines.

136

ECaS

Spatiotemporal penetration of zoonotic influenza A viruses through respiratory mucus gel networks

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The ongoing widespread of highly pathogenic avian influenza virus (HPAIV) H5N1 (clade2.3.4.4b) around the globe and the infection of novel hosts like dairy cattle and low frequency spillover into humans on the American continent is concerning. Furthermore, cattle may have potential as a mixing vessel for future influenza A viruses (IAV) and the current route of transmission is yet to be completely understood. For most viral infection routes, mucus is the first barrier against attachment or entry before virus-cell interaction occurs. In the airways, respiratory mucus forms a viscous network that mechanically traps inhaled particles, such as viruses, which are then transported outward from the lungs, airways or nasal cavity through mucociliary clearance. In addition to the mechanical barrier function, mucins possess a high glycan density, able to bind viral lectins. Hence, host and tissue-specific mucus may affect virus-host interaction by influencing virus attachment and antiviral defense.We investigate the interplay between different IAV strains and mucus collected from various species to understand its relevance for viral tropism and host adaptation. We started collecting primary cells and native mucus samples from organs of relevant species (e.g., cattle, swine, birds, humans). Further, we develop standardized biophysical and biochemical techniques to quantify the spatiotemporal virus penetration through the obtained species-specific mucus samples. Using these methods, we analyze the penetration properties of IAV particles and their subsequent infection potential. Preliminary results suggest that origin-specific mucus influences viral penetration and infection efficiency of H5N1 and H1N1 influenza strains. Our aim is to investigate the interaction between viral attachment proteins, mucin glycans and the physical properties of mucus that may shape viral penetration efficiency and infectivity. Understanding these mechanisms provides novel insights into the cross-species transmission potential of zoonotic and potentially pandemic IAV strains.

137

Single-cell analysis of influenza virus infection in adult stem cell-derived human airway organoids identifies target cells and alterations in immune response

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Background Influenza is one of the highly infectious acute respiratory diseases which can induce acute respiratory distress syndrome. Enhanced understanding of influenza virus infection and pathogenesis is critical for the development of therapeutics. There is no robust in vitro model for assessing the infectivity of influenza viruses in humans. In this study, we generated 3D airway organoids from adult stem cells (ASCs) in human lung to provide insight into viral replication, cell tropism, host–viral interactions and immune response after influenza virus infection.

Methods ASCs were isolated from the patients' lungs, and the cells were embedded in BME Type 2 to generate the human airway organoids. The cellular composition of organoids was analyzed by immunofluorescence staining, western blotting and single-cell sequencing. For viral infection, the organoids were incubated with influenza virus PR8 strain, and the viral replication and cell tropism were detected through RT-qPCR, western blotting and immunofluorescence. Additionally, the cytokines of organoids were detected to by RT-qPCR analyze the immune responses to influenza virus infection. Gene and pathway alterations in each cell type of the human airway organoids were detected by single-cell sequencing.

Results The 3D human airway organoids generated in this study accommodated four types of airway epithelial cells after 28 days of culture: ciliated, goblet, club and basal cells. After viral infection, increased organoid morphological changes, cell death, and viral NP protein were observed. Inflammatory cytokines such as IL-6, IL-29, CXCL10 and IFN-β1 were significantly increased after viral infection. It was also found that influenza virus could effectively replicate in goblet cells. And the number of ciliated cells and goblet cells were decreased, while basal cells and club cells were increased. Additionally, multiple pathways were altered in four cell types after influenza virus infection, and the altered pathways were different in each cell type, according to the results of single-cell sequencing. Changes in ribonucleoprotein complex biogenesis and mitochondrial inner membrane were observed in basal cells, and cilium organization were observed in ciliated cells. For club cells and goblet cells, mitochondrial inner membrane and Wnt signaling pathway were significantly altered.

Conclusions Overall, we successfully established human airway organoids using ASCs. The results indicated that ASCs-derived human airway organoids were susceptible to influenza virus and could activate immune response to influenza virus infection in different epithelial cell types. Thus, organoids would help us to better understand the pathogenesis of influenza virus infection to enable the development of alternative therapeutic interventions.

Figure 1

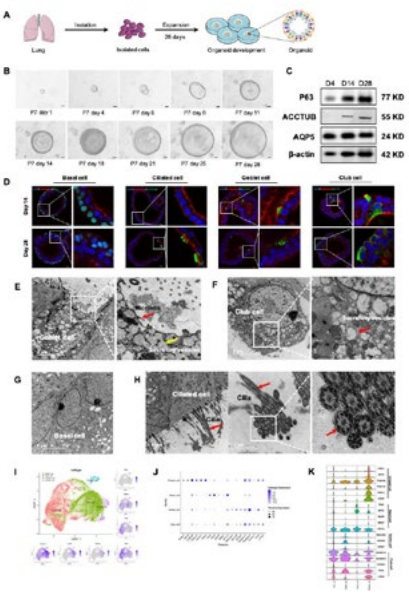


Figure 2

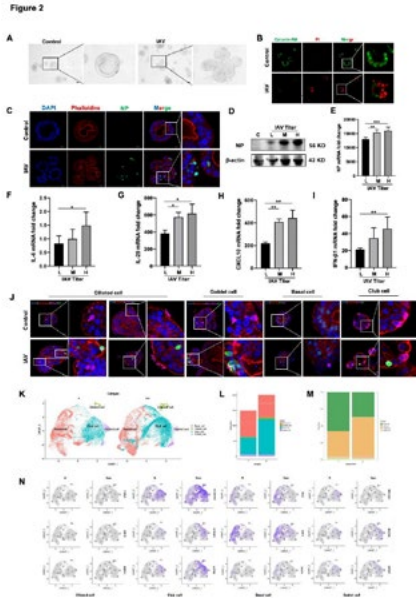
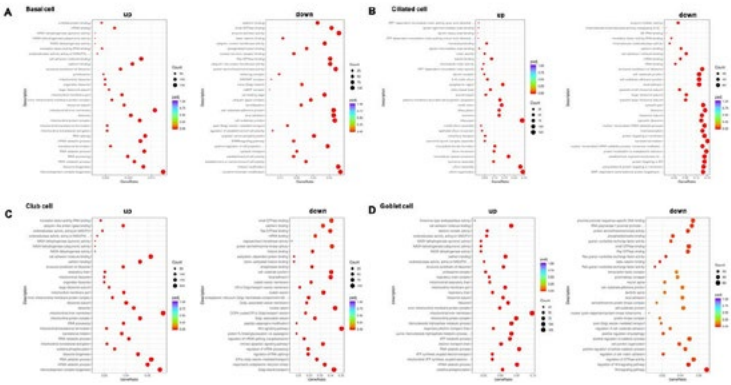


Figure 3



138

ECaS

Antiviral potential of Sec61 translocon inhibitors to block Respiratory Syncytial Virus envelope protein biogenesis

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Background. Respiratory syncytial virus (RSV) is a ubiquitous respiratory virus that infects nearly all children at least once by the age of two. It is a major cause of acute lower respiratory infections and hospitalization in young children, but also poses a significant threat to elderly, high-risk adults, and immunocompromised patients. Despite major progress regarding vaccines and RSV prophylaxis, the current arsenal of RSV antivirals is very limited. The RSV fusion (F) protein is a type I integral membrane protein that mediates fusion of the viral and host cell membranes to enable viral entry. Following infection, protein translation and transport of F to the cell membrane is necessary for the production of new infectious virus particles and viral spread. Intracellular transport of F is initiated by targeting of the translating ribosome to the endoplasmic reticulum (ER), followed by translocation of the signal-peptide (SP)-containing F preprotein through the Sec61 translocon channel and insertion of the protein into the ER membrane. Specific SP-dependent inhibition of this ER protein translocation process might prove an interesting antiviral strategy. In this study, we investigate the antiviral potential of selective Sec61 translocon inhibitors to suppress F protein expression and subsequent viral propagation.

Methods. The small-molecule translocon inhibitor CK147 was tested in Hep-2 cells for its cytotoxicity and antiviral activity against both RSV A and RSV B using an MTS viability assay. Expression of RSV F, G, and SH following CK147 treatment was evaluated in transfected HEK293T cells or infected Hep-2 cells by flow cytometry and western blot analysis. In vitro translation experiments were used to confirm the effect of CK147 on protein translocation and evaluate the role of the RSV F SP in CK147 sensitivity.

Results. CK147 demonstrated antiviral activity against both RSV A and RSV B with EC50 values of 0.10 µM and 0.08 µM, respectively. The CC50 value for CK147 in Hep2 cells was 1.44 µM, returning a meaningful selectivity index of 14. CK147 treatment of infected cells or cells transfected with individual RSV envelope proteins resulted in a profound decrease in RSV F cell surface expression, with a partial effect on cell surface RSV G expression and no effect on RSV SH or RSV N expression. These results point to a direct antiviral effect of CK147 by RSV F protein suppression. Moreover, transfection and in vitro translation experiments showed that the signal peptide of the RSV F preprotein is responsible and sufficient for conferring sensitivity to CK147, resulting in the inhibition of protein translocation across the ER membrane.

Conclusion. Selectively downmodulating the expression of the RSV fusion protein F via signal peptide-specific Sec61 translocon inhibitors can be a novel and innovative antiviral strategy for RSV disease.

144

ECaS

Limited toxicity and inhibition of influenza virus neuraminidases by 39 plant species used to treat respiratory diseases by South Africans

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Influenza A and B viruses is known to develop resistance against licensed antiviral drugs including the neuraminidase inhibitors oseltamivir and zanamivir. South African plant species with anecdotal data display high, non-scientific potential, for usage against respiratory viruses. This study aimed to determine if selected medicinal plant extracts can inhibit influenza A/B viruses' neuraminidases. Crude aqueous and organic plant extracts were prepared. Toxicity testing was done by treating **Artemia nauplii**(brine shrimp) with plant extracts for 24 to 48 hours, whereafter the 50% lethal dose was determined. The NA-XTD assay was used to assess if crude plant extracts can inhibit viral neuraminidase activity. Thereafter, micro-ultrasonic extraction-based fractionation of select plant extracts was done followed by neuraminidase inhibition assays. Most plant extracts (39/41), were non-toxic to brine shrimp. Approximately 46% of crude aqueous and organic extracts reduced influenza A and B viruses' neuraminidase activity by ≥95%. Among 12 plant extracts selected for fractionation 5/7 fractions of Schotia brachypetala (B) extract and 5/7 fractions of the Acacia karroo extract inhibited influenza A/B virus neuraminidase activity by ≥95%. In addition, 2 to 4 fractions from 8/12 (66.7%) plants also displayed ≥95% inhibition. Here we showed that a selection of Southern African medicinal plants based on ethnobotanical use to treat respiratory symptoms were able to inhibit influenza A/B viruses' neuraminidases. As the influenza viruses neuraminidases are essential for virus infection, the use of medicinal plant with anti-neuraminidase activity may explain the resultant alleviation of symptoms.

146

Development of a HiBiT-Tagged Virus-Like Particle Entry Assay for Highly Pathogenic Enveloped Viruses

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Background: Detection of neutralizing antibodies against highly pathogenic enveloped viruses typically relies on semi-quantitative neutralization tests or quantitative plaque reduction neutralization assays. While these methods offer high specificity, they are hindered by low throughput, long turnaround times, and the need for biosafety level 3 (BSL-3) laboratories due to the use of live viruses. This creates a strong demand for safer, faster, and more accessible alternatives that can be implemented in BSL-2 settings.

Methods: Virus-like particles (VLPs) are non-infectious and non-replicative structures that mimic the size and surface features of infectious virions. Engineered to present viral surface proteins without containing viral genetic material, VLPs can be safely handled under BSL-2 conditions. However, quantifying their cell entry and membrane fusion activities remains a key challenge.

To overcome this, we employed the HiBiT system—an 11-amino-acid peptide tag that binds with high affinity to its complementary partner, LgBiT, forming a highly active NanoLuc luciferase. The resulting bioluminescence can be detected using the Nano-Glo assay, enabling sensitive, rapid, and straightforward quantification of tagged proteins. The small size of HiBiT allows flexible tagging without disrupting protein function, making it an ideal tool for VLP-based entry assays.

Results: We developed a VLP-based entry assay using the HIV Gag protein fused to HiBiT for the generation of VLPs displaying entry proteins from influenza A virus (H5N1), Nipah virus (NiV), and SARS-CoV-2. We also tagged internal structural proteins—Matrix 1 (M1) of H5N1, the Matrix protein of NiV, and the Membrane protein of SARS-CoV-2—with HiBiT. This allowed us to create tailored VLPs for each virus, capable of reporting entry events via bioluminescence.

Conclusions: We present a VLP-based viral entry assay compatible with BSL-2 laboratories that enables the safe, rapid, and high-throughput assessment of entry mechanisms for highly pathogenic viruses such as H5N1, NiV, and SARS-CoV-2. This platform is suitable for screening neutralizing antibodies or antiviral compounds and offers valuable insights into viral entry processes in a secure and scalable format.

147

Surveillance-Based Detection Trends and Typing of RSV and Adenovirus in South Korea, 2023–2024

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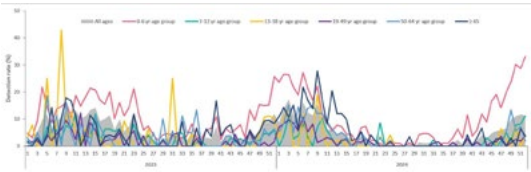
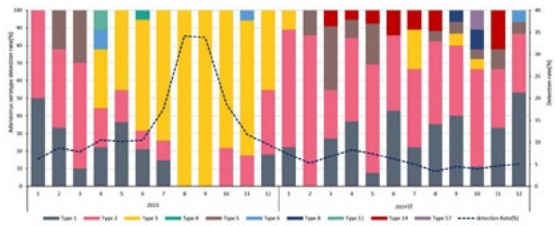
This study aimed to investigate the detection trends of respiratory syncytial virus (RSV) and adenovirus (AdV) in South Korea, with a focus on RSV subtypes and AdV serotypes. A total of 33,688 respiratory specimens were collected from patients with respiratory symptoms between January 2023 and December 2024 through the Korea Respiratory Viruses Integrated Surveillance System (K-RISS). RSV and AdV were identified using real-time reverse transcription polymerase chain reaction (RT-PCR), and AdV-positive samples were selected for nucleotide sequencing to determine serotypes based on age-specific detection rates.

The average detection rate of RSV was 6.4% in 2023 and 5.3% in 2024, with peak circulation observed during the winter season from December to February. In 2023, RSV subtype B accounted for 3.6% of total detections, slightly exceeding subtype A at 2.8%. In contrast, subtype A became predominant during the 2024 winter season. Children aged 0 to 6 years showed the highest RSV detection rate at 10.7%, followed by individuals aged 65 years and older at 5.2%. Detection rates in other age groups remained below 5%.

Adenovirus was detected in 14.8% of specimens in 2023, with a marked increase during epidemiological weeks 26 to 43, spanning summer and autumn. In 2024, the average detection rate declined to 5.8% and remained below 10% throughout the year. The 0 to 6-year age group again showed the highest positivity, with a rate of 27.5%. Serotyping revealed that type 3 was the most prevalent, accounting for 36.0 % of sequenced cases, followed by types 2, 1, and other minor serotypes. Specifically, type 3 was the major contributor to the 2023 peak, whereas type 14, previously unreported in South Korea, was continuously detected throughout 2024.

These findings highlight distinct seasonal and age-specific patterns in RSV and AdV circulation and underscore the necessity of continuous molecular surveillance for early detection of emerging variants and for guiding effective public health intervention.





148

Molecular mechanisms of action of LiteVax Adjuvant, a novel carbohydrate-based adjuvant that enhances influenza vaccine immunogenicity in older adults - A systems vaccinology approach

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1: Center for Vaccinology, Ghent University and Ghent University Hospital, Ghent, Belgium;2: Harmony Clinical Research, Melle, Belgium;3: Vismederi Srl, Siena, Italy;4: Laboratory of Pharmaceutical Biotechnology, Ghent University, Ghent, Belgium;5: LiteVax B.V., the Netherlands

Background. Influenza vaccines are the primary strategy to prevent severe influenza disease, however their efficacy is suboptimal, particularly in older adults. LiteVax Adjuvant (LVA), an oil-in-water emulsion containing Carbohydrate Fatty Acid Monosulphate Ester (CMS), has been previously shown to be safe and to enhance immunogenicity of seasonal influenza vaccines in a first-in-human phase 1 study. In this study the molecular mechanisms of action (MoA) of CMS and evaluates its immunogenicity when combined with a seasonal influenza vaccine were investigated.

Methods. A phase 1b, double-blind, active-controlled clinical trial was conducted from January to October 2024 at the Center for Vaccinology. Participants received either 1 mg of LVA added to VaxigripTetra (TETRALITE) or VaxigripTetra alone. Twenty-four younger adults (18-50 years) and 32 older adults (≥60 years) were randomized to each formulation.

Hemagglutination inhibition (HI) titres against four influenza strains were measured pre-vaccination and at Days 7, 28, and 180 post-vaccination. LVA's MoA was further explored through bulk RNA sequencing of whole blood and single-cell RNA sequencing of PBMCs collected pre-vaccination, Day 1 and Day 7 post-vaccination.

Results. TETRALITE and VaxigripTetra elicited similar kinetics of HI titers, with increases on Day 7, peaking at Day 28, followed by a decline that remained above baseline at Day 180. In younger adults, titers were comparable between vaccines; in older adults, TETRALITE induced significantly higher titers post-vaccination. On Day 1, LVA triggered a strong innate immune response characterized by interferon signaling activation mediated by monocytes and DCs. These innate immune cells showed increased interactions with CD4+ T cells on Day 1 but not on Day 7. Inflammatory gene signatures were not apparent on Day 7 and showed a limited but positive correlation with HI titers. LVA also increased the frequency and functionality of memory B cells on Day 1, while simultaneously elevating suppressive regulatory T cells negatively correlated with HI titers. Both vaccines induced an increase in the expression of all IgG-subtypes in plasmablasts on Day 7.

Conclusion. TETRALITE effectively enhances humoral immune responses in older adults but not in younger adults compared to VaxigripTetra. LVA activates transient innate immune response, promotes a rapid memory B cell response, and boosts IgG expression. However, the concurrent rise in regulatory mechanisms suggests that excessive inflammation may hinder optimal adaptive responses. These findings highlight LVA's capacity to activate multiple aspects of the immune response. Future studies will focus on detailed IgG responses, beyond HI, to better understand the mechanism of action of LVA and the interplay between innate and adaptive immune responses.

151

ECaS

Cold-adapted influenza B virus as a viral vector for designing multivalent vaccines against human respiratory viruses

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Institute of Experimental Medicine, Russian Federation

Background:

Acute respiratory viral infections (ARVI) are the most common group of acute infectious diseases. Currently, different types of vaccines are licensed worldwide against influenza, COVID-19 and respiratory syncytial virus (RSV), but not against other ARVI pathogens. One of the promising approaches for the development of combination vaccines against two or more respiratory infections is the use of live attenuated influenza vaccine (LAIV) strains to deliver immunogenic fragments of coronavirus and other ARVI pathogens to target cells. We have previously developed a system for modification of type A LAIV strains, and the development of a similar system for the vaccine component of LAIV type B is an urgent task, as it will allow increasing the total "capacity" of the genome of modified LAIV strains in order to expand the spectrum of protection against other ARVI pathogens.

Methods:

We used previously developed reverse-genetics system for B/USSR/60/69 LAIV master donor virus for designing LAIV/B viral vector. For this, various strategies of modifying of NA and NS genes were employed to incorporate foreign fragments of ARVI pathogens enriched with T-cell epitopes. For LAIV/RSV construct, the immunodominant CD8 T-cell epitope M282–90 (SYIGSINNI) was used to ensure correct delivery and processing of the inserted target epitopes. T-cell cassettes of other ARVI pathogens were also used to generate chimeric influenza B viruses. Recombinant B/LAIV viruses were studied in vitro for their genetic stability and replicative characteristics, as well as in a mouse model to assess immunogenic and protective potential against both influenza virus and the target respiratory pathogen.

Results:

Several T-cell cassettes of different respiratory viruses were inserted into genome of B/USSR/60/69-based LAIV virus using standard gene engineering approaches. In general, there was no significant impact of the foreign inserts on the main properties of the chimeric LAIV B virus, although the prolonged cassettes were shown to decrease the infectious activity of the recombinant virus. Importantly, insertions did not adversely affect the induction of influenza-specific immune responses, while inducing specific T-cell responses against the target ARVI pathogen. Noteworthy, these pathogen-specific immune responses correlated with protective potential of the chimeric vaccine against corresponding respiratory viruses.

Conclusions:

Here we developed a reverse-genetics system for designing chimeric influenza B viruses expressing foreign T-cell epitopes derived from a panel of ARVI pathogens. The development of such a vector system will significantly expand the possibilities for designing polyvalent vector vaccines against various ARVI pathogens through the use of a trivalent combined live attenuated influenza vaccine that fully imitates the standard licensed trivalent LAIVs both in terms of the method of industrial production and its practical application in the form of nasal spray.

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152

ECaS

Headache in respiratory syncytial virus: a retrospective cohort study in 1,113 patients

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Background

Respiratory syncytial virus (RSV) is one of the main agents causing bronchiolitis in children and low respiratory tract infections in adults. Headache prevalence in these patients is not well known, since only a few reports describe highly variable values. Thus, our goal in this study is to describe the prevalence of headache in patients with RSV infection in a large retrospective cohort.

Methods

Retrospective cohort study that included all consecutive RSV-confirmed cases between the 2016-2017 season and the 2021-2022 season in patients ≥2 years old. Information was obtained from electronic medical records. The prevalence of headache was compared depending on patients' sex, age group, and need for hospitalization.

Results

1,113 RSV-positive patients were enrolled. The prevalence of headache was 5.0% (Wilson confidence interval (CI): 95%; 3.9-6.5%). Prevalence along seasons is shown in Figure 1. Significant differences were found on headache prevalence by age between 18-59 years old (yo) and 2-5 yo groups (10.2% [6-16.6%] vs 3.7% [1.8-7.1%]; p=0.010) and between 18-59 yo and ≥60 yo groups (10.2% vs 3.9% [2.6-5.8%]; p=0.004). Differences were also found by hospitalization status (non-hospitalized, 8.7% [6.1-12.2%]; hospitalized, 3.3% [2.2-4.9%]; p<0.001) (Figure 3a). There were no differences depending on patients' sex (p=0.887) (Figure 2b).

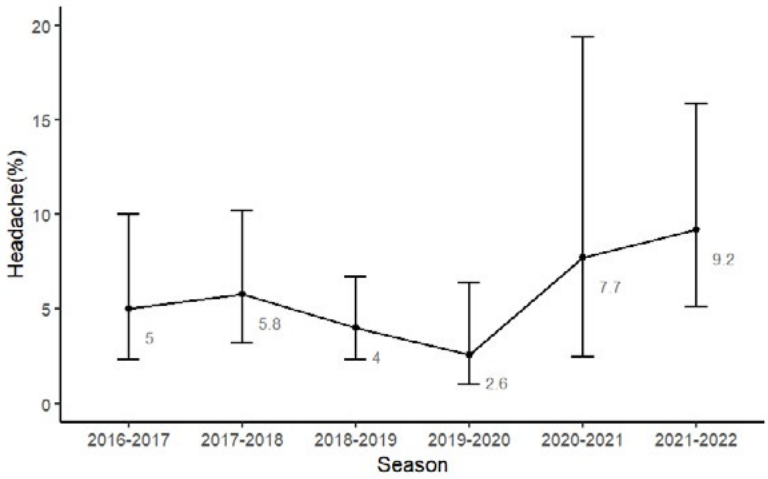
Figure 1. Prevalence of headache associated to RSV by season

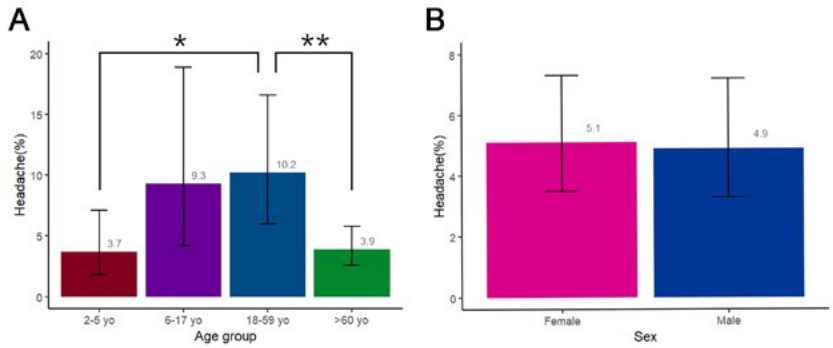
Figure 2. Prevalence of headache associated to RSV by a) age group and b) sex

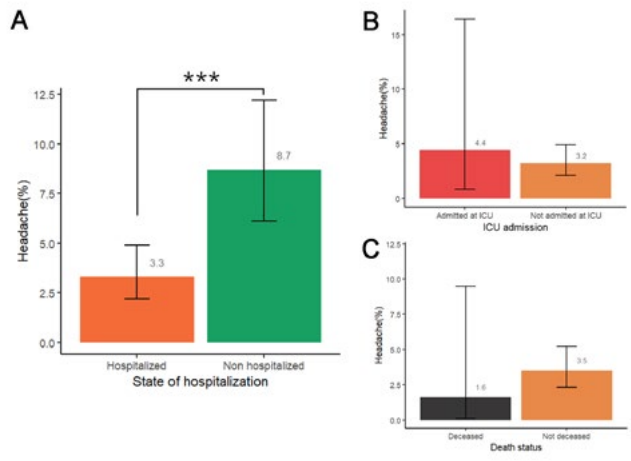
Figure 3. Prevalence of headache associated to RSV by a) hospitalization status; b) ICU admission in hospitalized patients; and c) death in hospitalized patients

Conclusion

Headache was reported only in one in twenty patients with confirmed respiratory syncytial virus infection, albeit it seemed more prevalent within non-hospitalized and young patients. This could be related with a more efficient immune response, but further research is needed to understand the molecular implications of this phenomenon.


Headache(%)
20
15
10
5
0
5
5.8
4
2.6
7.7
9.2
2016-2017
2017-2018
2018-2019
2019-2020
2020-2021
2021-2022
Season


A
*
**
Headache(%)
3.7
9.3
10.2
3.9
2-5 yo
6-17 yo
18-59 yo
>60 yo
Age group
B
5.1
4.9
Female
Male
Sex


A
***
Headache(%)
3.3
8.7
Hospitalized
Non hospitalized
State of hospitalization
B
4.4
3.2
Admitted at ICU
Not admitted at ICU
ICU admission
C
1.6
3.5
Deceased
Not deceased
Death status

153

Comparable Effectiveness of Adjuvanted and High-Dose Influenza Vaccines Against Test-Confirmed Influenza Outcomes, including Hospitalizations, in Overall and High-Risk Older Adults: A Test-Negative Design Study During 2022–2023 and 2023–2024

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1: CSL Seqirus, Canada;2: Optum, Eden Prairie, MN, USA;3: Boston Medical Center, Boston, MA, USA;4: CSL Seqirus, Amsterdam, The Netherlands;5: CSL Seqirus, Waltham, MA, USA

Background: Older adults are at increased risk of severe complications from seasonal influenza. Several European Union (EU) countries—including Germany, Denmark, and Austria—have included adjuvanted and high-dose influenza vaccines in their preferential recommendations for older adults. In the US, the Advisory Committee on Immunization Practices (ACIP) recommended older adults receive adjuvanted or higher-dose influenza vaccines since 2022. Evidence from the 2017–2020 and 2022–2023 influenza seasons demonstrates that adjuvanted trivalent/quadrivalent influenza vaccine (aTIV/aQIV) and high-dose trivalent/quadrivalent influenza vaccine (HD-TIV/HD-QIV) offer comparable protection against test-confirmed influenza among US adults aged ≥65 years. Expanding on prior evidence, this study evaluated the rVE of aQIV vs. HD-QIV in preventing test-confirmed influenza during the 2023–2024 season, including hospitalizations in overall and high-risk older adults in a pooled analysis with 2022–2023.

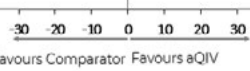
Methods: This retrospective TND study included US adults ≥65 years vaccinated with aQIV or HD-QIV who presented with acute respiratory or febrile illness in any setting as well as those with emergency department (ED) visits and/or hospitalizations. Test-positive cases and test-negative controls were grouped by vaccine received. rVE was estimated using a doubly robust model, combining inverse probability of treatment weighting and logistic regression to adjust for potential confounders. rVE was also evaluated for hospitalizations only through a pooled analysis of 2022–2023 and 2023–2024 seasons. A pooled analysis additionally estimated rVE in preventing hospitalizations in patients with ≥1 high-risk condition(s).

Results: After applying selection criteria, 37,377 vaccinated patients were included (3,174 positive cases and 34,203 negative controls). Comparable effectiveness between aQIV and HD-QIV was observed in preventing test-confirmed influenza with a rVE of -0.9% (95% CI: [-9.9, 7.3]) in any setting and 0.5% (-12.1, 11.6) for ED visits/hospitalizations (Figure 1). Similarly, comparable effectiveness was observed in preventing hospitalizations in the pooled analysis in both the overall (-0.5% [-13.4, 10.9]) and high-risk subgroup (-1.3% [-14.4, 10.4]) (Figure 1).

Conclusions: Consistent with prior evidence, these results demonstrate comparable effectiveness between aQIV and HD-QIV for preventing test-confirmed influenza in any setting as well as ED visits/hospitalizations among adults ≥65 years during the 2023–2024 season. Additionally, pooled overall and high-risk subgroup analyses for the 2022-2023 and 2023-2024 seasons showed comparable effectiveness of aQIV and HD-QIV in preventing hospitalizations specifically. These findings contribute to the growing body of evidence supporting the use of adjuvanted or high-dose influenza vaccines and offer valuable insights for EU countries implementing such strategies for older adults.

Figure 1. Adjusted rVE of aQIV vs. HD-QIV in preventing test-confirmed influenza

Season	Analysis/Outcome	aQIV Cases	aQIV Controls	HD-QIV Cases	HD-QIV Controls	Adjusted rVE% (95% CI)	Adjusted rVE% (95% CI)
2023-2024	Any Setting	1,244	13,622	1,930	20,581		-0.9% (-9.9 to 7.3)
	ED visits and/or Hospitalizations	569	7,595	841	11,303		0.5% (-12.1 to 11.6)
2022-2023 and 2023-2024 (pooled)	Hospitalizations only – Overall population	546	9,052	892	14,728		-0.5% (-13.4 to 10.9)
	Hospitalizations only – High-risk population	537	8,815	878	14,401		-1.3% (-14.4 to 10.4)



156

Genomic Epidemiology and Seasonality of Respiratory Syncytial Virus (RSV) in children less than 5 years of age in Bangladesh: Findings from Integrated Respiratory Disease Surveillance in Bangladesh.

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Background:

Respiratory syncytial virus (RSV) is one of the leading causes of respiratory tract infection among young children under 5 years of age. With a view to start Integrated Respiratory Disease Surveillance, Institute of Epidemiology, Disease Control & Research (IEDCR) as National Influenza Centre (NIC), Bangladesh has incorporated RSV in addition to Influenza and SARS-COV-2 in their National Influenza Surveillance platform (NISB) since 2022. Development of RSV vaccines and drugs are based on understanding RSV epidemic genotypes and their sequence variation. The aim of the study was to estimate the proportion of RSV positive cases among the Influenza like Illness (ILI) and Severe acute respiratory illness (SARI) enrolled cases following WHO case definition among children < 5 years of age, seasonality of RSV and to identify the circulating genotypes.

Methods:

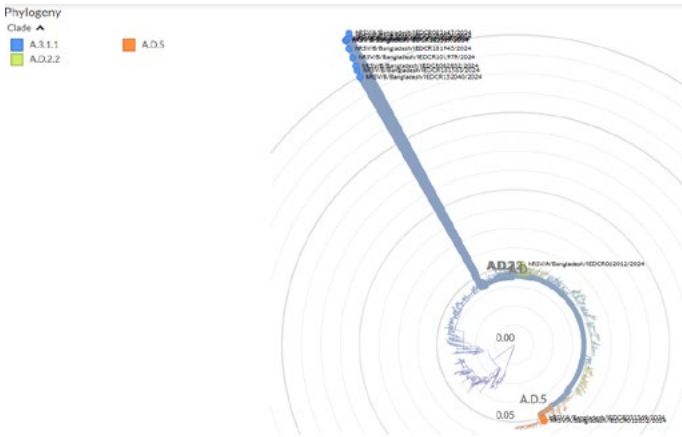
A total of 5986 suspected cases (ILI=2480 and SARI=3506) under 5 years of age according to case definition were enrolled in the study from 10 sentinel sites across the country from NISB platform during October 2022 to April 2025. The epidemiological and metadata were collected in electronic device. Nasal and throat swabs (NS and TS) were collected in VTM and shifted to IEDCR Virology laboratory weekly. Real Time RT PCR was done to detect RSV. A total of 205 RSV positive samples with ct value less than 28 were sequenced using Paragon Genomics library preparation kit in Illumina MiSeq V3 system. Phylogenetic analysis was done using BioEdit Software Version 7.0.5.3 and CLC Workbench software (Qiagen, Germany).

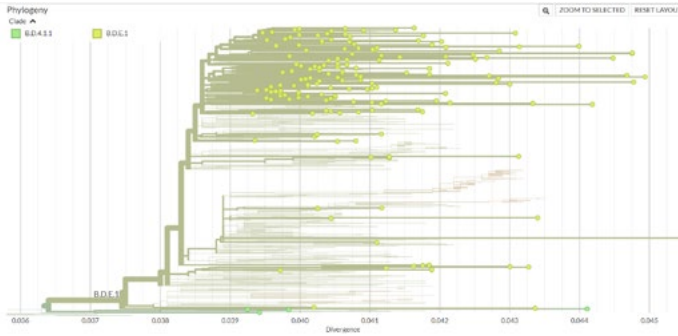
Results:

Among the enrolled cases, 1321 (22.1%) was positive for RSV. Positivity was 16.6% and 5.5% in SARI and ILI cases respectively. The incidence rate was more in male 828(62.7%) than in female37.3%). RSV seasonality was September through December with peak in November (57.3%) and October (42.1%). 65.1% positive cases were less than one-year age followed by 29.8% in 12 to 23 months and 5.1% in 24 months to 59 months age group. Among 205 sequenced samples, 192 (93.7%) belonged to RSV B Victoria (B.D.4.1.1, B.D.E.1) and 13 (6.3%) were RSV A (A.3.1.1, A.D.2.2, A.D.5). Nucleotide variations were observed in the RSV-B samples only. Phylogenetic analysis of RSV- B (F gene) with the KT992094(vaccine strain) 100 random Global samples from October 2022 to December 2025 with our 205 samples showed that sequences were distinctly clustered according to the geographical region and 115 SNPs were identified. Phylogenetic analysis of RSV- B (G gene) showed that they were distinctly clustered according to the geographical region, 192 BD sequences have created two distinct clusters and total 98 SNPs identified.

Conclusions:

Continuation of surveillance and large-scale whole-genome sequencing of RSV would help identifying its evolutionary dynamics and vaccine strain selection for the country.


Phylogeny
Clade
A.3.1.1
A.D.2.2
A.D.5
AD.23
0.00
A.D.5
0.05


Phylogeny
Clade
B.D.4.3.1
B.D.E.1
ZOOM TO SELECTED
RESET LAYOU
B.D.E.1
0.036
0.037
0.038
0.039
0.040
0.041
0.042
0.043
0.044
0.045
Divergence

161

ECaS

Broad Spectrum Antiviral Copolymers for Enveloped Respiratory Viruses

Hylemariam Mihiretie MENGIST (1), Paul DENMAN (2), Charlotte FROST (2), Julian SNG (1), Saskia LOGAN (2), Tejasri YARLAGADDA (2), Kirsten SPANN (2), Leonie BARNER (2), Kathryn FAIRFULL-SMITH (2), Nathan BOASE (2), Kirsty SHORT (1)

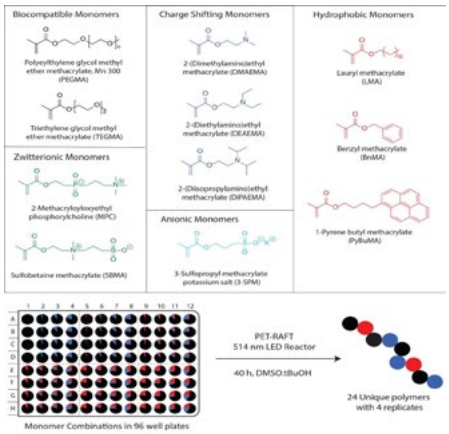
1: School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, 4072, Australia;2: Centre for Materials Science, Queensland University of Technology, Brisbane, 4000, Australia

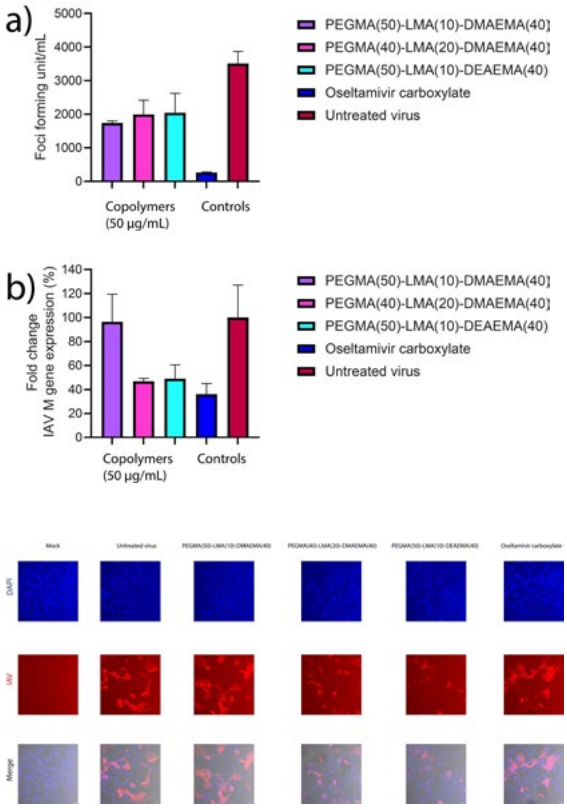
Introduction: It is impossible to predict which viral infection will cause the next pandemic. Accordingly, the development of broad-spectrum antivirals that can be stockpiled is essential for pandemic preparedness. The lipid envelope represents an attractive target for broad-spectrum antivirals as this is shared by multiple pandemic viruses and it is under the control of the host (hence not subject to antiviral resistant mutations).

Methods: Here, we use an oxygen tolerant photopolymerization technique to develop different combinations of biocompatible, hydrophobic, and charged polymers to target the viral lipid envelope. These polymers were tested **in vitro, ex vivo** and **in vivo** for toxicity and antiviral activity.

Results: High throughput antiviral testing of 468 copolymers identified three lead compounds that provided more than 90% protection against influenza A virus (IAV) (H3N2) **in vitro** at a concentration of 100 µg/ml. This polymer concentration was not toxic to MDCK cells. Polymers exhibited anti-IAV activities with IC50 values ranging from 0.3-0.47 µg/ml. Importantly, these polymers were effective against several different respiratory viruses (H3N2 IAV, H1N1 IAV and SARS-CoV-2) while leaving the non-enveloped human rhinovirus unaffected. Polymers inhibited the entry of enveloped virus into cells whilst virus binding was unaffected. Polymers remained antiviral when administered after viral infection (i.e. therapeutically) and showed virucidal activity by targeting the viral lipid envelope. Lead polymers remained non-toxic and highly antiviral when administered to primary human nasal epithelial cells. **In vivo** daily intranasal administration of the three lead compounds at a concentration of 100 µg/mL was found to be non-toxic.

Conclusion: Our findings suggest that the combination of high-throughput polymer synthesis and in vitro antiviral screening is a novel approach to identifying broad-spectrum virucidal synthetic polymers that could potentially be further developed in the clinic and ultimately stockpiled as part of a thorough pandemic preparedness plan.




a)
Foci forming unit/mL
5000
4000
3000
2000
1000
0
Copolymers (50 µg/mL)
Controls
PEGMA(50)-LMA(10)-DMAEMA(40)
PEGMA(40)-LMA(20)-DMAEMA(40)
PEGMA(50)-LMA(10)-DEAEMA(40)
Oseltamivir carboxylate
Untreated virus
b)
Fold change IAV M gene expression (%)
140
120
100
80
60
40
20
0
Copolymers (50 µg/mL)
Controls
PEGMA(50)-LMA(10)-DMAEMA(40)
PEGMA(40)-LMA(20)-DMAEMA(40)
PEGMA(50)-LMA(10)-DEAEMA(40)
Oseltamivir carboxylate
Untreated virus
Mock
DAPI
IAV
Merge

168

The contribution of pre-existing, broadly reactive T cells in heterosubtypic protection against A(H1N1)pdm09 infection in children

Marjolein STIP, Rik RUIJTEN, Leonie BROUWER, Lia DE ROND, Annemarie BUISMAN, Jørgen DE JONGE

RIVM (Dutch Institute for Public Health and Environment), Netherlands, The

Background

The 2009 influenza pandemic presented the interesting opportunity to study the immune response against a pandemic virus (A(H1N1)pdm09) in children having limited prior immunity against influenza. In our cohort of 9-year old children, many participants lacking antibody reactivity to seasonal H1 and H3 proteins before the pandemic, were later infected with A(H1N1)pdm09 (Baas et al, 2013). Conversely, in children with H3-specific B cells, induced by seasonal H3N2 during the 2008–2009 influenza season, A(H1N1) pdm09 infection rates were much lower. Studies in adults (Sridhar et al, 2015) already observed that pre-existing broadly-reactive T cells were correlated with less seroconversion for A(H1N1)pdm09 during the pandemic. Thus far it remained unclear whether such cross-reactive T cells played a protective role in children as well. Therefore, we investigated whether cross-reactive T cells induced by seasonal H3N2 and H1N1 strains recognize (H1N1)pdm09 and if these cells could have contributed to heterosubtypic protection against A(H1N1)pdm09. By leveraging unique longitudinal samples from our pediatric cohort with clean immune backgrounds, we aim to identify the phenotype and functionality of these cross-reactive T cells and their potential role in protection against novel influenza viruses that cause a pandemic.

Methods

For both pre- and post-pandemic samples, hemagglutination inhibition (HAI) assays were performed to evaluate the infection history of the study cohort (ISRCTN64117538) for H3N2 A/Uruguay/716/07, H1N1 A/Brisbane/59/07 and A(H1N1)pdm09. Influenza-specific T cells were quantified using IFNγ ELISpot assays and characterized using flow cytometry. For both assays, PBMCs were stimulated with H1N1 peptide pools targeting conserved influenza proteins (NP, M1 or PB1) or on of the three live viruses. Flow cytometry analyses comprised cytokine production, activation-induced markers (AIM) and chemokine receptors.

Results

From HAI assays, we observed that children with a recent seasonal influenza infection were less likely to have seroconversion for A(H1N1)pdm09. Next, using both adult PBMCs and those of 9-year old controls, ELISpot conditions were optimized to detect the low-frequency T cells in pediatric samples, for which activation could possibly take longer compared to adult T cells. Flow cytometry and ELISpot data revealed that T cells from control samples secrete cytokines (mostly IFNγ and TNFα) and upregulate AIM-markers (CD69, CD137, CD154 and OX40) upon NP, M1 or PB1 peptide pool or whole virus stimulation. ELISpot and flow cytometry results from the pediatric cohort are currently coming in and will be correlated to the obtained serological data. These results will be presented during the conference.

Conclusions

Studying cross-reactive influenza-specific T cells in this pediatric cohort will contribute to defining pediatric immune correlates of protection and offers unique insights, due to their relative clean immune background for influenza infections. Additionally, these results will be important for the development of vaccination policies for children and the design of universal influenza vaccines.

169

Safety and Efficacy of Baloxavir Marboxil for Influenza Treatment in Chinese Children Aged 1-<5 Years: A Single-Arm Multicenter Clinical Trial

Yuchuan LI (1), Peng GUO (1), Chenguang JIA (1,2), Ju YIN (1), Yuncui YU (1), Qiang QIN (1), Xiaoyan ZHANG (1), Shunying ZHAO (1), Haiming YANG (1), Weihua ZHANG (1), Yuguang LIANG (1), Qian DING (1), Man TIAN (3), Yiping CHEN (4), Chunmei ZHU (5), Fang WANG (6), Jing MA (7), Lisu HUANG (8), Yongping XIE (8), Jing BI (9), Sainan SHU (10), **Chengsong ZHAO** (1)

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Background. Influenza causes significant morbidity in children under 5, with high rates of hospitalization and complications. Baloxavir marboxil offers clinical benefits through its single-dose regimen and rapid antiviral activity in influenza treatment. While currently approved for individuals 5 years of age and older in China, the safety and efficacy data in children (1-<5 years) are limited. This study presents the primary analysis evaluating the safety and efficacy of baloxavir marboxil in Chinese children aged 1 to <5 years with influenza.

Methods. We conducted a single-arm, multicenter study in Chinese children aged 1 to <5 years with laboratory-confirmed influenza A and/or B (symptom onset ≤48 hours). Eligible participants received a single oral dose of baloxavir marboxil suspension based on body weight (2 mg/kg for <20 kg; 40 mg for ≥ 20 kg to < 80 kg). The primary endpoint assessed the incidence and severity of adverse events, including serious adverse events. Secondary endpoints evaluated the time to alleviation of influenza signs and symptoms (TTASS), fever duration, incidence of influenza-related complications, and virus detection in serial respiratory swabs.

Results. During the 2024-2025 influenza season, we enrolled 100 influenza patients. Overall, 34 adverse events (AEs) were reported in 29 (29%, 29/100) children, with SAEs occurring in 3% (3/100) of participants. The most common AEs included bronchitis (4%), diarrhea (4%), upper respiratory tract (4%), infection (3%) and cough (3%). Only one case (rash) was determined by the investigators to be drug-related AE, which was grade 1. The median TTASS was 150.17 hours (95% CI, 90.23-NE), with fever resolution occurring within a median of 38.65 hours (95% CI, 25.32-42.35). The median time to cessation of viral shedding by virus titer was 22.74 hours (95% CI, 22.20-24.58). Influenza-related complications developed in 5% of participants, while systemic antibiotics were administered in none of the cases.

Conclusions. In this multicenter evaluation, weight-adjusted baloxavir marboxil demonstrated a favorable safety profile and clinically meaningful efficacy for the treatment of influenza in Chinese children aged 1 to <5 years.

170

A highly protective pandemic H5N1 vaccine based on a clade 2.3.4.4b influenza virus

Eduard PUENTE-MASSAGUER (1), Thales Eduardo GALDINO ANDRADE (2), Michael Jonathan SCHERM (1), Kirill VASILEV (1), Hassanein ABOZEID (3), Josh YUEH (1), John Darren CAMPBELL (4), Dong YU (4), Richard J. WEBBY (5), Yoshihiro KAWAOKA (3), Gabriele NEUMANN (3), Julianna HAN (2), Andrew B WARD (2), Florian KRAMMER (1)

1: Icahn School of Medicine at Mount Sinai, United States of America;2: The Scripps Research Institute, United States of America;3: School of Veterinary Medicine, University of Wisconsin-Madison, United States of America;4: Dynavax Technologies, United States of America;5: St Jude Children's Research Hospital, United States of America

Background: H5N1 influenza viruses have the potential to cause the next influenza pandemic. Since its introduction into the Americas in December 2021, the clade 2.3.4.4b H5N1 highly pathogenic avian influenza virus (HPAIV) has spread at an unprecedented scale, raising significant concern. HPAIV has caused widespread mortality in wild birds and domestic poultry, along with confirmed infections across multiple mammalian species. Although most human cases associated with clade 2.3.4.4b have been mostly mild, recent reports of potential mammal-to-mammal transmission as well as several human deaths underscore the urgent need for effective countermeasures.

Methods: We developed an Alum/CpG adjuvanted, inactivated split-virus vaccine based on a clade 2.3.4.4b H5N1 strain. Mice were immunized intramuscularly, and immune responses were assessed across several parameters: cross-reactivity against diverse hemagglutinin (HA) and neuraminidase (NA) glycoproteins, **in vitro** H5N1 virus neutralization and hemagglutination inhibition, multiple antibody functions, antigen-specific T cell responses, and protection against challenge with different H5N1 viruses.

Results: Immunization with a low dose of this vaccine, delivered in either a single (prime) or two-dose (prime + boost) regimen, elicited broadly cross-reactive, neutralizing, and NA inhibiting antibodies targeting all tested H5 HAs and avian N1 NAs. The vaccine also induced strong systemic and local antigen-specific T cell responses. Serum antibody analysis by negative-staining electron microscopy revealed targeting of conserved epitopes in both the H5 HA and N1 NA glycoproteins. Notably, vaccinated mice were fully protected from lethal challenge with different strains, including heterologous clade 1 H5N1.

Conclusions: We have developed a highly protective, adjuvanted-vaccine candidate targeting potential pandemic H5N1 influenza viruses. This platform demonstrates strong cross-reactive immunity and complete protection in preclinical models, supporting its potential as a countermeasure in pandemic preparedness.

172

The protection of ferrets against influenza by nasal immunization with an LTh(αK) adjuvanted influenza vaccine

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Advagene Biopharma, Taiwan

Background

Mucosal immunology represents a frontier with significant potential, yet remains uncharted territory in vaccine development. While traditional vaccines typically focus on inducing systemic immunity through injection, mucosal vaccines aim to stimulate compartmental immune responses, which is particularly important for respiratory pathogens such as influenza. Addressing this critical gap, Advagene has developed an innovative seasonal influenza intranasal vaccine that leverages egg-derived split hemagglutinin (HA) antigens combined with LTh(αK), a detoxified enterotoxin, as a mucosal adjuvant. Advagene has completed a Phase 2 clinical trial demonstrating robust safety and immunogenicity, notably in populations often considered challenging to immunize effectively, such as elderly individuals (NCT03784885). Trial results showed that the intranasal vaccine elicited high levels of neutralizing secretory IgA (sIgA) antibodies, which are key to early virus neutralization at the mucosal surface before systemic infection occurs. The high HA-specific sIgA induction observed among both adults and elderly participants underscores the vaccine's potential to provide superior mucosal protection, reducing individual disease severity and potentially limiting transmission. Recognizing the difficulty associated with human challenge studies for influenza, Advagene has utilized the established ferret influenza model to evaluate vaccine efficacy.

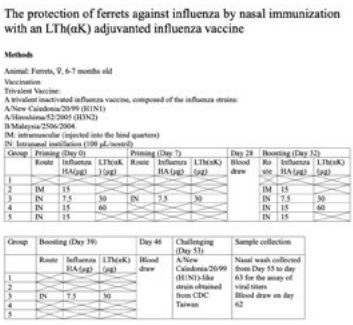
Method

Results:

Advagene's intranasal vaccine protected ferrets from developing influenza-induced fever and substantially reduced nasal viral shedding. These findings support the vaccine's ability to protect individuals from symptomatic illness and limit viral transmission.

Conclusions

The combined clinical and animal model data suggest that Advagene's intranasal influenza vaccine significantly advances mucosal vaccinology. By generating robust mucosal immune responses in elderly populations and demonstrating clear evidence of protection and reduced transmission in ferrets, the vaccine has proven its promise as an effective public health intervention.



The protection of ferrets against influenza by nasal immunization with an LTh(αK) adjuvanted influenza vaccine

Methods

Animal: Ferrets, ♀, 6-7 months old

Vaccination

Trivalent Vaccine:

A trivalent inactivated influenza vaccine, composed of the influenza strains:

A/New Caledonia/20/99 (H1N1)

A/Hiroshima/52/2005 (H3N2)

B/Malaysia/2506/2004

IM: intramuscular (injected into the hind quarters)

IN: Intranasal instillation (100 μL/nostril)

Group	Priming (Day 0)			Priming (Day 7)			Day 28	Boosting (Day 32)		
	Route	Influenza HA(μg)	LTh(αK) (μg)	Route	Influenza HA (μg)	LTh(αK) (μg)	Blood draw	Route	Influenza HA (μg)	LTh(αK) (μg)
1										
2	IM	15						IM	15	
3	IN	7.5	30	IN	7.5	30		IN	7.5	30
4	IN	15	60					IN	15	60
5	IN	15						IN	15	

Group	Boosting (Day 39)			Day 46	Challenging (Day 53)	Sample collection
	Route	Influenza HA (μg)	LTh(αK) (μg)	Blood draw	A/New Caledonia/20/99 (H1N1)-like strain obtained from CDC Taiwan	Nasal wash collected from Day 55 to day 63 for the assay of viral titers Blood draw on day 62
1						
2						
3	IN	7.5	30			
4						
5						

174

How to improve reporting for pandemic preparedness: an integrated surveillance dashboard for pandemic potential viruses

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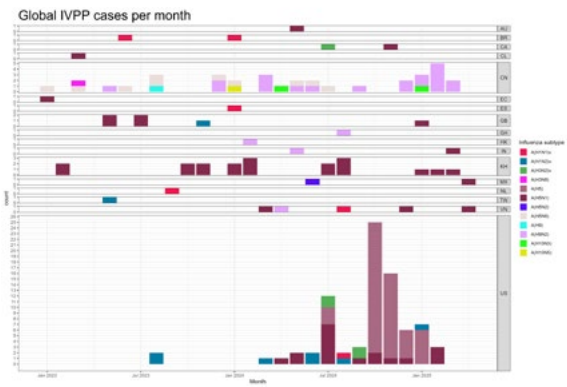
Background: Influenza A viruses of avian or swine origin occasionally infect humans, and are considered Influenza Viruses of Pandemic Potential (IVPP). Timely sharing of easily accessible data is crucial for pandemic preparedness. However, data on human cases are fragmented among different data sources. In this project we aim to analyse and reflect upon the current global status of IVPP reporting, and propose a comprehensive, timely, and user-friendly dashboard of available data.

Methods: We conducted a comprehensive online search to identify publicly available resources reporting IVPP cases, collecting information on the type of reporting (aggregated, case-based), medium (report, website, dashboard, database), publication interval, influenza types covered, geographical coverage, and additional information (e.g. detections in birds or mammals, or risk evaluation). The identified public data sources regularly reporting new human IVPP cases are consulted on a weekly basis to collect information on any new cases. For each case key clinical and epidemiological variables are collected where available. Retrospective data, starting January 2023, has also been collected, based on earlier work by the authors.

Results: We identified 14 data sources that met our reliability and accessibility requirements. Publications interval ranged from daily for websites and dashboards to three months for more comprehensive reports. Seven sources reported cases caused by any IVPP, three focused on avian influenza only and four focused on avian influenza A(H5N1). Most sources reported cases worldwide, although some reports focused the interpretation and contextualization on one specific region or country. Based on the combination of available sources and as of 25 April 2025, a total of 170 human IVPP cases have been identified since January 2023, in 17 countries (figure 1). These cases were caused by 12 different influenza subtypes, primarily influenza A(H5N1) and influenza A(H9N2).

Conclusions: Publicly available IVPP data is either presented in a case-based 'news' format, signalling new cases, or in longer reports with aggregated data and a longer publication interval. We combined the strengths of these different resources into one easily interpretable overview figure. This figure has the potential of being developed into an interactive dashboard, where more detailed data about each case can be accessed easily. Given the zoonotic origin of the virus, an essential future step is to integrate reporting of human IVPP cases with surveillance data on the virus circulating in birds, mammals and the environment, with possible expansion to other (zoonotic) pandemic potential viruses.



177

ECaS

Intranasal RSV vaccines offer protection against RSV in preclinical models

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Abstract

Introduction: All three currently approved RSV vaccines (Abrysvo, Arexvy, mResvia) target the pre-fusion F (pre-F) protein of RSV and are administered intramuscularly. While Abrysvo provides indirect infant protection through maternal immunization, existing vaccines are primarily approved for elderly populations, leaving infants without direct immunization options. Despite the critical role of the G protein in RSV infection and pathogenesis and evidence linking G-specific humoral immunity to infant protection, it remains underexplored as a vaccine target. To address the gap in RSV vaccine development, mucosal RSV vaccines—capable of blocking infection at entry sites and enhancing infant protection—are urgently needed.

Methods: This study explored intranasal RSV vaccine candidates based on a live attenuated influenza virus vector with deleted NS1 gene (DelNS1). **This vaccine system was used to develop an intranasal COVID-19 vaccine (DelNS1-RBD), which received regulatory approval in China in 2022.** The candidate vaccines express either the truncated Pre-F or the conserved central domain (CCD) of G from RSV subtypes A2 and B1, designated as **DelNS1-Pre Fhead** or **DelNS1-G2Nab**. The antigenicand safety profiles of these intranasal vaccine candidates are evaluated both **in vitro** and **in vivo** assessments.

Results: Both vaccine candidates elicited high levels of antigen specific IgG in serum and IgA in bronchoalveolar lavage fluid (BALF) and nasal wash. Additionally, they stimulated Th1-biased, RSV specific tissue-resident memory CD4+ and CD8+T cell responses in lung of mice following intranasal boosting. Intranasal administration of **DelNS1-Pre Fhead** and **DelNS1-G2Nab** provide effective protection against mouse-adapted RSV infection in Balb/c mice without inducing vaccine-enhanced respiratory diseases (VERD).

Conclusion: Intranasal immunization with influenza viral vector vaccines, DelNS1-Pre F head and DelNS1-G2Nab, induced robust immune response and effective protection against RSV. Mucosal immunity and T cell responses played a pivotal role in the efficacy of these vaccine candidates. The DelNS1 live-attenuate viral vaccine system presents a promising vaccination strategy for making mucosal vaccines against common cold viruses in combination with flu vaccine.

179

Cost Analysis and Public Health Impact of Enhanced Influenza Vaccination for Adults 65 years and above Compared to Current Programs in Nordic Countries

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Background: Immunosenescence reduces vaccine efficacy and increases infection susceptibility in older adults, leading to higher influenza-related morbidity and mortality. Enhanced influenza vaccines (EIV) offer better protection than standard-dose influenza vaccines (SD-IV). EIVs are used variably in Nordic countries: Denmark (≥70 years), Sweden and Norway (nursing home residents), Finland (≥85 years).

Objective: Compare the public health impact and costs of current vaccination strategies to expanding EIV use to all individuals aged ≥65 in Nordic countries.

Methods: A decision-tree model compared outcomes of a universal 65+ EIV program with existing programs, incorporating national demographics and vaccination coverage. SD-IV effectiveness was set at 51%, with EIV's relative effectiveness at 24.8% estimated based on calculation from the literature. Health outcomes included symptomatic influenza cases, GP visits, hospitalizations, inpatient days, and deaths. Costs included healthcare utilization due to influenza.

Results: Expanding EIV to all adults ≥65 years would prevent a significant number of annual influenza related events:

1. **Sweden:** 12,707 infections, 3,812 GP visits, 648 hospitalizations, 1,370 outpatient visits, 18 deaths.
2. **Norway:** 5,469 infections, 1,641 GP visits, 279 hospitalizations, 590 outpatient visits, 8 deaths.
3. **Finland:** 5,817 infections, 1,745 GP visits, 297 hospitalizations, 627 outpatient visits, 8 deaths.
4. **Denmark:** 2,206 infections, 662 GP visits, 113 hospitalizations, 238 outpatient visits, 3 deaths.

Broader access to EIVs would significantly reduce healthcare costs:

5. **Denmark:** Expanding EIV to include the 65-69 age group could avert an additional DKK 3M in healthcare expenses.
6. **Sweden:** SEK 58.5M savings.
7. **Norway:** NOK 11.9M savings.
8. **Finland:** EUR 1.76M savings.

Conclusion: Expanding EIV to all individuals aged 65 and above would significantly reduce influenza-related health burden and healthcare costs across the Nordic region. Improved outcomes already observed in Denmark with a 70+ program highlight the benefits of extending EIV coverage.

180

Evolution of respiratory viruses circulation in SARI patients in Tuscany, Italy, during the seasons 2023/2024 and 2024/2025

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Background:

Influenza A and B viruses are the main cause of one of the most contagious seasonal respiratory diseases. Human respiratory syncytial virus (HRSV) is an important cause of acute respiratory infection with more severe disease in the elderly and immunocompromised. Following the lifting SARS-CoV-2 restrictions, during the winter seasons 2023/2024 and 2024/2025, the circulation of HRSV, influenza and other respiratory viruses has fully returned to pre-pandemic levels, alongside the continued presence of SARS-CoV-2. In patients affected by Severe Acute Respiratory Infection (SARI), the presence of comorbidities may worsen the severity of the disease, and the risk of co-infection with other viruses could contribute to clinical deterioration and prolonged hospitalization. The aim of this study is to investigate, by analyzing oropharyngeal swabs, the presence of respiratory viruses in SARI patients evaluating their association with symptoms and the vaccination status of the patients.

Methods:

A total of 250 oropharyngeal swabs were collected at the Intensive Short Observation of Santa Maria alle Scotte University Hospital in Siena, Italy, from November 2023 to May 2025 by subjects affected by SARI. In particularly, 147 swabs during the season 2023/2024 and 103 in the season 2024/2025. Viral RNA was extracted and tested for the detection of 21 respiratory viruses, among these SARS-CoV-2, influenza A and B and HRSV, by RT-PCR. Some samples positive for influenza and SARS-CoV-2 were sequenced to characterize subtypes and circulating variants.

Results:

Among the 147 subjects enrolled in the 2023/2024 season, 62 (42%) tested positive for SARS-CoV-2; 18 (13%) tested positive for HRSV, 23 (17%) were infected with influenza A virus and 5 (3%) with influenza B virus. Similarly, swabs from 103 subjects recruited during the 2024/2025 season, 53 (52%) tested positive for SARS-CoV-2, 9 (9%) were tested positive for HRSV, 10 (10%) for influenza A virus and 1 (0.9%) for influenza B virus. For both seasons, samples tested positive for SARS-CoV-2 and Influenza with a cycle threshold (CT) value <40 were sequenced. For both seasons, the most observed chronic conditions were related to the cardiovascular diseases, hypertension and respiratory diseases.

Conclusions:

Our study shows that while Covid-19 positives remain the same, cases of respiratory syncytial virus and influenza has halved compared to the years 2023/2024. These findings highlight the prevalence of respiratory infections in both seasons and emphasize the need for continued surveillance and preventive measures. Primary prevention, through the implementation of vaccination strategies against microorganisms for which vaccines already exist, represents a crucial measure to prevent co-infections from pathogens for which no vaccines have yet been developed, such as RSV. Vaccination plays a critical role reducing the risk of complications, particularly in elderly or vulnerable individuals, such as patients with SARI, who are the focus of our study.

181

Infrared Thermography for Detection of SARS-CoV-2 Infection in a Human Challenge Model: Correlation Between Thermal Patterns and Respiratory Symptoms

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HVIVO services, United Kingdom

Background: Non-invasive detection of viral respiratory infections remains a challenge in clinical settings. Infrared thermography (IRT) shows promise for early detection by analyzing facial thermal patterns. This study evaluated IRT's effectiveness in detecting SARS-CoV-2 infection in a controlled human trial where participants were intentionally exposed to the virus.

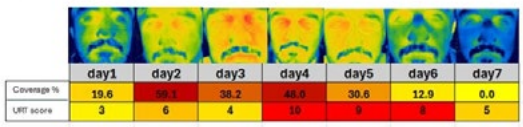
Methods: Thermal patterns were monitored in 8 quarantined participants inoculated with SARS-CoV-2 Omicron (BA.5). FLIR thermal cameras captured daily images over 7 days, three times a day. Temperature parameters (maximum and average) and coverage percentage (extent of elevated temperature on the face) were analyzed from facial regions. Daily upper respiratory tract (URT) symptoms were scored on a 0-20 scale. Excel-generated heat maps categorized timepoints based on temperature coverage and URT symptom scores. Data were analyzed across timepoints with and without symptoms.

Results: To better reflect the dynamic nature of symptom onset and resolution, we analyzed thermal metrics across individual timepoints. A total of **168 timepoints** (8 participants × 7 days × 3 timpoints) were included. Each timepoint was classified as **symptomatic (URT ≥ 3)** or **asymptomatic (URT<3)** based on the reported symptom scores for that day. **Symptomatic timepoints** (n = 18) exhibited higher thermal intensity compared to asymptomatic ones (n = 150). Visual inspection showed a consistent pattern of elevated **URTs** and expanded **coverage** during days with respiratory symptoms (Fig.1). **Tavg** was significantly higher during symptomatic timepoints (mean ± SD: 35.8 ± 0.6°C) compared to asymptomatic ones (34.3 ± 0.8°C); $p < 0.05$. **Coverage (%)** of high-temperature areas increased during symptomatic days (33.3% ± 12%) vs. asymptomatic (14% ± 6%); $p < 0.05$. Both **coverage and Tavg increase progressively** with higher URT symptom scores, suggesting a positive association between thermal intensity and self-reported symptom burden (Fig.2).

Conclusions: This preliminary analysis demonstrates that thermal imaging can visually differentiate symptomatic from asymptomatic timepoints. Despite fluctuating symptom severity, thermal images highlighted physiological changes correlating with reported symptoms. This supports the feasibility of IRT as a non-invasive adjunct to symptom diaries in controlled human infection models.

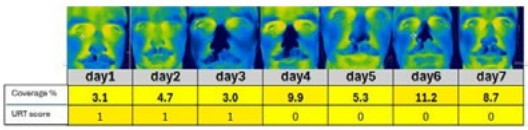
Figure 1. Top row: Thermal image progression over 7 days from a symptomatic participant (Case 1) and from an asymptomatic participant (Case 2). **Bottom row:** A heat map timeline matrix showing *coverage* % and URTs score (color-coded table). Notable increase in periorbital and nasal region temperature (red/yellow intensification) corresponds with symptom presence. Visual changes began on Day 2 and persisted through Day 5.

Case 1 (symptomatic):



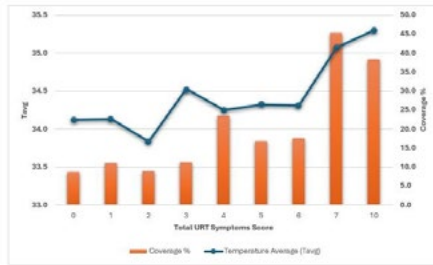
	day1	day2	day3	day4	day5	day6	day7
Coverage %	19.6	59.1	38.2	48.0	30.6	12.9	0.0
URT score	3	6	4	10	9	8	5

Case 2 (asymptomatic):



	day1	day2	day3	day4	day5	day6	day7
Coverage %	3.1	4.7	3.0	9.9	5.3	11.2	8.7
URT score	1	1	1	0	0	0	0

Figure 2. Relationship between upper respiratory tract (URT) symptom scores and facial thermal parameters. The x-axis shows the total daily URT symptom score (scale 0–10). The left y-axis shows the corresponding average facial temperature (Tavg, °C; blue line) and the right y-axis shows the average Coverage % (orange bars).



184

Long term evolution of seasonal influenza A/H3N2 virus in differentiated human airway epithelial pathways reveals diverse evolutionary potential

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Immunity-induced selection is a key driver of seasonal influenza A/H3N2 virus evolution. Therefore, research has focused heavily on characterizing the historically observed antigenic evolution of A/H3N2 viruses. However, we do not know how repeatable the observed evolutionary trajectory is nor what other phenotypes A/H3N2 might optimize without the constraints of antibody-mediated selection. Here, we studied pace, repeatability, and potential phenotypic consequences of the long-term evolution of A/H3N2 in the absence of immune selection pressures.

We established 12 lines of **ex-vivo** differentiated human airway epithelium (HAE) culture from 4 healthy donors and serially passaged the same A/H3N2 virus starting population for over 100 passages. We sampled each line at every tenth passage and analyzed the dynamics of molecular A/H3N2 virus evolution using whole genome sequencing.

Across all 12 lines, we detected an average of 54 variant substitutions of which 13 were eventually fixed within the line. Each line acquired substitutions at different rates. Of note, we observed novel fixed mutations in antigenic sites A, C and E of the H3 haemagglutinin protein. We also observed fixed mutations within the polymerase complex that are likely to increase protein stability. However, we found no evidence of enrichment in any particular segment, nor did we find signatures of convergent evolution across donors or within single HAE donor replicates.

Despite being in a near-natural environment without the constraints of antibody-mediated selection, A/H3N2 virus remains genotypically plastic with the fixation of multiple diverging substitutions across independent HAE lines and no clear signatures of convergent evolution. Our results suggest that the evolutionary space of the seasonal influenza A/H3N2 virus is large and remains relatively underexplored.

187

Development of a Rapid Multiplex Nucleic Acid Amplification Diagnostic Test for the Detection of Influenza Types A, B, C, and D with Influenza A Hemagglutinin-Subtype H1-H18 Delineation

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Background:

Influenza is a priority pathogen with high risk for future pandemics. New virulence and capacity to infect novel hosts is rapidly and continuously evolving via mutation and genetic reassortment. As learned during the global Coronavirus pandemic, a comprehensive, easy-to-use, rapid diagnostic test is needed immediately when a pandemic threat emerges. Employing automated multiplex PCR technology for simultaneous detection, we developed a diagnostic test for influenza detection, including existing pathogenic and potential emerging subtypes on the BIOFIRE® FILMARRAY® TORCH and SPOTFIRE® systems.

Methods:

Similar to the FDA-cleared BIOFIRE® Respiratory 2.1 (RP2.1) and SPOTFIRE® Respiratory/Sore Throat (R/ST) Panels, the design for this Emerging Influenza Panel utilizes a pouch-based nested, multiplex PCR capable of detecting and differentiating all four known genera of influenza and hemagglutinin subtypes H1-H18 within the genus **Alphainfluenzavirus**.

Influenza assays are designed principally to sequences deposited to the NCBI Virus database (previously NCBI Influenza Virus Resource). These assays are built using nested primers deliberately constructed to maximize sensitivity to the many disparate sequences for each influenza target and maintain on- and off-panel exclusivity.

Assays are evaluated **in silico** and on benchtop to ensure coverage and performance. Testing is conducted on both FDA-cleared platforms with synthetic target transcripts based on common sequences and inactivated, whole-virus genomes (BEI Resources, NIAID, NIH) to confirm function with high multiplex under pouch conditions.

Results:

In silico evaluation demonstrates detection of influenza A, B, C and D for more than 90% of all available sequences, inclusive of human and non-human hosts. **In silico** data also establishes hemagglutinin-specific detection and demarcation with 88% sequence inclusivity or greater for subtypes H1-H18, with exclusivity to human, coronavirus, and other common respiratory pathogens. Continuous **in silico** surveillance for H5N1 and other currently circulating sequences confirms ongoing inclusivity.

For all type and subtype assays, detection of designed synthetic nucleic acid is robust, typically down to at least 1000 copies per test. Pilot studies with whole influenza viruses are detected on both platforms for influenza from both human and non-human hosts, including H1, H3, H5 (Bovine), H5 (Avian), Influenza B, and Influenza C.

Conclusions:

Initial assay design and functional testing highlight feasibility of this influenza diagnostic panel which detects all four genera of Influenza, as well as differentiates between Influenza A Hemagglutinin subtypes on both FDA-cleared BIOFIRE® FILMARRAY® TORCH and SPOTFIRE® systems. Following FDA clearance and launch, this diagnostic will provide a critical tool in pandemic preparedness to quickly and effectively differentiate between all types of influenza and hemagglutinin subtypes.

* This project has been funded in whole or in part with Federal funds from the Department of Health and Human Services; Office of the Administration for Strategic Preparedness and Response; Biomedical Advanced Research and Development Authority, under Contract No. 75A50123C00071.

190

ECaS

Characteristics and course of disease of patients admitted to Dutch intensive care units in the first Post-COVID-19 Pandemic Influenza 2023-2024 influenza season

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1: Spaarne Gasthuis, Netherlands, The;2: Erasmus MC University Medical Centre, Netherlands, The

Introduction:

Influenza can lead to severe complications, with certain risk factors and comorbidities associated with poor outcomes. Certain patients face a higher risk of secondary infections, ICU admission, and mechanical ventilation. The first post-COVID-19 seasonal influenza surge placed a burden on Dutch ICUs. This study examines the disease course and outcomes of patients with influenza admitted to the ICU. **Methods:**

A retrospective influenza registry study was conducted across 34 Dutch ICUs, including patients aged 18 and older admitted between November 2023 and March 17, 2024, with a positive influenza real-time RT-PCR test.

Results:

A total of 498 patients were included in the study. The median age was 64 (IQR: 55-72) years and 58.8% of the patients were men. The most common comorbidities were cardiovascular disease (34.1%), chronic obstructive pulmonary disease (COPD) (31.5%), and diabetes (22.3%). Co-infections were present in 37.6% of the patients. Invasive mechanical ventilation (IMV) was necessary in 46.0% of patients, 38.0% of those requiring IMV were proned. A substantial mortality rate was observed, with an ICU mortality rate of 21.9% and an additional hospital mortality rate of 5.2%.

Conclusion:

This study described the characteristics and course of disease of all patients with laboratory-confirmed influenza infection admitted to one of the 34 participating Dutch ICUs between November 2023 and March 2024. The major findings of this study are the substantial mortality rate, a high proportion of patients with bacterial co-infections, and a significant percentage of patients requiring IMV and proning. Finally, patients without comorbidities that were to the ICU with an influenza virus infection showed severe disease, but had a lower mortality than patients with comorbidities.

191

Surveillance of avian influenza in poultry in antananarivo, madagascar: a longitudinal study in live bird markets and at importation, 2021 to 2024

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Avian influenza, first documented in 1878, remains one of the world's most widespread diseases and continues to cause significant economic damage to the poultry industry globally. Despite being less neglected than other avian diseases in Madagascar, data on avian flu circulation remains limited. Previous serological studies conducted around lakes and farms have confirmed the presence of avian influenza viruses in poultry, but failed to identify specific viral strains. In early 2021, we conducted a pilot study in live bird markets in Antananarivo and reported the circulation of influenza A/H9. Based on these results, we set up a longitudinal surveillance program in October 2021, systematically monitoring three live bird markets and border checkpoints in Antananarivo. Tracheal and cloacal swabs were collected from each poultry. All samples were screened for influenza A viruses by real-time RT-PCR. Positive samples were then subtyped for A/H5, A/H7 and A/H9, and a subset of amplification products was sequenced using next-generation sequencing (NGS) for genomic characterization.

From August 2021 to December 2024, we collected samples from 10,123 birds of three species, ducks (n=5,197), chicken (n=3,528) and geese (n=1,398). RT-PCR screening revealed a positivity rate of 6.5% (661/10,123) for Influenza A viruses. Avian influenza exhibited year-round circulation, generally peaking in winter. Almost 11.5% of ducks (596/5,197) were positive, 2.6% of goose (37/1,398) and 0.8% of chicken (28/3,528). Notably, while ducks constituted approximately half of the sampled birds, they accounted for 90.2% (596/661) of all positive cases. Based on qRT-PCR subtyping, 18.7% (124/661) of positive samples were identified as A/H9 and 1% (n=7/661) as A/H7. No cases of A/H5 were identified. The remaining positive samples were unsubtypeable. NGS sequencing allowed to identify additional subtypes, including A/H1, A/H4, A/H6, A/H7, A/H9 and A/H11. A/H9N2 was the most commonly detected subtype. A case of coinfection with the A/H1N2 and A/H6N1 viruses was also detected in a duck. These genomic data have been deposited in the GISAID database. Preliminary phylogenetic analysis indicated that the Malagasy strains were low pathogenic and belonged to the Eurasian lineage.

This surveillance system has allowed to track the circulation of influenza viruses in poultry and identify the spectrum of circulating subtypes over the last three years. Our surveillance has also enabled to establish a seasonal profile of circulating avian influenza viruses in Madagascar and to identify ducks as the species most affected. We plan to extend these activities to migratory birds to investigate potential introduction routes of new strains of avian flu and to assess the risk of zoonotic emergence in humans and animals. This work represents the first step towards implementing appropriate sanitary measures in the event of detection or suspicion of a highly pathogenic strain of avian influenza in Madagascar.

195

Co-seasonality of seasonal influenza and COVID-19 in the US, 2022-2024

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Background

Seasonal influenza viruses and SARS-CoV-2 contribute substantially to the fall and winter respiratory disease burden. Unlike influenza, SARS-CoV-2 has yet to establish consistent seasonal patterns, with activity surges occurring during and outside the typical winter respiratory season. As SARS-CoV-2 becomes endemic, understanding its patterns relative to influenza is important for predicting healthcare burdens and for guiding the seasonal vaccination strategy for multiple circulating pathogens.

Methods

We used national surveillance data for influenza and COVID-19 hospitalizations from the United States (US) Centers for Disease Control and Prevention (FluSurv-NET, COVID-NET) collected between September 1, 2022 and April 30, 2024. Temporal relationship between weekly hospitalization rates for influenza and COVID-19 was evaluated with lead-lag dynamics over a range of 35 weeks per season for all ages and by age groups (18-49, 50-64, 65 and above). Weeks between June and August were not included in this analysis focusing on winter respiratory season. The cross-correlation function (CCF) was computed across a 12-week lead-lag time window to assess the strength of correlation and the relative time shift of COVID-19 time series with respect to influenza time series.

Results

During the 2022-2024 respiratory virus seasons, weekly hospitalization rates for influenza and COVID-19 in all age groups were moderately correlated (Figure 1), with the maximum correlation observed at a one-week lag (CCF=0.58, Figure 2), indicating that the COVID-19 hospitalization trend lagged that of influenza by one week. This temporal relationship was consistent across all age groups, with highest correlations occurring within a 2-week lead-lag time window, indicating similar timing between COVID-19 and influenza hospitalizations across age groups. For adults 65 years and above, the strongest correlation was observed at zero time-lag, implying synchronous trends over the study period.

Conclusions

Influenza and COVID-19 associated hospitalization displayed overlapping seasonal patterns, with synchronized activities and peaks during fall and winter months. A modest increase in COVID-19 hospitalization rates occurring during late summer, distinct from the winter peak, warrants further investigation. Our findings support coadministration of influenza and COVID-19 vaccines or use of a combined vaccine to optimize protection before peak viral circulation in winter.

Figure 1. Weekly hospitalization rates for influenza and COVID-19 in the US, 2022-2024

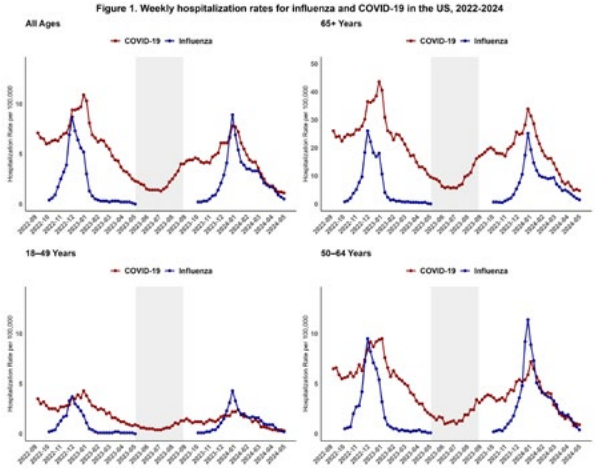
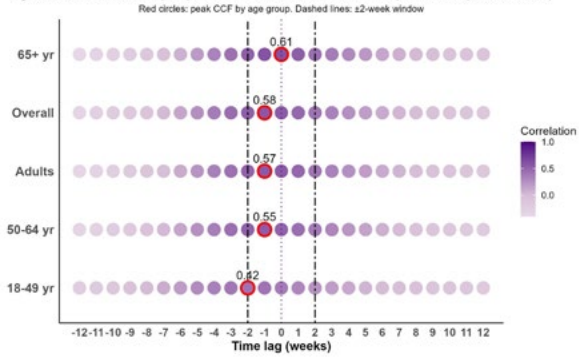


Figure 2. Cross-correlation (CCF) between influenza and COVID-19 hospitalizations (2022–2024, US)



197

Development of a mRNA vaccine against highly pathogenic H5N1 (2.3.4.4b clade) avian influenza virus

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The worldwide spread of the highly pathogenic H5N1 influenza virus, specifically clade 2.3.4.4b, has caused a significant panzootic, with infections in humans and numerous zoonotic outbreaks, leading to multiple instances of cross-species transmission. As more cases of transmission from livestock to humans occur, the risk of a potential pandemic increases, highlighting the urgent need for more effective vaccine development and preparedness strategies. Thus, we aimed to design antigens that reflect the multi-state structure for the development of influenza vaccine candidates by modeling the hemagglutinin (HA) protein using artificial intelligence (AI) softwares. The folded 3D structure of two forms (prefusion and transient form) of HA protein were analyzed, and the most significant differences between the two forms were identified. The antigenic protein was designed to ensure that the structure of the HA protein would remain in the prefusion conformation without undergoing a transition to the postfusion conformation. The optimized HA sequence was used to develop vaccine candidates using mRNA technology, enabling rapid production in response to a pandemic situation. Balb/c mice were immunized with mRNA vaccine candidates twice, with a three-week interval between each dose. The levels of binding and neutralizing antibody titers were elevated in the groups which immunized with candidates including optimized HA. Furthermore, these groups showed a significant improvement in protective efficacy against a 10-fold lethal dose of infection, compared to the PBS-immunized group. These results suggest that antigen sequence design for H5N1, informed by AI software analysis, could be a promising approach for the development of RNA-based vaccines.

201

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hMPV impact in the older adults: a retrospective study in both 2022-2023 and 2023-2024 seasons.

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Background

The impact of human Metapneumovirus (hMPV) in older adults is not well understood. This study analyses two consecutive hMPV seasons in older adults hospitalized and attending the emergency room (ER).

Methods

A retrospective cohort study was performed including all >60 years old consecutive hMPV-confirmed hospitalizations and ER attended cases in two consecutive seasons (2022-2023, 2023-2024) in the Hospital Universitario Rio Hortega of Valladolid, Spain. Information was obtained from electronic medical records. Description and comparison of demographic and clinical features was performed. Severe hMPV cases met one of the following criteria: ≥11 days of hospital length of stay (LoS); ICU admission; in-hospital death.

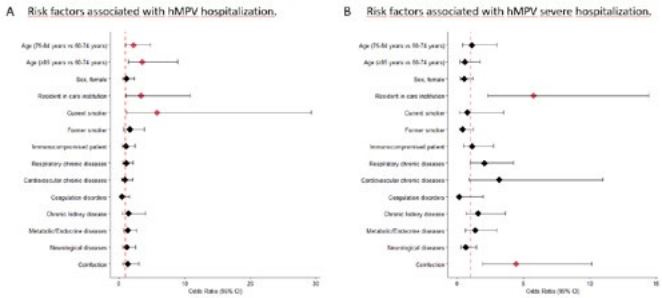
Results

276 hMPV-positive patients were enrolled (220 hospitalized, 79%; 56 ER, 21%). Compared to ER patients, hospitalized patients showed significantly higher mean age (80.7 vs 75.2 years) and were more frequently living in care institutions (27.0 vs 7.1%)(p<0.05)(Table 1). LoS was significantly higher in severe patients compared to non-severe patients (16.3 days, SD=10.5 vs 5.4 days, SD=2.3). 42(19.1%) of hospitalized patients spent ≥11 LoS, 10(4.5%) needed ICU-admission, 7(3.2%) needed mechanical ventilation, and 15(6.8%) died at the hospital. A logistic regression (Figure 1A) showed that patients had higher risk of hospitalization (compared with ER) if they were smokers (OR=5.79,CI95%=1.15-29.21;p-value=0.033), ≥85 years (OR=3.83,CI95%=1.78-8.23;p-value=0.001) or living in care institutions (OR=3.36,CI95%=1.04-10.83;p-value=0.042). Risk of severe hospitalization (compared to hospitalized patients)(Figure 1B) was higher in patients living in care institution (OR=5.75,CI95%=2.30-14.42;p-value=0.000) and coinfected (OR=4.43,CI95%=1.94-10.12;p-value=0.000).

Conclusion

The impact of hMPV infection in older adults is especially high in the older ones, smokers, those living in care institutions and those suffering coinfections.

Features	hMPV Hospitalized patients (n=220)	hMPV Emergency unit patients (n=56)	p-value
Age (years, SD)	80.7 (9.8)	75.2 (9.3)	0.000
Sex, female (n, %)	126 (57)	30 (54)	0.652
Resident in care institution (n, %)	59 (27)	4 (7.1)	0.001
Current smoker (n, %)	17 (7.7)	2 (3.6)	0.548
Former smoker (n, %)	49 (22.3)	13 (23.2)	0.548
Immunocompromised patient (n, %)	42 (19)	11 (20)	1.000
Respiratory chronic disease (n, %)	91 (41)	22 (39)	0.879
Cardiovascular chronic disease (n, %)	185 (84)	41 (73)	0.079
Coagulation disorders (n, %)	11 (5.0)	5 (8.9)	0.332
Chronic kidney disease (n, %)	50 (23)	6 (11)	0.061
Metabolic/Endocrine disease (n, %)	139 (63)	29 (52)	0.127
Neurological disease (n, %)	90 (41)	15 (27)	0.064


A Risk factors associated with hMPV hospitalization.
B Risk factors associated with hMPV severe hospitalization.

202

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Targeting host kinases in influenza A virus infection: antiviral potential of encapsulated GRK and PKC inhibitors

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Background: Worldwide circulating and emerging viruses represent a global burden for public health as known for the annual influenza A viruses (IAVs). Treating severe IAV cases remains an unresolved challenge as specific antiviral treatment strategies are rare and viruses can rapidly develop resistances. Since viruses depend on the host cell machinery for successful infection, host factors indispensable for the viral life cycle represent promising targets for therapeutic interventions. Recently, G protein-coupled receptor kinases (GRKs) or protein kinases C (PKCs) have emerged as potential players that support viral infection. However, the limited bioavailability, hydrophobicity and cytotoxic effects of some kinase inhibitors necessitate innovative approaches such as their incorporation into nanoparticles (NPs). Poly(lactic-co-glycolic acid) (PLGA) is one of the most widely used, FDA-approved, biocompatible, and biodegradable polymer. To enhance NPs performance, stealth polymers like polyethylene glycol (PEG) are commonly added. However, a growing number of individuals exhibit anti-PEG antibodies leading to hypersensitivity reactions. Therefore, alternative stealth polymers such as poly(2-ethyl-2-oxazoline) (PEtOx) are urgently needed. This project aims to incorporate existing kinase inhibitors into NPs for tailored treatment of host cell proteins essential for the IAV life cycle.

Methods: The impact of different GRK and PKC inhibitors on viral infection was investigated in cells infected with IAV (H1N1/A/Puerto Rico/8/34). Viral titers were analyzed via standard plaque assay and viral protein expression was determined by Western blot. Bisindolylmalamide-I (BIM-I), was encapsulated into PLGA-based NPs, which were further enhanced by incorporation of stealth polymers PEG or PEtOx. Particles were characterized (dynamic light scattering, UV–VIS spectroscopy, scanning electron microscopy) and evaluated for their cytotoxicity and antiviral potency. NPs were labeled with DY635 to analyze their uptake in different cell lines and in an **ex vivo** mouse lung model.

Results: Contrary to previous literature reports, the GRK inhibitors (paroxetine, CMPD101) did not decrease viral replication. In contrast, we identified two PKC inhibitors (Gö6983, BIM-I), which significantly inhibit IAV multiplication in A549 cells without affecting cell viability. The most effective inhibitor BIM-I was successfully incorporated into NPs based on different polymer compositions, exhibiting narrow size distribution and robust drug loading capacity. The encapsulation of BIM-I reduced cytotoxic effects of the compound while preserving its antiviral activity, especially for the PEG- and PEtOx-containing NPs. Particles were efficiently internalized by different cell lines (A549, Calu-3, MDCK cells) and **ex vivo** mouse lung tissue.

Conclusion: The NP-based delivery of BIM-I preserved its antiviral efficacy while improving its biocompatibility. These findings demonstrate the potential of targeting host kinases via encapsulated inhibitors as a novel treatment strategy to counter infections by respiratory viruses in future epidemics and pandemics, highlighting PEtOx as a viable alternative to PEG.

203

ECaS

RSV in older adults: a prospective multicentric cohort study in the primary care setting in Italy

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Background

Respiratory syncytial virus (RSV) is a leading yet under-recognised cause of acute respiratory infection (ARI) in older adults. Primary care data are indispensable to guide the introduction of newly licensed adult RSV vaccines in Europe.

Methods

A prospective cohort was set up in four Italian regions over three consecutive winters (2022–2025). Community-dwelling adults ≥60 years who consulted general practitioners (GPs) with WHO-defined ARI were swabbed and tested by multiplex RT-PCR for RSV plus 21 other viral and bacterial pathogens. Co-infection patterns across the most relevant pathogens were reported in Figure 1.. Telephone follow-up on day 14 and day 30 captured the clinical course of RSV cases.

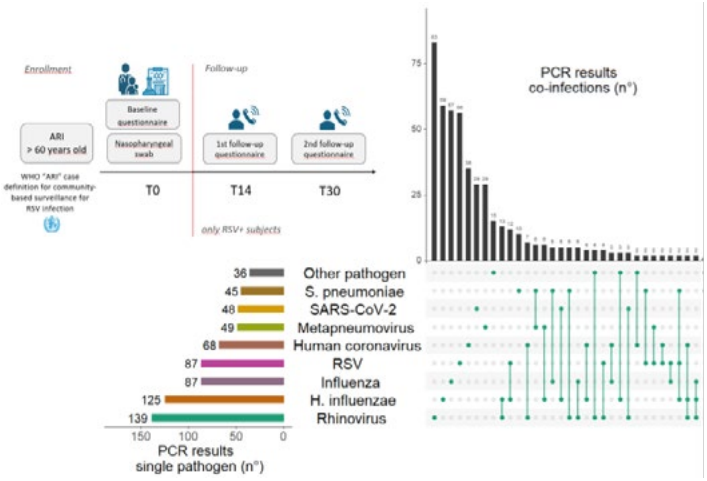
Results

Among 654 ARI episodes, RSV was identified in 87 patients (13%, median age 74 years); this proportion declined from 22% in 2022-23 to 10% in 2024-25. Single-pathogen detections predominated, yet 129/502 (25.7 %) episodes involved ≥2 agents. RSV featured in 31/129 (24%) mixed infections, most often paired with Haemophilus influenzae (n = 16). Other frequent RSV co-infections are highlighted by the intersections in Figure 1.

At enrollment RSV+ cases most commonly reported coryza (77%), productive cough (62%), fatigue (53%), and fever (35%). 89% of RSV subjects completed both follow-up interviews. Median RSV illness duration was 24 days (IQR 15–34), and 74 % of RSV patients re-contacted their GP, and 4 % visited an emergency department within day 30. All 2 RSV-related hospitalisations recorded by day 30 occurred in patients positive to RSV alone.

Conclusions

RSV accounted for 1/8 of all ARI recorded in adults ≥ 60 years and participated in 1/3 of co-infections, most frequently with H. influenzae. Taken together, vaccine-preventable pathogens were identified in almost 60 % of cases, highlighting the potential impact of integrated adult immunisation programmes. Coordinated deployment of newly licensed RSV vaccines alongside established ones could therefore meaningfully reduce the clinical burden of ARI in Europe's ageing population.


Enrollment
Follow-up
ARI
> 60 years old
WHO "ARI" case definition for community-based surveillance for RSV infection
Baseline questionnaire
Nasopharyngeal swab
1st follow-up questionnaire
2nd follow-up questionnaire
T0
T14
T30
only RSV+ subjects
PCR results co-infections (n°)
75
50
25
0
36 Other pathogen
45 S. pneumoniae
48 SARS-CoV-2
49 Metapneumovirus
68 Human coronavirus
87 RSV
87 Influenza
125 H. influenzae
139 Rhinovirus
150 100 50 0
PCR results single pathogen (n°)

206

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Assessing children's RSV hospitalization risk across 5 seasons in primary care setting in Italy

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Background

Respiratory syncytial virus (RSV) is a known cause of acute respiratory infection and long-term clinical burden in children, although information on the subject relies mostly on data collected from hospitals. Our study aims to explore RSV clinical burden in children under 5 years in the context of primary healthcare.

Methods

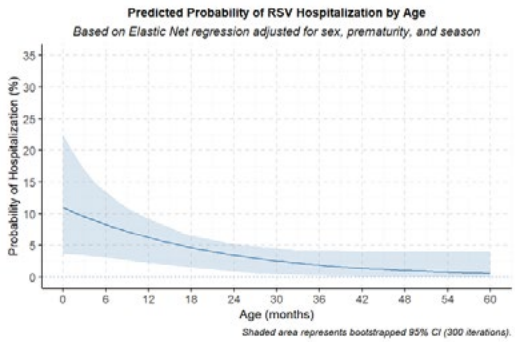
We conducted a prospective, multicenter cohort study across five Italian regions, enrolling children under 5 years old presenting to primary care pediatricians who met the WHO-ARI criteria. At enrollment (T0), naso-pharyngeal swabs were tested by multiplex RT-PCR for RSV and other respiratory pathogens. RSV-positive children underwent follow-up at 14 and 30 days. Primary outcomes included illness duration, symptom persistence, RSV-attributable hospitalization rate, and length of stay. We used an elastic-net logistic model to derive age-specific hospitalization risk with bootstrap-estimated 95% confidence intervals (CIs).

Results

1767 children were recruited during five winter seasons, from 2019-20 to 2024-25 (excluding 2020-21), of whom 711 (40.2%) tested RSV positive. At T0, RSV+ subjects most commonly had cough (95%), coryza (85%), and fever (56%), which is in line with the percentages of the overall ARI population. A different distribution was observed for dyspnea (36% overall, 44% RSV), wheezing (25% overall, 33% RSV), and sore throat (26% overall, 21% RSV). 636 (89%) parents of RSV+ children completed both follow-up interviews. The mean RSV symptom duration was 15.2 days (IQR 8 - 19), and hospitalization occurred in 32 (5%) cases, with a median stay of 6 days (IQR 4.5-7). Predicted age-specific hospitalization risk is reported in Figure 1.

Conclusions

Our data demonstrate that children presenting with RSV in primary care continue to face a notable risk of hospitalization. Accordingly, these results support considering RSV preventive strategies beyond the standard 24-month age limit to address the persistent vulnerability observed in older pediatric groups.



207

Exploring pan-antiviral targets for emerging viral infections: Mapping promising hot spots in viral RNA polymerases of influenza virus, betacoronaviruses and dengue virus

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Background:

Influenza, COVID-19 and Dengue were addressed as top global health threats (WHO), with scarce or non-existent therapeutic options. RNA Polymerases (Pol) have a central role in viral replication and pathogenesis. We recently disclosed targeting regions for new Pol inhibitors against influenza and COVID-19; and demonstrated that introducing mutations in these hotspots significantly impair virus replication **in vitro**. Considering the functional-structural similarities across viral Pol, we are currently focused on exploring influenza A virus (IAV) PB1, betacoronaviruses nsp12 and dengue virus (DENV) NS5-Pol.

Our study aims to identify-and-map conserved druggable regions (top hotspots) across these viral Pol that represent promising antiviral targets for the development of pan-Pol strategies.

Methods:

Viral Pol sequences from human-infection viruses were retrieved from public databases.

Final datasets included: 984 (IAV PB1); 1106 (betacoronavirus nsp12 - SARS-CoV, SARS-CoV-2, MERS-CoV) (until 2021); and 4127 (DENV1-4 serotypes NS5) (until 2023) sequences. Amino acid (aa) conservation was calculated by Valdar method and druggability was predicted for available Pol crystallographic structures using machine-learning algorithms in DoGSiteScorer and SiteFinder-MOE.

Results:

Pol are more conserved across distinct virus families when compared to other targeted proteins. We identified: 10 consensus druggable pockets (CDPs) within IAV PB1; 17 CDPs in betacoronavirus nsp12 – mostly placed in the fingers and palm protein subdomains. Notably, both CDP-9 (IAV) and CDP-10 (betacoronaviruses) represent putative binding pockets for favipiravir and molnupiravir – homologous target site; and have an important role in protein structure/function. In DENV NS5, 5 main CDPs with high druggability scores were identified, predominantly located in the fingers and thumbs subdomains. An ultimate sequence-to-structure protein comparison between the three viruses is in progress and will be presented at the conference.

Conclusions:

This study provides a structural basis for the development of broad-spectrum resistance-resilient antiviral strategies against distinct emerging viral diseases - that can prompt the discovery of next-generation pan-Pol inhibitors. Moving beyond the one-virus-one-drug approach, we seek to overcome current antiviral limitations and expedite the path from research to clinical applications. Our work has also the potential to enhance global health preparedness by advancing next-generation broad-spectrum antivirals for viruses with limited or no therapeutics, ensuring improved outbreak readiness.

212

A Blueprint for Far-UVC Use in Influenza Control

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Background: Seasonal influenza remains a leading cause of global illness and death, and the potential for a future influenza pandemic continues to pose a major threat to global health. While conventional approaches such as vaccines, therapeutics, ventilation, and filtration remain critical, they are not always sufficient to reduce exposure risk in shared indoor environments. Far-UVC (200–235 nm), a form of germicidal ultraviolet light, is an emerging technology that may provide an additional layer of protection by continuously inactivating pathogens suspended in indoor air—without requiring behavioral compliance or occupancy restrictions.

Methods: To assess the feasibility of far-UVC as a practical tool for influenza and pandemic preparedness, we conducted a multidisciplinary synthesis involving literature review, quantitative modeling, and structured input from more than 100 experts across virology, public health, indoor air quality, photobiology, and engineering. We evaluated far-UVC's performance across key domains, including viral inactivation, safety, air chemistry, materials compatibility, and regulatory alignment.

Results: Evidence to date suggests that far-UVC can effectively inactivate influenza viruses under exposure levels that remain within current safety thresholds. Its strong absorbance in proteins limits penetration into skin and eye tissues, enabling safe use in occupied spaces. Far-UVC also offers operational advantages—such as silent, low-maintenance operation and compact form factors—that may enable deployment in a wider range of environments than existing germicidal UV systems.

Nonetheless, important scientific and technical questions remain:

How well far-UVC inactivates influenza in human respiratory aerosols,

What measurement protocols should guide efficacy claims,

What biological responses are triggered at chronic, low-level exposures,

How far-UVC interacts with real-world indoor air chemistry, and

How best to optimize installation and dosing for diverse room geometries and ventilation regimes.

Conclusions: Far-UVC is unlikely to serve as a stand-alone intervention, but it may offer meaningful reductions in exposure to infectious aerosols when used alongside other controls. Its passive and scalable nature make it a promising candidate for risk reduction in high-traffic public spaces. This presentation will share our synthesis of the current evidence, highlight research priorities for the scientific community, and outline conditions under which far-UVC could contribute to long-term influenza mitigation and pandemic preparedness.

213

Comprehensive Clinical Characterization of RSV-A-Memphis 37b Human Challenge Model: Historical Analysis from 16 Clinical Trials (2017-2025)

Alexandre LIMA, Rachna GOBURDHUN, Brandon LÖNDT, Andrew CATCHPOLE, Mariya KALINOVA

HVIVO services, United Kingdom

Background: Respiratory Syncytial Virus (RSV) remains a significant cause of morbidity among vulnerable populations, particularly young children and elderly, highlighting the urgent need for effective vaccines and treatments. The human viral challenge model has emerged as a rapid and efficient approach for developing and assessing these interventions. Over an eight-year period (2017-2025), this study demonstrates the safety profile characteristics of the RSV-A-Memphis 37b challenge strain across multiple clinical trials, providing essential reference data for researchers and sponsors.

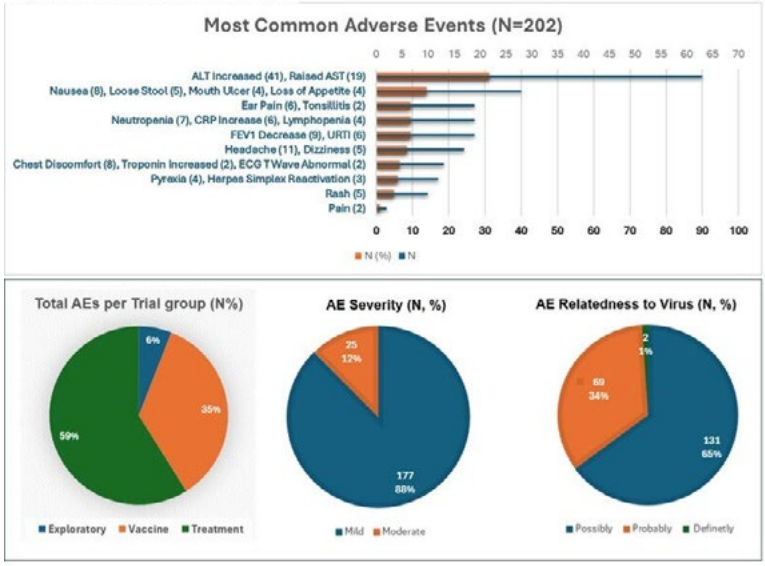
Methods: A retrospective analysis of HVIVO's historical data from 16 RSV challenge trials conducted between 2017 and 2025 included 1074 healthy adult participants inoculated with the RSV-A-Memphis 37b strain. Adverse events (AEs) following viral challenge were categorized by trial type (exploratory, vaccine, or treatment) to evaluate their distribution across study cohorts. Only AEs plausibly associated with the virus were analyzed, with their relationship classified as "possibly," "probably," or "definitely" related based on investigator judgment and exclusion of alternative causes. Six subclinical myocarditis cases related to RSV were reported without complications and detailed elsewhere. [(1)]

Results: Among 1074 inoculations, 202 virus-related AEs were recorded, with an incidence rate of 18.8%. The majority of AEs were mild (88%, n = 177), with moderate AEs comprising the remainder (12%, n = 25). AEs were most prevalent in the treatment group (59%), followed by the vaccine (35%) and exploratory (6%) groups. Analysis of AE relatedness to RSV revealed 65% (n = 131) as possibly related, 34% (n = 69) as probably related, and 1% (n = 2) as definitively linked. Elevated liver enzymes (ALT increased: n = 41; AST raised: n = 19) were the most frequently reported AEs, followed by gastrointestinal symptoms. Less common AEs included respiratory symptoms (e.g., FEV1 decrease) and systemic events such as headache, rash, and fever (Fig 1).

Conclusions: Data analysis demonstrates an excellent safety profile for RSV-A-Memphis 37b challenge studies, with a low incidence of AEs predominantly classified as mild. Transient elevations in liver enzymes represented the most frequent finding, all of which resolved without sequelae. The prominence of hepatic and gastrointestinal manifestations underscores significant extrapulmonary presentations of RSV infection, potentially overlooked in community settings. These findings validate the RSV challenge model as a robust platform for expediting interventions against this virus, particularly crucial for vulnerable populations.

1.Incidental Myocarditis in Healthy Adults Following RSV Inoculation in Human Infection Studies. 13th International RSV Symposium, 12-15 March 2025, Iguazu Falls, Brazil.

Figure 1. Adverse Events Characteristics



214

Matrix-M adjuvant – enhancing the fight against respiratory pathogens

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Background:

Adjuvants are key tools to improving immunogenicity and efficacy of vaccines by stimulating the immune system. Saponin-based Matrix-M® adjuvant induces potent and durable immunity through production of long-lasting memory B-cells and broad-based T-cell immunity, with the potential to be antigen-sparing,[1,2] across a variety of vaccine platforms targeting different disease areas. In particular, Matrix-M–containing vaccines have demonstrated success in addressing respiratory diseases (COVID-19 and influenza)[3-6] and malaria[7] with a foundation of evidence supporting favorable vaccine safety and tolerability critical to ensuring vaccine uptake and completion of multi-dose regimes.[8,9]

Methods:

We completed a targeted review of published randomized clinical trials (RCTs) in which Matrix-M–containing vaccines were evaluated to provide an overview of the clinical experience. For vaccines with published results from Phase 2–4 clinical studies, we summarize safety and tolerability. Pre-clinical studies were also reviewed.

Results:

The versatility of Matrix-M was demonstrated through its successful application as an adjuvant in a broad array of vaccine platforms and targeted diseases (Figure 1). The efficacy, safety, and tolerability of Matrix-M–containing vaccines was assessed with varying numbers of doses in primary immunizations, boosting of immunity, and at different amounts of adjuvant. Beyond pre-clinical studies,[10-12] the clinical experience features 38 RCTs including >62,000 participants who received at least 1 dose of Matrix-M–containing vaccines.

Among adults, Phase 2 and 3 RCTs of Matrix-M–containing vaccines have been conducted for COVID-19,[3,4] influenza,[5,13] and combination COVID-19/Influenza (CIC).[6] Results from placebo-controlled RCTs and active-comparator RCTs (comparing to marketed influenza vaccines)[5,6,13] revealed acceptable tolerability with predominantly mild-to-moderate and transient solicited local and systemic events. Rates of severe adverse events (SAEs), adverse events of special interest (AESI), and autoimmune AEs were similar between vaccine and comparison groups (placebo or influenza vaccine active comparator). Post-authorization studies of Matrix-M–adjuvanted COVID-19 vaccines indicated favorable reactogenicity profiles and lower negative impacts on quality-of-life measures compared to licensed mRNA COVID-19 vaccines.[14,15]

Matrix-M–containing vaccines have been evaluated in pediatric populations for prevention of COVID-19 (ages ≥12-year-old)[16,17] and malaria (ages 5–36-month-old)[7] with similar rates of unsolicited AEs, SAEs, and AESI observed between Matrix-M–containing vaccines and comparator groups.

Conclusions:

RCTs and phase 4 studies support the efficacy, safety, and tolerability of Matrix-M–containing vaccines across an array of vaccine types and target antigens. Ongoing research efforts are focused on improving our understanding of the adjuvant's mechanism of action, developing new (including combination) vaccines to address additional disease areas, and identifying novel applications (e.g., immuno-oncology).

Figure 1: Vaccine Platforms and Diseases Areas

Purified recombinant proteins[10,11,18-20]	• COVID-19 – Ph3 • Malaria – Ph2b • HSV Type 2 – Ph2 • West Nile – Pre-clinical • Rabies – Pre-clinical	Whole or detergent-split inactivated viruses[10]	• Influenza – Ph1
Adenoviral-vectored vaccines[10,21]	• Malaria – Ph2b • Influenza – Pre-clinical	Virosomes[10,22]	• H5N1 – Ph1
Nanoparticles[2,5,10,23,24]	• Influenza – Ph3 • COVID-19 – Ph1/2 • Ebola – Ph1 • Epstein-Barr – Ph1	Virus-like particles[7,10]	• Malaria – Ph3 • H7N9 – Ph1/2

215

Understanding the Risks and Benefits of Delaying Seasonal Influenza Vaccine Recommendations

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Background: The advent of new technologies for vaccination has generated significant discussion around the timing of the seasonal influenza strain recommendation. Multiple studies have assessed the potential for improvement for genetic, and antigenic match, of influenza vaccines to circulating viruses, by altering the timing of vaccine composition decisions. The window between the seasonal influenza vaccine recommendation and availability of influenza vaccines for immunisation, for any platform, is comprised of sequential processes required to produce vaccine components and reagents for manufacture and release, including regulatory approvals. These factors determine the time needed to ensure the manufacture of the large numbers of doses for administration prior to the peak of the influenza season.

Methods: Timing for key steps for candidate vaccine virus generation, manufacture and release of influenza vaccines were collated.

Results: We present a summary of requirements for enabling timely manufacture and release of seasonal influenza vaccine, detailing past challenges to vaccine delivery and collaborative strategies used to overcome these.

Conclusion: All influenza vaccine platforms require technical process steps and regulatory approvals, for the timely development and supply of vaccines, to protect the global population from seasonal influenza.

218

ECaS

Assessment of Genotypic and Phenotypic Resistance Profiles in Avian and Swine Influenza A Viruses Isolated in Korea

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Background

Influenza A viruses (IAVs) circulate widely among livestock populations in Korea, causing substantial economic losses and posing an increasing risk of zoonotic transmission. The intensification of livestock farming and increased inter farm contact facilitate viral dissemination across regions, enhance host adaptation, and promote the accumulation of genetic mutations. Given these concerns, this study aimed to investigate the antiviral resistance potential of IAVs from swine and avian sources in Korea.

Method

In this study, we investigated antiviral resistance profiles in 41 IAV isolates—21 of swine origin collected from domestic farms and 20 of avian origin from migratory birds in Korea. Possible resistance-associated mutations in the NA and PA genes were identified in a subset of isolates such as PA-I38V and PA-K34R in swine-origin H1N1/H3N2 viruses, and NA-D198E and NA-S246G in avian-origin H4N6/H3N6/H8N6 strains. Phenotypic susceptibility to oseltamivir was assessed using a MUNANA-based neuraminidase inhibition assay, and susceptibility to baloxavir was assessed through a plaque reduction assay IC50 values were determined and compared with the reference strain A/Puerto Rico/8/1934 (H1N1).

Results

Despite the presence of antiviral resistance mutations, none of the viruses exhibited significant resistance to either of the two antiviral agents, with IC50 values below 10nM.

Conclusions

These findings emphasize the importance of regular phenotypic testing alongside genetic surveillance for accurate antiviral resistance monitoring.

219

ECaS

Risk Assessment of the Zoonotic Potential of Swine Influenza A Virus in Korea

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Background

Swine influenza A viruses (swIAVs) are recognized as potential mixing vessels for interspecies transmission due to their ability to bind both avian- and human-type receptors. Since the 2009 H1N1 pandemic, over 300 cases of human infection with swIAVs have been reported in North America, raising concerns about the potential for a future pandemic caused by swIAVs. Therefore, assessing their zoonotic potential is essential for preparedness against the next pandemic.

Methods

In this study, we isolated swIAVs from domestic pigs in Korea between 2021 and 2024 and conducted a risk assessment using a decision-making tree developed based on the Influenza Risk Assessment Tool (IRAT). To evaluate the zoonotic potential of the isolated viruses, we first performed a solid-phase binding assay to determine receptor specificity. We then assessed viral replication kinetics in polarized human bronchial epithelial cells cultured at the air-liquid interface, followed by measuring fusion pH to determine acid stability. Additionally, we conducted hemagglutination inhibition (HI) assays using human sera from individuals who had experienced seasonal flu to evaluate the cross-reactive immunity between seasonal flu and swIAVs. We also analyzed antiviral resistance profiles with a focus on neuraminidase.

Results

Our results demonstrated that the swIAVs isolated in this study exhibited strong binding to α2,6-linked sialic acid (human-type) receptors and replicated efficiently in human airway epithelial cells. Moreover, swIAVs exhibited limited cross-reactivity with human sera, suggesting a lack of pre-existing immunity against swIAVs in humans.

Conclusions

These findings underscore the importance of ongoing surveillance and risk assessment of swIAVs to ensure timely control of cross-species transmission.

220

Enhancing RSV surveillance in older adults: A cluster randomized study to compare existing and to propose novel clinical case definitions

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Background: In Europe, surveillance of respiratory syncytial virus (RSV) has been recently incorporated into existing influenza monitoring platforms. Case definitions vary: some countries use influenza-like illness (ILI) definitions, other countries apply a broader acute respiratory infection (ARI) case definition, while still other countries use both ILI and ARI criteria. No RSV-specific case definitions are currently available. Here, we compared RSV detection rates between the ARI and ILI surveillance networks and developed an alternative RSV-specific case definition.

Methods: The study was conducted in Genoa (Italy) during the 2023/2024 and 2024/2025 seasons. Thirty-eight general practitioners (GPs) were randomized 1:1 to enroll individuals ≥50 years seeking care for ARI or ILI, respectively. The current ECDC criteria were used to define both ARI and ILI and all outpatients were molecularly tested for RSV and 23 other pathogens. To account for clustering, generalized estimating equations (GEE) were used to study the association between RSV positivity and surveillance group.

Results: Of 1,431 patients, 5.2% tested positive for RSV. Of 75 RSV cases, 62.7% were mono-detections. RSV detection rate in the ARI group (5.8%; 43/741) was higher than in the ILI group (4.6%; 32/690). In the base-case GEE model, the odds of any RSV and RSV mono-detections in the ARI versus ILI groups were 26% and 51%, respectively, higher. These results were robust in different sensitivity analyses. Notably, when GPs with unexpectedly low enrollment rates (<30) were excluded, the odds ratios (ORs) increased up to 1.80 (Figure 1). Conversely, the prevalence of SARS-CoV-2 (OR 0.57; 95% CI: 0.34–0.95) and influenza A (OR 0.83; 95% CI: 0.47–1.44) was higher in the ILI group. Positivity for RSV was associated with several respiratory but not systemic symptoms (Figure 2). Based on different classification rules, the RSV case definition of ARI with wheezing and/or productive cough and/or rhonchi and/or dyspnea performed reasonably well (sensitivity 92.0%; specificity 30.8%).

Conclusions: Compared to a highly sensitive ARI definition, ILI-based surveillance may underestimate the burden of RSV. However, specificity of the ARI criteria is low, which may hinder vaccine effectiveness studies. If externally validated, the proposed RSV case definition might be of value.

Figure 1. Association between positivity for respiratory syncytial virus (RSV) and surveillance group.

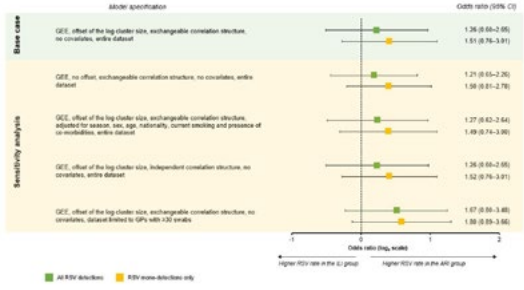
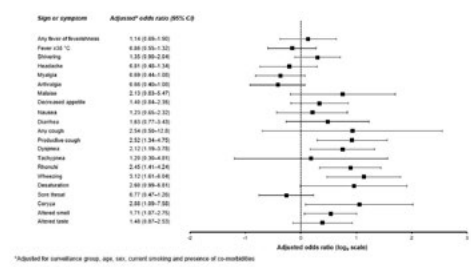


Figure 2. Association between positivity for respiratory syncytial virus (RSV) and signs and symptoms reported by adults aged ≥50 years.



221

ECaS

Machine Learning Assessment of Zoonotic Potential in Avian Influenza Viruses using PB2 Segment

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Background: Influenza A virus (IAV) is a major global health threat, causing seasonal epidemics and occasional pandemics. Particularly, Influenza A viruses from avian species pose significant zoonotic threats, with PB2 adaptation serving as a critical first step in cross-species transmission. A comprehensive risk assessment framework based on PB2 sequences is necessary, which should encompass detailed analyses of specific residues and mutations while maintaining sufficient generality for application to non-PB2 segments.

Methods: In this study, we developed two complementary approaches: a regression-based model for accurately distinguishing among risk groups, and a SHAP-based risk assessment model for more meaningful risk analyses. For the regression-based risk models, we compared various methodologies, including tree ensemble methods, conventional regression models, and deep learning architectures. The optimized regression model, combined with SHAP value analysis, identified and ranked individual residues contributing to zoonotic potential. The SHAP-based risk model enabled intra-class analyses within the zoonotic risk assessment framework and quantified risk yields from specific mutations.

Results: Experimental analyses demonstrated that the Random Forest regression model outperformed other models in most cases, and we validated the target value settings for risk regression through ablation studies. Our SHAP-based analysis identified key residues (271A, 627K, 591R, 588A, 292I, 684S, 684A, 81M, 199S, and 368Q) and mutations (T271A, Q368R/K, E627K, Q591R, A588T/I/V, and I292V/T) critical for zoonotic risk assessment. Using the SHAP-based risk assessment model, we found that influenza A viruses from **Phasianidae** showed elevated zoonotic risk scores compared to those from other avian species. Additionally, mutations I292V/T, Q368R, A588T/I, V598A/I/T, and E/V627K were identified as significant mutations in the **Phasianidae**.

Conclusion: These PB2-focused quantitative methods provide a robust and generalizable framework for both rapid screening of avians' zoonotic potential and analytical quantification of risks associated with specific residues or mutations.

222

ECaS

The Acquisition of the PB2 G590S Mutation Enhances Viral Replication of Canine H3N2 Influenza Virus

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Background

H3N2 canine influenza viruses (CIVs) have circulated in China and Korea, resulting in multiple intercontinental transmissions to North America. A/canine/Korea/SH6/2017 (SH6), previously isolated in South Korea, was identified as a precursor of the 2018 North American CIV outbreaks. Among the mutations acquired in South Korea, a glycine-to-serine substitution at position 590 (G590S) in PB2 has been hypothesized to enhance viral replication and adaptation in mammalian hosts.

Methods

To investigate the functional role of the PB2 G590S mutation, we used a reverse genetics system to generate recombinant SH6 viruses with either the wild-type PB2 (G590) or the mutant PB2 (S590). Viral replication kinetics were assessed in MDCK and Calu-3 cells at a low multiplicity of infection (MOI). Polymerase activity was examined using a mini-genome assay. In vivo replication was evaluated in a mouse infection model.

Results

Reversion of the mutation (S590G) significantly reduced viral polymerase activity and impaired replication in both MDCK and Calu-3 cells. In the mouse model, robust replication was observed only in animals infected with rgSH6, while no replication was detected in those infected with the rgSH6-S590G mutant.

Conclusions

Our findings demonstrate that the acquisition of the PB2 G590S mutation between 2016 and 2017 enhances viral polymerase activity and supports efficient replication in mammalian cells and mice. These results suggest that the PB2 G590S mutation may facilitate CIV infection in mammalian hosts by enhancing viral fitness under suboptimal conditions.

223

Emerging role of wild canids in the ecology of HPAI H5: insights from Croatian red foxes and golden jackals

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Background:

Highly pathogenic avian influenza (HPAI) viruses, in particular the subtype H5N1 from clade 2.3.4.4b, have recently been detected in various non-avian species, including wild canids. Among them, the red fox (**Vulpes vulpes**) has become a prominent host in Europe, with an increasing number of reported cases. Genomic studies of HPAI isolates from foxes have identified mutations in viral polymerase genes that enhance replication in mammalian cells and facilitate neuroinvasion. The golden jackal (**Canis aureus**), another wild canid that is expanding its range due to anthropogenic factors, is still largely unexplored in this context. Our aim was to investigate the possible infection with HPAI in apparently healthy wild canids, particularly golden jackals, collected as part of national rabies surveillance.

Methods:

Samples were collected between September 2024 and March 2025 in all Croatian counties. Brain tissue from 151 red foxes and 33 golden jackals was analysed for the influenza A virus (IAV) M gene using real-time RT-PCR. Muscle extracts for serology were collected from 368 dead wild canids — 265 red foxes and 103 golden jackals and screened for antibodies against the IAV nucleoprotein (NP) using ELISA. Positive samples were tested for haemagglutinin H5-specific antibodies by competitive ELISA.

Results:

During the six-month period, no IAV RNA was detected in the brain tissue of wild canids. However, serological evidence of previous infection with IAV H5 was found in 1.13% of foxes in the counties of Zagreb, Karlovac and Split-Dalmatia. One third of the foxes that tested positive for H5-specific antibodies were adult red foxes from Zagreb County. No IAV antibodies were detected in golden jackals.

Conclusions:

These results indicate a limited but measurable exposure of red foxes to IAV H5 viruses in Croatia, which is consistent with similar reports from across Europe. The absence of viral RNA in brain tissue suggests that there was no active IAV infection at the time of sampling. However, the H5-specific antibody-positive foxes originated from the counties where HPAI was continuously detected in mute swans (**Cygnus olor**) in 2024, emphasising the possible role of red foxes as incidental hosts. Although golden jackals showed no evidence of exposure in this study, their ecological spread and increasing overlap with avian habitats warrant further surveillance. Detection of IAV H5 antibodies in wild canids, regardless of the absence of symptoms, has implications for both wildlife conservation and public health, as further adaptation of the virus to mammalian hosts is possible. Continuous serological and virological monitoring of wild carnivores is essential for the early detection of spillover events and emerging zoonotic threats.

225

PCR diagnostics respiratory pathogens in routine and sentinel surveillance in Montenegro, 2024-2025

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BACKGROUND

Virological surveillance is performed by the National Influenza Centre (NIC), located at the Center for Medical Microbiology, Institute of Public Health Montenegro (IPHMN). Clinical samples are obtained from patients consulting GPs in sentinel sites, from non-sentinel sources and from hospitalized patients. Through sentinel surveillance, samples are tested for influenza, SARS-CoV-2 and RSV. The aim of this study was to describe what influenza strains were circulating, related to data received from epidemiological/ virological surveillance in population of Montenegro.

METHODS

This study was conducted as a part of the national sentinel surveillance system for influenza in Montenegro in 2024-2025. Laboratory diagnostics of all suspected human influenza cases are carried out in the Department for Molecular Diagnostics in IPHMN. Typing and subtyping of influenza were performed by CDC real-time RT-PCR assay for the **in vitro** qualitative detection and characterization influenza virus in respiratory specimens A (H1/H3/H5) as well as influenza B lineages (B/Yamagata and B/Victoria).

RESULTS

During 2024-2025, in total 2030 specimens were tested for influenza, out of them 1419 (69,9%) were non-sentinel and 611 (30,1%) sentinel samples.

Influenza virus infection was laboratory confirmed in 542 (38.2%) cases, out of the total tested routine non-sentinel samples (1419). Influenza A infection were detected in 62.18%, influenza B were detected in 37.82% (205/1419). A positive result for influenza A/H1 was dominant (85.7%); influenza A/H3 were detected in 14.3%. All Influenza B positive samples were Influenza B Victoria lineage.

All sentinel samples were tested for influenza, SARS-CoV-2 and RSV. Of the total 611 tested samples, 18.98% (116/611) were positive for Influenza A; 27.33% (167/611) for Influenza B; 9.82% (60/611) samples were positive for RSV, and 3.27% (20/611) samples were positive for SARS-CoV-2. Coinfection with SARS-CoV-2/Influenza was detected in 3 cases as well as Influenza/RSV coinfection in 6 cases. Among all confirmed influenza A sentinel cases, 87 samples (75%) were influenza A/H1 and 29 (25%) samples were A/H3. All Influenza B sentinel positive samples belong to the Victoria lineage.

CONCLUSIONS

The 2024-2025 influenza season in Montenegro was characterized by a dominance of influenza type A viruses (with mixed circulation of both A/H1 and A/H3 influenza subtypes) and for influenza B virus predominance Victoria lineage appearance. Laboratory monitoring of circulation of influenza and other respiratory viruses will provide realization of effective prophylactic and anti-epidemic measures to prevent and improve care to influenza-infected people, especially risk group part of population.

229

Ancestral SARS-CoV-2 vaccine induces strong long-lasting cross-reactive and polyfunctional T-cell responses to future strains in healthy older adults

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1: Norwegian Institute of Public Health;2: Oslo University Hospital

Background: Adults over 65 years are at higher risk of severe COVID-19 and death and therefore one of the main target groups of COVID-19 vaccination. Annual booster vaccination is currently recommended, often with reference to waning antibody responses, yet it is unclear how optimal this frequency is for cellular immunity against SARS-CoV-2. Vaccine induced T-cell responses are crucial for viral clearance and reducing disease severity. To inform further vaccine policy, we studied the magnitude and quality of T-cell and humoral responses after multiple boosters and infections until May 2024 in healthy older adults.

Methods: We established a longitudinal cohort of healthy adults aged 65–80 years (The Senior cohort) in 2020. Blood samples were collected at six consecutive time points from Dec 2020 (T0) to May 2024 (T6). Information on vaccinations and infections was obtained from national health registries and regular questionnaires. IgG levels against spike, nucleocapsid (T0-T6) and membrane (T6) were measured using a bead-based flow cytometric method and neutralising antibodies (T4-T6) in virus neutralisation assays. Spike specific CD4 and CD8 T-cell memory responses (T3-T6) to former and future virus variants were detected using activation induced marker (AIM) assays. Polyfunctionality (expression of two or more of the functional markers CD40L/CD154, CD137/4-1BB, IFN-γ, TNF-α, and IL-2) was estimated using a Bayesian statistical framework through the COMPASS algorithm. The influence of different factors on T- cell responses were evaluated with a generalized linear mixed model.

Results: All participants had taken three doses of mRNA vaccine by T3 (Jan 2022), and up to six doses by T6 (May 2024). 86.8% and 47.4% were seropositive for membrane and nucleocapsid respectively, whereas 60% of participants had a reported infection. Neutralising antibody titers against B1 were high (T4, T5, T6), and increased against XBB.1.5, but remained poor against JN1. Spike specific CD4 and CD8 T-cell effector memory responses were good at T3 (3 doses uninfected) against B.1, BA.1, XBB.1.5 and BA.2.86, and no significant differences were measured at T4, T5 and T6. Frequencies were similar for those with and without an XBB.1.5 booster or recent infection (T6). Polyfunctionality scores (PFS) were also comparable across time points, but significantly lower for BA.2.86 at T6.

Conclusions: After the first booster, CD4 and CD8 spike specific T-cell frequencies and quality of responses remained consistent in healthy older adults, during a period of several years with repeated boosters or infections. Cross-reactivity to new Omicron variants (XBB.1.5/BA.2.86) already existed in samples from early 2022, long before their circulation, indicating that three doses of the original Wuhan vaccine generated good T-cell cross-reactivity against future divergent strains. In contrast, neutralising antibody titres decreased with the emergence of new SARS-CoV-2 strains.

230

Unmasking The Trends: How The COVID-19 Pandemic Transformed Acute Respiratory Illness Testing and Diagnostics

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Background:

Clinical signs and symptoms for acute respiratory infections are not pathogen specific, and diagnoses are often supported by tests. An increase in the availability of tests due to the COVID-19 pandemic has likely improved pathogen specific diagnosis, however, to date, real world diagnostic and testing patterns for acute respiratory illness have not been well described in the US. This assessment of changing testing and diagnostic patterns is necessary to better inform evidence generation activities that rely on testing and diagnostic data from healthcare systems. A change in diagnostic testing practices could influence the interpretation of these analyses especially if practices change suddenly e.g. policy changes implemented during a pandemic. The purpose of this analysis is to describe the real-world patterns of testing and diagnosis coding of acute respiratory illnesses and assess how the patterns have changed in the US, especially in response to the COVID-19 pandemic.

Methods:

We will use claims data from Merative™ MarketScan® Commercial and Medicare Databases from January 2016 to June 2024 to describe ARI associated testing and diagnosis coding trends in outpatient and inpatient settings. We will use Current Procedural Terminology® and Healthcare Common Procedure Coding System codes to represent medical procedures (including testing), provided to patients seeking care for respiratory illness. We will explore an exhaustive list of different viral respiratory tests including but not limited to PCR (including multiplex), culture and rapid diagnostics. International Classification of Diseases (ICD-10) codes will be used to identify diagnosis codes for ARIs (syndromic approach) and viral aetiologies including influenza, SARS-CoV-2, Respiratory Syncytial Virus (RSV), human metapneumovirus (hMPV) and rhinovirus. Trends in testing and diagnoses will be compared over time, and especially between the pre- and post-COVID-19-pandemic period.

Results:

Results are not yet available, but we hypothesise that over the 8-year study period, we expect to see substantial increases in the number of tests ordered in the outpatient setting, particularly PCR tests. This will likely correspond with a rise in the use of specific virus-associated ICD diagnostic codes in the inpatient care setting, particularly for non-influenza viruses and adult age groups. We expect the COVID-19 pandemic to also be a key catalytic point leading to increased testing and more frequent use of specific ICD diagnostic codes.

Conclusion:

The COVID pandemic marked a shift in availability of laboratory tests, which we expect will have impacted diagnostic coding trends in outpatient and inpatient claims data. These changes need to be considered when utilizing testing and diagnosis data pre- and post-pandemic and evaluating trends in disease burden for respiratory viruses.

231

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Hospitalizations associated with circulation of influenza, RSV and other neglected respiratory viruses in the USA, 2016-2024

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Background:

The emergence of SARS-CoV-2 in late 2019 and the mitigation measures put in place to curb the pandemic's spread have affected the circulation pattern of other respiratory viruses. Establishing disease burden estimates for respiratory viruses is often difficult due to the non-systematic use of laboratory testing as part of standard of care. Instead, researchers use statistical approaches, such as time series modelling, to evaluate the burden of a pathogen. Understanding the contribution of different respiratory viruses to morbidity across age groups is invaluable for informing healthcare resource prioritization, especially with the expanding portfolio of seasonal vaccines for older adults.

Methods:

We will create age-specific time-series models from 2016 through 2024, to estimate the contribution of influenza and RSV to broader non-specific respiratory coded hospitalization in the US. This model will use ICD-coded hospitalization claims data from Merative™ MarketScan® and incorporate weekly influenza and RSV surveillance as covariates from the CDC/NVERSS system along with terms for seasonal and secular trends. We will also consider SARS-CoV-2 surveillance data to estimate the fraction of hospitalisations that could be associated with COVID-19, the inclusion of HMPV and rhinovirus data will also be explored. In addition, we will use testing and diagnostic data from 2016-2024 to understand any changes in routine testing and diagnostic practices in hospitals due to the COVID-19 pandemic and adjust the model accordingly.

Results:

Our integrated modelling approach will quantify the comparative disease burden of major respiratory viruses by combining specific discharge codes with modelled "excess" hospitalizations from broader diagnostic categories. Leveraging time periods marked by low virus circulation and out-of-season rebounds, will allow us to disentangle the joint burden of respiratory viruses that typically co-circulate. Age-stratified analysis could reveal distinct patterns of vulnerability across demographic groups, with notable seasonal variations in virus predominance. We expect this analysis to demonstrate that both influenza and RSV maintain substantial contributions to respiratory hospitalization burden even in the post-pandemic period. Preliminary exploration of HMPV and rhinovirus data will reveal underappreciated contributors to the overall respiratory disease landscape, warranting further investigation.

Conclusions:

This comprehensive time-series analysis will provide valuable insights into the comparative disease burden of major respiratory viruses across different age groups in the US. We expect our findings will underscore the continued significance of influenza and RSV and the importance of other respiratory viruses in this evolving landscape, where SARS-CoV-2 continues to circulate. Future work will further refine these models to reflect changing epidemiological patterns, ultimately supporting more effective respiratory disease prevention and management.

233

Salivary antibodies against SARS-CoV-2 in children and adults during the first waves of the COVID-19 pandemic in Norway

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Background

Infection with SARS-CoV-2 infection induces antibodies against several viral proteins, both in serum and in the mucosa. We conducted a COVID-19 transmission and immune response study between May 2020 and June 2021. Two main types of viruses were circulating, first ancestral Wuhan-like viruses and then Alpha B.1.1.7. Here, we report IgA levels in saliva against the two different virus types, and factors influencing the salivary antibody response.

Methods

SARS-CoV-2 infection was PCR-confirmed. Saliva and sera, questionnaires and symptom diaries were collected longitudinally starting on the day of inclusion (D0) until Day 42 (D42).. IgA levels in saliva were measured against the ancestral Wuhan antigens spike (subunit S1), receptor binding domain (RBD) and nucleocapsid (N) by an in-house multiplex immunoassay using the Bio-Plex 200 system (BioRad) with an anti-SARS-CoV-2 antibody (21/234 NIBSC, Potters Bar, England) as reference.

Results

In total, 205 of 216 participants had at least one IgA measurement in saliva; 68.3% were infected. Among the infected, 52.1% were females, the median age was 32.7 years (range 2-71 years) and 23.6% were children (<18 years). Children had 9-fold lower viral loads than adults (p=0.005). Anti-SARS-CoV-2 IgA levels in saliva were generally low, with a peak on D14, compared to D42 for IgG in serum. Fold-increase was lower for IgA against N (~2), than for spike and RBD (~5- and 3-fold, respectively). On D14, adults and children had similar levels of IgA against spike and RBD, but the level of anti-N was 2.5 times higher in the adults.. Anti-SARS-CoV-2 IgA levels on D0were twice as high in adults as in children, both among infected cases and uninfected individuals. Waning of anti-spike and anti-RBD IgA levels seemed faster in children than in adults. Virus type did not seem to influence antibody levels. Individuals reporting fever had higher anti-SARS-CoV-2 IgA levels on D14 than individuals without fever.

Conclusions

Anti-SARS-CoV-2 IgA levels were found in saliva during infection, and the IgA response peaked earlier after symptom onset compared to IgG in serum. Fever was associated with IgA levels, whereas virus type was not. Children had lower viral loads and lower levels of anti-N IgA both pre- and post-infection.

235

Disparities in Access to Enhanced Vaccines for Older Adults: Addressing Equity Gaps Between Developed Nations in the Northern Hemisphere and Latin America (LATAM)

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Introduction

The WHO's Decade of Healthy Aging (2021–2030) aims to improve life quality for older adults. The global population aged 60+ is expected to grow from 1 billion in 2019 to 1.4 billion by 2030, with significant increases in developing countries. By mid-century, 80% of older adults will live in less developed countries. This poses challenges especially in emerging economies, where disparities in longevity demand inclusive and equitable aging strategies. Older adults experience immunosenescence, impairing vaccine responses and increasing morbidity and mortality. The CDC and ACIP recommend enhanced influenza vaccines—such as MF59-adjuvanted or high-dose formulations—for individuals aged 65 and older. These vaccines show improved efficacy and are included in national immunization schedules in some countries, strengthening protection for vulnerable populations. This study aims to identify official recommendations for enhanced vaccines for older adults in developed Northern Hemisphere countries (NHC) and compare national immunization program (NIP) recommendations for enhanced influenza vaccines in NHC and Latin America (LATAM) countries.

Methods An online search was conducted from June to December 2024 to identify national immunization program (NIP) policies on influenza vaccination for older adults in developed Northern Hemisphere countries (NHC) and Latin America (LATAM). Official Ministry of Health websites were reviewed using keywords such as influenza vaccination, immunization schedules, and adult flu vaccination.

Results Ten countries in Europe and two in North America recommend enhanced influenza vaccines for older adults, with age thresholds varying—some starting at 60, others at 65 or 70. Two countries specifically target nursing home residents. NHC have gradually expanded recommendations to increasingly include enhanced vaccines for older adults. In contrast, only two out of 20 LATAM countries—Argentina (since 2021) and Paraguay (starting in 2025)—have incorporated enhanced vaccines into their NIPs.

Conclusion While advanced immunization strategies for older adults are implemented in the U.S., Canada, and several European nations, only Argentina and Paraguay have taken similar steps in Latin America. To ensure equity and protect older populations' rights, regional collaboration and public health policies promoting access to enhanced vaccines are essential. LATAM's growing aging population underscores the urgent need for improved vaccination strategies to reduce disease burden and strengthen health outcomes across the region.

Title: " Disparities in Access to Enhanced Vaccines for Older Adults: Addressing Equity Gaps Between Developed Nations in the Northern Hemisphere and Latin America (LATAM)"

Table 1. Recommendations by major health authorities in Latin America by country for adults aged ≥65 years within the category of enhanced vaccines (LATAM)

Country	Argentina	Paraguay
Recommendations	aTIV ≥65	aTIV ≥ 60

Table 2. Recommendations by major health authorities in Europe by country for adults aged ≥65 years within the category of enhanced vaccines

Country	Austria	Denmark	Finland	Germany	Greece	Italy	Norway	Spain	Sweden	UK
Recommendations	aQIV/HD-QIV preferred over aTIV respectively	aQIV 75+ cohort in NIP4/26	aQIV is the vaccine of choice in NIP4/26	aQIV, HD-QIV, ≥60	aQIV/HD-QIV ≥65	aQIV/HD-QIV ≥65	aQIV/HD-QIV issued by MoH, individuals living in nursing homes (≥65)	Most regional tenders favor enhanced vaccines ≥65	aQIV/HD-QIV/QIVr issued by MoH; endorses living in nursing homes.	aQIV/HD-QIV/QIVr in subjects aged ≥65 aQIV and QIVr are reimbursed

Table 3. Recommendations by major health authorities in North America by country for adults aged ≥65 years within the category of enhanced vaccines

Country	USA	CANADA
Recommendations	aQIV/HD-QIV and QIVr: ≥65	aTIV/HD-QIV/QIVr: ≥65.

236

How Respiratory Syncytial Virus Hospitalization Affects Quality of Life in the Ageing Population of the Valencia Region of Spain

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Background

Respiratory syncytial virus (RSV) and other infectious diseases can significantly impact health-related quality of life (QoL), particularly among older adults. This study aimed to assess the effect of hospitalization due to RSV-related severe acute respiratory infection (SARI) on QoL in ≥65-years-old patients.

Methods

Prospective, active-surveillance study in ≥65-years-old SARI-hospitalized patients within the Valencia Hospital Network for the Study of Infectious Diseases (VAHNSI) in 2023/2024 and preliminary 2024/2025 seasons. Participants were tested for RSV using real-time RT-PCR. RSV-positive patients completed the EQ-5D-5L questionnaire at hospital admission (day 0) and 30 days after discharge (day 30). We reported the mean and standard deviation (SD) of the Utility Index (UI; range: -0.416 to 1, where 1 indicates optimal health) and the Visual Analogue Scale (VAS; range: 0–100, with 100 indicating the best possible health status) at both time points, stratified by age group (65–79 years and ≥80 years). Paired t-tests were performed to assess statistical significant QoL improvement (p-value<0.05) at day 30.

Results

336 patients were SARI-hospitalized with RSV; 229 (68.1%) completed the EQ-5D-5L questionnaire. Baseline characteristics of the population were described in Table 1. Mean (SD) values for UI at day 0 vs. day 30 were 0.71 (0.26) vs. 0.68 (0.30) (p=0.33) in 65-79 years, and 0.53 (0.31) vs. 0.47 (0.31) (p=0.96) in ≥80 years, and for VAS 51.98 (20.52) vs. 57.88 (21.42) (p=0.02) in 65-79 years, and 50.85 (20.52) vs. 49.66 (21.72) (p=0.68) in ≥80 years.

Table 1: Baseline characteristics of the RSV positives overall and included in the EQ-5D-5L analysis.

Conclusions

RSV-related SARI hospitalization in adults ≥65 years was associated with a notable short-term impact on health-related QoL, particularly in ≥80 years, who showed no significant recovery at 30 days post-discharge. In contrast, patients 65–79 years exhibited a modest but statistically significant improvement in self-perceived health status (VAS), although no significant changes were observed in utility scores (UI). These findings highlight the potential long-term consequences on functional health, especially among the oldest adults. Preventive strategies and post-discharge follow-up care may be crucial to mitigate these effects.

Characteristic	RSV positives N = 336	RSV positives with EQ-5D-5L N = 229
2023/2024	172 (51.19%)	131 (57.21%)
2024/2025	164 (48.81%)	98 (42.79%)
65-79 years old	134 (39.88%)	106 (46.29%)
≥80 years old	202 (60.12%)	123 (53.71%)
Lung disease	109 (32.44%)	81 (35.37%)
Asthma	44 (13.10%)	33 (14.41%)
Diabetes	112 (33.33%)	84 (36.68%)
Cardiovascular disease	167 (49.70%)	112 (48.91%)
Kidney disease	64 (19.05%)	32 (13.97%)
Obesity	104 (30.95%)	69 (30.13%)

237

The Effects of High- and Moderate-Risk Conditions and Multimorbidity on COVID-19-Related Hospitalization: Real-World Evidence from German Claims Data

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Background: The presence of underlying conditions and multimorbidity ("risk-stacking", defined as two or more underlying conditions) is associated with poorer health outcomes in many diseases, including COVID-19. This study investigated the effects of high- and moderate-risk conditions and multimorbidity on COVID-19-related hospitalizations across different age groups.

Methods: In a retrospective observational study using anonymized German claims data from the WIG2 benchmark database, we stratified adult individuals into age groups (18–49, 50–64, ≥65 years) and classified them as low-, moderate-, and high-risk based on the WHO risk classification of pre-existing conditions ≤2 years prior to the study period, defined by Omicron XBB dominance (February 13 – November 20, 2023). We identified COVID-19-related hospitalizations during the study period and assessed the risk of COVID-19-related hospitalization using Cox proportional hazards regression, stratified by risk level, number of conditions, and age group and adjusted for age and gender.

Results: The study included 2,303,244 individuals (47.4% female; mean [SD] age, 52.4 [18.6] years [**Table**]). Multimorbidity increased with age (mean [SD] number of conditions: 1.0 [1.4], 2.4 [2.4], and 4.7 [3.3], for ages 18–49, 50–64, and ≥65, respectively). Hypertension (39.4%), depressive disorders (18.5%), and heart conditions (18.3%) were the most frequent conditions. Among individuals aged 18–49, 50–64, and ≥65 years, 0.10%, 0.22%, and 1.02%, respectively, had a COVID-19-related hospitalization. The risk of a COVID-19-related hospitalization was more than three times higher for individuals with ≥1 moderate-risk (HR [95% CI]: 3.36 [3.03–3.72]) or high-risk condition (HR: 3.32 [3.00–3.68]), compared with low-risk (**Figure**). Hospitalization risk also increased with the number of moderate- or high-risk conditions. Results were consistent across age groups, with the effect of multimorbidity generally greater in younger versus older age groups.

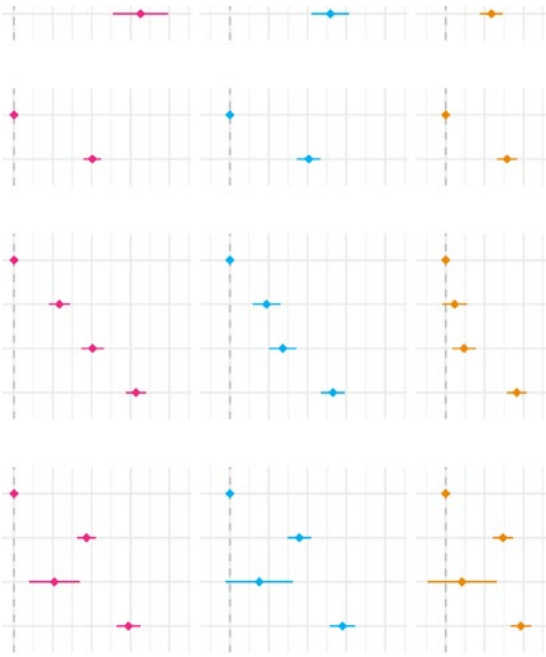
Conclusions: Our analyses demonstrated that risk of COVID-19 hospitalization was similar among adults with moderate- and high-risk conditions and further increased with multimorbidity across all age groups, highlighting a potential need for broader vaccination and treatment guidelines to mitigate severe COVID-19-related outcomes.

Table: Baseline Characteristics

	Aged 18–49 (N = 1,007,638)	Aged 50–64 (N = 662,088)	Aged ≥65 (N = 633,518)	Overall (N = 2,303,244)
Age in years, mean (SD)	34.8 (9.2)	57.1 (4.2)	75.4 (7.5)	52.4 (18.6)
Gender, female	463,796 (46.0%)	294,728 (44.5%)	332,333 (52.5%)	1,090,857 (47.4%)
WHO Risk Conditions:				
Low-risk*	477,286 (47.4%)	151,579 (22.9%)	44,949 (7.1%)	673,814 (29.3%)
Moderate-risk Conditions	450,053 (44.7%)	416,312 (62.9%)	467,415 (73.8%)	1,333,780 (57.9%)
High-risk Conditions	80,299 (8.0%)	94,197 (14.2%)	121,154 (19.1%)	295,650 (12.8%)
Number of conditions, mean (SD)	1.0 (1.4)	2.4 (2.4)	4.7 (3.3)	2.4 (2.8)

SD: standard deviation

* Low-risk indicates the absence of moderate- or high-risk conditions.



238

Estimated Additional Burden Averted With Use of MF59-Adjuvanted Influenza Vaccines Compared to Egg-Based Influenza Vaccines Among People 50-64 Years of Age

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Background:

Individuals aged ≥65 experience the highest rate of severe influenza complications, including hospitalizations and death. However, rates of severe complications begin to increase earlier, around 50 years of age. Immunosenescence, the decline in immune response with age, reduces the response to influenza vaccination. To address this, enhanced vaccines, like adjuvanted and higher-dose options, are preferentially recommended for adults aged ≥65 in many countries. However, enhanced vaccines have historically not been approved or widely used for individuals aged 50–64, a population that could also benefit from improved vaccine effectiveness. The MF59-adjuvanted influenza vaccine (aTIV/aQIV) was recently approved in the UK and EU for adults aged 50–64. Using an extension of a model from the US Centers for Disease Control and Prevention (CDC), this study modeled the potential public health impact of administering aTIV/aQIV to all vaccinated individuals aged 50-64 in the United States, compared to quadrivalent influenza vaccine (QIV), across the 2017–2020 and 2022–2024 influenza seasons.

Methods:

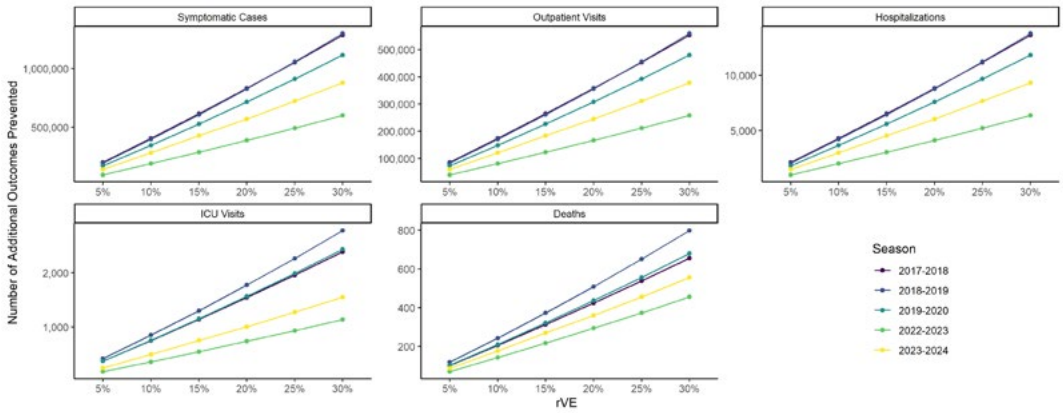
The model extended estimates of overall burden averted by vaccination into a relative vaccine effectiveness (rVE) context. It utilized CDC data on vaccine uptake, influenza incidence, influenza-related healthcare resource use and deaths. CDC estimates of absolute vaccine effectiveness (aVE) (any vaccine) were used as the aVE of QIV. While the rVE of aTIV/aQIV vs QIV has not yet been evaluated among people 50–64 due to recent approvals, it has been studied extensively among adults ≥65. To reflect seasonal variation in effects and uncertainty in how ≥65 rVE estimates translated to the 50–64 population, rVE values ranging from 5%–30% were included. Base-case results were validated with deterministic (DSA) and probabilistic (PSA) sensitivity analyses.

Results:

Figure 1 shows the anticipated additional influenza-related cases and complications that would be averted if all vaccinated people 50–64 received aTIV/aQIV vs QIV. Across the influenza seasons evaluated, on average each 5% increase in the rVE of aTIV/aQIV vs QIV, would prevent an additional 172,738 symptomatic illnesses, 74,277 outpatient visits, 1,832 hospitalizations, 343 ICU admissions, and 105 deaths. This corresponds to an average increase in burden averted of 15.2%, with a range of 5.9% to 37.2%. DSA revealed greatest variability tied to rVE and burden estimates. PSA results were normally distribution.

Conclusion:

Individuals 50–64 years of age could benefit from the use of aTIV/aQIV over QIV, with an average increase in the number of influenza outcomes prevented of 15.2% per 5% improvement in vaccine effectiveness.


Symptomatic Cases
Outpatient Visits
Hospitalizations
ICU Visits
Deaths
Number of Additional Outcomes Prevented
rVE
Season
2017-2018
2018-2019
2019-2020
2022-2023
2023-2024

241

ECaS

Infection of domestic cats with H5Nx clade 2.3.4.4b predisposes these viruses to replicate more efficiently in the human airway

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A(H5) viruses of the clade 2.3.4.4b have been the predominating highly pathogenic avian influenza virus (HPAIV) circulating in birds globally since 2020. In recent years, this clade has also been detected in multiple mammalian species, including cattle, seals, mink, various feline species, and even recently, sheep. These viruses have also proven their ability to spill into the human population, particularly in occupationally exposed workers, with over 45 incidences of human detections since January 2024. These spill-over events into the mammalian population raises concerns about how readily transmissible these viruses may be within the human population, and whether infection of mammals increases the fitness of the virus to infect humans, potentially leading to the next pandemic.

This study investigates whether the infection of domestic cats with HPAIVs increases the ability of the virus to replicate in human cells, therefore demonstrating a potential zoonotic risk within the domestic household.

Preliminary growth curves in A549s, a human lung epithelial-cell line, using a virus isolated from a cat, compared with two A(H5) HPAIVs isolated from avian species from similar time periods indicates that the cat virus has an enhanced ability to replicate in this human cell line compared to the avian isolates. Further investigation is planned for other isolates obtained from cats, tested against their respective avian equivalents of the same genotype to further elucidate whether infection via a domestic cat enhances the fitness of the virus in human cell lines. These experiments will be initially conducted in A549s and we will then aim to repeat the comparison in a primary human bronchial epithelial airway model.

Given that some estimates suggest over a quarter of UK households possess cats, these investigations highlight a potential high-risk pathway at the human-domestic animal interface that could increase the risk of human infections. Genomic analysis of these viruses and those produced through these investigations is key to understanding the potential impact and adaptive mechanisms that spill-over of H5 clade 2.3.4.4b viruses into domestic cats from avian species may have. This evidence contributes to pre-pandemic preparedness efforts and will assist in predicting the potential impact such viruses could have on public health.

242

MODELLING THE GREENHOUSE GAS EMISSIONS IMPACT OF RSV IMMUNIZATION WITH NIRSEVIMAB IN SPAIN: A HEALTH CARBONOMICS ANALYSIS

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Objectives: Healthcare systems significantly contribute to greenhouse gas (GHG) emissions, with Spain's healthcare sector accounting for 4.4% of national emissions. Spain has embraced initiatives at both national and regional levels to decarbonize its healthcare system towards net zero by 2050. Respiratory Syncytial Virus (RSV) is a leading cause of infant hospitalization, creating substantial pressure on healthcare resources during autumn/winter seasons. Since the 2023-2024 RSV season, Spain has successfully implemented an RSV immunization program using nirsevimab, a long-acting monoclonal antibody approved for RSV prevention in infants. This study estimates the net GHG emissions impact of the immunization programme with nirsevimab infants compared to standard of care (SoC) in Spain.

Methods: A health Carbonomics© framework was applied to model the carbon footprint across the entire patient care pathway for 337,400 infants in Spain. The study compared emissions from the nirsevimab immunisation programme (including product-related emissions) with emissions avoided through reduced healthcare resource utilization (HCRU). Data were collected from multiple sources including health economic models, official environmental agencies, clinical trials, and were validated through expert consultations. An immunization coverage of 97.6% of the infant cohort was considered, with 61% immunized at birth in hospital and 39% in primary care settings. Emission factors were derived from Spanish hospital data and extrapolated from NHS England using a healthcare carbon intensity ratio. Sensitivity analyses were conducted to account for uncertainty in emission factors, patient travel patterns, and healthcare infrastructure efficiency.

Results: In Spain, RSV-related healthcare utilisation emits approximately 10 kt CO_2eq annually (32kg CO_2eq per infant). Immunizing infants with nirsevimab would generate 2.9 kt CO_2eq attributable to the product lifecycle, patient travel and administration emissions. Avoided RSV burden would translate in a 6.5 kt CO_2eq reduction, associated with reduced HCRU, which would result in a net reduction of 3.6 kt CO_2eq annually (−35% vs SoC). This reduction is primarily driven by avoided primary care visits (−2.4 kt CO_2eq) and hospitalizations (−2.3 kt CO_2eq). On a per-patient basis, the RSV programme would reduce emissions by 11kg CO_2eq compared to SoC. These savings equate to avoiding 10,000 Madrid-New York return flights or the annual GHG emissions of 200 ambulances in Spain.

Conclusions: This study demonstrates that implementing a high-coverage RSV infant immunization programme with nirsevimab can deliver substantial environmental benefits in addition to public health benefits. The 35% reduction in RSV-related GHG emissions significantly contributes to healthcare decarbonization, highlighting how preventive interventions can deliver co-benefits for health systems and the environment. These findings support inclusion of environmental impact assessments in immunization policies and reinforce Spain's leadership in sustainable healthcare as it advances towards 2050 net zero goals.

243

ECaS

The expanding avian influenza panzootic: skua die-off in Antarctica

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Background:

High pathogenicity avian influenza H5 viruses (H5 HPAIV) of the A/goose/Guangdong/1/1996 lineage, clade 2.3.4.4b, is expanding its host and geographical range. In 2023, H5 HPAIV invaded Antarctica and is a potential threat to the lives of millions of wild birds and mammals there. Although mortality in Antarctic wildlife from H5 HPAIV has been suspected, mainly based on virological analysis of swabs collected from dead animals, it has not been unequivocally diagnosed.

Results:

Here we show that H5 HPAIV caused high mortality in a breeding colony of skuas at one of ten sites in Antarctica we visited in March 2024. Whereas it caused multi-organ necrosis and rapid death in skuas, it did not cause mortality in other H5 HPAIV-positive species examined, such as Adélie penguins, for which we diagnosed different causes of death.

Methods:

We collected swabs and tissues from dead animals and performed molecular analyses to detect influenza A viral RNA and histopathological analyses to assess the exact cause of death. The multi-basic cleavage site was sequenced to confirm the presence of highly pathogenic strains. Additionally, Pasteurella multocida diagnostics was performed to consider avian cholera as an alternative cause of death.

Conclusions:

We diagnosed HPAI as the probable cause of death of an unusual mortality event in skuas at Beak Island, Antarctica. Taken together with recent data, skuas in Antarctica are at risk of continued mortality from H5 HPAIV infection, threatening their already small populations. Conversely, because of their wide distribution and ecological relevance, skuas may play a substantial role in spread of the virus across Antarctica. Transdisciplinary surveillance is needed in coming years both to monitor the impact of this poultry-origin disease on Antarctica's unique wildlife, and to provide the factual basis for policies that prevent new HPAIV emergence and spillover events from poultry.

244

Correlation of Humoral Immunogenicity Results elicited by mRNA-1010 Seasonal Influenza Vaccine in Adults Aged ≥18 Years: A Comparative Phase 3 Analysis

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Moderna, Inc., Cambridge, MA, USA

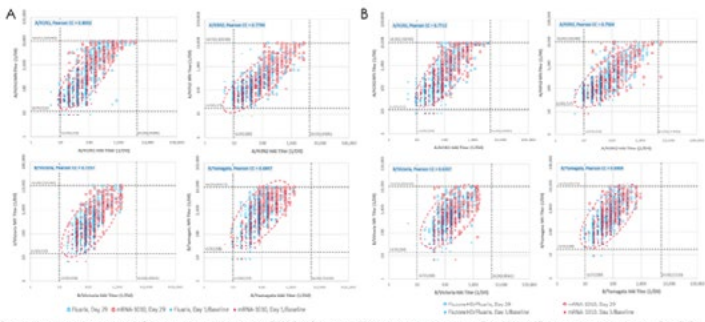
Background: mRNA-1010 is a novel mRNA-based quadrivalent influenza vaccine targeting vaccine-matched influenza A and B strains. The hemagglutination inhibition (HAI) and microneutralization (MN) assays are both used to detect antibodies against influenza viruses. mRNA-1010 has previously demonstrated superior immunogenicity compared to age-appropriate licensed comparators (standard dose, SD for participants aged 18-64, or high dose, HD, for participants aged ≥65), as measured by the HAI assay. Here, we present exploratory MN data from a subset of participants and correlate with HAI.

Methods: This Phase 3, randomized, observer-blind, active-controlled trial evaluated humoral responses for 4 influenza strains (A/H1N1, A/H3N2, B/Victoria and B/Yamagata). Adults were randomized to mRNA-1010 (50 µg) or licensed QIV comparators (SD, Part B: adults aged 18 to <65 years) or HD, Part C: adults aged ≥65 years). MN titers at baseline and Day 29 were measured in 1000 participants (250 per arm in each part, 17% of study participants). Pearson correlations were calculated based on log-transformed MN and HAI titers.

Results: In Part B, mRNA-1010 demonstrated superior MN immune responses compared to SD QIV across all four strains. Day 29 geometric mean titer (GMT) levels and geometric mean fold rise (GMFR) from Baseline at Day 29 were higher in mRNA-1010 compared to SD QIV. The overall GMT ratios of mRNA-1010 compared to SD QIV were 2.36, 2.40, 1.72 and 1.56 for A/H1N1, AH3N2, B/Victoria and B/Yamagata, respectively. Similarly, Part C showed that mRNA-1010 elicited superior or comparable MN immune responses for all 4 strains compared to HD QIV. Day 29 geometric mean titer (GMT) levels and geometric mean fold rise (GMFR) from Baseline at Day 29 were higher in mRNA-1010 compared to HD QIV. The overall GMT ratios of mRNA-1010 compared to HD QIV were 1.75, 1.32, 1.30 and 1.06 for A/H1N1, AH3N2, B/Victoria and B/Yamagata, respectively. Pearson correlations demonstrated MN and HAI titers were positively correlated in all strains (Figure 1). High correlation coefficients of ≥0.70 were seen for both A strains in Parts B and C as well as B/Victoria in Part B.

Conclusions: MN responses to mRNA-1010 are comparable to or higher than licensed QIV comparators and are strongly correlated with HAI titers across all strains. The strong correlation between MN and HAI provides evidence that MN and HAI can each serve as adequate surrogate endpoints for prevention of influenza regardless of the type of vaccine.

Figure 1. Correlations between MN and HAI Titers in mRNA-1010 P303 Study



Correlations between MN and HAI titers in study participants from P303 Part B (A, aged 18-64, randomized to either mRNA-1010 or SD licensed comparator) and Part C (B, aged 65+, randomized to either mRNA-1010 or HD licensed comparator). Participants in this analysis had nonmissing Baseline (Day 1) and Day 29 neutralizing antibody data. HAI hemagglutination inhibition; MN microneutralization; LLOQ lower limit of quantification; PPS per protocol immunogenicity set; ULOQ upper limit of quantification.

248

Comparative Healthcare Burden of COVID-19, Influenza, and RSV in Canada: Pre- and Post-Pandemic Analysis

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Abstract

Background: Historically, influenza and respiratory syncytial virus (RSV) placed substantial strain on the Canadian healthcare system each winter. With COVID-19 now circulating in the winter months, it is important to understand the burden of the viruses on the health care system from a resource utilization perspective. This work is looking at healthcare utilization trends by patients with influenza, RSV or COVID-19 to inform hospital preparedness and public health response strategies.

Methods: We conducted a retrospective analysis of weekly hospitalization data from the Canadian Institute for Health Information (CIHI) Discharge Abstract Database (DAD), focusing on influenza, RSV, and COVID-19 among five age groups (0–4, 5–19, 20–44, 45–64, and 65+ years). Patients were classified by virus-specific flags (using ICD-10 codes), and bed-days were calculated to accurately allocate length of stay (LOS) across epidemiological weeks. We examined intensive care unit (ICU) admission rates, total hospital LOS (including ICU) and ICU LOS across two periods: pre-pandemic (September 2010 to February 2020) and post-pandemic (September 2022 to February 2025).

Results: In the post-pandemic period, COVID-19 accounted for the highest burden, with 67,588 hospital admissions (655,643 hospital bed-days) and 5,387 ICU admissions (36,043 ICU bed-days). Influenza patients accounted for 20,641 hospitalizations (123,211 hospital bed-days) and 1,644 ICU admissions (9,428 ICU bed-days). RSV accounted for 22,811 hospitalizations (102,954 hospital bed-days) and 3,036 ICU admissions (12,412 ICU bed-days).

COVID-19 patients had an ICU admission rate of 8.0%, and a median hospital and ICU LOS of 5 days and 4.5 days, respectively. Compared to the pre-pandemic period, influenza showed a decrease in ICU admission rates (from 10.7% to 8.0%), median hospital LOS (from 4 to 3 days) and median ICU LOS (from 4.6 to 3.8 days) and RSV showed an increase in ICU admission rates (from 11.8% to 13.3%), median hospital LOS remained stable at 3 days, and median ICU LOS declined slightly (from 3.7 to 3.3 days).

General linear model (GLM) analysis showed that virus type and time period were statistically significant predictors of hospitalization burden across age groups. The most pronounced post-pandemic increases were observed in hospitalizations due to RSV, particularly among children aged 0–4 years.

Conclusion:

Our findings highlight the differences in healthcare utilization by patients with respiratory viruses during the post-pandemic period. COVID-19 appears to cause the most severe outcomes; however, there were notable changes in burden by patients with influenza and RSV compared to the pre-pandemic period. While difficult to determine whether these changes are due to a shift in disease severity or to balance finite healthcare resources, these trends underscore the importance of ongoing and enhanced surveillance strategies for managing viral respiratory illnesses in Canada.

251

Respiratory Viruses Differentially Influence Streptococcus pneumoniae Colonisation in a Gambian Household Cohort

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1: The University of Sheffield, United Kingdom;2: The Medical Research Council Unit The Gambia at the London School of Hygiene & Tropical Medicine;3: The University of Cambridge

Background: Respiratory viruses are thought to influence the acquisition and proliferation of key bacterial pathogens, yet real-world evidence is limited. We examined virus-specific effects on Streptococcus pneumoniae (pneumococcal) colonisation in the upper respiratory tract using a large Gambian household cohort.

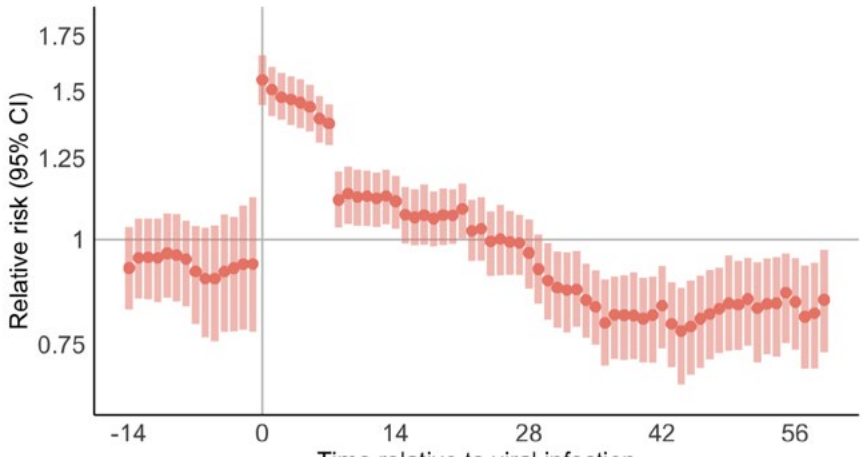
Methods: We analysed 14,545 nasal/oral swabs from 349 individuals in 52 households, collected weekly between March 2021 and June 2022. Viral infections were identified using a validated multiplex PCR and pneumococcus by lytA PCR. A nested mixed-effects logistic regression model assessed the species-specific effects of recent viral infection (first PCR-positive within 14 days) on pneumococcal detection. The model was adjusted for age, sex, and season (natural spline, 5df). Random intercepts were included for individuals nested within households.

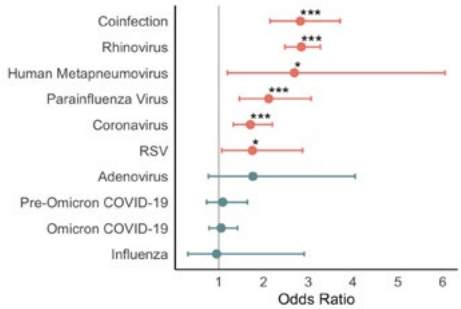
Results: We detected 1,402 distinct viral episodes, 88% of which were asymptomatic. In unadjusted analysis, the risk of pneumococcal detection was greatest immediately after detection of any virus, returning to baseline by one month (Figure 1). Mixed effects modelling showed clear differences in the odds of pneumococcal detection by viral species. Odds were increased with RSV (OR 1.75, 95% CI 1.07–2.85), rhinovirus (OR 2.84, 95% CI 2.43–3.33), human metapneumovirus (OR 2.69, 95% CI 1.18–6.16), parainfluenza (OR 2.11, 95% CI 1.41–3.15), and seasonal coronaviruses (OR 1.70, 95% CI 1.31–2.20) (Figure 2). No association was observed for SARS-CoV-2 (either pre-Omicron or Omicron). Too few episodes of influenza or adenovirus were detected to draw reliable conclusions.

Conclusions: Several respiratory viruses, including RSV, markedly increased pneumococcal carriage risk, whereas SARS-CoV-2 did not—highlighting virus-specific modulation of bacterial colonisation. Notably, most viral infections were asymptomatic, suggesting that the role of silent viral infection in pneumococcal dynamics may be underappreciated. These findings may help explain pathogen-specific patterns of secondary bacterial disease and support virus-targeted strategies to reduce pneumococcal transmission and disease.

Figure 1: Risk of viral infection relative to most proximal viral infection, utilising a -7/+7 sliding window.

Figure 2: Virus-specific adjusted odds ratios for pneumococcal detection within 14 days of infection.


Relative risk (95% CI)
1.75
1.5
1.25
1
0.75
-14
0
14
28
42
56


Coinfection
Rhinovirus
Human Metapneumovirus
Parainfluenza Virus
Coronavirus
RSV
Adenovirus
Pre-Omicron COVID-19
Omicron COVID-19
Influenza
***
***
*
***
***
*
1
2
3
4
5
6
Odds Ratio

252

Quantifying the role of common viruses on severe acute respiratory infections admissions to the intensive care unit: a modeling approach

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1: RIVM (Centre for Infectious Disease Control Netherlands, National Institute for Public Health and the Environment), Netherlands, The;2: National Intensive Care Evaluation (NICE) Foundation, Amsterdam, the Netherlands;3: Department of Medical Informatics, Amsterdam UMC, Location AMC, Amsterdam Public Health research institute, Amsterdam, The Netherlands;4: Department of Intensive Care Medicine, University Medical Center, University Utrecht, Utrecht, the Netherlands;5: Department of Medical Informatics, Amsterdam UMC, Location AMC, Amsterdam Public Health research institute, Amsterdam, The Netherlands

Background

Due to cost constraints and limited treatment applications, routine exhaustive testing of severe acute respiratory infections (SARI) patients is uncommon, with testing often limited to specific patients or seasons. With SARI mostly comprised of pneumonias, exhaustive testing for a wide range of respiratory pathogens is neither typically performed nor mandated by pneumonia guidelines. Even when performed, identifying a causative pathogen is challenging in roughly half of pneumonia cases. Setting up SARI surveillance remains a challenge for many Western European countries. While recently established surveillance systems aim to capture pathogens, the full burden of respiratory viruses in SARI remains unclear. The pre-COVID-19 proportion of SARI Intensive Care Unit admissions (SARI-ICU) attributable to respiratory viruses was estimated using a modelling approach. For future situational awareness in epidemics and outbreaks it is not only important to set up SARI surveillance but also to understand the historic contribution of different viruses to SARI trends.

Methods

We examined the association between SARI-ICU admissions and the circulation of common respiratory viruses using regression modelling with two data sources (2007-2017): (1) weekly counts of all adult SARI-ICU admissions from the Dutch National Intensive Care Evaluation registry, and (2) weekly counts of positive laboratory detections of respiratory viruses from the national virological laboratory surveillance (mostly hospital submissions), including: influenza virus types A and B (IAV, IBV), rhinovirus, respiratory syncytial virus (RSV), adenovirus, common human coronaviruses (CH-coronaviruses), parainfluenzaviruses, and human metapneumovirus (hMPV). We applied a binomial generalized linear model to associate adult SARI-ICU admissions with virus counts.

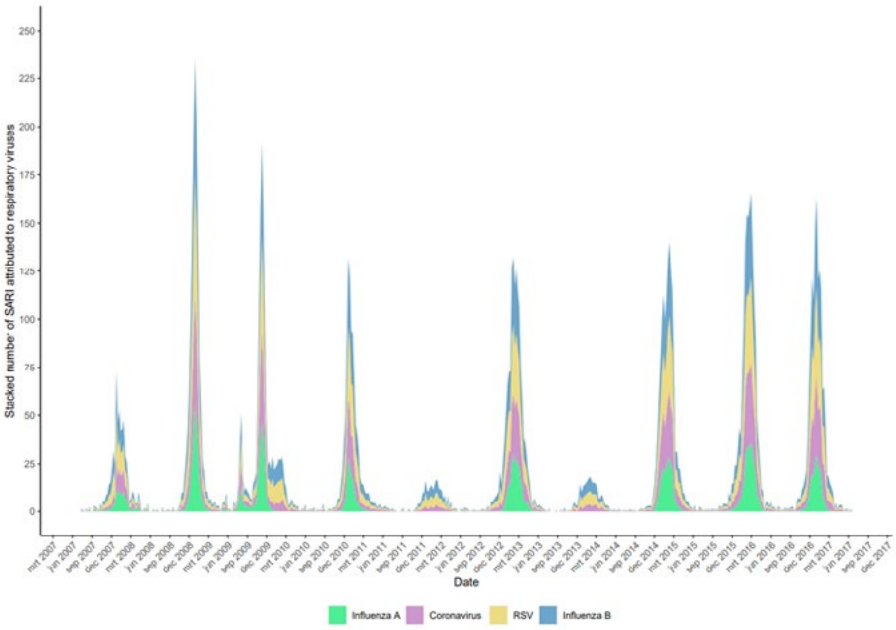
Results

8.2% of all adult SARI-ICU admissions were associated with IAV and IAB, CH-coronaviruses, and RSV; (seasonally ranging from 2.4% (2011/2012) to 14% (2015/2016) (figure). No significant association with adult SARI-ICU was found for rhinovirus, adenovirus, parainfluenzavirus, and hMPV. The predicted proportion of adult SARI-ICU attributed to viruses was consistently greater than originally clinically reported as 'pneumonia-viral', with the absolute difference in the yearly percentages ranging between 0.03-8.0%. The largest contributor to virus-attributable SARI-ICU was IAV in eight of ten seasons. SARI-ICU trends preceded virus detections in laboratory surveillance, except for RSV and for four out of ten IAV seasons.

Conclusions

The actual prevalence of viral SARI may be underestimated by clinically diagnosed 'pneumonia-viral'. In the pre-COVID-19 era, IAV contributed the most to virus-attributabed SARI-ICU admissions, with CH-coronaviruses and IBV playing occasional roles, and RSV showing a stable, lower impact.

Figure: Estimated number (stacked) of SARI-ICU admissions attributable to viruses.


Stacked number of SARI attributed to respiratory viruses
250
225
200
175
150
125
100
75
50
25
0
mrt 2007
jun 2007
sep 2007
dec 2007
mrt 2008
jun 2008
sep 2008
dec 2008
mrt 2009
jun 2009
sep 2009
dec 2009
mrt 2010
jun 2010
sep 2010
dec 2010
mrt 2011
jun 2011
sep 2011
dec 2011
mrt 2012
jun 2012
sep 2012
dec 2012
mrt 2013
jun 2013
sep 2013
dec 2013
mrt 2014
jun 2014
sep 2014
dec 2014
mrt 2015
jun 2015
sep 2015
dec 2015
mrt 2016
jun 2016
sep 2016
dec 2016
mrt 2017
jun 2017
sep 2017
dec 2017
Date
Influenza A
Coronavirus
RSV
Influenza B

257

Distribution of Influenza Subtypes and Lineages, and availability of enhanced Influenza vaccines in Latin America countries in the post pandemic period.

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Background: Influenza viruses (IV) are one of the leading causes of severe respiratory infections worldwide, significantly affecting vulnerable populations. Effective epidemiological surveillance is essential for the timely implementation of preventive and control measures. The aim of this study is to analyse post pandemic influenza virus circulation and identify countries in Latin America (LATAM) that are adopting new interventions to assess their potential future impact.

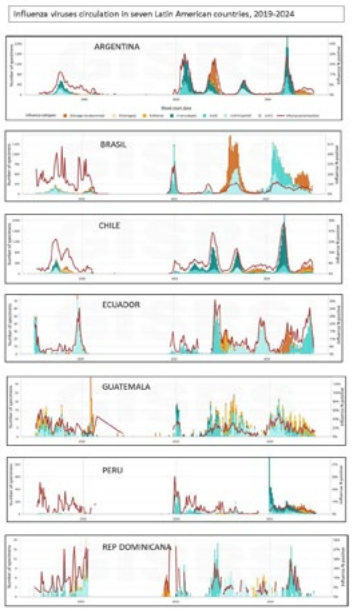
Materials and Methods: A retrospective, descriptive study was conducted on influenza virus circulation in Argentina (AR), Brazil (BR), Chile (CHI), Ecuador(EC), Guatemala (GUA), Peru (PE), and the Dominican Republic (D REP) from 2019 to 2024. Viral circulation data were collected through reports from different LATAM countries to FluNet, GISRS, from 2019 to 2024. The study evaluated annual circulation periods and viral circulation percentages from each of the seven countries Additionally, information on new immunizations with enhanced influenza vaccines authorized in LATAM was included.

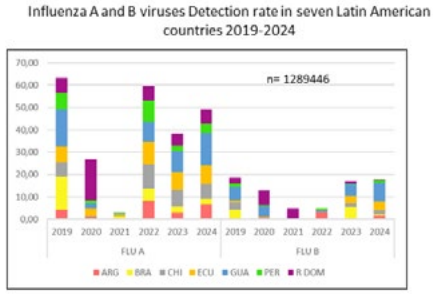
Results: Analysis of influenza virus circulation across seven Latin American countries, AR, BR, CHI, EC, GUA, PE and D REP from 2019 to 2024 shows marked variability in the activity of influenza A and B viruses. In 2020, viral circulation in the Southern Hemisphere declined due to isolation measures implemented during the COVID-19 pandemic. However, as adherence to these measures decreased in late 2021, influenza virus circulation return. In post pandemic period, peaks in circulation generally coincide with colder months in the Template Climate countries, although seasonal patterns vary by region, In Central America, the viral circulation continues year around. An early outbreak of A(H3N2), in 2022 started the influenza circulation in South America before stabilizing at lower levels in the period, A(H1N1) and B Victoria viruses also circulated later in the 2022-2024 period. Notably, since early 2020, the B/Yamagata lineage was not detected, see Figure 1. The distribution of the Detection Rates is shown in Figure 2. In Latin America, only Argentina, Brazil, Paraguay and Mexico have approved the use of enhanced influenza vaccines, see Figure 3.

Conclusion:

Monitoring influenza virus circulation is key for selecting vaccine strains and scheduling immunization in LATAM. The approval and implementation of new and improved influenza vaccines in more countries across the region is essential to reduce morbidity and mortality,particularly at high-risk populations.

Acknowledgments: The authors thank Seqirus Laboratories for their support.


Influenza viruses circulation in seven Latin American countries, 2019-2024
ARGENTINA
BRASIL
CHILE
ECUADOR
GUATEMALA
PERU
REP DOMINICANA


Influenza A and B viruses Detection rate in seven Latin American countries 2019-2024
n= 1289446
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
2019 2020 2021 2022 2023 2024 2019 2020 2021 2022 2023 2024
FLU A
FLU B
ARG BRA CHI ECU GUA PER R DOM


Seasonal Influenza vaccines in Latin American countries
Flu vaccination
Standard formulation
Improved vaccines
Not introduced
Excluded
Enhanced Flu Vaccines
Adjuvated
High Dose
Cell Culture
Brazil
Approved
Approved & available in private sector
Approved
Mexico
Approved
Approved
Argentina
Approved & available in public sector
Approved & available in private sector
Approved & available in private sector
Paraguay
Approved & available in public sector

259

ECaS

A Systematic Review of Estimation Methods for Non-Respiratory Severe Influenza Burden

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1: World Health Organization Collaborating Centre for Infectious Disease Epidemiology and Control, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region, China, Hong Kong S.A.R. (China);2: Faculty of Life Sciences & Medicine, King's College London, London, United Kingdom;3: Global Influenza Programme, Epidemic and Pandemic Threat Management, World Health Organization, Geneva, Switzerland;4: Laboratory of Data Discovery for Health, Hong Kong Science and Technology Park, New Territories, Hong Kong Special Administrative Region, China

Background: Despite being primarily classified as a respiratory infection with notable impacts on respiratory hospitalization and mortality, influenza can also lead to a wider range of consequences beyond respiratory presentations. Excluding these outcomes from burden estimates could underestimate influenza's full impact, potentially compromising the prioritization of influenza vaccination policy. A comprehensive understanding of non-respiratory influenza burden is essential for an integrated perspective on its public health implications.

Methods: A systematic review of peer-reviewed articles was conducted using PubMed and Embase. Studies estimating non-respiratory influenza-associated severe outcomes, such as hospitalizations and mortality, were included. These outcomes covered cardiovascular diseases, malignant neoplasms, diabetes, renal diseases, chronic liver diseases, and broader categories such as respiratory plus cardiovascular (R+C) and all-cause measures. Details on study designs including data sources and definitions, statistical methods and outcome measures were summarized, while heterogeneity in estimates by study design features was explored using meta-regression models.

Results: Of the 238 articles included, the majority focused on mortality (207/238, 86.9%) and hospitalization (48/238, 20.2%), with all-cause (187/238, 78.6%), R+C (60/238, 25.2%), and cardiovascular-related (94/238, 39.5%) outcomes being the most studied. Most studies were conducted in the European Region (90/238, 37.8%), the Region of the Americas (74/238, 31.1%), and the Western Pacific Region (71/238, 29.8%). The elderly population (170/238, 71.4%) was the most frequently studied age group.

Regression models, including linear, Poisson and negative binomial models, were the most commonly used statistical approaches, followed by rate difference methods. Over half of the included studies incorporated influenza activity proxies (145/238, 60.9%), adjusted for long-term trends and/or seasonality (183/238, 76.9%), RSV activity (194/238, 81.5%) and meteorological factors (176/238, 73.9%).

Beyond all-cause and R+C outcomes, cardiovascular-related burdens appeared to be higher than those for other non-respiratory causes. For instance, among individuals aged 65 years or older, the median mortality rate per 100,000 reported in the included studies was 108.0 for all-cause outcomes, 66.1 for R+C outcomes, and 56.5 for cardiovascular-related outcomes respectively, while it was 5.38 and 3.3 for cancer and diabetes-related outcomes.

Conclusions: Different modelling approaches have been used to estimate non-respiratory severe influenza disease burden, with certain variations in the reported estimates observed across different causes, age groups and studies. Understanding how modeling techniques affect non-respiratory burden estimates and standardizing methodological approaches is crucial to improving comparability and updating burden estimates.

260

Immunosuppressive capabilities of Influenza A Virus might be related to the origin of the viral NS1 protein

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Background: The viral non-structural protein 1 (NS1) plays a key role in modulating the innate immune response during influenza A virus (IAV) infection enabling the virus to suppress early host defences to establish infection. NS1 is a multifunctional protein that primarily antagonizes type I and type III interferon (IFN) responses by interfering with viral RNA recognition and by disrupting the function of key IFN-stimulated genes (ISG). The immunosuppressive capabilities of NS1 affects how individuals respond to and recover from an infection, but the fundamental mechanisms of how immune evasion by NS1 differ between IAV strains remain to be fully understood.

Methods: Human and porcine airway epithelial cells grown in 24-well plates were infected with four IAVs with a MOI of 0.01 and incubated for 1 hour at 37 °C and 5% CO2. The viruses were of swine origin differing in their NS gene segments (pandemic (pdm09) origin or Eurasian avian-like swine origin, see Table 1). After incubation, virus inoculum was removed and fresh infection media was added. Cells and media were collected at 24, 48, and 72 hours post infection. Antiviral gene expression was analyzed by microfluidic qPCR (Biomark, Fludigm) and viral titers were determined by plaque assay.

Results: Assuming that better immune evasion is correlated with higher viral titers, the pandemic NS1 has an advantage over the Eurasian avian-like swine NS1. Viral pattern recognition receptors (PRRs), cytokines, and ISGs were induced in both human and porcine cells after all infections, but cells infected with IAV with NS gene segments of pandemic origin demonstrated high expression levels of several antiviral immune genes. In contrast, IAV with NS gene segments of Eurasian avian-like swine origin barely expressed any antiviral factors despite infectious virus were demonstrated after all infections.

Conclusions: The immunosuppressive capabilities of IAV might be related to the origin of the viral NS1 protein, as the innate antiviral transcriptional response profiles remained largely conserved when the origin of the NS gene segment was the same.

Table 1: Gene variants of the four selected viruses. Pandemic IAV gene segments (pdm) are marked with green and Eurasian avian like swine (av/sw) IAV segments are marked with blue.

	HA	NA	PB2	PB1	PA	NP	M	NS
H1pdmN1pdm-1	pdm	pdm	pdm	pdm	pdm	pdm	pdm	pdm
H1pdmN1pdm-2	pdm	pdm	pdm	pdm	pdm	pdm	pdm	av/sw
H1pdmN1av-1	pdm	av/sw	pdm	pdm	pdm	pdm	pdm	pdm
H1pdmN1av-2	pdm	av/sw	pdm	pdm	pdm	pdm	pdm	av/sw

261

ECaS

Incidence and transmission of symptomatic and asymptomatic respiratory viral infections in The Gambia: results from a prospective household cohort study

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1: Medical Research Council The Gambia at the London School of Hygiene and Tropical Medicine,, United Kingdom;2: Division of Clinical Medicine, School of Medicine and Population Health, The University of Sheffield;3: Centre for Mathematical Modelling of Infectious Diseases, London School of Hygiene and Tropical Medicine;4: Institute for International Health, Charité Universitätsmedizin,

Background: Data on the true community burden of respiratory viruses in resource-limited settings are scarce, with most studies limited to cases presenting with symptomatic infections to healthcare facilities. We aimed to evaluate the incidence, asymptomatic fraction, and household transmission of multiple respiratory viruses in The Gambia.

Methods: We conducted a prospective household cohort study in The Gambia. Between March 2021 and June 2022, 52 weekly upper respiratory tract swabs were collected from 349 participants in 52 urban/peri-urban households. The study period included SARS-CoV-2 Delta and Omicron BA.1/BA.2 waves in The Gambia. A multiplexed RT-PCR assay was used to identify 12 respiratory viruses.

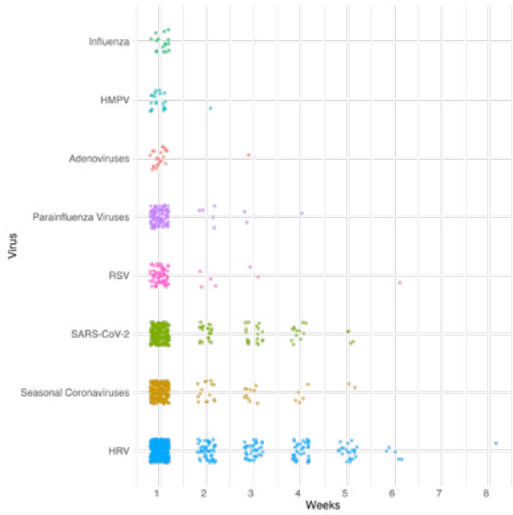
Results: 1423 discrete respiratory viral episodes were detected in 14550 swabs, including 664 human rhinovirus/enterovirus (HRV) infections, 275 SARS-CoV-2 and 237 seasonal coronavirus infections, 105 infections due to parainfluenza viruses 1-4, 79 RSV-A and 15 influenza-A infections. Most episodes were detected for one week only, with longest shedding durations seen with HRV (maximum 8 weeks), RSV (maximum 6 weeks) and seasonal coronaviruses/SARS-CoV-2 (maximum 5 weeks). 88% of all episodes were asymptomatic, with influenza-A (25%), RSV (18%) and SARS-CoV-2 (16%) causing the greatest proportion of symptomatic infections. There was strong evidence of an association between incidence and age ($p<0.0001$). Incidence was highest in children <5 years at 9.0 (95% CI 7.8 – 10.3) episodes per person-year (ppy), falling to 4.0 (3.2 – 5.0) ppy in adults over 50 years. This pattern was observed for HRV, RSV, seasonal coronaviruses and parainfluenza viruses, but not for SARS-CoV-2 in which the incidence was constant across age groups. Household secondary attack rate (SAR) was associated with the specific virus ($p<0.001$), length of viral shedding ($p<0.001$) and the RT-PCR Cycle Threshold($p=0.03$). The SAR was higher for HRV (8.7%, 7.3-10.2), RSV (7.5 %, 95% CI 4.7-11.4) and SARS-CoV-2 (7.6%, 5.7-10.0) compared to seasonal coronaviruses (6.4%, 4.6-8.6).

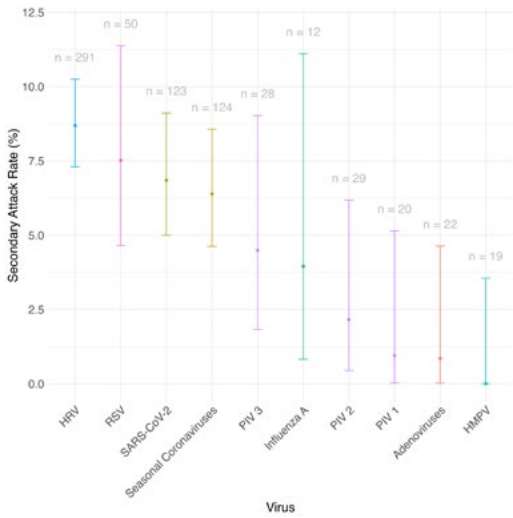
Conclusions: We describe a high burden of largely asymptomatic respiratory viral infections in an African setting which drives household transmission, with age-related reduction in incidence. The symptomatic proportion and the secondary attack rate varied by the infecting virus.

Figure 1 - Infection incidence by age for each virus

Figure 2 - Duration of viral shedding

Figure 3 - Seconary attack rate (SAR) by virus


Influenza
HMPV
Adenoviruses
Parainfluenza Viruses
RSV
SARS-CoV-2
Seasonal Coronaviruses
HRV
Virus
1
2
3
4
5
6
7
8
Weeks


12.5
10.0
7.5
5.0
2.5
0.0
Secondary Attack Rate (%)
n = 291
n = 50
n = 123
n = 124
n = 28
n = 12
n = 29
n = 20
n = 22
n = 19
HRV
RSV
SARS-CoV-2
Seasonal Coronaviruses
PIV 3
Influenza A
PIV 2
PIV 1
Adenoviruses
HMPV
Virus

265

Molecular insights into the binding properties of two broadly inhibiting anti-N1 monoclonal antibodies

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Background:

The influenza A virus has two major surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). Recently two broadly inhibiting monoclonal antibodies (mAbs) against the N1 NA have been characterized, originating from a human patient infected with the 2009 pandemic H1N1 influenza virus (Hansen **et al.**, 2023, Immunity). These antibodies, named 3H03 and 2H08, have shown broad inhibition of HxN1 viruses **in vivo** and **in vitro**. The study also included experimental structures of the mAbs bound to influenza virus neuraminidase from A/Brevig Mission/1/1918 (H1N1). However, in further measurements it was observed that for more recent H1N1 vaccine strains the binding and inhibiting efficiency of these mAbs can decrease. These findings were surprising, as 3H03 and 2H08 have shown binding to and inhibition against various H1N1 strains from 1918 to 2015.

Methods:

We set out to explore the molecular reasons behind this binding difference. We used AI-aided structural predicting methods based on AlphaFold to find molecular reasons for the decreased affinity observed in later years. Then we used **in vitro** wet-lab methods enzyme-linked immunosorbent assay (ELISA) and enzyme-linked lectin assay (ELLA) to characterize the binding affinity and inhibition capabilities of 2H08 and 3H03 with selected HxN1 strains, based on our initial models. We used further computational structural models and molecular dynamics (MD) simulations in an attempt to fully elucidate the underlying molecular effects governing the binding and inhibition of these mAbs to various N1 NAs.

Results:

Our initial structural models have indicated that the decreased binding of the mAbs is due to a change in the electrostatic surface potential of the epitope surface in recent years, and four amino acid mutations important for this charge change were identified. In our **in vitro** measurements we observed that both the binding affinity and inhibition efficiency decreased towards H1N1 variants isolated from 2019 on, including strains with all four of these mutations. Interestingly, the binding affinity measured by ELISA has also shown a difference based on the tetramerization domain used for the recombinantly expressed the NA head. To explain this effect beyond the electrostatic change, we are also doing MD simulations and further **in vitro** measurements.

Conclusions:

An influenza virus NA epitope targeted by mAbs isolated from a 2009 pH1N1 patient has changed in H1N1 variants isolated post-2019, causing a decrease in binding affinity and inhibition efficiency. The molecular reason behind this change was characterized using structural modeling and MD simulation methods. Our results can provide an insight into adaptive NA evolution and important considerations for vaccine and antibody design. The latest results will be presented at the conference.

266

A series of diagnostic contamination events from seasonal influenza vaccines.

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Background: Polymerase chain reaction (PCR) is widely used for the diagnosis of influenza infections due to its high sensitivity and ability to distinguish between influenza A and B viruses, as well as their respective subtypes and lineages. However, this sensitivity also makes PCR vulnerable to false-positive results from environmental contamination. During the 2022–2023 influenza season in Norway, an unusual pattern emerged, with pre-epidemic diagnostic samples testing positive for both influenza A and B, or for B/Yamagata lineage—which is presumed extinct—raising concerns about possible external contamination. These anomalies were temporally associated with the national influenza vaccination campaign.

Methods: A retrospective review of influenza diagnostic data was conducted to assess the frequency and distribution of unusual detection patterns. The study focused on diagnostic samples collected between weeks 40 and 52 of 2022, with particular attention to weeks 42 to 46, coinciding with the national influenza vaccination campaign. The investigation included analysis of PCR results, frequency of co-detections (influenza A and B), and identification of B/Yamagata lineage cases. Environmental surface swabs were collected from multiple vaccination sites and analyzed for influenza virus RNA using PCR. Sequencing of selected suspect diagnostic samples was also performed to identify the likely source of contamination.

Results: An increased frequency of diagnostic samples showing atypical detection patterns, particularly dual influenza A and B positivity and B/Yamagata detection, was observed during weeks 42 to 46 of 2022. The prevalence of such suspected contamination cases peaked at 3.45% of all samples during this period. Environmental sampling from vaccination clinics confirmed the presence of both influenza A and B viral RNA, supporting the hypothesis of vaccine-derived contamination. Molecular sequencing of select diagnostic samples demonstrated influenza RNA sequences consistent with components of the seasonal influenza vaccine, further implicating vaccine contamination as the source.

Conclusions: This investigation highlights the risk of diagnostic sample contamination with influenza RNA originating from seasonal virion-derived influenza vaccines. Such contamination can compromise diagnostic accuracy and distort influenza surveillance data, including the false appearance of co-infections and the detection of lineages such as B/Yamagata that are no longer in circulation. Awareness of this contamination risk is essential during vaccination campaigns, especially in shared clinical spaces. Preventative measures, including enhanced sample handling protocols and environmental hygiene, are critical to maintaining the integrity of influenza diagnostic testing and public health surveillance systems.

267

Drivers of Influenza Vaccination Behavior Among Adults in Türkiye: A Cross-Sectional Study Using Discrete Choice Experiment Method

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Background: Despite the free provision of influenza vaccines to risk groups in Türkiye, adult vaccination coverage remains remarkably low. There is limited research using quantitative preference elicitation methods to evaluate how behavioral, informational, and structural barriers contribute to vaccine hesitancy. Effectively designed discrete choice experiments (DCE) can supply high-quality, policy-relevant results. We aimed to identify the key drivers influencing adult influenza vaccination behavior in Türkiye and assess the relative importance of vaccine attributes using a DCE methodology.

Methods:A DCE survey was conducted at a Family Health Center in Ankara in February 2025. Respondents evaluated hypothetical flu shot programs defined by six attributes: efficacy, serious side effect risk, co-payment discount, access location, place of production, and price. A random parameter logit model was applied to estimate attribute-level preferences. Additional sociodemographic and vaccination behavior data were analyzed using a logit model to determine correlates of vaccine uptake.

Results: Data of 149 valid surveys were analyzed. The mean age of the participants was 42.2 years (SD 15.5). Only 10% received a flu shot in 2023-2024, and 4% in 2024-2025, and 72% had never been vaccinated and had no intention of doing so. Older age (OR: 1.08 per year, **p**<0.05), higher income (OR: 1.20, **p**<0.10), and presence of chronic illness (OR: 8.77, **p**<0.05) were significantly associated with vaccination in at least one of the seasons. Factors influencing the likelihood of receiving the influenza vaccine were analyzed through the responses to DCE questions. Participants valued domestic vaccine production ("Made in Türkiye"), higher efficacy, and lower side effect risk most strongly. Willingness to pay for locally produced vaccines was 4,924 TL ($135) higher than for imported ones. Emotional messaging (e.g., images of children affected by influenza) and risk-based education substantially increased self-reported vaccination intention. Access-related interventions, such as evening/weekend availability or financial incentives, had minimal influence. Protest voters (n=12), who refused vaccination in all scenarios, were more likely to have lower education, and be susceptible to misinformation although having chronic illness.

Conclusions:

Ongoing monitoring of drivers of vaccination behavior via DCEs and other similar preference elicitation tools can aid policymakers in the development and implementation of responsive, effective and locally tailored immunization programs. The results of this study showed that utilization of trusted healthcare settings (e.g., family health centers), emotionally resonant communication, and promotion of locally manufactured vaccines can be effective strategies to promote the influenza vaccination acceptance of adults. Routine integration of vaccination for chronic disease patients and targeted interventions for protest vaccine-hesitant groups is essential to overcoming behavioral barriers.

269

Characterisation of highly pathogenic avian influenza A H5N1 virus in human nasal and bronchotracheal epithelial cells

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Background:

The recent global spread of the highly pathogenic avian influenza (HPAI) H5N1 virus (clade 2.3.4.4b) to diverse mammalian species, including dairy cows, highlights its cross-species adaptability. However, the molecular mechanisms underlying this adaptability and the tissue tropism of clade 2.3.4.4b HPAI H5N1 remain poorly understood. This study comprehensively compares the replication dynamics, tropism, and host response of contemporary HPAI H5N1 variants, including the ancestral 2004 strain (VN1194), clade 2.3.4.4b genotypes B3.13 and D1.1, and seasonal influenza virus, using human airway organoids (HAOs). This comparative approach aims to elucidate clade 2.3.4.4b HPAI H5N1 has evolved to overcome species barrier and establish infection in mammalian hosts.

Methods:

Normal human nasal and bronchial epithelial cells were differentiated to generate HAOs for infection experiments. H5N1 viruses, including a mink-derived isolate and genotype B3.13 strains from cattle and humans, and genotype D1.1 variants from human cases, were generated via reverse genetics. Seasonal H3N2 was isolated from a patient in 2022. HAOs were infected with these viruses and replication kinetics were assessed. Immune responses were evaluated using single-cell RNA sequencing. Viral tropism was analysed by immunofluorescence staining.

Results:

We found that clade 2.3.4.4b H5N1 isolates from cattle and human cases replicated more efficiently than the mink-derived strain. Seasonal H3N2 induced the strongest immune responses, particularly type I interferons, IL-6 and IL-1β, which were delayed during H5N1 infections. Single-cell RNA sequencing confirmed distinct transcriptomic profiles between H3N2- and H5N1-infected organoids. Immunofluorescence imaging revealed that H5N1 preferentially infected airway club cells, while H3N2 infected both club and ciliated cells. Finally, since mutations at positions 190 and 226 in hemagglutinin (HA) from the A/British Columbia/PHL-2032/2024 (D1.1) human isolate were identified, analysis with reverse genetically derived viruses carrying these substitutions showed that E190D mutation enhanced viral infection in HAOs.

Conclusion:

While previous animal studies suggested limited airborne transmissibility of clade 2.3.4.4b H5N1, our findings demonstrate that both B3.13 and D1.1 genotypes replicate robustly in HAOs, more efficiently than the VN1194 strain or the mink-derived isolate. This suggests these viruses from the clade 2.3.4.4b (genotypes B3.13 and D1.1) have acquired the enhanced capacity to infect human cells. The delayed immune response to H5N1 may indicate immune evasion capabilities that facilitate crossing species barriers. Notably, infection with the human-derived TX37 isolate induced a transcriptomic profile similar to seasonal H3N2 infection, supporting the hypothesis that bovine H5N1 viruses may be acquiring human-like characteristics. Finally, our findings reveal that the E190D mutation in HA, rather than substitution at position 226, appears critical for the human adaptation of clade 2.3.4.4b H5N1 viruses.

271

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Repeat influenza vaccination effects in 2021/22 and 2022/23 in a community-based cohort in Hong Kong

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Background: Repeated annual influenza vaccination has been associated with attenuated immune responses and reduced clinical effectiveness. Most studies of repeat vaccination immunogenicity have focused on the responses at 30 days after vaccination.

Methods: In this community-based cohort study in Hong Kong, we investigated the impact of repeated annual influenza vaccination on HAI titer boosting and waning rates of 5 vaccine strains, namely A/Brisbane/2/2018 (H1N1), A/Victoria/2570/2019 (H1N1), A/Hong Kong/4801/2014 (H3N2), A/Cambodia/e0826360/2020 (H3N2), B/Phuket/3073/2013 (Yamagata), in 2021/22 and 2022/23. We divided participants into three groups based on vaccination history in the prior six years: no prior vaccination, low uptake (1-2 vaccinations) and higher uptake (3+ vaccinations). An immunogenicity score was calculated to separate ceiling effects from repeat vaccination effects during titer boosting. A power model was used to characterize HAI titer waning rates after day 14 post-vaccination and differences by vaccination history.

Results: We found reduced mean-fold rises in the higher vaccination uptake group for A/Victoria/2570/2019 and B/Phuket/3073/2013 in 2021/22. Prior year vaccination (2020/21) was associated with reduced HAI titer boosting for A/Victoria/2570/2019, A/Cambodia/e0826360/2020 and B/Phuket/3073/2013. The higher vaccination uptake group had lower estimated antibody titers at day 14 post-vaccination, but slower waning after 2021/22 vaccination for A/Victoria/2570/2019 and B/Phuket/3073/2013, and reached similar titers at six months post-vaccination compared to the groups with less vaccination history.

Conclusions: Our findings suggest that repeated influenza vaccination is associated with attenuated antibody responses at day 14 post-vaccination, but has less impact on antibody levels at six months post-vaccination.

273

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Differential transmission dynamics of two different H1N1 swine influenza viruses and a respiratory coronavirus in swine

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1: Ghent University, Belgium;2: Pirbright Institute;3: University of Leeds;4: Universidad Pontificia Comillas

9. **Background:**
Influenza and coronaviruses have caused some of the deadliest human pandemics, and pigs are important virus reservoirs. The COVID-19 pandemic has caused a surge in virus transmission studies, but there are few experimental transmission studies or comparative studies with different respiratory viruses. Here, we aimed to compare the transmission efficiency of 3 endemic respiratory viruses of swine: the porcine respiratory coronavirus (PRCV) and the 2 most important European swine influenza A viruses (swIAV), the 2009 pandemic H1N1 (pH1N1) and the Eurasian avian H1N1 (EA H1N1) virus.

10. **Methods:**
We performed separate transmission experiments with each of the 3 viruses. In each experiment, 8 donor pigs were inoculated intranasally with 7 log10 TCID50 of virus. Two days later, 8 direct contact pigs were placed in the same pen as the donor pigs. Eight indirect contact pigs and 2 indirect contact ferrets were placed in separate pens but in the same room and only had airborne contact with the donor pigs. Nasal swabs were collected daily from each animal (0-12 days post inoculation (DPI), 0-18 days post contact (DPC)). Eighteen environmental samples were collected every other day. Virus titers were determined by titration in MDCK cells. Blood for serological analyses was collected at multiple timepoints.

11. **Results:**
The mean duration of nasal virus excretion of the donor pigs was 5 (swIAV) or 6 (PRCV) days. All 3 viruses transmitted to contact pigs. The average lag period was <2 days in direct contact pigs and 4-5 days in indirect contact pigs. Virus excretion became undetectable in the direct contact pigs by 10 (swIAV) or 11 (PRCV) DPC, and in the indirect contact pigs by 12 (EA H1N1) or 14 (PRCV, pH1N1) DPC. All pigs showed seroconversion. Both swIAVs transmitted from pigs to ferrets, while PRCV did not. All 3 viruses could be isolated from a few environmental samples in direct contact with the donor pigs, such as chewing ropes, feeders and drinkers, gloves and boots of the caretakers. Highest virus titers were detected in chewing ropes. Objects out of reach of the animals were generally negative for virus.

12. **Conclusion:**
The results suggest that the EA H1N1 swIAV is more efficiently transmitted through the air than the other 2 viruses. PRCV was excreted for a longer period than the influenza viruses. In contrast to PRCV, swIAV have zoonotic potential, as shown by their transmission from pigs to ferrets. We will use the longitudinal nasal shedding data to calibrate a mathematical viral kinetics model which captures pig-level growth, saturation and decay, yielding a more detailed mechanistic quantification of viral shedding dynamics and duration for each virus. These estimates can be integrated into a Wells-Riley approach to quantify virus-specific airborne transmission under realistic ventilation scenarios.

275

ECaS

Shielding the Vulnerable: Single domain antibody (sdAb) based influenza defense for high-risk populations

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Background

Every year, influenza poses a serious health risk to vulnerable populations, particularly the elderly and severely obese. These high-risk groups encompass millions of individuals who not only face heightened anxiety during the influenza season but also represent a potential strain on the healthcare system. While current influenza vaccines offer moderate protection in healthy individuals, their efficacy drops substantially in those most at risk. To address this critical gap, we propose the development of a broadly neutralizing influenza treatment based on single-domain antibodies (sdAbs) for early, targeted intervention. Such an approach could offer rapid, effective protection for vulnerable populations at a global scale.

Methods

To identify and characterize candidates for an sdAb-based broadly neutralizing antiIAV drug for early intervention and prophylactic use. The methodology used was branched into 3 stages:

(i) Generation of IAV antigens through a multi-faceted approach, including **in silico**-identified conserved peptides; production of naturally glycosylated influenza proteins; and work with whole inactivated viruses.

(ii) Phage display generation and characterization of sdAbs based on our inhouse synthetic human sdAb libraries.

(iii) Assessing neutralization capabilities of a selection of the sdAbs. Testing of the neutralization capability or reduced infectivity of the sdAb in relevant respiratory cells **in vitro**.

Results

Two viral surface proteins, hemagglutinin (HA) and neuraminidase (NA), were chosen as targets for a phage display campaign, with the goal of interfering with IAV infection at multiple stages of the viral life cycle.

To guide target selection, a comprehensive sequence analysis was carried out to identify conserved regions within proteins of several influenza A virus subtypes. These conserved peptide sequences were subsequently synthesized and expressed to mimic human-like glycosylation patterns, and used as targets in a phage display campaign against human sdAb libraries.

We anticipate identifying high-affinity binders towards all conserved epitopes. Candidate sdAbs will be selected based on their sequence diversity and predicted binding regions. Binding affinity assays will be performed via ELISA and neutralization assays will confirm **in vitro** sdAb potency.

Conclusion

This study aims to identify and develop broadly-acting sdAbs targeting conserved regions HA and NA across multiple IAV subtypes. Through extensive sequence conservation analysis and a targeted phage display campaign, we anticipate isolating sdAbs capable of recognizing structurally and functionally relevant epitopes that remain stable across antigenic variation. These findings are expected to provide a foundation for the development of novel antiviral candidates with cross-strain activity, serving as a stepping stone towards a universal influenza therapy.

276

Comparing molecular and serological methods for detecting respiratory syncytial virus (RSV) infection in children aged 6–23 months in South Africa, 2022

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Background

Respiratory syncytial virus (RSV) is a leading cause of lower respiratory tract infection and hospitalisation in young children. As RSV immunization strategies are introduced, accurate detection of infections becomes increasingly important for measuring real-world effectiveness of interventions and understanding virus circulation. We aimed to compare detection of RSV infections through molecular and serological techniques.

Methods

We enrolled healthy children aged 6-23 months in a prospective cohort study from May through October 2022 in South Africa, collecting thrice weekly mid-turbinate nasal swabs, irrespective of symptoms, for RSV rRT-PCR testing. Serum was collected at baseline and end of follow-up (exit). Anti-RSV IgG was detected using enzyme-linked immunosorbent assay (ELISA) (EUROIMMUN, Germany) and a multi-pathogen bead-based immunoassay (MIA) (Bio-plex 200 System: Bio-Rad Laboratories Ltd., USA; RSV (pre-F) antigen: Sino Biological, China). Baseline sero-positivity defined as ELISA titre ≥22 relative units [RU]/ml. We calculated sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Cohen's kappa statistic (κ) of PCR (≥1 positive swab), ELISA (four-fold titre rise) and MIA (four-fold rise plus exit titre ≥1 arbitrary units [AU]/ml) to detect recent RSV infection (irrespective of baseline seropositivity) compared to positivity on ≥2 assays.

Results

We included 80/93 (86%) enrolled children (excluded four without paired sera and nine where baseline bloods collected during/after PCR-confirmed RSV infection). The 2022 RSV season started mid-February. At baseline, 22/80 (28%) children were RSV seropositive, 6/22 (27%) had subsequent infections (baseline ELISA GMT range 28-143RU/ml): two each positive on PCR-only, MIA-only, and PCR and MIA. We detected 29/80 (36%) infections by PCR, 33/80 (41%) by ELISA, 35/80 (44%) by MIA, and 40/80 (50%) by all assays combined. Of 29 PCR detections, 23 (79%) were detected on both serological assays, two by ELISA only, two by MIA only, and two on neither. 11/80 (14%) infections were detected by serology and not PCR (three MIA, one ELISA, seven both). ELISA had 92% (95%CI 78-98%) sensitivity, 100% (95%CI 92-100%) specificity, 100% (95%CI 89-100%) PPV, 94% (95%CI 82-99%) NPV and κ of 0.9 (95%CI 0.8-1.0). MIA had 94% (95%CI 81-99%) sensitivity, 93% (95%CI 81-99%) specificity, 92% (95%CI 78-98%) PPV, 95% (95%CI 84-99%) NPV and κ of 0.9 (95%CI 0.8-1.0). PCR had 78% (95%CI 61-90%) sensitivity, 98% (95%CI 88-100%) specificity, 97% (95%CI 82-100%) PPV, 84% (95%CI 71-93%) NPV and κ of 0.8 (95%CI 0.6-0.9).

Conclusions

Molecular detection, even with intense follow-up, may miss a third of infections in children. MIA and ELISA correlated well, with high sensitivity, specificity, PPV and NPV. Serological assays offer complementary tools for identifying RSV infections in children. As interventions are rolled out, incorporating serology into surveillance could improve infection burden estimates. Validating serological definitions of infection in immunized populations, older children and adults is needed.

277

Natural Killer cells in obese children have a 'trained' phenotype to ex vivo influenza stimulation which does not reduce with weight loss

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Children living with obesity have an increased risk of severe influenza virus infection, although the underlying mechanisms are unclear, especially in children. Natural Killer (NK) cells play a key role in the innate immune responses to influenza A virus (IAV) and clearance of IAV-infected cells. 'Trained immunity' of NK cells refers to an initial stimulus inducing metabolic and epigenetic changes that can make these cells hyperinflammatory upon restimulation. Here, we test whether obesity induces trained immunity in paediatric NK cells that results in a hyperinflammatory response to IAV (which is associated with immunopathology). We further assess whether weight loss is sufficient to reverse any observed training. Here, we show that NK cells isolated from venous blood of children with obesity have a hyperinflammatory phenotype, with increased proportions of TLR4, HLA-DR, CD11c and CD11b expressing cells relative to children without obesity. Additionally, we demonstrate that NK cells children with obesity are 'trained' with a heightened IFN-γ response to stimulation with IAV **ex vivo**. At a follow-up timepoint after engagement with a weight loss clinic, a decrease in body mass index (BMI) z- did not result in normalization of IFN-γ responses, suggesting that weight loss is not sufficient to normalize NK cell function in response to IAV stimulation. Better understanding of obesity-related immune modulation in children is critical for risk-stratification, targeted prevention and improving preparedness for the next influenza virus pandemic.

278

Genomic characterisation of highly pathogenic avian influenza H5N1 Eurasian genotype EA-2024-DI to inform zoonotic risk

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Background

The A(H5N1) clade 2.3.4.4b panzootic represents an increasing risk to global public health as the number of zoonotic events increase and mutations conferring adaptation to mammalian hosts are acquired. The reassortment of internal gene segments with other influenza A viruses, particularly segments encoding the polymerase complex, can also impact zoonotic risk. Understanding the ancestry of each segment can inform the assessment of emerging genotypes. Currently, clade 2.3.4.4b Eurasian EA-2024-DI is the most prevalent H5N1 genotype detected in genomic data from European birds. Here we aimed to identify the reassortment pattern of EA-2024-DI gene segments to assess public health risk.

Methods

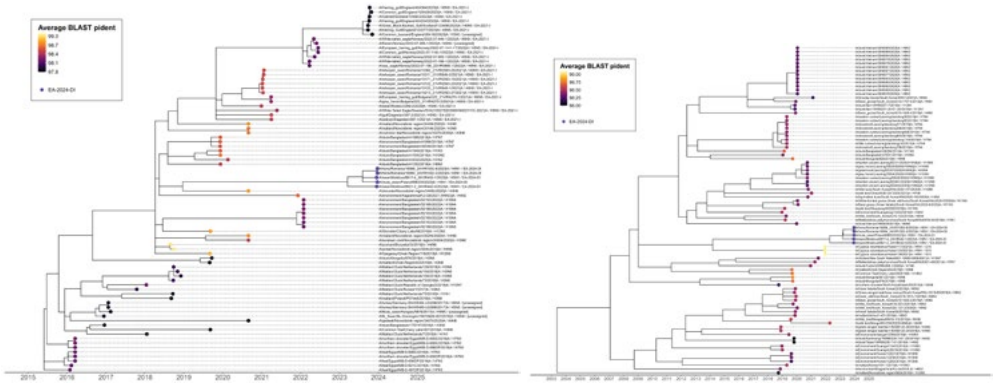
Genomes for all influenza A subtypes with collection dates between January 2016 and November 2023 were downloaded from the Global Initiative on Sharing All Influenza Data (GISAID) to build a custom Basic Local Alignment Search Tool (BLAST) database. Using BLAST, we identified sequences of high identity to genomes downloaded from GISAID designated as EA-2024-DI by Genin2. The top 50 high identity sequences were used to construct time-scaled phylogenies for each segment using the TreeTime package.

Result

We identified that the EA-2024-DI genotype consists of a Eurasian EA-2023-DA backbone with reassorted PB1 and PB2 segments. The PB1 segment is closely related to avian strains from the Russian Federation circulating between 2018-2021. It is estimated the PB1 segment has a H3N8-like progenitor with the most recent common ancestor (MRCA) placed at the end of 2018. The PB2 segment showed intra-subtype reassortment, with an avian H5N1-like virus progenitor of Asian origin with the MRCA estimated in 2014.

Conclusions

Here we discovered that EA-2024-DI has reassorted PB1 and PB2 segments creating a novel polymerase complex. The ancestral polymerase genes circulated in avian hosts prior to reassortment, limiting opportunity for mammalian adaption. The EA-2024-DI genotype also shows no indication of increased mammalian infections with only sporadic self-limited spillover events. Public health management of spillover infections in humans can therefore be managed proportionately, improving patient experience and reducing the burden on Health Protection Teams. Continual genomic surveillance of avian H5N1 infections is key to inform zoonotic risk of emerging strains to the UK population.



279

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Robust Antibody Responses After Influenza Vaccination in Young Children in Rural Bangladesh

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Background: Young children in low- and middle-income countries (LMIC) are vulnerable to influenza, and underlying malnutrition may impact immune responses. This study investigates antibody responses to quadrivalent influenza vaccination (QIV) in young children in rural Bangladesh, with a focus on the influence of malnutrition.

Methods: In this phase IV clinical trial (NCT04998344), 2,736 children aged 6–59 months were randomized by village to receive either QIV or inactivated polio vaccine (IPV) as control. Pre- and post-vaccination blood samples were collected to assess hemagglutination inhibition (HI) antibody responses. Nutritional status was evaluated using WHO growth standards.

Results: Despite a high prevalence of malnutrition (36% of children met WHO criteria for stuntung, wasting or underweight), QIV induced robust antibody responses to all vaccine strains, with post-vaccination serumprotection rates ranging from 47.2% to 93.8% depending on the vaccine strain. Nutritional status did not significantly impair HI titers, with preserved immunogenicity even among severly malnourished children.

Conclusion: Quadrivalent influenza vaccination elicits strong antibody responses in young children in LMIC, including those who are malnourished. These results highlight the feasibility and potential impact of seasonal influenza vaccination in vulnerable pediatric populations in resource-limited settings.

286

Using routine infection control active surveillance swabs for respiratory viral detection

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Recent influenza, human metapneumovirus and the global COVID-19 pandemic have highlighted the need for urgent rapid detection and epidemiological investigations into respiratory viral infections. These are difficult to operationalize quickly in large busy tertiary hospitals and often outbreak detection is delayed. We studied whether routinely collected Methicillin resistant Staphylococcus aureus (MRSA) surveillance swabs used for infection control can be used to detect respiratory viral infections.

The National University Hospital (NUH) is a 1000 bed tertiary hospital in Singapore with an active surveillance program to identify MRSA carriers on admission for isolation or cohorting for infection control purposes. We obtained leftover MRSA e-Swab (Copan) sample aliquots from 39 adult subjects who had consented to participate in an influenza study collected between July 2024 till March 2025 (DSRB ethics approval reference number 2023/00365). Subjects were identified as positive for a respiratory virus by their treating clinical teams using the Cobas Influenza A/B & RSV test by Roche, or the Biofire RP2.1 panel by bioMérieux.

The swabs were stored at -80 degrees till thawed and then subjected to the Biofire RP2.1 test conducted in our College of American Pathologists accredited hospital clinical laboratory.

This study found that leftover MRSA swab samples had an overall 61.5% (24 of 39) sensitivity rate for detection of respiratory viruses.

This varied from 54.5% (12 of 22) for influenza A to 75% for influenza B (6 of 8), 80% for RSV (4 of 5), and 50% for hMPV (2 of 4).

Although the numbers are small, it does appear that routine MRSA active surveillance swabs collected for infection control purposes can be used for detection of respiratory viruses using a commercially available platform. This will greatly simplify outbreak investigations for respiratory viruses and could be a useful epidemiological tool to determine baseline rates of respiratory viruses in various populations (especially in long term care facilities where MRSA infection control surveillance has been routine for years) to guide vaccination strategies and understand the burden of respiratory illnesses.

289

Genomic Surveillance of Respiratory Syncytial Virus (RSV) during Nirsevimab deployment, Eastern Spain, early results

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Introduction. In October 2023, Spain launched a new universal immunization program with nirsevimab for all children under one year of age facing their first RSV season. The program achieved over 90% uptake and demonstrated high effectiveness, reducing both hospitalizations and primary care visits. Genomic viral surveillance data in Spain for RSV breakthrough infections after nirsevimab administration is not yet available.

Aims. Our goal was to identify changes in the viral F protein that might be associated with alterations in nirsevimab susceptibility.

Methods. We conducted whole-genome sequencing of RSV-A and RSV-B isolates from 83 children under one year of age with confirmed RSV infection during the 2023/2024 season. All cases were diagnosed by reverse transcription PCR (RT-PCR) for RSV RNA from oro/nasopharyngeal swabs. Samples were collected prospectively from two clinical settings: (i) children presenting with influenza-like illness (ILI) or severe acute respiratory infection (SARI) (n=59) who required hospitalization at five hospitals in eastern Spain —one in Barcelona and four in the Valencia region; and (ii) children presenting with low respiratory tract infections (LRTI) at a primary care network in the Valencia Region (n=24).

Results. There were no significant differences in RSV subtype distribution (overall 81.9% RSV-A) between outpatients and inpatients, nor between nirsevimab recipients and non-recipients. Sequencing was successful for RSV clade assignation in 68/83 samples: 41.7% of samples from primary care and 98.3% from hospital inpatients. The 54 RSV-A sequences belonged to clades A.D.1 (n=32), A.D.3 (n=12), and A.D.5 (n=10), and the 14 RSV-B sequences identified all belonged to the B.D.E.1 clade. Overall, amino acid diversity in the F protein was low, with 543/575 (94.4%) sites conserved in RSV-A isolates and 557/575 (96.9%) in RSV-B isolates. For RSV-A there were only 2/54 sequenced isolates with mutations in the Site Ø F2 subsite (one from a nirsevimab recipient) without known effect in nirsevimab binding, whereas all RSV-B isolates showed the three substitutions (I206M+Q209R+S211N) characteristic of the currently circulating B.D.E.1 clade, irrespective of nirsevimab administration.

Conclusions. We found no significant differences in the distribution of RSV clades or amino acid mutations/polymorphisms in nirsevimab binding sites on the F protein independent of immunization or care setting. Genomic surveillance of RSV will continue to be important for monitoring potential changes in nirsevimab binding sites, and for RSV vaccine monitoring. Genome-wide RSV data analyses from the 2024/2025 season nirsevimab campaign in our region are warranted.

290

The impact of influenza on healthcare utilization in patients with diabetes

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Background:

Individuals with diabetes are at increased risk of influenza disease severity, warranting annual vaccination. However, the full impact of influenza on this population and on related healthcare utilization is understudied. This study aims to assess the impact of laboratory-confirmed influenza (LCI) on the risk and severity of diabetes decompensation and associated healthcare use among adults aged 50 years.

Methods: We conducted a retrospective cohort study over an 8-year period (from January 2011 through December 2018) using regional electronic healthcare databases in Valencia, Spain. Patients with diabetes were identified through a systematic search of International Classification of Diseases (ICD) codes in both ambulatory and hospital discharge databases. Other selected chronic conditions included chronic obstructive pulmonary disease (COPD), asthma, obesity, heart failure (HF), liver disease, kidney disease, and immunocompromised status. LCI cases were identified from microbiological registries. Influenza-related consultations or admissions occurring on the same day as diabetes-related visits were excluded from the analysis. Diabetes-related healthcare resource utilization (including the number of primary care and specialist visits, hospitalizations, length of stay, and ICU admissions) was compared during the six months before and after influenza virus infection. Poisson, binomial, and log-normal regressions were used to compare rates of healthcare utilization, incorporating robust (sandwich) standard errors to account for intra-subject correlation.

Results: The study cohort included 3,753 subjects with LCI and diabetes, with a mean age of 69 years; 54% were male. The most frequently identified chronic conditions in this population were COPD (49.56%), kidney disease (38.1%), and immunocompromised status (39.14%). Healthcare resource utilization was higher during the six months following influenza virus infection. There were increased rates of primary care visits by 19% (RR: 1.19, 95% CI: 1.13–1.26) and specialist visits by 74% (RR: 1.74, 95% CI: 1.47–2.06) after the LCI episode. No significant differences were observed in hospital admissions, length of hospital stay, or ICU admissions between the two periods (Table 1).

Conclusions: This study quantify the impact of influenza in patients with diabetes, documenting the increased demand for healthcare resources following LCI. Efforts to increase influenza vaccination uptake in this population can improve individual-level protection against influenza-related complications and reduce strain on the healthcare system, particularly during respiratory seasons.

Table 1. Estimates of RR, OR, and MR (95% CI) for diabetes-related healthcare utilization before and after LCI.

	PRE influenza	POST influenza	
Nº PC Visits	1	1.19(1.13-1.26)	RR(95% CI)
Nº PC Visits (Specialists)	1	1.74(1.47-2.06)	RR(95% CI)
Hospital admission	1	0.94(0.73-1.21)	OR(95% CI)
Length of hospitalization (days)	1	0.95(0.77-1.26)	MR(95% CI)
ICU admission	1	1.14(0.51-2.57)	OR(95% CI)

ICU: Intensive Care Unit; PC: primary care; RR: Relative Risk; OR: Odds ratio; MR: Mean Ratio; LCI: Laboratory-confirmed influenza.

291

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Tracking the evolution of swine influenza A viruses in Belgium, 2022-2024

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Background: Swine influenza A virus (swIAV) is endemic in the swine population globally. This virus exhibits substantial genetic and antigenic diversity due to reassortment and antigenic drift. The geographical segregation of pig populations, combined with repeated introductions of human influenza viruses, drives the continuous evolution and diversity of swIAV across continents. Outdated vaccine strains may limit current vaccine effectiveness. Swine influenza viruses also pose a significant public health risk due to their zoonotic potential. However, surveillance for swIAV in Europe lags behind that in the USA, or surveillance for avian or human influenza viruses. This study aimed to identify the prevailing swIAV subtypes in Belgian swine from 2022 to 2024 and study their genetic and antigenic characteristics.

Methods:

Between 2022 and 2024, 53 swIAV isolates were obtained by virus isolation in Madin-Darby Canine Kidney (MDCK) cells. Isolates originated from lung tissue or nasal swabs of pigs with respiratory illness. The swIAV subtypes were determined by multiplex reverse transcription quantitative PCR (qPCR) specific for hemagglutinin (HA) and neuraminidase (NA) genes. Genetic characterization involved whole genome sequencing (WGS) to determine genetic constellations. Phylogenetic analysis of HA1 and NA sequences was performed to determine evolutionary relationships and clades. Antigenic characterization was performed by cross hemagglutination inhibition (HI) assays with reference influenza A virus strains from swine and humans, including vaccine strains from both species. Monospecific antisera were produced in swine, by vaccination with matched strains or commercial vaccines, or by ferret infection.

Results:

H1N2 (53%) and H1N1 (47%) were the predominant subtypes, H3N2 was not detected. Three distinct HA lineages were identified: the European avian-like H1 lineage (1C) (48%), the 2009 pandemic H1 lineage (1A) (28%) and the European human-like H1 lineage (1B) (27%). The isolates belonged to four distinct H1 clades: 1C.2.1, 1C.2.2, 1B.2.1 and 1A.3.3.2. The 1C.2.2 clade was dominant. NA genes clustered into three lineages: avian-like N1 (45%), human-like H3N2-derived N2 (47%, swG84-like), and human-like H1N2-derived N2 (8%, swSC94-like). Internal gene analysis showed that 50% of the isolates were reassortants between lineages 1A and 1C. Antigenic characterization showed greater similarity to European strains than North American strains. The recent swIAV retained some cross-reactivity with most swine vaccine strains. However, there was low or no cross-reactivity with human vaccine strains from lineages 1A and 1B.

Conclusion:

These findings demonstrate the continuous evolution of swIAVs and the cocirculation of multiple lineages and reassortants. As in previous Belgian studies, the 1C lineage was predominant. However, the prevalence of clades 1C.2.2 and 1A.3.3.2 has increased. While the identified clades are also found in neighboring countries, their distribution and relative dominance exhibit distinct patterns in Belgium**.** Many isolates were antigenically distinct from the current human vaccine strains, suggesting poor cross-protection and a potential zoonotic risk.

292

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Bivalent mRNA or self-amplifying mRNA-based vaccines induce antigen-specific T-cell responses in ferrets

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Background:

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and influenza virus continue to pose a public health threat, predominantly to immunocompromised individuals and the elderly. Vaccines are the most effective means of preventing severe disease; however, vaccines have not been successful in preventing infection and transmission. Breakthrough infections occur in high numbers, highlighting the need for novel vaccines. The ferret model is widely used to evaluate the efficacy of novel influenza vaccine candidates, due to the similar distribution of sialic acids in the ferret respiratory tract compared to that in humans. Additionally, ferrets have been used to evaluate the efficacy of coronavirus disease-2019 (COVID-19) vaccines. Whereas vaccine efficacy can easily be studied in ferrets by evaluating susceptibility to challenge infections, vaccine immunogenicity has been understudied in this model. Especially measuring cellular immune responses is difficult due to the limited availability of ferret-specific reagents. Improved assays to measure these responses would provide valuable tools in the preclinical evaluation of newly-developed influenza and COVID-19 vaccine candidates.

Methods:

Here, we used an IFNγ ELISpot assay in conjunction with a newly-designed flow cytometry panel to quantify and phenotype antigen-specific T-cell responses in ferrets. Next, we validated these assays by evaluating the immunogenicity of novel lipid nano-particle (LNP)-encapsulated bivalent mRNA and self-amplifying mRNA (saRNA) vaccines, each consisting of a mixture of two constructs encoding either the influenza virus hemagglutinin (HA) or the SARS-CoV-2 spike (S) protein.

Results:

In addition to the induction of functional antibody responses, intramuscular vaccination with either the mRNA or saRNA bivalent vaccine induced strong HA- and S-specific T-cell responses, predominantly CD8+ T cells, in ferrets.

Conclusions:

Taken together, we developed novel assays to detect antigen-specific T cells in the ferret model and show that these can be used to evaluate vaccine immunogenicity. The availability of such assays provides valuable tools to study cellular immune response in ferrets after vaccination, improving the toolbox for preclinical evaluation of novel vaccines.

293

Immune responses following repeated mRNA booster doses targeting the SARS-CoV-2 Omicron variants in immunocompromised individuals – a 3.5-year follow-up

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Background Immunocompromised individuals have shown reduced responses to SARS-CoV-2 mRNA vaccines, necessitating recommendations for additional boosters. Continued monitoring of this population is essential with respect to introduction of new adapted SARS-CoV-2 vaccines; e.g bivalent original/Omicron BA.4/5 adapted vaccines in 2022, monovalent Omicron XBB.1.5 vaccines in 2023, and monovalent Omicron JN.1 vaccines in 2024. However, long-term data on immunity induced by repeated vaccine doses against emergent variants remains limited. We evaluated immune responses to repeated booster vaccinations during a 3.5-year follow-up in immunocompromised individuals. **Methods** We studied a subgroup of immunocompromised individuals (n=25) with varying immunodeficiency conditions; chronic kidney disease and organ transplant (CKD group, n=11) or rheumatological disease (RD group, n=14, with low-responders n=7). The participants had received 7 to 10 doses of SARS-CoV-2 mRNA vaccines and 79% had at least one documented SARS-CoV-2 infection. IgG to SARS-CoV-2 spike receptor binding domain of ancestral strain (wt) (Abwt, multiplex immunoassay), neutralizing antibodies (NAb, microneutralization test) and T-cell responses (secreted IFN-γ levels) against wt, BA.5, XBB.1.5 and JN.1 strains were evaluated before and one month after SARS-CoV-2 mRNA boosters targeting original/Omicron BA.4/5 (n=25) and Omicron JN.1 (n=14) variants. Abwt was additionally determined one month after the 4th vaccine dose. A healthy control group provided samples one (n=35) and 18 months (n=13) after 3rd SARS-CoV-2 mRNA dose. **Results** Immune responses to adapted booster doses did not correlate with the number of vaccine doses/infections. The original/BA.4/5 booster increased median Abwt levels by 1.3–2.6-fold resulting in significantly higher Abwt levels in the CKD and RD-low responder groups compared to levels after 4th vaccine dose (**P**<0.05). The original/BA.4/5 booster also increased the percentage of participants with NAbs by 1.1 to 2.5-fold in all groups to 91–100%, 82–100%, 71–86%, and 43–71% against wt, BA.5.1, XBB.1.5, and JN.1 strains, respectively. T-cell responses had been induced against all strains to a level comparable to healthy controls and the original/BA.4/5 booster did not enhance T-cell response further. The subsequent JN.1 booster increased median Abwt levels by 1.2–1.7-fold, comparable to the level after the previous BA.4/5 booster. Following the JN.1 booster, the percentage of participants with NAbs against wt, BA.5.1, XBB.1.5, and JN.1 strains ranged between 75–100% in all groups. Importantly, the percentage of participants with NAbs against JN.1 variant increased from 40–75% after BA.4/5 booster to 80–100% after JN.1 booster. Data will be supplemented with T-cell responses following the JN.1 booster. **Conclusions** Repeated booster doses in immunocompromised individuals did not further enhance Abwt levels, but increased the percentage of participants with NAbs against Omicron variants. T-cell mediated responses following the original/BA.4/5 booster were also observed against Omicron variants but were not further enhanced. T-cell immunity is likely to persist and provide protection without additional boosting.

294

Safety of Priming and Booster Vaccinations with MF59-Adjuvanted Cell Culture-Derived H5N8 or H5N6 Influenza Vaccines in Healthy Subjects Aged ≥18 Years

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Background

Avian influenza H5N1 and its genetic reassortants are highly pathogenic viruses with potential to infect humans. As such, they pose a threat to the world population, which lacks immunity to H5 viruses. Two MF59-adjuvanted, cell culture–derived H5 vaccines (aH5N8c and aH5N6c) are included in the Biomedical Advanced Research and Development Authority's pandemic influenza vaccine stockpile. This study assessed the safety of a homologous and heterologous prime-boost regimen with aH5N8c or aH5N6c in adults aged ≥18 years.

Methods

In a Phase 2, randomized, observer-blind, multicenter study, 479 adults 18–64 years (n=240) or ≥65 years (n=239) were randomized 2:1:1 to one of three priming regimens given on Days 1 and 22: Arm A, homologous priming with two doses of aH5N8c (n=239); Arm B, heterologous priming with aH5N8c→aH5N6c (n=120); Arm C, heterologous priming with aH5N6c→aH5N8c (n=120). All subjects received aH5N8c on Day 202 (6 months post-2nd priming). Safety endpoints included solicited local and systemic adverse events (AEs), unsolicited AEs, serious AEs (SAEs), AEs of special interest (AESI), AEs leading to study withdrawal, and medically attended AEs (MAAEs). Data were analyzed by vaccination and age cohort.

Results

Rates of solicited and unsolicited AEs were comparable among the 3 vaccine groups. Most solicited AEs (up to 7 days post-vaccination) were mild or moderate, occurred soon after vaccination, and resolved within 3 days. Rates of solicited AEs after the second priming and booster vaccinations were generally similar to or lower than rates after the first priming vaccination. Solicited AEs were reported by fewer subjects aged ≥65 than 18–64 years. The most frequent solicited local AE was injection site pain and the most frequent systemic AE was fatigue (Table 1). Rates of related unsolicited AEs (up to 3 weeks post-vaccination) were low in all treatment groups. MAAEs were reported by 36.7–45.8% of subjects over the course of 12 months, with no reports of related SAEs, AESIs, or deaths. One subject each from Arms A and B withdrew due to an AE.

Conclusions

Multiple vaccinations with MF59-adjuvanted H5 vaccines (aH5N8c and aH5N6c) demonstrated an acceptable safety profile. Solicited AEs were common but transient and of mild or moderate intensity. Lower reactogenicity was observed in older adults. Rates of solicited AEs did not increase after a second or third vaccine dose. The overall reactogenicity profile for aH5N8c/aH5N6c was consistent with observations from studies of other MF59-adjuvanted pandemic influenza vaccines. No safety concerns were identified in this study.

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Table 1: Solicited AEs

	Arm A (N=120) n (%)			Arm B (N=59) n (%)			Arm C (N=59) n (%)		
	Vacc. 1 aH5N8c	Vacc. 2 aH5N8c	Booster aH5N8c	Vacc. 1 aH5N8c	Vacc. 2 aH5N6c	Booster aH5N8c	Vacc. 1 aH5N6c	Vacc. 2 aH5N8c	Booster aH5N8c
18-64 years	118	109	98	59	56	53	58	59	52
Injection Site Pain	58 (49.2)	45 (41.3)	44 (44.9)	20 (33.9)	17 (30.4)	21 (39.6)	27 (46.6)	22 (37.3)	19 (36.5)
Fatigue	48 (40.7)	31 (28.4)	27 (27.6)	23 (39.0)	12 (21.4)	15 (28.3)	21 (36.2)	18 (30.5)	19 (36.5)
≥65 years	117	111	104	60	61	59	57	60	59
Injection Site Pain	27 (23.1)	21 (18.9)	22 (21.2)	12 (20.0)	9 (14.8)	9 (15.3)	9 (15.8)	11 (18.3)	7 (11.9)
Fatigue	40 (34.2)	21 (18.9)	19 (18.3)	15 (25.0)	10 (16.4)	10 (16.9)	12 (21.1)	12 (20.0)	11 (18.6)

295

30-days post-discharge mortality following RSV-associated hospitalizations in older adults: insights from four Spanish regions (2023–2024)

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Background

Growing evidence shows that respiratory syncytial virus (RSV) poses a major health burden among older adults. However, limited data exists on the complications and mortality following an RSV-related hospitalization in this population. This study assessed the 30-days post-discharge mortality in patients aged ≥65 years hospitalized with RSV across four Spanish regions during the 2023/24 RSV season.

Methods

A retrospective observational study based on medical records reviews of patients aged ≥65 years hospitalized for at least 24 hours due to RSV and/or with a laboratory-confirmed RSV infection by RT-PCR between October 27, 2023, and May 3, 2024. The study included data from Catalonia, Navarre, Seville and Valencia with an estimated total population of adults' ≥65 years covered of approximately 2 million. Depending on the region, either all RSV hospitalizations were included or a target sample size of 138–150 patients was set to ensure robust outcome estimates. A descriptive analysis of the case fatality rate overall and by age group was conducted. Mortality was defined as deaths occurring during hospitalization or within 30-days post-discharge, directly or indirectly related to RSV. Data were collected through medical reviews of hospital, specialist, and/or primary care records, depending on the region.

Results

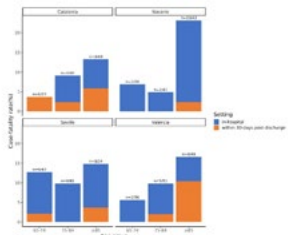
A total of 552 RSV hospitalizations were included, ranging from 113 in Navarre to 139 in Catalonia with a median age of 81 (IQR 74, 88). Case fatality rates ranged from 10% to 12%, with differences in the mortality setting (in-hospital vs. post-discharge) observed across regions (Table 1). The highest rates were consistently observed in patients ≥85 years. However, the proportion of deaths that occurred in-hospital versus post-discharge within each age group differed by region (Figure 1).

Conclusions

RSV case-fatality rates were similar across all regions, with an average of 12% of the hospitalizations resulting in death. The case-fatality setting and age group variations could reflect differences in the data sources available per region, as well as clinical practice differences in palliative care.

Table 1. Case fatality rates in individuals aged 65 years or older hospitalized due to respiratory syncytial virus or with a laboratory-confirmed infection.

	Catalonia	Navarre	Seville	Valencia	Total
N	139	113	162	135	549
Age (median (IQR))	84 (76, 89)	81 (74, 88)	81 (73, 87)	80 (74, 87)	81 (74,88)
Case-fatality rates	14 (10%)	14 (12%)	20 (12%)	15 (11%)	63 (11%)
In-hospital	8 (5.8%)	13 (12%)	17 (10%)	9 (6.7%)	47 (8.6%)
within 30-days post-discharge	6 (4.3 %)	1 (0.9%)	3 (1.9%)	6 (4.4%)	16 (2.9%)



296

Evaluating clinical judgment and ILI case definitions in sentinel surveillance of influenza and other respiratory viruses in Slovenia

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Background In Slovenia, upper respiratory tract (URT) swabs have initially been collected from patients with influenza-like illness (ILI) as part of the sentinel influenza surveillance program. At first, testing was limited to influenza viruses and respiratory syncytial virus (RSV). Starting with the 2012/13 season, the testing panel was expanded to include eight additional respiratory viruses. From 2020 onward, SARS-CoV-2 was added. With the expansion of the testing panel, clinicians began collecting swabs from patients presenting with a broader range of respiratory symptoms beyond the strict ILI case definition. This study aimed to better understand the clinical characteristics of patients selected for sampling in the primary care sentinel network.

Methods During the 2022/23, 2023/24, and 2024/25 seasons, 50 sentinel clinicians were asked to complete a questionnaire for each patient from whom an URT swab was collected. The questionnaire included tick boxes for ILI clinical signs (according to the EU case definition) and the clinician's opinion on the likely viral cause—namely influenza, RSV, SARS-CoV-2, or another respiratory virus. Swabs were collected year-round, with the majority obtained between October and April. All specimens were tested using real-time RT-PCR for the presence of influenza A and B, RSV, adenoviruses, enteroviruses, rhinoviruses, human metapneumoviruses, parainfluenza viruses (types 1–4), seasonal coronaviruses (NL63, HKU1, OC43, 229E), human bocaviruses, human parechoviruses, and SARS-CoV-2.

Results Over the three seasons, 4,826 URT specimens were tested. Of these, 4,801 were accompanied by clinical data, and 4,087 included the clinician's opinion on the likely cause of illness. Among the 4,009 patients who met the ILI clinical criteria, 29% tested positive for influenza, 8% for RSV, 6% for SARS-CoV-2, 41% for other respiratory viruses, and 15% were negative for all tested pathogens. Among 1,230 patients clinically suspected of having influenza, 49% tested positive for influenza, 5% for RSV, 4% for SARS-CoV-2, 26% for other viruses, and 17% tested negative. Of the 174 patients clinically suspected of having RSV infection, 28% were laboratory-confirmed. Among the 120 patients suspected of having SARS-CoV-2, 33% were confirmed. Of the 2,473 patients whom clinicians believed had another viral infection, 46% had a non-influenza, non-RSV, non-SARS-CoV-2 virus confirmed.

Conclusions Despite the expanded panel of respiratory viruses tested in the sentinel surveillance system, clinicians primarily collect specimens from patients who meet the ILI case definition or are suspected to have influenza based on clinical judgment. Clinicians' judgment was found to be more accurate in identifying influenza infections than strict adherence to the ILI case definition. Their diagnostic accuracy was also relatively high for SARS-CoV-2 and other respiratory viruses but lower for RSV.

297

Integrated Respiratory Virus Surveillance in Madagascar: Epidemiological Patterns and Genomic Dynamics of Influenza viruses, SARS-COV-2 and RSV (2021-2024)

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Madagascar has implemented an integrated sentinel surveillance system for influenza-like illness (ILI) and severe acute respiratory infections (SARI), which monitors the co-circulation of respiratory viruses including influenza, SARS-CoV-2, and respiratory syncytial virus (RSV). This system supports national response strategies and contributes to global pandemic preparedness efforts

The surveillance network includes 31 ILI and 5 SARI sentinel sites. Respiratory samples collected from suspected patients are tested for influenza viruses, SARS-CoV-2, and RSV. Positive influenza samples are sent to WHO Collaborating Centres (CCs) for antigenic and genetic characterization. Genomic sequencing of influenza, SARS-CoV-2 and RSV is also conducted at the National Influenza Center (NIC) using NGS platforms. Epidemiological and virological data are periodically shared with policy decision-makers. Genomic data are submitted to GISAID.

Between January 2021 and December 2024, 14064 samples were tested for influenza, yielding a positivity rate of 15% (2036/14064): 9% for influenza A and 5% for influenza B. Influenza activity was low in 2021, reflecting the impact of COVID-19 mitigation measures. A resurgence of influenza occurred in 2022, with a peak around mid-year, followed by sustained circulation in 2023 and 2024. Overall, influenza activity tented to be seasonal, with major peaks typically occurring in mid-year, between May and August. Distinct annual peaks were observed, especially in 2022, 2023, and 2024. Among 573 positive samples shipped to WHO CCs and 72 sequenced locally, 5350 sequences were obtained. Evolutionary analyses revealed divergent post-pandemic dynamics: B/Victoria likely persisted at low levels during non-pharmaceutical interventions, while A(H1N1)pdm09 and A(H3N2) disappeared and were subsequently reintroduced.

SARS-CoV-2 surveillance showed a positivity rate of 12% (1544/12880). Genomic data from 2148 sequences revealed early circulation of the Beta variant in 2021, followed by dominance of Delta, and replacement by Omicron and its sublineages from 2022 onwards.

RSV surveillance involved 3712 hospitalized children under five. The RSV detection rate was 35% (1288/3712), with peak transmission occurring in February-March. Predictive symptoms included cough, dyspnea, and intercostal retractions ($p<0.001$). Infants aged 0–3 months had the highest infection risk (OR=2.08). Phylogenomic analysis based on 132 G gene sequences revealed co-circulation of RSV-A (genotype GA.2.3.5) and RSV-B (genotypes BA9 and GB.5.0.5). RSV-A showed endemic evolution whereas RSV-B appeared to be repeatedly introduced from external sources. Seasonal RSV circulation correlated positively with rainfall and inversely with humidity.

Integrated surveillance in Madagascar enables real-time monitoring of respiratory virus trends and genomic evolution. These data guide vaccine strain selection, inform outbreak response strategies, and contribute to global networks like GISRS and GIHSN. The RSV genomic and epidemiologic findings supported Madagascar's participation in the WHO GISRS Phase 3 RSV program. Our findings demonstrate the importance of an integrated approach to sustain respiratory virus surveillance in low-resource settings, both for seasonal control and pandemic preparedness.

298

mRNA Vaccines for Broadly-Reactive T-cell Immunity Against Influenza and Coronavirus: Insights from Animal Models

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Background: For the protection of humankind from continuously changing seasonal and occasional pandemic influenza and coronavirus outbreaks, broadly-reactive vaccines are essential. Harnessing T-cell responses targeting conserved internal viral proteins could contribute to protection from a broad range of influenza and coronaviruses, by clearing infected cells through recognition of shared virus epitopes.

Methods: We investigated novel T cell–inducing nucleoside-modified messenger RNA (mRNA) vaccines against influenza and SARS-CoV-2 viruses. The mRNA-Flu vaccine encodes conserved nucleoprotein, matrix protein 1, and polymerase basic protein 1 of an H1N1 influenza virus. mRNA vaccines against SARS-CoV-2 encode nucleoprotein (mRNA-N) or nonstructural proteins -5, -12, -13, -16 (mRNA-nsp) of SARS-CoV-2. Utilizing animal models to mimic both naïve and virus-experienced scenarios, we evaluated prime-boost regimens to mimic real-world vaccination strategies.

Results: In ferrets, mRNA-Flu not only elicited but also boosted broadly-reactive T-cell responses across systemic and mucosal compartments. Notably, vaccination of influenza-experienced ferrets conferred enhanced protection against a heterosubtypic highly pathogenic avian influenza infection. Similarly, in HLA-A2 transgenic mice, mRNA vaccines against SARS-CoV-2 induced robust T-cell responses in relevant tissues. However, upon challenge with a pathogenic Beta SARS-CoV-2 variant, vaccine-associated enhanced disease was observed, with more prominent weight loss and lung pathology in omicron-experienced mice.

Conclusion: These findings underscore the potential of mRNA vaccines encoding conserved internal viral proteins for broad immune protection against influenza. Yet, they also raise caution regarding vaccine-associated enhanced disease, urging further mechanistic studies to inform the development of safe and effective broadly-reactive vaccines.

299

Differential Neutralizing Antibody Responses to Repeated Vaccination with Influenza A Vaccine Strains A/Victoria/2570/2019 (H1N1)pdm09 and A/Darwin/9/2021 (H3N2)

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Background: Seasonal influenza vaccine (SIV) is recommended annually because both vaccine and infection-induced immunity wane over time and influenza viruses frequently undergo antigenic drift. However, concerns have been raised that annually repeated vaccination may compromise immunogenicity and effectiveness. Weaker antibody responses have been observed in repeatedly immunized subjects, when the influenza strains in the previous season's vaccine were identical or highly similar to those in the current season's vaccine.

Healthcare workers (HCW) form a highly vaccinated group as SIV is not only recommended but required for them as a priority profession. We invited HCWs from Helsinki University Hospital to participate in a follow-up study conducted in 2017–2025 in Finland, including eight influenza seasons (EU CT number: 2024-515372-12-00). In this study, we measured neutralizing antibodies (NAb) against the corresponding influenza A vaccine strains to evaluate the effect of repeated vaccination when the influenza vaccine strain remained the same in two consecutive seasons.

Methods: We included two cohorts in the study (1) HCWs from seasons 2021–2022 and 2022–2023 (n=42), and (2) HCWs from seasons 2022–2023 and 2023–2024 (n=55), when A/Victoria/2570/2019 (H1N1)pdm09 and A/Darwin/9/2021 (H3N2) vaccine viruses were included in tetravalent SIV in two consecutive seasons, respectively. Blood samples were collected prior to vaccinations, and one, six and 12 months post vaccination. We assessed NAb responses from sera using microneutralization assay to the above-mentioned vaccine virus strains.

Results: A notable increase in geometric mean titer (GMT) was observed one month after the first vaccination compared to pre-vaccination NAb levels: 11.5-fold to A/Victoria/2570/2019 (H1N1)pdm09 and 6.8-fold to A/Darwin/9/2021 (H3N2) vaccine viruses. Although GMTs gradually decreased during the 12-month follow-up, the 12-month GMTs remained elevated compared to pre-vaccination NAb titers. One month after vaccination in the subsequent season, the GMT increased by 1.3-fold for the A(H1N1)pdm09 and 2.1-fold for the A(H3N2) vaccine strain. During the second season, post-vaccination GMTs were either lower or higher compared to those of the first season against the A(H1N1)pdm09 and the A(H3N2) vaccine strains, respectively. The 12-month GMTs of the second season remained elevated compared to the pre-vaccination NAb GMTs of the first season, with a 2.5-fold increase for the A(H1N1)pdm09 and 4.9-fold increase for the A(H3N2) vaccine strain.

Conclusions: Vaccine-induced NAb titers against the A/Victoria/2570/2019 (H1N1)pdm09 strain were weaker in the second season compared to the first, suggesting hyporesponse due to repeated vaccination. Despite this, repeated vaccination remained more effective than no vaccination. On the other hand, repeated A/Darwin/9/2021 (H3N2) vaccination induced stronger NAb titers in the second season compared to the first. The differences in NAb kinetics between the vaccine strains may reflect the strain-dependent antigenic properties of viruses and highlight the need for further research into the clinical significance of these findings.

300

Serological surveillance of fur farm workers exposed to highly pathogenic avian influenza A(H5N1) in Finland, July-October 2023

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Background: Finland faced a large highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b epidemic in wild birds that spread to several dozen fur farms in 2023. PCR testing of humans exposed to the virus during the outbreak, which was recommended as a public health measure, did not identify any human cases. In this study, our aim was to use serological methods to retrospectively assess whether any unidentified infections might have occurred following exposure to the virus circulating in fur animals during the epidemic.

Methods: Serum samples were collected from 90 workers exposed to fur animals infected with A(H5N1), between July and September 2023. We used microneutralization (MN) and haemagglutination inhibition (HI) assays to measure serum antibodies against influenza A viruses, including A(H5N1) clade 2.3.4.4b strains isolated from farmed foxes and a black-headed gull in Finland during the 2023 epidemic, as well as the avian influenza vaccine strain A(H5N8) A/Astrakhan/3212/2020. We investigated the presence of serological indications of infection based on antibody titers, defining seropositivity as a neutralizing antibody titer ≥40 against A(H5N1) clade 2.3.4.4b in two independent assays, MN and HI.

Results: Based on the antibody results, we could not confirm that any infections had occurred among workers, despite documented exposure at the fur farms where they were employed. Some individual serum samples showed titers ≥40 by one assay, but none were confirmed by both methods.

Conclusions: Despite the spread of H5N1 on fur farms, no human cases were detected, indicating that the virus circulating in 2023 did not transmit easily to humans, even though transmission between mammals was observed. Due to cross-reactive antibodies, interpretation of serological findings is challenging, and no conclusions about past infections can be drawn from a single test result. Serological tests should be maintained in preparedness for situations where avian influenza viruses cause epidemics in animals and human exposures occur. Additionally, these tests should be pre-validated for epidemic strains, and contingency plans should be in place to confirm results with a second test.

303

Adherence to influenza vaccination in primary cardiovascular prevention: results from the Epidemiological Information Study of Communities

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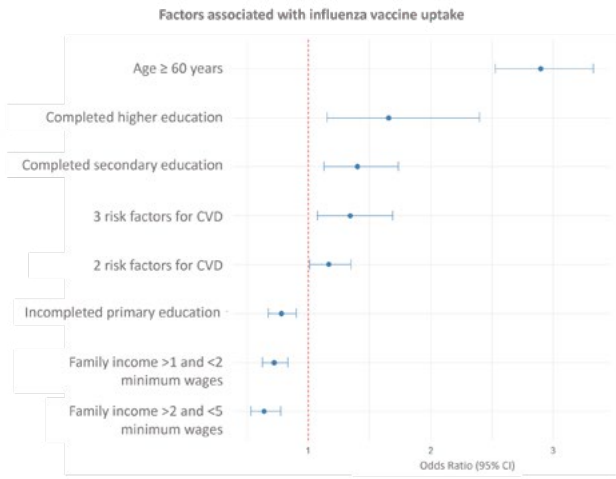
Background: Cardiovascular diseases (CVD) account for the deaths of 17.7 million people each year. There is special interest in the potential relationship between respiratory infections and cardiovascular risk. Identifying factors associated with adherence to influenza vaccination in individuals at risk of CVD is crucial for several reasons, including public health protection. The study aims to identify factors associated with influenza vaccination adherence in individuals undergoing primary cardiovascular prevention.

Methods: The study was based on data from the 'Epidemiological Information Study of Communities' - EPICO, an observational and cross-sectional study that included a total of 7,936 individuals who were treated at primary healthcare units in 32 cities in the state of São Paulo and had at least one cardiovascular risk factor (hypertension, diabetes mellitus, and/or hypercholesterolemia). The analysis considered individuals aged over 18 years who had necessarily reported their age and had no previous cardiovascular event (myocardial infarction and/or Stroke), totaling 7,164 individuals. The binary logistic regression model was used. The dependent variable was influenza vaccine adherence (determined by self-reported vaccination of at least three times during the study period – 2015-2018), and the independent variables were demographic data, adherence to antihypertensive, hypoglycemic, and lipid-lowering medications, stratified systolic and diastolic blood pressure, stratified glucose levels, lipid profile, tobacco consumption, level of physical activity, and presence of cardiovascular risk factors (hypertension, type 2 diabetes mellitus, and hypercholesterolemia). For all analyzes a significance level of 5% was considered.

Results: The regression model included the following predictor variables for influenza vaccination during the study period: age ≥ 60 years OR=2.90 (95% CI: 2.53-3.32, p<0.001), education – completed secondary education OR=1.40 (95% CI: 1.13-1.73, p<0.01), completed higher education OR=1.66 (95% CI: 1.15-2.40, p<0.01) and incompleted primary education OR=0.78 (95% CI: 0.67-0.91, p<0.01) – family income >1 and <2 wages OR=0.72 (95% CI: 0.62-0.83, p<0.01) and >2 and <5 minimum wages OR=0.64 (95% CI: 0.53-0.77, p<0.01) – and risk factors for CVD – minimum 2 factors OR=1.17 (95% CI: 1.01-1.35, p<0.01) and 3 factors OR=1.34 (95% CI: 1.07-1.69, p<0.01).

Conclusions: It was possible to investigate the determinants influencing the decision to adhere or not to seasonal flu vaccination. Factors such as advanced age, higher socioeconomic status, presence of chronic diseases, and higher education levels were identified as predictors of vaccination adherence. The identification of factors associated with adherence to influenza vaccination in individuals undergoing primary cardiovascular prevention is of utmost importance for public health promotion and the reduction of the burden of cardiovascular diseases.


Factors associated with influenza vaccine uptake
Age ≥ 60 years
Completed higher education
Completed secondary education
3 risk factors for CVD
2 risk factors for CVD
Incompleted primary education
Family income >1 and <2 minimum wages
Family income >2 and <5 minimum wages
1
2
3
Odds Ratio (95% CI)

304

Cellular immune responses (IFN-γ) to MF59-Adjuvanted Inactivated Influenza A(H5N8) Vaccine in High-Risk Groups in Finland

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Background: Highly pathogenic avian influenza (HPAI) clade 2.3.4.4b A(H5N1) caused a large outbreak in wild birds and farmed fur animals in Finland in 2023. The MF59-adjuvanted inactivated A(H5N8) vaccine from Seqirus was offered to high-risk occupational groups as a preventive public health measure. As part of our immunogenicity study, we measured virus-specific T-cell responses, including interferon-gamma (IFN-γ) secretion in peripheral blood mononuclear cells (PBMCs) following MF59-adjuvanted A(H5N8) vaccination. Antibody responses can predict the immune protection induced by the vaccine, but cellular immunity likely plays a significant role in providing long-term protection against severe disease.

Methods: We investigated IFN-γ responses following a two-dose vaccination regimen in 18 participants. Subjects were categorized into two groups, previously unvaccinated (n=13) and previously vaccinated (n=5). Previously vaccinated subjects had received two to six doses of A(H5N1) vaccines between 2009 and 2018. PBMCs were collected before vaccination and three weeks after the second dose. We analysed T cell activation and IFN-γ secretion from PBMC supernatants after a 36-hour stimulation with A(H5N8) hemagglutinin (H5) and neuraminidase (N8), A(H1N1) (H1), and PR8 nucleoprotein (NP) peptide pools. Additionally, PBMC were stimulated with SARS-CoV-2 JN.1 spike peptide pool and tetanus toxoid as positive control and dimethyl sulfoxide (DMSO) as negative control. IFN-γ secretion in the culture supernatant was measured by Luminex MAGPIX magnetic bead analyzer.

Results: Before vaccination, IFN-γ responses to influenza virus antigens were already detectable in both unvaccinated and vaccinated participants. After two doses of the A(H5N8), IFN-γ responses to the vaccine H5 and N8 antigens were detected, as well as responses targeting PR8 NP and H1, in both previously unvaccinated and vaccinated participants. In previously unvaccinated participants, after the second vaccine dose, IFN-γ levels increased by 5.55-fold and 5.02-fold compared to IFN-γ levels before vaccination, when PBMCs were stimulated with H5 or N8, respectively. However, the increase was statistically significant only for the H5N8 NA ($p=0.0024$, Wilcoxon matched-pairs signed-rank test). Similarly, the statistically significant increase in IFN-γ levels was seen in stimulation with H1N1 HA and PR8 NP ($p=0.0479$ and $p=0.0012$, Wilcoxon matched-pairs signed-rank test, respectively). In previously vaccinated participants, IFN-γ levels increased by 4.75-fold and 3.47-fold when PBMCs were stimulated with H5 or N8, respectively; however, these increases were not statistically significant due to the small sample size. Stimulation with JN.1 and tetanus toxoid showed strong IFN-γ responses, confirming that the methodology can successfully assess IFN-γ responses to different antigens.

Conclusions: We showed that influenza A(H5N8) vaccination induces T-cell responses against both the hemagglutinin and neuraminidase antigens, which could complement antibody-mediated immunity and thereby may mediate long-term protection. Before vaccination, IFN-γ responses to influenza virus antigens were already detectable, indicating some degree of pre-existing cross-reactive T-cell immunity likely due to prior infections and/or previous influenza vaccinations.

307

Development and in vivo evaluation of Modified Vaccinia virus Ankara-based vaccines delivering hemagglutinin of highly- or low-pathogenic influenza A virus H7 subtypes

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Background:

Vaccination remains one of the most effective countermeasures against influenza A viruses (IAV), but licensed human IAV vaccines target predominantly subtypes A(H1N1) and A(H3N2), as they are responsible for seasonal epidemics. In addition, some IAV vaccines, which target subtypes A(H5N1) or A(H5N8), have a restricted authorization, e.g., for pandemic preparedness or administration during an avian influenza virus outbreak [1]. Human infections with other IAV subtypes, such as H7, are uncommon, but have occurred sporadically in the past, with reported case fatality rates of more than 40% in hospitalized patients [2]. Efficacious vaccines against A(H7) subtypes are lacking and thus, we aimed to generate and characterize two candidate vaccines targeting A(H7) viruses using the highly attenuated Modified Vaccinia virus Ankara (MVA) as viral vector.

Methods:

We designed two consensus sequences based on publicly available full-length hemagglutinin protein sequences of highly-pathogenic (HP) A(H7N9), A(H7N7), A(H7N3) or low-pathogenic (LP) A(H7N9), A(H7N2), A(H7N7), A(H7N4) subtypes, which were isolated from infected humans during local outbreaks. The consensus sequences were codon-optimized for vaccinia virus usage and inserted into deletion site III of MVA by homologous recombination to obtain recombinant MVA-H7-HP and MVA-H7-LP. Genetic stability, protein expression and replicative capacity were analyzed by PCR, western blot and viral growth kinetics, respectively. To test immunogenicity, C57BL/6J or humanized **HLA-A2.1-/HLA-DR1-transgenic H-2 class I-/class II-knockout** mice [3] were immunized twice (days 0 and 21) with 10^7 PFU of recombinant MVA-H7-HP or MVA-H7-LP. On day 35, mice were euthanized and isolated splenocytes were restimulated with H7-specific peptides. T cell immunity was measured by intracellular cytokine staining followed by FACS analysis and IFN-γ ELISPOT assay. In addition, serum H7-specific IgG binding antibodies were determined by ELISA on days 18 and 35 post prime immunization.

Results:

Both MVA-H7-HP and MVA-H7-LP showed high genetic stability, unimpaired protein expression over time, viral growth in avian DF-1 cells and replicative deficiency in human HaCat cells. Upon prime-boost vaccination, the two vaccines elicited a CD4+ T cell response in both mouse strains. In addition, A(H7) subtype-specific IgG antibodies in sera of C57BL/6J mice, immunized with either MVA-H7-HP or MVA-H7-HP, were already detectable after one immunization and were boosted after the second immunization.

Conclusions:

We demonstrate the capability of MVA-H7-HP and MVA-H7-LP to induce A(H7)-specific T cell responses and A(H7)-specific serum IgG binding antibody responses in mice. Further studies are warranted to evaluate the protective efficacy of the two vaccines in a suitable challenge model.

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312

A high throughput antiviral screening assay for influenza A & B viruses based on an A549 reporter cell line

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Background: Worldwide circulating and emerging viruses, such as influenza A virus (IAV), still pose a significant global health threat due to seasonal epidemics and sporadic pandemics. Current strategies to overcome this burden are not as reliable since the development of new vaccines is time-consuming and the use of antiviral drugs is often limited due to the emergence of drug-resistant strains. Hence, new effective treatment strategies are needed to circumvent these complications. This study aimed to develop a robust high-throughput screening (HTS) assay to facilitate the identification of broad-spectrum antiviral compounds effective against Influenza A and B viruses.

Methods: We developed the assay using an A549 reporter cell line engineered to express a mi670 far-red fluorescent marker. The use of this cell line enables us to easily determine cell viability at different time-points, this enables us to very precise determine the most appropriate readout time and follow-up on virus induced cell death or toxicity of the screened compounds. Optimization was carried out on the Yokogawa CV8000 high-content imaging platform in a 384-well format, enabling automated and scalable screening. To prove the efficacy of our assay we validated the assay in reference plates containing proven antivirals against influenza A & B subtypes.

Results: In this study, a reporter A549-mi670 cell line was used to set up an HTS screening assay to identify anti-influenza drug candidates. This cell line proved to be susceptible to influenza A and B infection, showing a significantly reduced far-red (mi670) signal upon virus-induced cell death. Using high-content imaging, cell viability was assessed in a real-time manner to determine the best infection conditions (cell count and virus input) as well as the optimal timepoint for quantification of the virus-induced cell death in the infected non-treated control wells. The assay proved to be robust, demonstrating consistent and reproducible results across multiple screening runs, with a Z'-factor greater than 0.5. Validation of the assay with a panel of reference anti-influenza A and B drugs (e.g. baloxavir) showed the expected antiviral activity, confirming the assay's suitability for large scale compound screening.

Conclusion: We successfully established a high-throughput, cell-based imaging assay suitable for large-scale screening of antiviral compounds targeting influenza viruses. This platform enables the potential identification of broad-spectrum inhibitors and represents a valuable tool for high throughput screening purposes. As a part of epi-/pandemic preparedness for future influenza virus infections, this assay can be a pivotal tool in the effort towards new antiviral discoveries.

314

Wastewater-Based Surveillance within a One Health Framework: Connecting Wildlife, Agriculture, and Humans

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The emergence of novel influenza A viruses (IAV) with a pandemic potential often results from genetic reassortment events occurring at the aquatic birds, livestock and human interface. Traditional surveillance methods rely on clinical sampling and wildlife monitoring, which is often invasive, resource-intensive, costly, and may fail to detect early signals of viral circulation and evolution. Wastewater-based surveillance is cost-effective and has demonstrated its utility in early detection of outbreaks and new variants emergence during the COVID-19 pandemic, and recently enabled the detection of H5N1 IAV in city wastewater months prior to its detection in dairy cattle.

This project aimed to develop an integrated One Health surveillance approach in the province of Québec in Canada at the aquatic birds, livestock and human interface to improve the prediction emerging IAV with zoonotic or pandemic potential. Sampling protocols, nucleic acid purification, and viral RNA detection by qRT-PCR were optimized for city wastewater and sludges and for wetlands. During the 2024-2025 flu season, IAV was detected in city wastewater 2 weeks prior to the peak in human population. The relative proportions of H1 and H3 subtypes detected in city wastewater closely reflected those observed in clinical surveillance data. Additional samples will be collected in two cities during the 2025-2026 season to confirm these findings. Wetland sampling adjacent to farms is scheduled for Fall 2025, coinciding with aquatic bird migration. Current efforts are focused on the Montérégie and Eastern Townships regions, due to their proximity to the U.S. border and the abundance of livestock farms. Sequencing, using the Illumina technology, will be optimized for municipal wastewater and sludges, as well as for wetland samples. IAV genomes will be analyzed to identify potential mutations associated with mammalian adaptation, antiviral resistance, and increased virulence. Concomitantly, clinical data obtained from patients across the province will be analyzed to assess the predictive value of environmental samples.

All together, this integrated One Health approach will strengthen the surveillance of circulating IAV subtypes at the aquatic birds–livestock–human interface — a known hotspot for the emergence of potentially pandemic viruses.

316

ECaS

Comprehensive Influenza A Virus Hemagglutinin Binding Maps reveal Antibody Binding Gaps and Epitope Dynamics

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Neutralizing antibodies are crucial for preventing and clearing Influenza A virus infections and are therefore being evaluated as novel therapeutics in the form of monoclonal antibodies (mAbs). However, influenza viruses rapidly evolve to escape the human immune response, which can quickly render mAbs ineffective. Assessing the limitations of mAb-mediated protection is critical for evaluating their therapeutic potential and understanding the epitope dynamics that significantly impact the success of antibody-based therapies. In this study, we developed a novel cell-based multiplex binding assay that rapidly determines antibody binding to hemagglutinin (HA), the primary target of influenza antibodies, using flow cytometry. By establishing a comprehensive influenza HA panel comprising historical and circulating H1N1, H2N2, and H3N2 strains, we generated a detailed binding map for 24 monoclonal antibodies, including several currently in clinical trials. Our data confirm that HA head-targeting antibodies are typically strain-specific, whereas HA stalk antibodies are more likely to cross-react with different HA subtypes. Additionally, we identified binding gaps in antibodies undergoing clinical trials, which could significantly affect their development and application. The presented assay is quickly adaptable to novel influenza strains, underscoring its value for continuously monitoring potential immune evasion by influenza viruses.

317

Application of phylotype analysis to infer patterns of pathogen evolution

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Background

Understanding the evolution of lineages and accumulation of mutations in a phylogenetic context is important for public health surveillance, as this can help identify changes in pathogen behaviour and signs of increasing risk to human health. In many cases, phylogenetic information is used to systematically track pathogen evolution and to assign consistent nomenclature to clades of interest. Interpretation of phylogenies often involves time-consuming visual inspection, unsuitable for large datasets. Here we show how phylotypes can be used for efficient analysis of phylogenetic relatedness, providing insights into patterns of pathogen evolution and the accuracy of lineage assignment based on phylogenetic relatedness. Specifically, we apply these methods to trace the evolutionary histories of mutations in influenza A H5N1 genotypes, and to assess the impact of genome coverage on SARS-CoV-2 lineage assignment.

Methods

Avian influenza: 1226 complete genomes from the North American B3.13 outbreak were used. Amino acid mutations were called using our in-house pipeline. A phylogeny was built with FastTree using aligned concatenated sequences, and corresponding phylotypes were assigned using Clusterfunk. A custom python script was developed to analyse the phylotypes and report whether each mutation fits a single origin or multiple origin pattern.

SARS-CoV-2: 191 high-quality UK sequences representing 46 lineages were chosen, with coverage loss modelled by masking combinations of amplicons observed in the previous 12 months of data. Pangolin lineage assignments for the masked sequences were compared to the 'expected' assignments from the unmasked sequences. A custom python script was developed to find the most recent common ancestor for each lineage pair and to evaluate the degree of difference.

Results

We show that avian influenza mutations with similar prevalence can have very different evolutionary histories; e.g. HA:P337L shows a single origin and subsequent clade expansion, whereas HA:A172T occurs across several clades, indicating multiple independent origins. As expected, the proportion of correct SARS-CoV-2 lineage assignments decreases with lower genome coverage and showed two distinct profiles: 'Non-specific' results where the observed lineage is contained within the expected lineage (e.g. XBB.1 vs XBB.1.16) and 'incorrect' results where the observed value is not contained within the expected value (e.g. XBB.2 vs XBB.1). The distance between observed and expected lineages was greater for 'incorrect' than 'non-specific' results, but in most cases was small (<4 steps).

Conclusions

By using phylotype analysis in pathogen surveillance we can efficiently classify the phylogenetic pattern of hundreds of mutations, without needing visual interpretation. This allows rapid identification of convergently evolved mutations, which may confer phenotypic advantage and indicate increased public health risk. This method also provided insights into the lineage-specific impact of coverage loss in SARS-CoV-2. Convergent mutations obscure the true phylogenetic relationships between lineages and can result in incorrect lineage assignment when genomic data is incomplete.

318

Improving Influenza Vaccine Efficacy Trials by Optimizing Virus Recovery from Suspected Influenza-like Illness Subjects

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Influenza vaccine efficacy trials are critical to evaluating new vaccine formulations or platforms (i.e., sa-mRNA). As part of these study, nasal swabs are collected from participants with Influenza-like illness (ILI) and tested for the presence of influenza by PCR followed by virus culture for antigenic typing. Although both methods have been used extensively, growing influenza virus to sufficient titers for typing (e.g., HAI or MN) has often been shown to be challenging. As such, the goal of this study was to improve the virus yields from culture but optimizing several critical steps in the process such as the impact of freeze/thaw cycles on viral viability and the choice of cell substrate for virus recovery. The latter focused on evaluating variants of MDCK cells that had various levels of cell surface expression of glycans. Overall, the results showed that storage and processing conditions (e.g., 4°C vs. freeze/thaw) did not have an impact on virus recovery. However, when comparing various MDCK cell lines, the data revealed that MDCK 33016PF and SIAT1 cells were particularly well-suited for recovering influenza viruses, and in particular for recent H3N2 strains. These differences in recovery were particularly noticeable when low concentrations of virus (as low as ~10 FFU) were used as the inoculum. Interestingly, lectin staining of the tested cell lines corroborated the hypothesis that increased levels of cell surface NeuAc2.6 correlated with the higher viral titers for influenza and especially for H3N2 viruses. Finally, reverse genetics was also evaluated as an alternative method to rescue viruses isolated from NP swabs by sequencing of the HA region from PCR-positive samples. We have successfully rescued influenza A reassortants each reaching high titers while maintaining 100% sequence integrity. Ongoing experiments are being performed to confirm that the wild-type viruses' antigenicity has not been altered by the reverse genetics approach.

319

Development of an influenza virus human challenge model (HCM) portfolio with diverse contemporary subtypes for the assessment of new vaccine and anti-viral therapies

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Background: Influenza virus infections remain a significant disease burden globally causing high levels of acute respiratory infections, morbidity and mortality. Challenge studies have played an important role in the development of respiratory virus vaccines and treatments. Here we describe the development of influenza human challenge models for three recently circulating influenza subtypes – H1N1 & H3N2 and Influenza B (Victoria lineage)

Methods:

Three new influenza viruses – A/France/21 H1N1, A/England/22 H3N2 and B/Connecticut/21 - underwent extensive **in vitro** characterization prior to GMP production. Human virus challenge studies were conducted to characterise the new strains in groups of 18-55 yrs, healthy, serosuitable adult participants inoculated intranasally with different virus doses to establish safe, tolerable and robust infections and develop appropriate endpoints for future investigational medicinal product studies. Participants were monitored 24/7 within a quarantine unit for up to 9 days post viral inoculation. Regular assessments were performed in quarantine including: ECG, bloods (haematology and biochemistry), vital signs and spirometry for safety. Nasopharyngeal and throat swabs were collected twice daily and viral load assessed by both qPCR and viral culture. Symptom diary cards were collected three times daily. Immunological samples including serum and peripheral blood mononuclear cells were collected for immunological assessments. Participants returned for a final Day 28 follow up visit. The results were compared to hVIVO's established influenza challenge strains.

Results: Inoculation with all virus doses was safe and well tolerated with no SAEs or AEs of concern. All influenza strains showed robust symptomology associated with upper respiratory and systemic symptoms similar to field-reported influenza symptoms. qPCR and infectious viral assays showed infection rates between 90% to 63%. Moderate to severe infection rates ranged from 75% to 37%, while febrile disease rates ranged between an unprecedented 60% and the more typical 11%, thus providing a strong symptomatic infected platform for the assessments of new vaccines and antiviral treatments. These results show the power of the use of contemporary influenza strains in the human challenge model to effect positive outcomes for new disease intervention strategies.

Conclusions: A new contemporary portfolio of wild type influenza virus challenge strains for the human challenge model has been established, shown to be safe, tolerable and produce robust viral and disease endpoints suitable for the future development of vaccine and treatment products.

321

ECaS

Molecular and Phenotypic Landscape of RSV in Children During the Pre- and Post-Nirsevimab Eras

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Background : Respiratory Syncytial Virus (RSV) is a major cause of acute lower respiratory tract infections in both infants and the elderly worldwide. It is estimated that RSV affects approximately 33 million children under the age of five each year, leading to over 100,000 deaths globally. Recently, a new monoclonal antibody (mAb), nirsevimab (Beyfortus®), targeting the RSV Fusion (F) protein, has been introduced. This mAb has become the primary choice in many countries to reduce RSV burden in newborns entering their first RSV season. Its widespread use raises concerns about potential impacts on RSV viral fitness. Therefore, it is essential to monitor both genomic and phenotypic variations of RSV over time to understand its evolutionary trajectory. Here, we investigate historical F gene changes and viral fitness of RSV using clinical isolates collected since 2017.

Methods: F gene variations were analyzed from 16,895 publicly available RSV sequences, including both RSV-A and RSV-B subtypes, focusing on key genomic features linked to well-characterized F protein antigenic sites. For phenotypic characterization, over 30 RSV clinical isolates were collected from children under seven years old during winter seasons from 2017/18 to 2024/25. Characteristics such as fusogenicity, growth kinetics, thermal stability, and neutralization by RSV F-specific mAbs were evaluated.

Results: Multiple sequence alignments of F protein amino acid sequences revealed higher variability in the RSV-A subtype compared to RSV-B, particularly in the signal peptide, p27 peptide, and heptad repeat B (HRB) domains. Specific mutations potentially associated with escape from mAbs such as nirsevimab and palivizumab (Synagis®) were identified. Phenotypically, RSV-A strains exhibited larger mean syncytium size than RSV-B, whereas RSV-B showed a significantly higher frequency of syncytium formation. Additionally, RSV-A strains achieved higher viral titers than RSV-B strains 72 hours post-infection. Upon stability testing, some RSV clinical isolates had more stability than others at different temperatures across time independently of their strain. Finally, the clinical isolates were tested against a panel of mAbs targeting distinct antigenic sites. Notably, nirsevimab consistently neutralized all RSV strains.

Conclusion: Our analysis reveals limited variation in the RSV F sequence among recent strains, whereas older sequences showed greater diversity that has diminished over time. Despite these limited genomic changes, distinct phenotypic traits were observed in some isolates, particularly in fusogenicity, thermal stability, and mAb neutralization. These findings underscore the importance of continuous RSV surveillance, especially in the context of widespread nirsevimab use, to detect emerging escape mutants and inform for the development of next-generation prophylactics for infants and young children.

322

ECaS

Comparative Analysis of RSV F Protein-Specific Antibody Responses in Naturally Infected Children and Adults

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Respiratory syncytial virus (RSV) is the leading cause of acute lower respiratory tract infections (ALRI) in children under the age of 5 worldwide. Nearly all children are infected by RSV by the age of 2, and in 5-10% of hospitalized cases, intensive care is required. Globally, it is estimated that RSV leads to 33 million cases, 3.4 million hospitalizations, and 150,000 deaths annually.

Three adult vaccines, a maternal vaccine, and the novel monoclonal antibody, Nirsevimab were recently brought on the market. These interventions all target the prefusion structure of the RSV F protein, which was shown to contain highly neutralizing epitope sites, such as sites Ø and V, this in contrast to the post-fusion structure with less neutralizing epitopes. Nevertheless, an effective pediatric RSV vaccine remains elusive. In this study, differences in RSV-specific antibody responses between young children and adults will be explored.

Some studies have already observed differences, for instance sera from children under 2 years of age show strong binding to peptides located in RSV F aa 101-121, partially overlapping with RSV F p27, which is less prominent in adults. Furthermore, antibodies targeting antigenic sites Ø and V are scarce in children, particularly in those under 3 months old. This suggests that while these sites are critical for neutralizing responses later in life, infants may rely on other antigenic regions, such as site III, for protection.

To investigate these immunological differences, we are conducting an analysis of 50 serum samples from children aged 0 to 2.5 years and 50 serum samples from adults. We are first assessing anti-RSV antibodies to determine prior infection. This is followed by detailed mapping of neutralization capacity against reference strains and contemporary clinical isolates, as well as the specificity to prefusion and postfusion conformations of the F protein, and antibody repertoire profiling. In children, high maternal antibody levels and neutralization capacity at birth decline by 4–6 months, followed by a rise in titers and stabilization of neutralization, likely reflecting natural RSV infection and immune maturation. Additional analyses in the pediatric cohort—including epitope-specific responses and antibody repertoire profiling—are ongoing. Results from adult samples are currently being processed and are expected to be available by the time of the conference.

Understanding these possible key differences in the immune landscape between young children and adults, could provide valuable insights for developing more effective pediatric RSV vaccines.

323

Predictors of severity among rhinovirus/enterovirus associated respiratory hospitalizations: A global perspective from the Global Influenza Hospital Surveillance Network 2017-2024

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Background

The Global Influenza Hospital Surveillance Network (GIHSN) collects standardized data on hospitalized patients with respiratory illnesses across multiple countries. Rhinovirus/enterovirus (RV/EV) is increasingly recognized as an important cause of severe lower respiratory tract infections, yet comprehensive analysis of severity risk factors across age groups remains limited.

Methods

We analyzed data from patients hospitalized with laboratory-confirmed RV/EV infection across 14 countries in the GIHSN (2017-2024). Countries were group as high-income countries (HICs), upper middle-income countries (UMICs), and lower middle-income countries (LMICs). Age-stratified (<5, 5-64 and ≥65 years) mixed-effects logistic regression models identified risk factors for severity markers defined as in-hospital mortality, mechanical ventilation, and intensive care unit (ICU) admission, adjusting for demographics, comorbidities, influenza coinfection, country income level, and season.

Results

Complete case analyses were conducted on 4,836 children (<5 years), 1,915 individuals aged 5-64 years, and 1,033 older adult patients (≥65 years).

In-hospital mortality varied significantly by age (0.4% in <5 years; 2.3% in 5-64 years; 8.1% in ≥65 years). In children (<5 years), presence of one comorbidity increased the odds of death (adjusted odds ratio [aOR]=3.46, 95% confidence interval [CI]:1.32-9.09), and those from LMICs were 9.55 (95%CI:1.81-50.32) times as likely to die than those from HICs. Among adults, those 50-64 years were twice as likely to die than their younger counterpart (18-49 years) (aOR=2.31, 95%CI:1.08-4.93). Older adults ≥75 years were almost three times as like to die compared to 65-74 years (aOR=2.76, 95%CI:1.50-5.09).

For mechanical ventilation, infants (<1 year) were three times as likely to require respiratory support than 3-4 year-olds (OR=3.18, 95%CI:1.42-7.12). For all children <5 years there was an association with presence of comorbidities (one comorbidity aOR=2.06, 95% CI: 1.27-3.34; ≥2 comorbidities aOR=4.58, 95% CI: 2.28-9.19). Those 5-17 years were half less likely than those 18-49 years to require mechanical ventilation (aOR=0.49, 95%CI: 0.24-0.96).

Factors associated with ICU admission were being from LMICs compared to HICs for those 5-64 years (aOR=7.45, 95%CI: 1.09-50.76), and those ≥65 years (aOR=9.32, 95%CI: 2.76-31.46).

Conclusions

Our analysis identified three key risk factors across multiple severity outcomes among hospitalized

patients with RV/EV-related respiratory disease: extremes of age, presence of comorbidities, and hospitalization in LMICs. Comorbidities strongly predicted severity in younger populations, while advanced age became the dominant mortality risk factor in older adult patients. Rhinovirus/enterovirus circulates year-round and should be considered a contributor to hospitalizations, especially during respiratory virus seasons. Future studies to differentiate the contribution of rhinovirus from the combined RV/EV respiratory disease burden separately are warranted, and improving testing capability at hospital levels could better inform patient management and infection control protocols.

324

Understanding acceptance and preferences for RSV infant interventions

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Background

Respiratory Syncytial Virus (RSV) is a leading cause of hospitalization amongst newborn in Sweden and globally. A maternal RSV vaccine was licensed in Europe in 2023 and protects newborns from severe RSV infections during at least their first six months. The RSV maternal vaccine is currently available at self-cost through private vaccination clinics in Sweden. A new monoclonal antibody (mAB) (e.g., Nirsevimab) requiring one injection each RSV season has also been approved in Europe and will be available in Sweden during the fall of 2025. Public Health Authorities in Sweden and elsewhere are analyzing implementation strategies of these new RSV interventions. Preferences among pregnant women are unknown and it is important that the roll-out of new interventions is informed by target communities to ensure effective and equitable uptake. We are therefore currently performing a qualitative study with the aim to determine the social and behavioral enablers and barriers to RSV interventions for infants, and to identify preferences among pregnant women for maternal vaccination compared to seasonal infant mAB injection.

Methods

In depth interviews (IDI) is being performed with pregnant women until saturation of data is reached, expected sample size is 20 IDI. Purposeful sampling is being used to capture a diverse sample regarding age and first versus second or later pregnancy. A structured interview guide based on the Behaviour and Social Drivers (BESD) framework has been developed, piloted and is being used in the ongoing data collection phase. Participants are specifically asked about their views of the maternal RSV vaccine and infant RSV mAB, their information needs, and factors that would influence their decision to receive the maternal RSV vaccine and the infant RSV mAB.

Interview are recorded using a digital recorder and transcribed et verbatim. Thematic analysis will be used to define presence of concepts and themes. Two researchers will repeatedly read the transcripts to familiarize themselves with the data. Meaning units with content relevant for the study aim will be identified and labeled with descriptive codes. Codes will then be organized into sub-categories and categories. The underlying meaning of these categories will be presented as themes in the results.

Results

Findings will be presented in updated text or conference presentation if approved.

Conclusions

Pregnant womens views and preferences on the new available RSV infant interventions are important to explore and identify, data needed to inform program implementation of these interventions to ensure effective and equitable uptake in the target population and ultimately lead to significant decrease in RSV among infants.

325

Selecting a respiratory syncytial virus subtype B strain for the development of a Controlled Human Infection Model (CHIM)

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Human respiratory syncytial virus (RSV) is the most common cause of acute lower respiratory infections (ALRI) in children younger than 5 years of age and is a significant contributor to severe respiratory disease in older adults and immunosuppressed patients. Although three RSV vaccines (GSK: Arexvy; Pfizer: Abrysvo; Moderna: mRESVIA) have now been approved for use in older adults, largely uncontrolled RSV epidemics occur annually, resulting in high levels of morbidity and mortality worldwide. RSV treatment options in such cases are limited to supportive care. The only approved monoclonal antibodies for the prophylactic prevention of RSV infection are Palivizumab (Synagis, AstraZeneca) and more recently Nirsevimab (Beyfortus, AstraZeneca & Sanofi) and Clesrovimab (Enflonsia, MSD) were approved. Approved antiviral therapies are currently limited to aerosol or oral administration of ribavirin, which is suboptimal due to a nonspecific antiviral mechanism, toxicity issues, and bystander effects.

Several **in vitro** and **ex vivo** models of respiratory tract epithelium have provided important insights into different aspects of the RSV life cycle. In parallel, the development of the Controlled Human Infection Model (CHIM) for RSV has provided additional opportunities to study virus pathogenesis, associated immune responses and enabled better testing of candidate vaccines or therapeutics. However, new contemporary RSV challenge strains are needed, especially from subtype B, to be developed for use in this model as RSV B strains have shown more diversity in recent years. The development of an RSV strain has been facilitated by Inno4Vac, a public-private partnership involving academic institutions, SMEs and pharma companies funded by the IMI/IHI.

A panel of contemporary RSV-A (x3) and -B (x2) clinical isolates (P1 on HEp-2 cells, November 2022) were characterized with respect to sequence analysis of the F and G genes and assessment of virus replication and cytopathic effects on HEp-2, MRC-5 and Vero cells. This enabled the consortium to select a contemporary RSV-B strain (RSV-B-I54) for further characterization including next generation sequencing (NGS) to determine the whole genome sequence with associated phylogenetic analysis placing this strain in the B.D.E.1 clade, NGS testing for adventitious agents, and determination of the optimal multiplicity of infection for virus growth on Vero cells which had been selected as the cell substrate most suitable for generation of a Master Virus Seed (MVS) and subsequently the GMP-grade Master Virus Bank (MVB). Re-isolation of RSV-B-I54 from the original clinical sample was performed on Vero cells (Nuvonis Technologies) with approved reagents with the passage 1 stock used as seed virus for generation of the MVS and MVB by a CDMO, using GMP-grade Vero cells. The resulting MVB has passed all quality controls, with a virus titer of $10^{6.4}$/mL and is now available for use in CHIM studies.

326

Filling the gaps in sentinel surveillance: Estimating hospitalisation rates of respiratory infectious diseases using wastewater surveillance

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BackgroundMonitoring hospitalisations is a fundamental component of epidemiological surveillance, essential for assessing disease burden, guiding public health interventions, and evaluating countermeasure effectiveness. However, in many countries, sentinel surveillance systems are limited or absent due to insufficient reporting infrastructure or reduced clinical testing. For example, in the United States, national COVID-19 hospitalisation metrics are currently derived from a limited network of sentinel hospitals, with most states no longer reporting detailed case data. This hampers the ability to detect and interpret localised disease trends.

Wastewater-based epidemiology offers a promising complementary approach that has been widely adopted in countries such the US, Germany and France as a cost-effective and unbiased method that does not rely on individual testing behaviour. While currently utilised primarily to monitor transmission trends, it is not routinely employed to estimate disease burden.

This study presents a methodology to estimate COVID-19 hospitalisation rates from wastewater data in real time. The approach is generalisable and could be extended to other respiratory infectious diseases, including influenza and RSV.

Methods A Bayesian hierarchical model was developed to estimate state-level and national hospitalisation rates from wastewater. The model was trained on CDC data from 12 states for which both SARS-CoV-2 concentrations and hospitalisation data was available, covering the period from June 2024 to March 2025. Data was extracted in real-time and was subsequently applied to generate predictions of hospitalisations both in the training states and in additional states where only wastewater data, but no hospitalisation data was available, yielding point estimates and credible intervals for hospitalisations.

ResultsAcross the 12 states with both hospitalisation and wastewater data available, a strong linear correlation between SARS-CoV-2 concentrations and hospitalisation rates was observed (Pearson correlation coefficients ranged from 0.60 to 0.94). This robust relationship underpins the model's ability to predict hospitalisations in states lacking clinical surveillance data.Model predictions closely matched observed hospitalisation data, with predicted values deviating by only 2% over the study period. The model effectively captured both the timing and magnitude of peaks in COVID-19 hospitalisations. When applied nationwide, the model enabled estimation of hospitalisation burden in an additional 29 states, thereby significantly expanding COVID-19 surveillance coverage and estimate of burden of the healthcare system.

ConclusionsWe demonstrate a proof-of-concept approach for estimating hospitalisations from SARS-CoV-2 wastewater viral concentrations using a Bayesian modelling framework. This method enhances real-time surveillance capability in regions with limited or delayed hospitalisation reporting and provides more accurate estimates of disease burden. The model is adaptable to other geographies and infectious diseases, including influenza and RSV, and can serve as a valuable tool for timely estimation of hospitalisation trends, supporting more effective allocation of healthcare resources and preparedness efforts. This enhances situational awareness and supports data-driven decision-making for public health authorities and treatment providers.

329

Safety and immunogenicity of Butantan's split-virion inactivated quadrivalent influenza vaccine in children and adolescents: A phase III randomized, double-blind clinical trial.

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Background: The quadrivalent influenza vaccine (QIV) has demonstrated additional benefits in vaccine effectiveness and improved influenza control compared to the trivalent influenza vaccine (TIV), in an epidemiological context involving co-circulation of two influenza B lineages (Victoria and Yamagata). The Instituto Butantan, Latin America's largest producer of TIV, developed a QIV for children and adolescents, considering its relevance in a potential re-emergence of the B/Yamagata lineage.

Methods: This Phase III, randomized, double-blind, multicenter clinical trial conducted in Brazil evaluated the safety and immunogenicity of QIV compared to TIV in two childhood age groups: children (3 to 8 years old) and children/ adolescents (9 to 17 years old), during the 2021 and 2023 influenza seasons. Participants were allocated in a 4:1:1 ratio to receive QIV, licensed TIV (Victoria B lineage), and alternative TIV (Yamagata B lineage), respectively. For unprimed children aged 3 to 8 years, a two-dose vaccination schedule was administered at an interval of 28 days, while a single-dose regimen was applied for all other participants. Blood samples were collected pre- and 21 days post-vaccination for the hemagglutination inhibition assay (HI). The safety follow-up period was 6 months after the last vaccination.

Results: 1,021 children aged 3 to 8 (681 received QIV) and 1,019 children/ adolescents aged 9 to 17 (680 received QIV) were randomized and vaccinated. The frequency of solicited and unsolicited adverse reactions (ARs) was lower after second dose than first dose, among unprimed children. The most frequent AR following QIV was pain/ tenderness at the administration site, observed in 52.7% of children aged 3 to 8 years after the first dose and 19.6% after the second dose, and in 70.6% of children/ adolescents aged 9 to 17 years. In children aged 3 to 8, other frequent ARs included swelling at the administration site (14.1%) and headache (14.1%). In participants aged 9 to 17, the solicited ARs such as headache (27.5%) and fatigue (18.8%) were more frequent in the QIV group compared to TIV (p=0.006). In children aged 3 to 8 years, QIV met the non-inferiority criteria for the A(H1N1), A(H3N2), and B(Victoria) strains. For the B/Yamagata lineage, however, the lower limit of the geometric mean ratio (GMR) confidence interval was borderline at 0.66 in the per-protocol analysis. Among the 9-17 age group, QIV demonstrated non-inferiority for all four concordant strains compared to TIVs. Additionally, QIV demonstrated superiority for the non-concordant B lineages in both age groups.

Conclusions: The incorporation of a second B lineage into the quadrivalent influenza vaccine platform maintained a safety profile comparable to TIV and did not compromise the immune response of the other three influenza strains. The QIV may promote additional protection in an epidemiological setting characterized by the co-circulation of influenza B lineages.

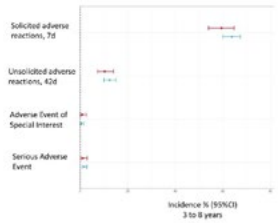
Table 1. Baseline demographic and anthropometric characteristics and medical history of Phase III study children and adolescent participants, by age group and according to intervention group.

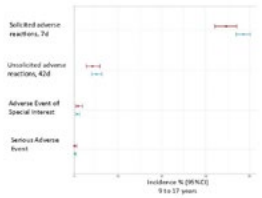
VARIABLES	QIV		Licensed TIV (Victoria B lineage)		Alternative TIV (Yamagata B lineage)	
3 to 8 years old						
Age (years)						
Median (Q1 - Q3)	6.1	(4.5 - 7.6)	6.0	(4.6 - 7.9)	6.2	(4.3 - 7.5)
Sex, n (%)						
Female	352	(51.8)	85	(50.3)	83	(48.5)
Male	327	(48.2)	84	(49.7)	88	(51.5)
Race/Color, n (%)						
White	224	(33.0)	56	(33.1)	60	(35.1)
Black	37	(5.4)	13	(7.7)	11	(6.4)
Mixed	400	(58.9)	95	(56.2)	98	(57.3)
Indigenous	1	(0.1)	1	(0.6)	1	(0.6)
Asian	15	(2.2)	4	(2.4)	1	(0.6)
Unknown or refused to answer	2	(0.3)	0	(0.0)	0	(0.0)
BMI (kg/m²)						
Median (Q1 - Q3)	15.9	(14.9 - 17.4)	16.0	(14.8 - 17.4)	15.7	(14.9 - 17.4)
Primary vaccination for influenza, n (%)						
Yes	619	(91.2)	157	(92.9)	153	(89.5)
No	60	(8.8)	12	(7.1)	18	(10.5)
9 to 17 years old						
Age (years)						
Median (Q1 - Q3)	13.2	(11.1 - 15.4)	13.0	(11.0 - 15.2)	12.7	(10.9 - 15.7)
Sex, n (%)						
Female	339	(49.9)	89	(52.7)	87	(51.2)
Male	341	(50.1)	80	(47.3)	83	(48.8)
Race/Color, n (%)						
White	287	(42.2)	65	(38.5)	70	(41.2)
Black	63	(9.3)	15	(8.9)	16	(9.4)
Mixed	317	(46.6)	87	(51.5)	82	(48.2)
Indigenous	0	(0.0)	0	(0.0)	0	(0.0)
Asian	9	(1.3)	2	(1.2)	2	(1.2)
Unknown or refused to answer	4	(0.6)	0	(0.0)	0	(0.0)
BMI (kg/m²)						
Median (Q1 - Q3)	20.0	(17.2 - 23.1)	19.6	(17.3 - 23.2)	19.3	(16.6 - 23.7)

Table 2. Analysis of non-inferiority (concordant strains) and superiority (non-concordant B strains) of quadrivalent influenza vaccine versus trivalent influenza vaccine, 21 days (+7) post-vaccination, by hemagglutination inhibition assay, by strain and age group: per-protocol analysis.

Strain	Non-inferiority		Superiority	
	QIV/TIVs GMR (IC95%)	Non-inferiority	QIV/TIV GMR (IC95%)	Superiority
3 to 8 years old				
A/H1N1	0.90 (0.77 ; 1.06)	Yes	-	-
A/H3N2	0.88 (0.75 ; 1.05)	Yes	-	-
B/Yamagata	0.86 (0.66 ; 1.12)	No	4.43 (3.51 ; 5.58)	Yes
B/Victoria	0.99 (0.82 ; 1.20)	Yes	2.47 (1.91 ; 3.20)	Yes
9 to 17 years old				
A/H1N1	0.87 (0.73 ; 1.03)	Yes	-	-
A/H3N2	0.90 (0.71 ; 1.15)	Yes	-	-
B/Yamagata	1.11 (0.92 ; 1.35)	Yes	3.92 (3.17 ; 4.86)	Yes
B/Victoria	0.90 (0.74 ; 1.09)	Yes	3.15 (2.56 ; 3.88)	Yes

Figure 1. Incidence of adverse reactions and adverse events in participants aged 3 to 8 years and 9 to 17 years, by intervention group.





332

Whole-genome sequencing of influenza viruses in Ukraine during the 2024–2025 epidemic season: Implications for laboratory diagnostics and surveillance

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Background. The WHO strategy emphasizes the need for integrated and sustainable sequencing systems at all levels to incorporate genomic surveillance into routine public health practice. Ukraine, despite the ongoing war, continues to integrate whole-genome sequencing into its national epidemiological monitoring system. Genetic characterization of viruses, particularly influenza viruses, allows timely detection of changes in circulating strains and supports informed public health decision-making at the national and global levels. In particular, these data help to update the composition of seasonal vaccines, assess the risks of new strains and make informed forecasts of possible outbreaks.

Methods. Nasopharyngeal swabs were collected from patients with suspected influenza or other viral respiratory infections. RT-PCR was used to confirm viral presence, and positive samples underwent whole-genome sequencing using validated protocols. Phylogenetic analysis was performed to assess genetic diversity.

Results. The 2024/2025 epidemic season is the second season during which the PHC has implemented whole-genome sequencing of influenza A and B viruses, as well as other acute respiratory viral infections (ARVI). A total of 435 RT-PCR–positive samples were sequenced, covering 21 regions of Ukraine and providing broad geographical representation. Of these, 381 samples were confirmed as influenza viruses. The remaining 49 samples were identified as non-influenza respiratory viruses. Phylogenetic analysis showed high genetic similarity between the sequenced strains and WHO vaccine reference strains recommended for season 2024-2025: A/Massachusetts/18/2022 (82 samples), A/Wisconsin/67/2022 (104 samples), and B/Austria/1359417/2021 (194 samples). It should be noted that one case of co-infection with A and B viruses was identified with B/Austria/1359417/2021 and A/Massachusetts/18/2022 viruses.

Conclusion. The data obtained by the PHC indicate that the circulating influenza virus strains sequenced in Ukraine are genetically similar to the WHO reference strains recommended for season 2024-2025. This work demonstrates Ukraine's sustained effort to implement nationwide genomic surveillance during wartime conditions and contribute valuable influenza genomic data to global platforms, such as GISAID.

338

Pre-Pandemic Preparedness for H5 Avian Influenza Viruses: Antigenicity, Serology and Antiviral Susceptibility

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H5 highly pathogenic avian influenza viruses (HPAIVs) have caused sustained global outbreaks in domestic and wild bird populations since 2020, with spillovers into mammals, including humans. As such, these viruses represent a significant threat to public health. As a WHO Collaborating Centre, one of the key roles of the Worldwide Influenza Centre is to link with public and animal health institutes globally, to perform horizon scanning and phenotypic assessments of HPAIVs with zoonotic potential, as part of pre-pandemic preparedness activities. This work directly feeds into the WHO's Global Influenza Surveillance and Response System (GISRS) and the bi-annual Vaccine Composition Meetings.

Through this work, the Worldwide Influenza Centre has collaborated across a number of countries to perform antigenic, serological and antiviral susceptibility. This has included working with colleagues in Cambodia, to characterise the H5 clade 2.3.2.1e (formerly clade 2.3.2.1c) HPAIVs, which have caused 19 human infections, with several fatal cases, since February 2023. Antigenic assessment of viruses from human and related poultry found that they were not well recognised by ferret antisera raised towards existing H5 candidate vaccine viruses (CVVs). Antiviral susceptibility of these viruses towards neuraminidase inhibitors (Oseltamivir and Zanamivir), as well as a cap-dependent endonuclease inhibitor (Baloxavir marboxil) was assessed, and it was found that these viruses remained susceptible to antivirals.

In addition, the Worldwide Influenza Centre has also characterised a number of H5 HPAIV clade 2.3.4.4b viruses from Europe, Asia and North America. Antigenic assessment found these viruses were well recognised by existing H5 clade 2.3.4.4b CVVs. Serological assessment using a panel of human sera collected pre- and post-vaccination with the human seasonal influenza vaccine found that there was no evidence of cross-reactivity with these viruses. Antiviral susceptibility assessment found that the majority of these viruses remained susceptible to neuraminidase inhibitors, all of which demonstrated normal susceptibility.

339

ECaS

Development of a Plant-Expressed Universal Influenza Vaccine Targeting Conserved Viral Domains

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Influenza continues to pose a significant global health challenge, with current commercially available vaccines primarily targeting the highly variable globular head of the haemagglutinin (HA) glycoprotein. As a result, vaccine effectiveness diminishes over time due to antigenic drift and shift, necessitating annual reformulation. In response, the development of universal influenza vaccine candidates targeting conserved regions of the virus, such as the extracellular domain of the matrix 2 protein (M2e) and the HA stalk region, offers promise for broader and longer-lasting protection.

Accordingly, this study aimed to develop a candidate universal influenza vaccine candidate by combining five tandem repeats of the M2e epitope (5xM2e) with the self-assembling HA stalk domain of influenza A, which is also highly conserved. The chimeric fusion protein was expressed in **Nicotiana benthamiana** using an **Agrobacterium tumefaciens**-mediated transient expression system. Protein expression was confirmed by western blotting, and self-assembly into virus-like particles (VLPs) was validated by transmission electron microscopy (TEM).

The chimaeric VLPs were purified using iodixanol density gradient ultracentrifugation, yielding approximately 400 mg per kg of fresh leaf weight (FWL). These VLPs will be evaluated in mice to assess immunogenicity, including both humoral and cellular immune responses, as well as cross-reactivity against diverse influenza strains.

To our knowledge, this is the first demonstration of a plant-based system successfully expressing and assembling a chimaeric 5xM2e-HA stalk fusion protein. This platform presents a promising approach for the development of universal influenza vaccines with scalable and cost-effective production.

340

The 2024-2025 highly pathogenic avian influenza H5N1 epidemic in Italy: origin, evolution and spatial spread

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Background:

Since 2021, the Highly Pathogenic Avian Influenza (HPAI) subtype H5Nx belonging to clade 2.3.4.4b has spread worldwide, remaining entrenched in the wild bird population in several countries. These viruses have shown the ability to infect mammals, including humans, posing a significant zoonotic threat. Genomic surveillance is crucial to track viral evolution, spatial spread and the emergence of variants with increased zoonotic potential, which in turn is key to implement timely and effective control measures. The epidemic that occurred in 2024-2025 had a significant impact, particularly in terms of the number of outbreaks and the number of animals involved. From September 2024 to January 2025, Italy notified 152 outbreaks in wild (N=97) and domestic (N=55) birds, and in three mammals. This study aims to reconstruct the origin, geographic spread and the role of different host for the 2024-2025 epidemic in Italy.

Methods:

Complete genome sequences of 112 H5N1 viruses collected from wild birds (N=55), domestic birds (N=54), and mammals (1 domestic cat and 2 red foxes) from October 1st, 2024 to January 31st, 2025 were obtained and phylogenetically analysed together with related sequences available in GISAID, using IQtree. A metadata set containing geographical, host, and temporal information was created. Continuous phylogeographic analysis was performed using a Bayesian approach through BEAST software and Markov Jumps was estimated for the Host trait. We searched for zoonotic molecular markers using FluMut (https://github.com/izsvenezie-virology/FluMut).

Results:

The BEAST results suggest that the HPAI H5N1 virus was introduced to Italy several times via wild birds (likely swans, wild duck and gulls) moving from north-eastern Europe to north-eastern Italy, from central Europe to northern Italy and from eastern Europe to central Italy. These results unveil that Italy played a role not only as a sink, but also as a source for the spread of the virus to nearby countries. Several spill over events from wild to domestic birds, likely occasionally followed by farm-to-farm transmissions, were identified. Furthermore, the genetic analysis indicates that the virus was likely transmitted directly or indirectly from chickens to a domestic cat that was found dead in a backyard farm where a poultry outbreak had been reported a few days earlier. Mutations in the PB2 protein, ie.627K/V and 701N, which are indicative of adaptation to mammals, were identified in 9 viruses from wild (N=4) and domestic (N=5) birds as well as in the viruses collected from the domestic cat and one wild red fox.

Conclusions:

Our study demonstrated the complex transmission pathways of the A(H5N1) virus in Italy during the 2024-2025 epidemic wave and the emergence and spread of viruses with molecular markers associated with increased zoonotic potential, providing critical information to improve the design of effective surveillance strategies from a One Health perspective.

343

Impact of Serum Pre-Treatment on HAI Titers against the A/Astrakhan (H5N8) Virus

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Background: This study aimed to evaluate the impact of serum pre-treatment on Hemagglutination Inhibition (HAI) titers, specifically for the A/Astrakhan (H5N8) RG71A virus. To optimize the HAI assay we investigated serum pre-treatment, which can differ between protocols. Specifically, we examined whether the sequence—Receptor Destroying Enzyme (RDE) treatment followed by blood adsorption or vice versa—affects HAI results for A/Astrakhan (H5N8). Although both methods are standard for removing non-specific inhibitors and agglutinins, their order is often unspecified, suggesting it may be unimportant for most influenza strains. However, this has not been confirmed for the A/Astrakhan (H5N8), making it the focus of this study.

Methods: The study was conducted at two laboratories: Vismederi (Siena, Italy) and CSL (Waltham, MA). Serum samples from 120 subjects immunized with an A/Astrakhan (H5N8) cell-based vaccine were collected on Day 1 and Day 43 post-vaccination. HAI assays compared two pre-treatment sequences: blood adsorption then RDE, or RDE then blood adsorption. Geometric mean titers (GMTs) and seroconversion rates (SCRs) were analyzed.

Results: Serum pre-treated with blood adsorption followed by RDE yielded approximately 10-fold higher GMTs than the reverse sequence, with no effect on background levels. This order also better matched MN titers, confirming its reliability for HAI.

Conclusions: The sequence of serum pre-treatment significantly affects HAI titers for A/Astrakhan (H5N8), highlighting the need for optimized protocols, especially for avian influenza strains with scarce data, to support public health responses to respiratory virus outbreaks.

345

Prevalence, clinical characteristic and pattern of distribution of Respiratory syncytial virus associated acute respiratory tract infections among adults and children in the Central Province of Sri Lanka from January 2021 - October 2022

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Background: Seasonal epidemics of respiratory syncytial virus (RSV) is one of the leading causes of morbidity and mortality in children and increasingly recognized as important pathogen among adults. So far, there are three approved vaccines (ABRYSVO, Arexvy, and mRESVIA) and two monoclonal antibodies (Nirsevimab and Palivizumab) available for the prevention and control of RSV infection. However, epidemiology of RSV-related ARTIs is largely undetected in adults and substantially underestimated in most of the Asian countries including Sri Lanka.

Method: In the current study, a total of 1021 respiratory samples from adults and children with acute respiratory tract infections (ARTIs) were tested to detect respiratory pathogens including RSV using a real time reverse transcriptase polymerase chain reaction (RT-PCR) from January 2021 to October 2022 in the Central Province of Sri Lanka.

Results: Respiratory pathogens were detected in 51.32% patients. Of the samples tested 9.5% patients were positive for RSV including 5.68%, 1.27%, 2.54% of RSV A, RSV B and both RSV A & B respectively. RSV infection was more prevalent in males (57.73%) than females. RSV infections were predominantly detected in children aged 0-<5 years. Fever, cough and sore throat were the most common symptoms. Very less number (≤2) of RSV were detected until April 2021 and a delayed surge of RSV was observed. RSV A was the predominant subtype circulated in our study period.

Conclusion: In conclusion, this study shows a prevalence of 9.5% for RSV infections in patients with ARTI. The circulation pattern of RSV and their subtypes varied during the study period. Despite the recent progress in vaccine development, still there are limitation due to the high cost of medication and the accessibility of the vaccines particularly for middle income countries. Hence, this study will help policymakers to plan appropriate preventive strategies for RSV and will act as an epidemiological data resource for the future RSV vaccination programs in Sri Lanka.

346

ECaS

Method choices and their impact on influenza vaccine effectiveness estimations

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Background:

Estimating influenza vaccine effectiveness (IVE) presents ongoing methodological challenges. To enhance the interpretation and reliability of IVE results, it is critical to understand how estimates vary across different analytical approaches. This study evaluates IVE against laboratory-confirmed influenza among older adults targeted for vaccination, applying two distinct statistical methods across nine influenza seasons.

Methods:

Prospective, active-surveillance study in influenza-like-illness (ILI)hospitalized patients aged ≥60 years and target for influenza vaccination within the Valencia Hospital Network for the Study of Infectious Diseases (VAHNSI) across 2012/2013 and 2022/2023 seasons. All participants meeting inclusion criteria were tested for influenza using real-time RT-PCR. Seasonal IVE was calculated as (1−odds ratio) ×100, using two analytical approaches: 1) Multivariable logistic regression, adjusting for age, sex, calendar week, Barthel Index, and number of comorbidities. 2) Inverse probability of treatment weighting (IPTW) was used to balance vaccinated and unvaccinated individuals based on propensity scores estimated from the same covariates. IVE was then estimated using logistic regression weighted by the IPTW, with robust standard errors (sandwich method).

Results:

A total of 9352 patients were included, of which 2237 (24%) tested positive for influenza. Vaccination coverage ranged from 51% to 63% among controls and from 34% to 55% among cases. Notably, 67.4% of cases and 64.28% of controls had mild or minimal functional dependence, 9.96% of cases and 8.2% of controls had no comorbidities, and 33.16% of cases and 35.74% of controls had three or more comorbid conditions. IVE results by method and season are presented in Table 1 and 2.

Conclusions:

The IVE estimates showed substantial variability across the seasons. Moderate protection was primarily observed in individuals with a vaccine-virus match.

Confidence intervals were often wide, reflecting high uncertainty that was likely influenced by sample size and seasonal differences in virus circulation.

In matched seasons, the difference between the multivariable regression and IPTW estimates was generally small, suggesting that both methods adequately controlled for confounding factors.

However, IPTW consistently yielded slightly higher IVE estimates, likely because it balanced baseline characteristics between groups more effectively and was less sensitive to errors in the specification of the multivariable model.

Table 1: Number of cases and controls vaccinated and unvaccinated subjects by season.

Season	Total	Cases	Controls	Vaccinated	No vaccinated	Antigenic matching
2012/13	820	134	686	462	358	Yes
2013/14	904	160	744	550	354	Yes
2014/15	2,150	539	1,611	1,393	757	Yes
2015/16	862	149	713	521	341	Yes
2016/17	948	172	776	561	387	No
2017/18	1,407	415	992	777	630	No
2018/19	1,304	197	1,107	763	541	Partial
2019/20	418	41	377	276	142	No
2022/23	539	75	464	331	208	Yes

Table 2: IVE values by different methods by season.

Season	Crude IVE	Multivariable IVE	IPTW IVE	Antigenic matching
2012/13	42.72% (16.93%,60.64%)	32.73% (-0.95%,55.25%)	39.85% (11.44%,59.15%)	Yes
2013/14	43.47% (20.26%,59.95%)	34.94% (6.17%,54.91%)	45.59% (22.4%,61.85%)	Yes
2014/15	30.47% (15.01%,43.07%)	31.02% (14.16%,44.55%)	31.71% (16.23%,44.32%)	Yes
2015/16	23.61% (-9.28%,46.47%)	8.22% (-35.78%,37.72%)	22.3% (-12.93%,46.54%)	Yes
2016/17	24.72% (-5.08%,45.99%)	25.45% (-6.76%,47.89%)	24.15% (-6.93%,46.2%)	No
2017/18	6.9% (-17.23%,26.03%)	1.21% (-28.5%,23.99%)	10.65% (-13.51%,29.66%)	No
2018/19	38.79% (17.03%,54.89%)	39.97% (16.63%,56.83%)	38.23% (15.52%,54.83%)	Partial
2019/20	43.93% (-8.32%,70.75%)	44.62% (-10.72%,72.13%)	43.75% (-10.5%,71.36%)	No
2022/23	43.7% (7.97%,65.6%)	42.56% (-2.3%,67.79%)	48.73% (12.2%,70.06%)	Yes

IVE: influenza vaccine effectiveness; IPTW:Inverse probability of treatment weighting

347

ECaS

Metabolomic signatures of two major respiratory pathogens in the reconstituted human airway epithelium model

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Background

SARS-CoV-2 and influenza A viruses (IAV) are major pathogens for human respiratory infections. Several studies describe the impact of both viruses on host's metabolism (Ayres et al. 2020, Bahadoran et al. 2020, Rubayet Hasan et al. 2021). Nevertheless, none of those studies have exhaustively compared both viruses in a similar experimental set-up. Our study examines the impact of these viruses on the metabolism of human lung epithelium with an untargeted metabolomics approach.

Methods

Reconstituted human airway epithelium (HAE) model was infected with SARS-CoV-2 (Wuhan strain) or IAV (H1N1 2009 pandemic strain) during 24 to 96 hours, and treated 48 hours after infections start or not with **Pseudomonas aeruginosa** flagellin. Infections were characterised by measuring TEER, inflammatory statement (Il-6), viral replication and transcriptomics profiles. Following cells collection, endometabolome was measured with an orbitrap LC-HRMS. Metabolomics data were processed with MZmine and Metabo-Analyst, then annotated with GNPS and Compound Discoverer in association with a homemade spectral database.

Results

Phenotype acquisition (TEER, Il-6, viral replication) and a K-means clusterisation method enabled us to define severity groups. LC-HRMS/MS analysis resulted on thousands of variables that were filtered (CV QC pool <30%, r2>0,7 for each variable). Non-supervised (PCA) or supervised (O-PLSDA) multivariate statistical analysis leaded to the identification of a VIP list associated to SARS-CoV-2 and/or IAV.

Conclusions

We have identified metabolic disruptions distinctive for each virus and associate to a VIP list. Disrupted metabolic pathways may lead to the identification of new biomarkers specific to each infection types or severity levels.

349

Pre-symptomatic, post-symptomatic and asymptomatic viral shedding of respiratory viruses in community-dwelling individuals

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Background: The importance of infectious individuals without apparent symptoms in respiratory virus transmission has been highlighted during the COVID-19 pandemic. Viral shedding is thought to be associated with transmission, however data on pre-symptomatic, post-symptomatic and asymptomatic viral shedding is scarce.

Methods: We conducted a household transmission study ("TReV") in Hong Kong. We identified index cases who meet the following criteria: ≥ 2 years old; outpatients who presented with ≥2 signs/symptoms of acute respiratory illness (ARI); within 48 hours of symptom onset; and living with ≥2 household members who did not have ARI in the past 14 days. We followed and collected separate nasal swabs and throat swabs from the index and household members with home visits at enrolment, 4 and 10 days after regardless of their symptom presentation. Multiplex viral panel and subsequently PCR was used to identify respiratory viruses from all swabs collected.

Results: From October 2023 to September 2024, we enrolled 55 households with 163 participants. Based on preliminary PCR data from 77 participants in 27 households, we observed (i) multiple introductions of different respiratory viruses in the same household during the 10-day follow-up period; (ii) pre-symptomatic viral shedding in individuals infected with influenza or SARS-CoV-2 virus; (iii) post-symptomatic viral shedding in individuals infected with influenza virus, SARS-CoV-2 and rhinovirus; and (iv) asymptomatic viral shedding in individuals infected with SARS-CoV-2, rhinovirus, human metapneumovirus (hMPV) and respiratory syncytial virus (RSV).

Conclusions: In this preliminary analysis, we observed viral shedding from infectious individuals without apparent symptoms for most respiratory viruses. Enrolment is continuing to expand the sample size which may allow the assessment of the relationship between pre-symptomatic and asymptomatic viral shedding and transmission for major respiratory viruses in circulation.

350

The role of the stalk in protective immunity to H1N1 influenza haemagglutinin of pandemic strains

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Background: After the 1918 influenza pandemic, H1N1 strains continued to circulate until 1957, being re-introduced in 1977, and disappearing again with the 2009 H1N1 influenza pandemic. The protective immunity against the 2009 H1N1 virus found in elderly people is thought to be due to previous exposure to older H1N1 strains with similar antigenic epitopes. Since the immune response to influenza is shaped by the history of exposure to antigenically similar strains, the role of the HA stalk in protective immunity to H1N1 influenza pandemic strains was investigated.

Methods: Human serum samples were tested for the presence of functional antibodies against the haemagglutinin of H1N1 strains A/South Carolina/1/1918 and A/California/7/2009 by a pseudotype-based micro-neutralization assay. The contribution of antibodies against the stalk of the haemagglutinin was investigated using a chimeric pseudotype (Head H11/Stalk H1/1/18). Samples were divided into groups based on the circulating H1N1 strains, according to the birth cohort of the subjects and the year of sampling.

Results: The response towards A/South Carolina/1/1918 seems to maintain protection over time and towards A/California/7/2009, although the same cannot be observed when considering the reverse relationship i.e. A/California/7/2009 does not protect towards A/South Carolina/1/1918. Furthermore, antibodies against the stalk gives a 'residual' response to A/South Carolina/1/1918 in groups exposed to A/California/7/2009, which precisely allows protection against the later strain.

To determine an explanation for the observed cross-protection, the amino acid sequences between strains representative of the H1N1 viruses circulating from 1918 to 2009 were compared. The stalk region of the 1918, 1977, and 2007 viruses is highly similar (about 95%), indicating that this region is less prone to variation compared to the head portion, which is more involved in antigenic drift. In contrast, the similarity between the 1918 virus and the 2009 virus is lower, explaining why samples collected after the 2009 pandemic show little to no protection against the 1918 pandemic H1N1 virus.

Conclusions: Significant differences in response to pandemic H1N1 strains between different age groups with a different history of exposure to H1N1 strains were observed. The use of a chimeric pseudotype virus allowed the selection of the antibody response towards the stalk, subtracting its immunogenic impact and selecting the antibody response towards the head portion. Antibodies directed against the stalk allow protection against following strains to be maintained, although this does not appear to be bi-directional, suggesting that mutations in the stalk have impact on the maintenance of protection. The findings of this study lend support to the presence of an immune imprinting that influence the responses to diverse H1 strains. The way in which this imprinting responds to strains belonging to the same phylogenetic group, such as H5, should be considered moving forward.

351

Impact of sex hormones on macrophage-driven immune response to SARS-CoV-2

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Background

Since the beginning of the COVID-19 pandemic, male sex has been considered to be one of the main risk factors for disease severity and outcome. Of note, numerous studies have highlighted the correlation of reduced testosterone levels in male patients with admission to intensive care units and death (1). Indeed, the enzyme CYP19A1 (also known as aromatase), which converts testosterone to estradiol, is increased in the lungs of men who died from SARS-CoV-2 infection, but not in women (2). The use of the clinically approved aromatase inhibitor Letrozole, was able to improve the lung health in the hamster model and restore dysregulated sex hormones, specifically in males. Interestingly, most of the cells expressing aromatase in the male lung are alveolar macrophages, already known to be responsible for the imbalanced immune response and cytokine storm in SARS-CoV-2 patients via the activation of the inflammasome pathway (3).

Material and methods

Lung macrophages were isolated from male and female hamsters and treated with different concentration of Poly I:C to simulate acute virus infection. Subsequently, mRNA expression levels of aromatase and cytokines were assessed by qRT-PCR.

Results

Upon Poly I:C treatment, lung macrophages from male hamsters were able to upregulate aromatase in a dose-dependent manner. However, after inhibition of the **Nlrp3** inflammasome gene, male-derived lung macrophages lost the ability to upregulate aromatase along with a reduced expression of IL-1β.

Discussion

These findings suggest that activation of the inflammasome could increase aromatase expression, resulting in hormonal dysregulation in patients upon SARS-CoV-2 infection, potentially leading to severe disease outcome and long-term consequences.

1. Schroeder M, Schaumburg B, Mueller Z, Parplys A, Jarczak D, Roedl K, et al. High estradiol and low testosterone levels are associated with critical illness in male but not in female COVID-19 patients: a retrospective cohort study. Emerg Microbes Infect. 2021;10(1):1807-18.

2. Stanelle-Bertram S, Beck S, Mounogou NK, Schaumburg B, Stoll F, Al Jawazneh A, et al. CYP19A1 mediates severe SARS-CoV-2 disease outcome in males. Cell Rep Med. 2023;4(9):101152.

3. Sefik E, Qu R, Junqueira C, Kaffe E, Mirza H, Zhao J, et al. Inflammasome activation in infected macrophages drives COVID-19 pathology. Nature. 2022;606(7914):585-93.

353

ECaS

Hybrid capture viral metagenomics enables detection and subtyping of influenza species in emergency department indoor air

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Background: Traditional influenza surveillance relies on symptomatic patients and diagnostic testing, which may miss asymptomatic or pre-symptomatic infections and requires additional tests for genomic surveillance. We evaluated whether hybrid-capture–enhanced viral metagenomics could identify influenza viruses directly from indoor air in an emergency department waiting room.

Methods: Four separate one-week samplings were conducted in the emergency department waiting area using an AerosolSense™ air sampler (Thermo Fisher Scientific), operating at 200 L/min. Samples were processed with the Comprehensive Viral Research Panel (TWIST Biosciences), which targets over 3,000 viral species and 15,000 strains. Libraries were sequenced on the AVITI platform to yield ~20 million reads per sample. Overall viral detection was performed by reference-guided assembly against all known human and animal viruses in GenBank (November 2022), and influenza subtyping used all reference sequences available through June 2024, followed by competitive read mapping against each subtype.

Results: Influenza A and B were detected in every sample. Three samples were dominated by influenza A and one by influenza B (**Figure 1A**), matching contemporaneous clinical genomic data. Influenza B belonged to the Victoria lineage. Among influenza A detections, one sample contained only H3N2, one only H1N1, and two samples harbored both subtypes (Figure 1B). No H5N1 or other subtypes were identified. Beyond influenza, we identified reads belonging to rhinovirus C11 and A28, rotavirus A, norovirus GII.17, and seasonal coronaviruses in multiple samples (Figure 2).

Conclusions: Hybrid-capture viral metagenomics of indoor air samples can detect and subtype influenza viruses—and uncover other respiratory and enteric pathogens—offering a promising supplement to conventional surveillance systems.

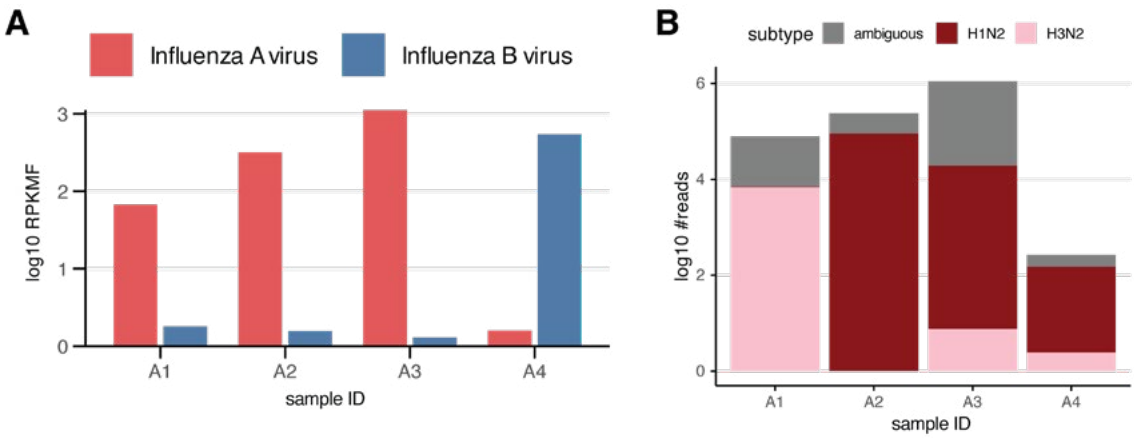

Figure 1. A. Influenza species identified in four samples. **B.** Influenza A subtypes identified in four samples.

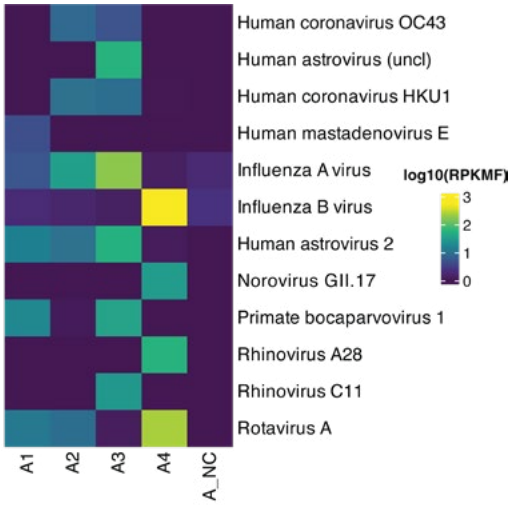

Figure 2. Other viral pathogens identified in four samples and negative control.

354

Seasonal Pattern and Burden of COVID-19 Compared to Influenza in the Post-Pandemic Period: A Comparative Ecological Analysis in the US and Europe, 2022–2025

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Background:

Influenza exhibits well-characterized winter peaks in the northern hemisphere. Although some studies hinted at emerging COVID-19 winter seasonality, surveillance still shows irregular multiple peaks. We analyzed the comparative post-pandemic pattern of SARS-CoV-2 and influenza virus circulation and associated hospitalization burden.

Methods:

We conducted a retrospective ecological study using influenza and SARS-CoV-2 weekly surveillance data from 2022-W13 to 2025-W12. Virus circulation was assessed by calculating detection proportion from sentinel primary-care (Europe, England) and NREVSS laboratories (USA). Severity was assessed using sentinel SARI detection rate (Europe), and hospitalization rates from RESP-NET (USA) and SARI Watch (England). Countries in Europe were grouped into WHO influenza-transmission zones. Country inclusion required ≥80% weekly data availability and consistent sentinel test volumes (r>0.9). Weekly detection proportion was smoothed using Generalized Additive Models (**mgcv**) with autocorrelation structure. Peaks were detected with **pracma** algorithm. Hospitalization rates were presented as weekly and season cumulative-crude rates.

Results:

Twenty-two countries met inclusion criteria for circulation analysis and ten for the severity analyses. Preliminary results indicate influenza maintained similar winter peaks across regions (median peak week 52, range 50–11) with detection peaks ranging 16–56%. SARS-CoV-2 peaked one to three times per season; highest overall peak was 30.4% (95% CI: 23.6–38.0) in Eastern Europe (Aug 2024), and highest winter peak was 28.1% (95% CI: 24.8–31.6) in the same zone (Dec 2023) (Figure 1).

SARI detections mirrored circulation patterns, with SARS-CoV-2 peaking predominantly outside winter months. Hospitalization data suggests a decrease of COVID-19 burden over the past three years, with cumulative COVID-19 hospitalization rates in the US (125.7 per 100,000) and England (110.2 per 100,000) similar to those of influenza (US: 124.6, England: 143.0 per 100,000) during 2024-2025 period (Figure 2).

Conclusions:

Preliminary analyses suggest that SARS-CoV-2 has not yet adopted a consistent winter seasonal pattern, with increases in summer/fall in US and Europe and no clear winter peak in most recent season. After a progressive decline in COVID-19 burden, recent cumulative hospitalization rates approximate those of influenza. Further analysis will offer insights on potential circulation drivers. This analysis informs immunization programs as several influenza/COVID-19 combination vaccines are in development.

Figure 1: Weekly SARS-CoV-2 and Influenza Virus Activity: US, England and European Regions

Based on NREVSS (US), ERVISS sentinel primary-care surveillance (Europe) and GP sentinel swabbing scheme (England)

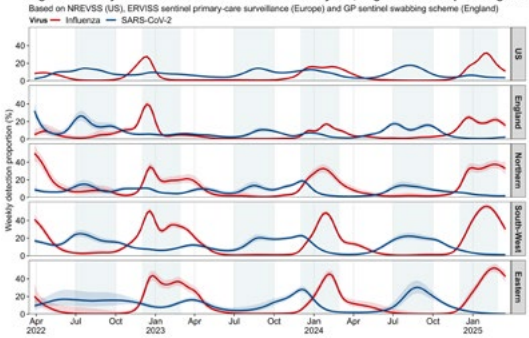
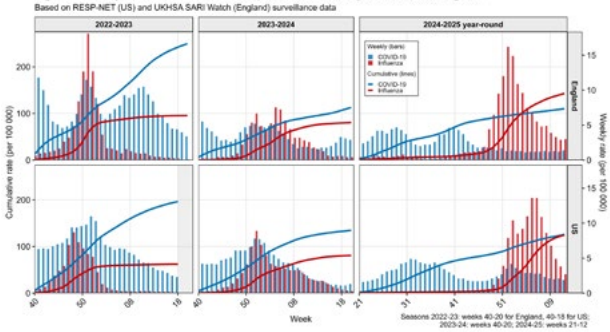


Figure 2: Weekly Trend vs. Cumulative Hospitalization Rates by Virus, US and England

Based on RESP-NET (US) and UKHSA SARI Watch (England) surveillance data



355

The safety, tolerability, and immunogenicity of trivalent modified RNA vaccines against seasonal influenza in healthy adults

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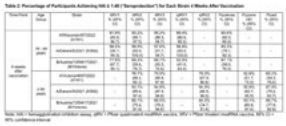
Background: Influenza remains a major cause of morbidity and mortality globally. Modified nucleoside messenger RNA (mRNA) vaccines induce robust humoral and cellular immune responses and allow for a closer match to circulating strains than licensed egg-based influenza vaccines. This Phase 2 study characterizes trivalent mRNA influenza vaccine candidates in younger and older adults.

Methods: Healthy adults ≥18 years of age were enrolled and evaluated for safety, tolerability, and immunogenicity following vaccination with trivalent mRNA influenza vaccines compared to licensed influenza vaccines and Pfizer quadrivalent mRNA influenza vaccines. Hemagglutination inhibition assays (HAI) were performed pre-vaccination, 4 weeks after vaccination, and 6 months after vaccination. Adverse events were collected through 4 weeks after vaccination and medically attended or serious adverse events were collected through the end of the study.

Results: 481 adults 18-64 years of age were randomized 1:1:1:1:1 and 479 (99.6%) were vaccinated with one of four mRNA influenza vaccines or a licensed influenza vaccine. 450 adults ≥65 years of age were randomized 1:1:1:1:1 and vaccinated (100%) with one of three mRNA influenza vaccines or one of two licensed influenza vaccines (high-dose or adjuvanted). mRNA influenza vaccines were well tolerated with injection site pain, fatigue, and headache most frequently reported after vaccination. Adverse event rates were similar across vaccine groups and no unexpected, related adverse events were reported. Geometric mean HAI titers (Table 1) and the percentage of participants with HAI titer ≥ 1:40 ("seroprotection"; Table 2) elicited 4 weeks after vaccination by trivalent mRNA influenza vaccines were comparable to or higher than licensed influenza vaccines and Pfizer quadrivalent mRNA influenza vaccines for A/H1N1, A/H3N2, and B/Victoria influenza strains.

Conclusion: Pfizer trivalent mRNA influenza vaccines were safe, well tolerated, and elicited geometric mean HAI titers and seroprotection rates comparable to or higher than licensed influenza vaccines.



358

Covid-19 Vaccine Hesitancy Prevalence and its associated factors among Health Care Workers in Tertiary Health Care Hospital of Sindh-Pakistan

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Background: Vaccine hesitancy is a major public health problem that can undermine efforts to control the COVID-19 pandemic, particularly among health care workers (HCWs). Being lead in public health, HCWs plays a critical role in promotion for vaccine uptake or refusal, as they possess a higher risk of exposure and further transmission to COVID.19 infections. Despite having medical background, some HCWs remain hesitant to receive the COVID-19 vaccine. This study was conducted to determine the prevalence and associated determinants of COVID-19 vaccine hesitancy among HCWs in tertiary health care settings of Sindh.

Methods: A cross-sectional study was conducted from June to September 2022 across three tertiary care hospitals in Sindh. A structured and pretested questionnaire was used to collect data from doctors, nurses, and allied health staff. The data collected on socio-demographic characteristics, vaccination status, beliefs about vaccine safety and efficacy, believe in health authorities, and reasons for hesitancy. Vaccine hesitancy was defined as delay in acceptance or refusal of vaccination despite availability. Data were analyzed using epi-info 7.2. Logistic regression was used to identify independent predictors of vaccine hesitancy.

Results: Among 450 participants, the prevalence of vaccine hesitancy was 29%(n=131). Common reasons identified included concerns about vaccine safety (63%), fear of side effects (54%), and lack of trust in vaccine trials (41%). Factors significantly associated with vaccine hesitancy included younger age (AOR=3.1; 95% CI: 2.3–5.4), female gender (AOR=2.8; 95% CI: 2.1–4.7), low trust in health authorities (AOR=2.5; 95% CI: 1.5–6.9), and reliance on social media for COVID-19 information (AOR=3.7; 95% CI: 1.5–10.9).

Conclusion: COVID-19 vaccine hesitancy among HCWs in tertiary care settings is significant and is affected by both personal beliefs and external information sources. Targeted interventions addressing vaccine safety concerns, promoting evidence-based communication, and having faith in public health authorities are essential to increase vaccine uptake among health care workers.

360

Surveillance of common respiratory viruses, including Influenza and SARS-CoV-2 during the COVID -19 pandemic in the Central province of Sri Lanka

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Background: Influenza virus (Inf-V) is one of the common respiratory viruses infecting humans worldwide. It is a winter virus in the temperate zone and has year-round circulation in tropical countries. The COVID-19 pandemic preventive measures aimed at SARS-CoV-2 may influence the transmission of other respiratory viruses including Inf-V. The current study aimed to identify the prevalence of common respiratory viruses including Inf-V during the COVID-19 pandemic.

Method: A total of 608 respiratory samples, received to the Virology Laboratory of the National Hospital, Kandy from COVID-19 suspected symptomatic patients, were simultaneously tested using real time RT-PCR for SARS-CoV-2 and real time PCR melting curve analysis for COMMON respiratory pathogens including Inf-V from January 2021 to October 2022. The demographic and clinical data were acquired from the medical records of the patients.

Results: Respiratory pathogens were detected in 40.46% patients and 6.25% patients were positive for SARS-CoV-2. Of the samples tested 5.92% patients were positive for Inf-V. Among them 87.2% of Inf- A and 18.2% of Inf-B were detected. Inf-V infection was more prevalent in males (69.44%) than females. Inf-V infections were predominantly detected in adults aged 18-<65 years. Fever, cough and sore throat were the most common symptoms. There was a single Inf-V detected until September 2021 and a delayed surge of Inf-V was observed in this study. Moreover, three Inf-V co-infections were noted in this study including one with SARS-CoV-2.

Conclusion: This study shows a prevalence of 5.92% for Inf-V infections in patients with ARTI. The circulation pattern of Inf-V and their subtypes varied during the study period. The current findings highlight the importance of diagnosing the other respiratory viruses including Inf-V during the COVID-19 pandemic and these will support to initiate appropriate management plans.

361

STUDY OF RESPIRATORY VIRUSES IN WASTEWATER IN THE METROPOLITAN AREA OF BUENOS AIRES, 2025

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Background: Wastewater surveillance has become established as a complementary tool for the early detection and monitoring of respiratory viruses in populations. The National Influenza Center located in Buenos Aires is starting to standardize and implement the detection of respiratory viruses such as influenza and SARS-CoV-2 as part of a cross-sectional study carried out by the Virology Department of INEI - ANLIS Malbrán in which other pathogens such as hepatitis, poliovirus, norovirus, among others, are studied. The objective of this study was to evaluate the presence of SARS-CoV-2, Influenza A, and Influenza B in wastewater collected in the Buenos Aires Metropolitan Area (AMBA) in 2025.

Methods: A total of 24 wastewater samples were collected (six per month) from different treatment plants operated by Agua y Saneamientos Argentinos (AySA), the public water and sanitation utility in the AMBA, between January and April 2025. Samples were concentrated using the polyethylene glycol-6000 (PEG 6000) precipitation method, and successful concentration was verified by detecting MS2, which was used as an internal positive control. Total RNA was extracted using the QIAamp Viral RNA Mini Kit (QIAGEN). Respiratory viruses were detected using a multiplex RT-qPCR capable of discriminating among SARS-CoV-2, Influenza A, Influenza B, and the RNAse P gene as an internal amplification control. MS2 was used as an internal positive control to assess sample quality.

Results: Twelve of the 24 samples tested positive for SARS-CoV-2. Influenza A and Influenza B were not detected in any of the samples. The RNAse P gene was amplified in 10 of 24 samples, indicating good performance of the extraction process. The concentration efficiency was assessed based on the viral recovery rate of the MS2 internal positive control, which met the accepted standards in all samples, confirming the effectiveness of the concentration method.

Conclusions: The results revealed a substantial presence of SARS-CoV-2 in wastewater from the AMBA region during the study period, highlighting the utility of this approach for monitoring respiratory viruses at the community level. The absence of Influenza A and B detection may be attributed to their seasonal circulation, as the sampling period preceded winter, when these viruses typically become more prevalent. The detection method is still being optimized to improve RNA recovery and enhance detection of the internal control gene. It is expected that, in the near future, the increasing number of samples studied and the optimization of the analysis method will allow for a better understanding of viral behavior, enabling its anticipation in the human population and thus facilitating the adjustment of public health measures.

363

Surveillance of RSV infections in Norway

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Background: RSV infection is a leading cause of hospitalizations due to respiratory infections in children below one year of age and also cause a significant burden of disease in older individuals. While RSV infections have been monitored in real-time via the Norwegian microbiology laboratory database, genetic characterization and subtyping has been lacking. To remedy this situation, the Reference Laboratory for RSV was established Norway in 2024 and integrated into the surveillance of influenza and SARS-CoV-2 starting from the 2024-25 winter season.

Methods: The established surveillance of RSV includes typing of RSA-A and -B by RT-PCR in samples received through the Norwegian sentinel surveillance system and from microbiological labs throughout Norway. Genetic characterization of a subset of RSV-A and -B samples were performed by nanopore sequencing. In addition to samples collected during the 2024-25 season, a smaller number of historical samples from 2022-23 and 2023-24 were retrospectively subtyped and sequenced. We have also established a multiplex analysis to evaluate immunity towards RSV-A and -B in the Norwegian population.

Results: Typing of RSV samples indicate a dominance of ~ 2/3 RSV B positive samples during the 2024-25 season. Subtype distribution was similar in hospitalized and out-patients, although we did observe geographical differences with more RSV-B in the north and a higher percentage of RSV-A in the central eastern part of Norway. Subtype distribution was also more skewed towards RSV-B in samples from older patients, while more evenly distributed between RSV-A and -B in the youngest patients. Analysis of historical samples indicate an alternating pattern of RSV-A and -B dominance from 2022 – 2025. Genetic characterization identified predominantly RSV-B samples of the B.D.E.1 clade in 2024-25, while RSV-A samples were more diverse including clades A.D.1, A.D.3 and A.D.5. Immunity was determined for the 0 – 4 years age group before and after the COVID-19 pandemic.

Conclusion: The establishment of a comprehensive surveillance system for RSV has provided a more detailed picture of circulating RSV subtypes and clades in Norway. Our data indicate geographical, age related and seasonal differences in subtype distribution and provide a valuable starting point for continued monitoring of RSV.

365

First-in-human Controlled Human Infection with RSV-B virus

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Background: Respiratory Syncytial Virus (RSV) is a major cause of acute respiratory illness, particularly affecting infants, the elderly, especially those with comorbidities, and immunocompromised individuals. While controlled human challenge trials have been instrumental in advancing RSV vaccine and therapeutic candidates—especially using the RSV-A Memphis 37 strain—no challenge model exists for RSV-B. Given the significant disease burden posed by RSV-B, this study aimed to develop and characterize the first cGMP-compliant RSV-B human challenge virus and evaluate its safety and infectivity in a first-in-human trial.

Methods: A wild-type RSV-B strain was isolated in London in 2022 from a respiratory tract sample obtained with informed consent. The virus was amplified to passage 3 using WI-38 human diploid lung fibroblast cells and further expanded in GMP-grade WI-38 cells (passage 4) to produce a GMP compliant Challenge Virus stock. Inoculum dilutions ranging from 4.4 to 5.5 Log_{10} PFU/mL were prepared.

Twenty healthy adult participants were enrolled in a controlled quarantine environment and challenged intranasally with RSV-B. Participants were monitored 24/7 within a quarantine unit for up to 9 days post viral inoculation. Regular assessments were performed in quarantine including ECG, bloods (haematology and biochemistry), vital signs and spirometry for safety. Nasal washes were collected twice daily and viral load assessed by both qPCR and viral culture. Symptom diary cards were collected three times daily. Serum samples were collected for immunological assessments pre and post challenge. A final follow-up was conducted on Day 28.

Results: Next Generation Sequencing (NGS) confirmed the full genomic identity of the RSV-B challenge virus. Extensive adventitious agent testing verified the absence of bacterial, fungal, mycoplasma, mycobacterial, and other viral contaminants, confirming the inoculums' sterility and safety.

Infection was achieved in most participants, with no adverse events (AEs) of clinical concern reported. Thus, the virus was well-tolerated by participants and demonstrated the ability to establish controlled infection suitable for the testing of Vaccine and Drug candidates.

Conclusions: This study marks the first successful development and clinical use of a GMP-compliant RSV-B human challenge virus using a qualified human cell line. The virus was shown to be safe and infectious in a controlled human model, providing a critical tool for advancing RSV-B-specific vaccine and treatment research. This milestone supports the broader goal of improving prevention and treatment strategies for acute respiratory infection linked to RSV.

370

Seroprevalence of Avian Influenza A Among Workers at a Vaccine Manufacturing Facility

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Background: Since 2003, over 1,000 human cases of influenza A/H5N1 and A/H7N9 infections have been reported globally, with a mortality rate of approximately 40%, highlighting the need for strong preventive measures. Although no human cases have occurred in Brazil, the potential for a pandemic remains. In response, the Butantan Institute began developing candidate vaccines for A/H5N1, A/H5N8, and A/H7N9 in 2023, using recombinant viruses generated with the PR8 backbone. This study evaluated the immune response of IB vaccine factory workers to these strains, considering their annual seasonal flu vaccination and likely exposure to influenza A vaccine strains, which may promote cross-neutralizing antibody development.

Methods: Serum samples from 148 workers, routinely vaccinated against seasonal influenza and presumed to be exposed to A/H5N1, A/H5N8, and A/H7N9 vaccine strains, were tested using the hemagglutination inhibition (HI) assay.

Results: Four individuals showed HI titers of 40 against A/H5N1, indicating seroconversion, while 61 had titers of 20 for A/H5N1 and A/H5N8, suggesting a mild antibody response. All samples had HI titers below 10 for A/H7N9.

Conclusions: The detection of antibody titers against A/H5N1 and A/H5N8 in some workers suggests possible seroconversion due to presumed exposure to vaccine strains or cross-reactivity from seasonal influenza vaccination, particularly with H1N1, which shares phylogenetic similarity with H5. The lack of response to A/H7N9 highlights the need for ongoing serological surveillance and pandemic preparedness. These preliminary findings open perspectives for future studies on monoclonal antibodies targeting avian influenza viruses.

371

Designing and development of multi-epitope T cell vaccine against acute respiratory viruses based on live attenuated influenza vaccine viral vector

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Background: Acute respiratory infections such as respiratory syncytial virus (RSV), metapneumovirus (MPV) and parainfluenza virus type 3 (PIV3) have been circulating among humans for many decades. Traditional vaccine approaches have proven to be unsuccessful for these pathogens. On the field of vaccine development, the most efforts are made to induce neutralizing antibodies, although the effective T-cell-inducing vaccines also represent a promising alternative. T cells have greater cross-reactivity, which is important to consider in case of mutations arising in the virus during its natural evolution. In this regard we focus on the development of T-cell epitope-based vaccine targeting these viruses. As live attenuated influenza vaccine (LAIV) viruses are known inducers of T-cell responses, we use these viruses as a vector to generate recombinant influenza viruses expressing immunogenic cassette enriched with T-cell epitopes of RSV, MPV and PIV3.

Methods: To determine the composition of immunogenic cassette we comprehensively analyzed circulating strains of RSV, MPV and PIV3 in Europe and Asia. We used bioinformatics tools for the alignment of proteins and to determine the most conserved region. The IEDB catalogs of published experimental epitopes were considered. In addition, T-cell epitopes were predicted using NetMHCpan EL 4.1 and NetMHCIIpan EL 4.1 focusing on antigenicity and conservancy analysis, avoiding allergenic regions. The immunogenic cassettes were screened to exclude undesirable neo-epitopes formation. The RSV, MPV or PIV3 cassettes were inserted into NA genes of H1N1 and H3N2 LAIV using the 2A self-cleavage site using reverse genetics methods. We also evaluated antigen-specific T-cell responses to the rescued viruses using in vitro assays on PBMCs of HLA-typed blood donors.

Results: According to our analysis the most conserved proteins were F, N and M proteins of RSV, MPV and PIV3. We selected most promising regions of each protein which included a large number of experimental and predicted T-cell epitopes capable to recognizing the diversity of HLAs**.** According to this strategy we expect that the T-cell vaccine may elicit potent T-cell immune responses in the general population. We generated eight vaccine candidates: one against PIV3, four against MPV and three against RSV. T-cell vaccines were genetically stable and the immunogenic cassettes were retained after the 10 sequential passages in chick-embryo. The activation of CD8+ T-cell response was confirmed using PBMC from HLA-A*02 and HLA-B7 healthy donors.

Conclusions: The T-cell-based vaccine candidates from this study are aimed to target several important respiratory viruses –seasonal influenza viruses and RSV, MPV and PIV3. This study proves the feasibility of multi-epitope vaccines, where a single vaccine formulation can potentially protect against multiple viral infections by targeting shared epitopes.

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373

Development of a comprehensive genomic surveillance platform for respiratory virus pathogens

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BACKGROUND

Over the last four years, genomic sequencing has been extensively applied in Slovakia to track the evolution of the SARS-CoV-2 virus. However, a broader, systematic approach to sequencing other infectious pathogens has not yet been established. This initiative aims to strengthen epidemiological surveillance by developing a comprehensive genome analysis platform that provides detailed insights into the diversity, origin, and evolution of infectious agents.

METHODS

Samples (nasopharyngeal swabs) were collected from individuals of all age groups and various locations. The samples were tested for respiratory viruses using RT-qPCR at the National Influenza Reference Centre. Only positive samples were selected for genomic library preparation, followed by whole-genome sequencing on an Illumina next-generation sequencing platform. Sequencing reads were assessed, trimmed, and mapped to reference genomes using comprehensive bioinformatics analyses. Generated consensus sequences were evaluated using Nextclade. All computational analyses were performed within the Snakemake framework.

RESULTS

For the preliminary testing of the digital genomic surveillance platform, we evaluated slightly over 300 samples positive for respiratory viruses. Of these, 272 successfully sequenced samples were analyzed using a comprehensive bioinformatics pipeline. The analysis revealed that over 57% of the samples were Influenza B/Victoria lineage (clade V1A.3a.2), while over 25% were Influenza A/H1N1 subtype, predominantly clade 6B.1A.5a.2a. Among all evaluated samples, we detected hRSV-positive cases, primarily RSV/A (7%). Additionally, two samples tested positive for human coronaviruses (HCoV-OC43 and HCoV-229E).

CONCLUSIONS

Our data on influenza and RSV viruses, used for testing and developing a genomic surveillance platform, provide a strong example of an effective and sustainable tool for long-term genomic monitoring of infectious diseases. This approach offers more comprehensive data on circulating pathogens, not only during the main winter influenza season. Early detection and monitoring of emerging threats can provide detailed insights about respiratory pathogens, helping to mitigate future adverse effects. This highlights the importance of establishing a robust genomic surveillance platform in Slovakia. This strategy aligns with ECDC recommendations to strengthen genomic surveillance systems, which have proven to be efficient tools for obtaining high-quality data.

FUNDING

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375

ECaS

Comparing air sampling instruments for environmental surveillance of respiratory viruses

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Background: Genetic material from SARS-CoV-2 and other respiratory viruses can be detected and sequenced from air samples collected in congregate settings. There has been limited performance benchmarking of newly introduced instruments that can be used for air sampling.

Methods: We compared the capture efficiency of four air sampling instruments: the Thermo Fisher AerosolSense, the InnovaPrep Cub, the InBio Apollo, and an in-house prototype. We disseminated inactivated SARS-CoV-2 in an engineering-controlled chamber. We disseminated two concentrations of the virus each with two nebulizers that produced distinct droplet-size distributions. We quantified the amount of virus genetic material captured by digital PCR and compared it with the estimated total amount dispersed in the chamber to determine capture efficiency.

Results: We found that the InnovaPrep Cub had the highest capture efficiency, followed by the Thermo Fisher AerosolSense, our in-house sampler, and then the InBio Apollo. This trend was consistent across test conditions of different virus concentrations and droplet size distributions.

Conclusions: The results suggest that the InnovaPrep Cub would be the most sensitive air sampler for detecting SARS-CoV-2 viral genetic material in real-world settings such as healthcare facilities and schools. Data from other instruments such as the ThermoFisher AerosolSense likely underestimate the concentration of viruses in these settings.

380

CHARACTERIZATION OF THE CIRCULATING RESPIRATORY SYNCYTIAL VIRUS IN ARGENTINA FOLLOWING VACCINE IMPLEMENTATION, 2024-2025

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BACKGROUND: Respiratory Syncytial Virus (RSV) is the main cause of bronchiolitis and pneumonia in young children and is also a significant cause of respiratory illness in adults and high-risk populations. Argentina incorporated the RSV vaccine for pregnant women into the National Vaccination Schedule in December 2023. The vaccine was administered from March 1 to August 31, 2024, prior to RSV circulation, and its administration resumed in January 2025. The adult vaccine was also available in the country from early 2024. The objective of this study was to analyze circulating RSV strains in Argentina during 2024 and 2025, integrating clinical, epidemiological, and molecular data considering the incorporation of the RSV vaccine.

Methods: The National Influenza Center (NIC) received respiratory samples from both inpatients and outpatients of different age groups from multiple locations across the country. Real-time RT-PCR and duplex rtRT-PCR were carried out for RSV detection and subtyping, respectively. Whole-genome sequencing by NGS was performed on a set of selected RSV A and RSV B samples. Nextclade and RSVsurver were used for clade assignment and amino acid analysis of the F protein (PreF), respectively.

Results: In 2024, RSV activity was moderate with a peak in late May. At the NIC, 945 RSV-positive specimens from 23 different jurisdictions of the country were analyzed, of which 577 (61%) were obtained from hospitalized children ≤ 2 years of age. Of this age group, approximately 66.7% (150/225) presented with fever, according to available data. Regardless of febrile status, 96.6% (199/206) presented with chest indrawing and 91% (61/67) with wheezing. Regarding vaccination status, 20.1% (55/273) were infants born to vaccinated mothers. Subtyping of 936 samples revealed 98 (10.5%) RSV A and 838 (89.5%) RSV B. Sequencing of 38 viruses (10 RSV A and 30 RSV B) showed that RSV A belonged to the Nextclade clades A.D.1, A.D.1.5, A.D.5.2 and A.D.5.4, while RSV B belonged to B.D.E.1, B.D.E.1.1 and B.D.4.1.1. Comparing the RSV B strains detected from immunized and non-immunized children, it was observed that they shared 4 mutations located in antigenic sites, as has been observed worldwide. By epidemiological week 20 of 2025, 134 RSV samples had been collected, with the vast majority classified as subgroup A. The 2025 sequences are still in progress.

Conclusions: In 2024, most cases were RSV B clade B.D.E.1 and, to a lesser extent, RSV A clade A.D.5.4. All RSV B PreF mutations detected have also been reported in other countries. So far in 2025, unlike the previous year, a predominance of RSV A has been observed. Monitoring the impact of RSV vaccination is essential to assess its effectiveness in reducing RSV infections in the population. The data reported here is expected to significantly contribute to national surveillance initiatives.

381

Perspectives of cardiology fellows and specialists on the safety and efficacy of influenza vaccination in cardiovascular patients: preliminary findings from the FLUence survey

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INTRODUCTION

Annual influenza vaccination has been shown to reduce major cardiovascular events and all-cause mortality in individuals with heart disease while maintaining an excellent safety profile. Nevertheless, several studies and registries consistently report suboptimal vaccination rates among this high-risk population.

OBJECTIVES

To assess the knowledge, attitudes, and practices of cardiology trainees and specialists regarding the safety and efficacy of influenza vaccination in patients with cardiovascular disease.

MATERIALS AND METHODS

From September 2023 to September 2024, an electronic survey was conducted following the Checklist for Reporting Results of Internet E-Surveys (CHERRIES). Participants' opinions were assessed using Likert-type scales. A non-probabilistic convenience sampling method was used, and duplicate entries were prevented using the IP-tracking features of the SurveyMonkey® platform.

RESULTS

A total of 1462 cardiologists from 59 countries answered the survey. The mean age was 46.0 ±14.0 years; 36.5% were women and 22.1% were in training (fellows). During the previous year, 65.6% of fellows and 76.9% of specialists reported having received the influenza vaccine (p<0.001). Furthermore, 34.1% of fellows and 13.7% of specialists admitted they never recommend the vaccine to their patients (p<0.001).

Although 97.4% of participants stated that the influenza vaccine was available in their country, 28.5% of fellows and 22.7% of specialists were unaware of clinical practice guidelines concerning influenza vaccination in patients with heart disease (p=0.033). Additionally, 42.7% of fellows and 33.7% of specialists were unsure whether different types of influenza vaccines were available in their country (p=0.003). Table 1 summarizes participants' perspectives on the safety of this intervention.

Only 28.2% of fellows and 38.4% of specialists considered it safe to vaccinate during hospitalization or within the first month following an acute coronary syndrome (p=0.001). Similar caution was noted regarding vaccination during hospitalization or within the first-month post-decompensation of heart failure (39.9% versus 46.8% respectively, p=0.029). Notably, only 37.8% of trainees and 49.6% of specialists recognized the role of influenza vaccination in preventing acute myocardial infarction (p<0.001; Table 2).

Finally, 66.6% of fellows and 74.3% of specialists deemed influenza vaccination highly relevant to their daily practice (p=0.006), while 56.4% of fellows and 45.5% of specialists expressed a need for further training in this area (p=0.001).

CONCLUSION

Our findings suggest that influenza vaccination remains underutilized among cardiologists—particularly in the context of recent cardiac events. A considerable proportion of clinicians lack awareness of clinical guidelines, underestimate the role of vaccination in secondary prevention, and are hesitant to vaccinate during hospitalization or early recovery. These gaps are more prominent among trainees, but also present among specialists. Targeted educational strategies and guideline dissemination are urgently needed to close the knowledge–practice gap and ensure patients with cardiovascular disease receive timely, evidence-based preventive care.

Table 1: perspective of cardiology fellows and speciallist regarding the safety of influenza vaccine in patients with heart disease.

	Cardiology fellows (n=323)	Cardiologist (n=1139)	p-value
The vaccine is very safe in adults	92.3%	92.8%	0.742
The vaccine is very safe in individuals on antiplatelet or anticoagulant therapy	81.1%	87.6%	0.003
Influenza infection is very severe in individuals with heart diseases	65.6%	71.5%	0.043
Adverse effects are very common with the influenza vaccine	8.6%	12.7%	0.027
Severe adverse effects are very common with the influenza vaccine	4.3%	7.4%	0.023
It is very important to vaccinate individuals over 65 years old with heart disease	90.1%	85.1%	0.012
It is very important to vaccinate individuals under 65 years old with heart disease	76.1%	71.8%	0.115

Table 2: opinion of cardiology fellows and specialist regarding the benefits of influenza vaccination in patients with heart disease.

	Cardiology fellows (n=323)	Cardiologist (n=1139)	p-value
Vaccine is very beneficial in preventing influenza infection	90.4%	88.4%	0.315
Vaccine is very beneficial in reducing the occurrence of acute myocardial infarction	37.8%	49.6%	<0.001
Vaccine is very beneficial in reducing the risk of stroke	32.2%	41.6%	0.002
Vaccine is very beneficial in preventing mortality	75.9%	79.3%	0.185
Vaccine is very beneficial in preventing hospitalizations	74.0%	82.5%	0.001

383

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Influenza surveillance in Argentina 2024-2025

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Background: In Argentina, influenza (FLU) circulation was disrupted, with detection levels reaching their lowest in 2020 and 2021. An unusual increase in activity was observed starting in December 2021, followed by a second peak between October and November 2022. Since 2023, FLU seasonality has nearly returned to pre-pandemic patterns, with an earlier peak of activity in 2024 and detections persisting throughout the summer of 2025. This report describes the viral characterization of the 2024–2025 FLU seasons, based on available clinical and epidemiological data.

Methods: Influenza A and B viruses collected nationwide by the National Laboratory Network through sentinel and non-sentinel surveillance were analyzed. Samples were obtained from pediatric and adult patients diagnosed with influenza-like illness (ILI) and severe acute respiratory infection (SARI) and sent to the National Influenza Center (NIC) for further subtyping of FLU A, lineage identification of FLU B, and genomic characterization. A subset of samples was selected for whole genome by next-generation sequencing (NGS), and phylogenetic analysis was conducted using IRMA, BioEdit, and Iqtree, applying the Neighbor-Joining method with 1,000 bootstrap replicates. Sequences obtained were submitted to the GISAID database. This analysis was complemented with the clinical and epidemiological information available from the National Health Surveillance System database (SNVS 2.0).

Results: Influenza activity in 2024 was detected year-round, with a peak between epidemiological weeks (EW) 21 and 24. The NIC received a total of 2,174 samples, most of which originated from non-sentinel surveillance of hospitalized patients. The highest number of samples was obtained from children aged 5 to 9 years. Viruses were characterized as follows: 1,372 FLU A(H3N2), 81 FLU A(H1N1)pdm09, and 549 FLU B Victoria. Genomic analysis of 119 FLU A(H3N2), 8 FLU A(H1N1)pdm09 and 19 FLU B Victoria viruses revealed that the majority of FLU A(H3N2) belonged to clade 2a.3a.1, subclade J.2; most FLU A(H1N1) pdm09 belonged to clade 5a.2a, subclades C.1.1 and C.1.9; and all FLU B Victoria belonged to clade V1A.3a.2, subclades C.5.1 and C.5.7.

As of EW20 2025, FLU activity remained low. Most samples were collected from outpatients aged 45 to 64 years. A total of 311 viruses were studied and the majority were characterized as FLU A(H1) (225), followed by FLU B Victoria (54), and only 4 as FLU A(H3). So far, most FLU A(H1N1)pdm sequenced (5 out of 7) belonged to clade 5a.2a.1, subclade D.

Conclusions: During the 2024–2025 period, influenza activity was detected year-round, with a peak between May and June 2024 dominated by FLU A(H3N2). So far, in 2025 influenza activity remains low with a predominance of FLU A(H1N1)pdm. Genomic characterization showed good correlation between the circulating strains and the vaccine components. The variability in timing and viral characteristics highlights the need for sustained, year-round local surveillance.

385

ECaS

Five Years of SARS-CoV-2 Genomic Surveillance in Argentina: Dynamics and Evolution of Variant Circulation.

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Introduction: Since the onset of SARS-CoV-2 in Argentina, the National Influenza Centre (NIC), in collaboration with the National Genomics and Bioinformatics Unit, both part of the ANLIS "Dr. Carlos G. Malbrán", has conducting continuous genomic surveillance of SARS-CoV-2 in accordance with WHO and Ministry of Health guidelines. The purpose of the national genomic surveillance is the early detection of new variants and to define lineages and sublineages in order to implement measures to reduce their transmission and mortality. This study aims to describe the genomic evolution and circulation dynamics of SARS-CoV-2 variants in Argentina between epidemiological week (EW) 11, 2020, and EW13, 2025.

Methods: Respiratory samples that resulted positive for SARS-CoV-2 by RT-qPCR (CT value ≤ 28), collected from paediatric and adult patients, outpatients, and inpatients, received at the NIC from different locations of the country were analyzed. Whole-genome sequencing was performed using NovaSeq® 6000 System and MiSeq® System, following a protocol adapted from Coviseq® (Illumina®). Bioinformatics analysis was conducted to identify variants, and results were reported to the National Health Surveillance System, and the generated sequences were deposited in the GISAID database. Phylogenetic analysis was also performed.

Results: A total of 17,149 (83%) sequences were obtained from 20,570 samples received coming from the 24 jurisdictions of the country. During the first year of the pandemic, Argentina experienced circulation of the original SARS-CoV-2 strains. No specific variant classifications were reported at that time. In 2021, the Alpha (B.1.1.7) and Gamma (P.1) variants were predominant. Starting in 2022, Omicron BA.1 and its descendant lineages became the only variants circulating in Argentina. This marked a significant shift in the genetic landscape of the virus. In early 2023, the BQ.1 lineage and its descendants were the most prevalent. Later in the year, they were gradually displaced by the XBB.1.5 and EG.5 lineages. In the first weeks of 2024, the JN.1 variant was the most prevalent in the country. In the second half of 2024 the KP.3.1.1 and XEC variants became the most prevalent. In early 2025 is characterized by the emergence and increasing circulation of the LP.8.1 variant. Over the five-year period, a total of 14,715 high-quality sequences were deposited in GISAID, accounting for 53% of all sequences submitted from Argentina.

Conclusions: Through the analysis of samples received from all 24 jurisdictions of the country, the surveillance secured a comprehensive and representative view of viral circulation nationwide. Through making possible the timely identification of emerging variants and tracking their evolution, genomic monitoring supports evidence-based public health decision-making at national levels. Genomic surveillance of SARS-CoV-2 has proven to be an essential tool for guiding public health strategies throughout the pandemic.

387

Are the elderly less susceptible to influenza A(H3N2) than before?

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Background:

The different seasonal influenza viruses differ in their impact in different age groups. Influenza A(H3N2) viruses have regularly caused large epidemics since their emergence in humans in 1968, and has been regarded as affecting the elderly particularly hard. In agreement with this, we have for many years in Norwegian virological surveillance observed that the A(H3N2) viruses were strongly represented among elderly patients when compared to other seasonal influenza viruses.

Methods:

As part of influenza surveillance and preparedness, the National Influenza Centre in Norway solicits weekly forwarding of representative influenza virus positive specimens from diagnostic laboratories across the country. Received viruses are tested for subtype/lineage and many of them further characterised. We analysed the age distribution of detected viruses of the different influenza types and lineages in the surveillance specimens for a series of influenza seasons.

Results: For a number of previous seasons, a pattern has been observed where the elderly are the most likely to be diagnosed with A(H3N2), the infants and toddlers (0-4 years) with A(H1N1)pdm09, and school-age children (5-14 years) with B/Victoria-lineage viruses.

E.g., during the 2017/2018 and 2018/2019 seasons, persons 60 years and older were more than twice as likely to be diagnosed with H3N2 than the all-ages average. During the 2024/2025 season, however, in our data the 60 years and older were not more likely than the all-ages average to get the H3N2 diagnosis. During the two preceding seasons, 2022/2023 and 2023/2024, the relative likelihood of the elderly to get an H3N2 diagnosis was also lower than in the pre-2020 seasons, but not as low as during 2024/2025.

Age profiles for A(H1N1)pdm and B/Victoria-lineage viruses, on the other hand, did not change much during the same time period, suggesting that the difference did not result from a change in the selection of specimens we received from the diagnostic laboratories.

Conclusions:

Norwegian surveillance data appear to indicate that the age profile of persons who get diagnosed with influenza A(H3N2) have changed during the last few years, with relatively less impact in persons 60 years and older. A corresponding change was not observed for the other circulating influenza viruses.

We aim to explore further if this apparent change is limited to a more specific age/birth cohort, and if such a change may be correlated with the evolution of the viruses, e.g. with reappearance of epitopes that may have resulted in immune imprinting in certain birth cohorts, or with increasing numbers of people who had A(H3N2) infection in early life joining the ranks of the elderly.

390

SARS-CoV-2 infection produces chronic inflammation in obese mice that can be partially reduced by therapeutic SARS-CoV-2 vaccination

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Background:

Long COVID, or post-acute sequelae of COVID-19 (PASC), currently affects an estimated 64 million people globally and manifests with over 200 symptoms across multiple organ systems. Although its pathogenesis is debated, emerging evidence indicates that PASC is a chronic proinflammatory state. Epidemiological studies have identified female sex, older age, comorbidities, and socioeconomic factors as risk enhancers, whereas prior vaccination appears protective. The role of therapeutic vaccination (i.e. vaccination after the development of PASC symptoms) remains highly controversial.

Methods:

We established a novel murine model to recapitulate pulmonary PASC sequelae using a mouse-adapted SARS-CoV-2 Beta variant. Female mice, beginning at four weeks of age, were subjected to a high fat diet for 10 weeks to reflect key risk factors such as sex, obesity and age. The therapeutic efficacy of vaccination was evaluated through vaccination with a Beta-HexaPro (Beta-HP) SARS-CoV-2 vaccine delivered by high-density microarray patch (HD-MAP), in comparison to intradermal injection and mock treatments.

Results:

Here, we show that SARS-CoV-2 infection of older, obese female mice results in systemic and pulmonary inflammation at 28 and 56 days post-infection. This same phenotype was not observed in younger male mice fed a standard chow diet. Interestingly, HD-MAP Beta-HP vaccination at 7 days post-SARS-CoV-2 infection partially attenuated the pulmonary inflammation previously observed in obese mice at 28 days post-infection.

Conclusion:

This study presents a practical murine model for pulmonary PASC that incorporates pertinent clinical risk factors. HD-MAP delivery of the Beta-HP vaccine shows potential promise as a therapeutic strategy against PASC, warranting further investigation in preclinical and clinical settings.

422

T-Cell Responses to Conserved SARS-CoV-2 Proteins: Implications for Durable Immunity and Pediatric Vaccination

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Background

The implementation of pediatric COVID-19 vaccination remains under debate. While current vaccines effectively induce neutralizing antibodies against spike, their protective effect is limited towards new variants and novel coronavirus outbreaks. In contrast, viral infection elicits cross-reactive T-cell responses targeting conserved internal viral proteins and has been associated with increased protection against SARS-CoV-2. Observational studies for influenza have revealed that unvaccinated children exhibit age-dependent increases in virus-specific $CD8^+$ T-cell responses, an effect not seen in vaccinated children.(Bodewes et al, 2011) These findings suggest that vaccination may interfere with the development of cross-reactive T-cell immunity. This study aims to characterize the prevalence and phenotype of cross reactive T-cell responses to conserved viral proteins following SARS-CoV-2 infection, particularly in the context of prior vaccination.

Methods

To detect and profile low-frequency cross reactive T cells specific for conserved viral proteins, we stimulated PBMCs with overlapping peptide pools derived from Nucleoprotein (Ncap) and non-structural proteins (Nsps) including Nsp3, Nsp12, and Nsp16 of SARS-CoV2 and analyze by IFNγ ELISpot or AIM/ICS followed by flow cytometry. Using PBMCs from SARS-CoV-2 positive healthy donors, assay parameters were systematically optimized, including PBMC seeding density, IL-2 concentration and peptide pool concentrations. Due to the limited availability of pediatric cohorts, we selected PBMCs from adult participants which experienced a SARS-CoV2 infection with or without prior vaccination with Pfizer/BioNTech vaccine. PBMCs were collected at different timepoints including prior to infection and at least 190 days after infection.

Results

We observed optimal IFNγ responses in healthy donors upon stimulating 1.5 M cells with optimal concentration of specific peptide pools, IL2 addition after 4 days, restimulation after 6 days followed by ELISpot. We applied the optimized settings of the proliferation ELISpot upon a small pilot batch of our cohort participants (n=4 unvaccinated and n = 7 vaccinated) prior to a SARS-CoV2 infection. We detected IFNγ production (>30 spots/wells) to Ncap and Nsps in PBMCs collected 190-360 days after infection in both unvaccinated and vaccinated participants. Moreover, in PBMCs collected at least 190 days after infection we observed a trend of higher IFNγ response to Ncap and Nsp12 in unvaccinated donors compared to vaccinated donors.

Conclusion

Our findings confirm that low frequency T-cells targeting conserved SARS-CoV-2 proteins remain detectable after ex vivo proliferation in a long term setting. Our preliminary results indicate more robust T cell responses to Ncap and Nsp12 in unvaccinated participants compared to vaccinated participants post SARS-CoV2 infection. We are currently testing more donors to confirm our findings and profile phenotype of cross reactive T cells using flow cytometry. These findings can provide indications towards the long term effect of vaccination on the durability of cross-reactive T cell responses and inform future vaccination strategies for children.

449

ECaS

Residue 346 modulates cleavage of the H1 and H16 hemagglutinin subtypes

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The specific residues that determine the cleavage activation of influenza virus hemagglutinin (HA0) by host cell proteases are not well understood. In our random mutagenesis study of H1 HA0, we identified a significant role for residue Asp346, which is conserved across most of the 19 subtypes but replaced by Asn in avian H11 (waterfowl), and H13 and H16 (seagulls). Notably, H16 is poorly activated and features a small alpha helix in its cleavage loop.

We assessed the entry efficiency of pseudoviruses bearing wild-type or mutant H1 and H16 HA, activated by (i) exogenous trypsin, (ii) co-expression of TMPRSS2, or (iii) transduction into TMPRSS2-positive MDCK cells. HA cleavage was confirmed using automated western blotting. Additionally, we evaluated the impact of these mutations on fusion pH using a split-GFP cell-cell fusion assay and we reverse-engineered viruses with wild-type or mutant HAs (with cognate NA) on a PR8 backbone to study growth kinetics.

In pseudovirus entry assays, the D346N mutation in H1 HA0 significantly reduced susceptibility to exogenous trypsin but not to cellular TMPRSS2. Conversely, the N346D mutation in H16 HA0 partially restored trypsin susceptibility without affecting TMPRSS2-mediated activation. Intriguingly, non-activated pseudoviruses bearing either wild-type or mutant H1 HA could enter TMPRSS2-expressing MDCK cells, whereas their H16 counterparts could not enter if not pre-activated. However, co-transfection of a TMPRSS2 expression plasmid during H16N3 pseudovirus production enabled efficient entry and HA activation was confirmed by automated western blot.

The mutations had minimal impact on HA fusion pH (5.5 for H1 WT and mutant; 4.8 for H16 WT and 4.9 for the H16 mutant), though the low fusion pH of avian H16 HA remains intriguing. Growth kinetics revealed a delay for the H1 mutant compared to WT in regular MDCK cells, but not in TMPRSS2-expressing cells. No significant differences were observed between H16 WT and mutant viruses in either cell line. Sequencing at 72 hours post-infection revealed the acquisition of N346D in the H16 WT virus in both cell lines, indicating adaptation to mammalian cell culture.

Alphafold modeling suggests that the H1 D346N mutation alters cleavage loop folding, shielding the scissile arginine with a small helical turn, similar to the structure observed in H16.

This study is the first to demonstrate that Asp346 is a key determinant of HA0 cleavability, underscoring its importance for virus host adaptation. Gull-derived H16N3 virus, which carries Asn346, is highly resistant to extracellular cleavage activation. Together with its pH stability, this leads us to hypothesize that H16N3 virus gains improved fitness from having such a stable HA.

459

Benefit of Empiric Antiviral Treatment for Patients with Influenza-Associated Pneumonia Admitted to the Intensive Care Unit: A Retrospective Cohort Study

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Influenza remains a clinically significant viral cause of community-acquired pneumonia in adults. Without timely antiviral treatment, severe influenza complicated by pneumonia may lead to poorer outcomes. However, the effect of empiric antiviral treatment on serious or life-threatening influenza is not well documented from clinical trials. We performed a retrospective study of patients with severe influenza complicated by pneumonia as confirmed by bronchoalveolar lavage polymerase chain reaction (PCR) in intensive care units at a tertiary hospital in Taiwan from 2009 to 2019. We collected demographic and clinical data from medical records. We compared survival outcomes between those with or without empiric antiviral treatment (before or after PCR diagnosis) with the Cox proportional hazard model. Of the 77 patients enrolled in this study, the survival rates were higher among those treated empirically with antivirals before diagnostic test results were obtained than for those who were not (P = 0.024). Compared with the nonempiric treatment group, the empiric antiviral treatment group had a 68.3% lower risk of death. Meanwhile, the risk of death increased by 3.8% for each unit increase of the acute physiology and chronic health evaluation (APACHE) III score. Empiric antiviral treatment of influenza may lead to improved survival rates. For patients with suspected severe influenza, antiviral medication should be initiated as soon as possible without waiting for laboratory confirmation of influenza virus infection.

460

Integrated Urban Virus Surveillance and Community Engagement for Pandemic Preparedness: The LBI SOAP Approach

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Background: Densely populated urban areas are critical hotspots for zoonotic spillover and the emergence of novel viruses due to close interactions between humans and animals in shared environments. Yet, the urban animal-human interface has remained a relatively underexplored gap in virus surveillance efforts. Compounding this biological risk are societal vulnerabilities: limited public trust in science, low levels of scientific literacy, and fragmented communication between science, policy, and the public can significantly hinder timely and effective outbreak responses. The Ludwig Boltzmann Institute for Science Outreach and Pandemic Preparedness (LBI SOAP) in Vienna, Austria, addresses these challenges through an integrated approach that combines biomedical and environmental surveillance, participatory citizen science, applied psychological research, and interdisciplinary collaboration to establish a comprehensive framework for pandemic preparedness that bridges both biological and societal dimensions.

Methods: LBI SOAP operates across four interlinked focus areas in cooperation with the City of Vienna, the Austrian National Public Health Institute, and several academic and civic partners. Specifically, focus area 1 "Virus Surveillance" monitors the emergence of pathogens through environmental sampling of urban birds (e.g., for avian influenza), rodents (e.g., hantaviruses), and wastewater for novel or re-emerging pathogens. Focus area 2 "Virus Characterization" performs molecular and genomic analysis of viruses, assesses zoonotic and pandemic potential, and develops biomedical countermeasures including vaccine candidates and monoclonal antibodies. Focus area 3 "Participatory Community Science" involves citizens in environmental sampling and data generation to be used for focus areas 1 and 2, while strengthening scientific literacy and promoting public understanding of virology and epidemiology. Finally, focus area 4 "Science Outreach and Science Education" evaluates and co-develops concepts for public engagement and science outreach strategies based on insights from motivational psychology.

Results: While full-scale operations of LBI SOAP will begin in 2026, initial workshops, stakeholder consultations, and pilot outreach activities have confirmed the feasibility and societal relevance of this interdisciplinary urban surveillance model. Stakeholders' and community interest and participation indicate strong potential for improving public awareness and trust through co-created learning experiences. Ongoing evaluation focuses on the psychological effectiveness of engagement strategies across demographic groups and the role of scientific literacy in shaping informed health behaviors.

Conclusions: LBI SOAP exemplifies a broad and comprehensive, inter-, and trans-disciplinary approach to pandemic preparedness by integrating virology, biomedical research, psychological science, and community engagement. It not only strengthens early detection capabilities but also fosters public agency through scientific literacy and trust in science. By bridging biomedical and societal readiness, LBI SOAP contributes to building resilient, informed populations capable of proactive, health-conscious decision-making. This scalable model has the potential to offer vital insights for enhancing prevention, pandemic preparedness, and inclusive response strategies against emerging viruses, especially influenza viruses, worldwide.

461

Host Antimicrobial Peptide Dermcidin Targets Influenza Virus Hemagglutinin to Prevent Infection

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Background: Some individuals infected with influenza virus remain asymptomatic, yet the underlying host factors that confer this resilience remain largely undefined. We investigated dermcidin, a human antimicrobial peptide, as a potential host factor contributing to resistance against influenza infection. Its antiviral activity had not been previously described.

Methods: We performed hemagglutination inhibition (IHA),binding assays, and molecular docking to evaluate dermcidin's interaction with influenza hemagglutinin (HA). Antiviral effects were assessed through plaque assays in cell culture. Dermcidin levels were measured in respiratory samples (saliva, nasopharynx, tears). In vivo efficacy was tested in a murine influenza model.

Results: Dermcidin binds to influenza HA and inhibits hemagglutination, resulting in reduced viral infection in vitro. It exhibited antiviral activity against all tested influenza A virus strains, including both H1N1 and H3N2 subtypes. Docking analysis suggested interaction with the HA stalk region, although further studies are needed to confirm the structural mechanism. In mice, dermcidin reduced disease severity and mortality following influenza challenge. It was consistently detected at respiratory entry sites and increased during infection. Notably, asymptomatic individuals showed significantly higher dermcidin levels than symptomatic peers.

Conclusions: We identify dermcidin as a novel human antiviral peptide that interferes with influenza virus infection across multiple A subtypes by targeting HA. Its constitutive presence at mucosal entry sites, increase during infection, and association with asymptomatic cases highlight its role in host-pathogen interaction and support its potential as a host-derived therapeutic candidate.

462

ECaS

Influenza vaccination effectiveness against influenza-associated hospitalization in children: Impact of Repeated Vaccination

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BACKGROUND

Hong Kong's subtropical climate and high population density facilitate prolonged influenza transmission with complex seasonality. The Department of Health provides free or subsidized influenza vaccination to children aged 6 months to 17 years, with school-based programs since 2018/19 (initially 3-11y; expanded 2023). 2024/25 coverage was 55% (6mo–5y), 74% (6–11y), and 57% (12–17y). We assessed influenza vaccine effectiveness (VE) against hospitalization and repeat vaccination effects.

METHODS

We used a test-negative design at three Hong Kong public hospitals (October 2015–September 2023), enrolling children ≤17 years hospitalized with acute respiratory illness. Epidemic periods covered October–September annual cycles to capture winter/summer influenza seasons. Multilevel logistic regression estimated adjusted odds ratios (ORs) for influenza positivity by vaccination status, with fixed effects (age spline, sex, hospital, prior vaccination) and nested random effects for between-season and biweekly within–seasons variation. VE = (1 − aOR) × 100%. We estimated overall VE, stratified by influenza type/subtype and prior-season vaccination status to evaluate repeat vaccination effects.

RESULTS

Among 23,550 children aged 9 months–17 years analyzed (184 SARS-CoV-2 exclusions), 3,881 (16.5%) tested influenza-positive: 1,570 (40.4%) A(H1N1), 1,285 (33.1%) A(H3N2), and 1,023 (26.4%) B cases. Among 6,008 vaccinated children (25.5%), 5,045 (84.0%) received quadrivalent, 381 (6.3%) trivalent, and 574 (9.6%) unknown vaccine types. Previous-season vaccination was reported by 4,439 children (18.8%), with 3,685 (83.0%) revaccinated during the current influenza season.

VE against influenza-associated hospitalization was 57.8% (51.8%–63.1%) across all subtypes and epidemic periods. Random effects revealed greater within-season biweekly variability (SD=1.07) than between-season variation (SD=0.51), indicating short-term fluctuations drove seasonal differences. VE was higher for A(H1N1) (69.3% [62.1%, 75.1%]) than B (50.1% [35.1%, 61.6%]) and A(H3N2) (32.0% [15.1%, 45.5%]). Repeated vaccination was associated with a 18.2% lower VE compared to non-repeated vaccination, with the greatest reduction against A(H3N2) (ΔVE=38.2%; -8.8%–88.5%). The protective effect of repeated vaccination against A(H1N1) decreased significantly (ΔVE=25.2%; 5.4%–46.5%), and showed moderate reduction against B (ΔVE=10%; -23.3%–44.8%). While repeated vaccination maintained substantial protection against A(H1N1) (53.1%) and B (43.3%), it significantly reduced protection against A(H3N2) (VE=4.9%; -52.2%–40.6%).

CONCLUSIONS

Overall VE against pediatric influenza hospitalization was 57.8% (51.8%–63.1%) in Hong Kong (2015–2023). Subtype–specific VE was highest against A(H1N1) (69.3%) and lowest against A(H3N2) (32.0%) . Repeated vaccination reduced VE by 18.2% versus non-repeated vaccination, with greatest reduction against A(H3N2). While repeated vaccination maintained substantial protection against A(H1N1) and B, it diminished A(H3N2) protection to 4.9% (-52.2%-40.6%). VE was consistently higher in children unvaccinated during the preceding season versus consecutively vaccinated. Current programs provide substantial protection, but require optimization through broader strain coverage and adjusted vaccination frequency.

474

ECaS

Investigating the role of two-pore channels in SARS-CoV-2 infections

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IntroductionThroughout the SARS CoV-2 pandemic the virus has been continuously evolving, in particular affecting the viral spike protein. Early variants of SARS-CoV-2 such as EU.1, Alpha and Delta have been reported to use both membrane fusion and the endosomal pathway to enter host cells, while the Omicron variant relies more on the endosomal pathway. In the endosome-mediated pathway, acidic conditions of endosomes and lysosomes activate proteases that cleave the viral envelope protein and release packaged viral genomes, which then enter the nucleus for replication. The release of the virus from the late endosomes/endolysosomes into the cytosol is presumably regulated by a few ion channels present in the endo-lysosome compartment. One of the major types of ion channels are the Two-Pore Channels (TPC). Only TPC1 and TPC2 are found in humans. TPC1 is primarily located in early endosomes and lysosomes while TPC2 is found in late endosomes and lysosomes.

ObjectivesWe sought to dissect the host cell entry pathways of replication-competent SARS CoV-2 variants and to assess the role of TPC proteins in SARS-CoV-2 infection and pathogenicity.

Material and methodsFor this study, TPC1, TPC2, and TPC1/TPC2 gene double knockout cell lines were generated in human lung cell-derived A549 cells overexpressing human ACE2 (A549-ACE2) using the CRISPR/Cas9 knockout system. Viability assays and confocal microscopy were performed to study virus cell entry and infectivity.

ResultsDepletion of TPC proteins prevented infection and cytopathic effects of Omicron XBB.1.5 more effectively than of earlier pandemic variants EU.1 and Delta. Confocal microscopy revealed that Omicron-infected cells contained markedly higher levels of early and late endosomes compared to the two earlier variants.

ConclusionOur data support the notion that Omicron infection relies more on the endosomal pathway than the Delta virus and that TPC1 and TPC2 regulate SARS-CoV-2 entry in a variant-specific manner.

479

ECaS

Novel luminescent and fluorescent systems for studying coronavirus Mpro dimerization and inhibitor-driven enhancement

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The main protease enzyme (M^{pro}) of SARS-CoV-2 (SARS2) cleaves the viral polyprotein into functional units essential for virus replication. Prior work has demonstrated that M^{pro} functions as a homodimer. However, studies on the actual mechanism of dimerization have been challenging because the purified protein is mostly dimeric, dimerization-defective mutants lack proteolytic activity, and cell-based assays have yet to be reported. To enable work on M^{pro} dimerization, we have developed a quantitative luciferase-based biosensor that accurately reports protein dimerization in living cells and, upon purification, also **in vitro**. Co-transfection of cells with a construct expressing M^{pro} fused C-terminally to the 18 kDa LargeBiT of luciferase (LgBiT) and a second construct with M^{pro} fused to the 1 kDa SmallBiT of luciferase (SmBiT) results in a reconstitution of luciferase activity in a dose-manner that requires conserved residues within the dimerization interface. Proteolytic activity is dispensable for dimerization and, uniquely, a C145A catalytic mutant exhibits higher dimerization signal likely due to lower cytotoxicity. M^{pro} enzymes from other coronaviruses also dimerize in this system indicating mechanistic conservation. Interestingly, this dimerization biosensor also provides a quantitative read-out of inhibitor facilitated dimerization. Covalent inhibitors such as nirmatrelvir cause a 3- to 5-fold increase in luciferase activity. Similar results were obtained with split eGFP construct. Together with corroborating structural (X-ray, SAXS), biophysical (SEC), and computational data sets, our studies support a model in which covalent M^{pro} inhibitors such as nirmatrelvir simultaneously block catalytic activity and induce an allosteric stabilization of the dimeric complex.

480

Using Personalized AI-Guided Strategies to Improve Influenza Immunity Across the Human Lifespan

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Background: While influenza vaccination remains our most effective strategy to prevent morbidity or mortality, vaccine effectiveness is not uniform. Age, exposure history, genetic background, and socio-environmental factors can substantially impact the vaccine response, yet we lack methods that can quantitatively characterize and leverage these effects. We hypothesized that a dynamic, individualized approach – using machine learning trained on longitudinal data – could identify predictors of robust vaccine responses at each life stage and suggest actionable strategies to enhance immunity.

Methods: We analyzed a compendium of serological and clinical data in over 5,000 individuals, combined with electronic health records from 500,000 patients. These longitudinal datasets track antibody responses, transcriptomics, HLA genotype, health traits, exposure history, and vaccine outcomes, collectively spanning the full human lifespan from infancy through old age. Using these rich datasets, we developed an integrated AI model that predicted the antibody response following vaccination and identified several modifiable drivers of immunity across age groups.

Results: Vaccine responsiveness followed distinct patterns across the lifespan. In infants, immune responses are shaped by the time between their date of birth and the influenza season. For example, infants that turn six months old at the end of the influenza season (Feb-May in the northern hemisphere) benefit from immediately getting their first influenza vaccine, as it significantly improved their vaccine response the following season. In adults and the elderly, immune trajectories were shaped by prior exposures. Machine learning models revealed that pre-vaccination antibody inhibition against multiple variants accurately predicted the magnitude, breadth, and durability of the post-vaccination antibody response. The sequence of prior influenza vaccine formulations also mattered, with live-attenuated vaccines priming stronger responses to an inactivated vaccine the following year.

Conclusions: Our findings demonstrate that vaccine responses are predictable across the lifespan and that interventions exist to optimize them. While longitudinal antibody profiles provided strong predictive power, many other features – such as health behaviors and comorbidities – remain underexplored. In particular, the longitudinal dynamics of lifestyle factors (e.g., exercise, chronic conditions) and their interaction with vaccinations and infections may offer additional opportunities for adaptive, personalized interventions. Together, these insights lay the foundation for adaptive, individualized framework to improve influenza vaccine effectiveness and inform both clinical practice and public health policy.

482

The effects of COVID-19 vaccination based on win analysis in patients after an acute coronary syndrome: a secondary analysis of the VIP-ACS trial

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Background

The win ratio (WR) method enables the integration of diverse outcome types, enhancing statistical power and improving the analysis of composite endpoints. Applying this approach offers a novel perspective on the effects of COVID-19 vaccination on cardiorespiratory outcomes. This study aimed to assess the impact of COVID-19 vaccination on cardiorespiratory events among high-risk individuals**.**

Methods

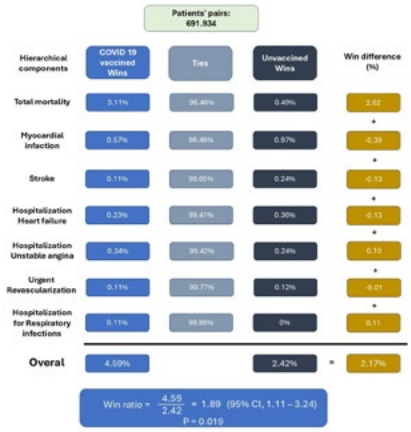
We conducted an exploratory analysis of data from the VIP-ACS trial (NCT04001504), a randomised controlled study in which patients were assigned 1:1 to receive either double-dose or standard-dose quadrivalent inactivated influenza vaccine. The double-dose was administered at hospitalisation, as soon as possible after randomisation, while the standard dose was given 30 days later. As a pre-specified secondary analysis, we evaluated the impact of COVID-19 vaccination on the primary hierarchical composite outcomes: all-cause death, myocardial infarction, stroke, hospitalisation for unstable angina, hospitalisation for heart failure, urgent coronary revascularisation, and hospitalisation for respiratory infections. Patients who received at least one dose of a COVID-19 vaccine during follow-up were classified as "COVID vaccinated" from the date of vaccination and subsequently censored from the "unvaccinated" group. A landmark analysis excluded patients who experienced any study endpoints within 90 days of randomisation. The win ratio statistical method was applied to compare hierarchical cardiorespiratory outcomes between COVID-19 vaccinated and unvaccinated patients. Only events adjudicated by the Clinical Events Classification Committee were included in the analysis.

Results

Of 1,801 patients enrolled in the original trial, 1,665 were included in the 90-day event-free cohort. The median age was 57 years, and 70% were male. The COVID-19 vaccinated group demonstrated a higher proportion of wins for the primary hierarchical composite outcome compared to the unvaccinated group (4.59% vs 2.42%; win ratio [WR] 1.89; 95% CI 1.11–3.24; p=0.019). Sensitivity analysis including all participants yielded consistent results (WR 2.99; 95% CI 1.08–4.88; p=0.001). However, no significant difference was observed between vaccinated and unvaccinated groups for hierarchical major cardiovascular events (1.84% vs 1.43% wins; WR 1.37; 95% CI 0.63–2.95; p=0.42).

Discussion

In this pre-specified secondary analysis of the VIP-ACS trial, win ratio analysis indicates that COVID-19 vaccination reduces cardiorespiratory events in high-risk patients. The win ratio is an increasingly adopted statistical method in clinical trials, especially within cardiovascular medicine and infectious diseases, owing to its capacity to incorporate hierarchical clinical outcomes in vaccinology studies.


Patients' pairs:
691.934
Hierarchical components
COVID 19 vaccined Wins
Ties
Unvaccined Wins
Win difference (%)
Total mortality
3.11%
96.49%
0.40%
2.62
+
Myocardial Infaction
0.57%
98.46%
0.97%
-0.39
+
Stroke
0.11%
99.65%
0.24%
-0.13
+
Hospitalization Heart failure
0.23%
99.41%
0.36%
-0.13
+
Hospitalization Unstable angina
0.34%
99.42%
0.24%
0.10
+
Urgent Revascularization
0.11%
99.77%
0.12%
-0.01
+
Hospitalization for Respiratory infections
0.11%
99.89%
0%
0.11
Overal
4.59%
2.42%
=
2.17%
Win ratio = 4.59 / 2.42 = 1.89 (95% CI, 1.11 – 3.24)
P = 0.019

483

Differences in glycoproteins and potential for early pro-tective efficacy of LAIV based on antigenically drifted A/H1N1pdm09 influenza virus variants

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Federal State Budgetary Scientific Institution 'Institute of Experimental Medicine'

Background: The evolution of the influenza virus is closely related to the process of antigenic drift, which is manifested in changes in the structure of the glycoproteins hemagglutinin (HA) and neuraminidase (NA). The A/H1N1pdm09 strain, which appeared in 2009, has undergone significant antigenic changes, necessitating the updating of vaccine strains. Live attenuated vaccines (LAIV) are a promising preventive measure that stimulates a comprehensive immune response. Unlike inactivated vaccines, LAIV are able to interrupt the transmission of an infectious virus, which has been repeatedly shown in large-scale studies.

Methods: The study examined vaccine strains based on two variants of the A(H1N1)pdm 09 drift virus: A/South Africa/3626/13 and A/Guangdong-Maonan /SWL1536/2019. Structural biology methods were used to study the HA glycosylation patterns of the vaccine strains. **In** The production of type I interferon and MX1 protein was assessed **in vitro.** Early protection against homologous and heterologous infection was studied in mice 6 days after intranasal immunization.

Results: The A/17/South Africa/2013 and A/17/ Guangdong – Maonan/2019 vaccine viruses showed different HA glycosylation patterns. At the same multiplicity of infection (MOI) in A549 cell culture, the A/17/South Africa/2013 virus induced increased production of interferon-α and MX1 protein compared with A/17/ Guangdong – Maonan/2019, although the differences were not statistically significant. In the respiratory tract of mice, the reproductive activity of the A/17/ Guangdong – Maonan/2019 strain was not lower, and sometimes higher, than that of A/17/South Africa/2013. Immunization with the A/17/South Africa/2013 strain stimulated a significant increase in IgM and IgG already in the first week and provided 70% protection of mice from mortality when infected with a homologous influenza virus adapted to mice.

Conclusions: Differences in HA glycosylation can potentially affect the cytokine and antibody response and the effectiveness of immunoprophylaxis. Strains that induce a more pronounced interferon response and antibody response early after immunization provide better protection against infection with a homologous influenza virus. These features should be taken into account when choosing vaccine strains to enhance immunity and protect against rapidly evolving influenza viruses.

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486

Bioinformatics tools for efficient genomic analyses of H5N1 viruses

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Background

The persistent circulation of clade 2.3.4.4b highly pathogenic avian influenza (HPAI) H5 virus in Europe starting from October 2020, its panzootic spread among wild birds and host range expansion - with an ever-growing number of infections in mammals - has increased the risk for zoonotic infection. This virus has also shown a strong inclination towards reassortment, which has led to the emergence of multiple genotypes. Real-time genomic surveillance is crucial to efficiently monitor the circulating European variants, hence the need for fast and automatic software able to accelerate manual analyses. In this context, we have developed Genin2, an open-source and cross-platform utility to quickly classify gene constellations based on nucleotidic sequences and predict a genotype.

Moreover, to monitor the emergence of mutations associated to an increased zoonotic risk or antiviral resistance, we developed FluMut, an open-source and cross-platform software able to efficiently process large volumes of nucleotidic sequences and identify molecular markers with any relevant effect among those reported in scientific literature.

Materials and Methods

Genin2 is a Python software based on a machine-learning approach implemented with Support Vector Machines (SVMs); this yields a lightweight classifier that eliminates the need for large sequence datasets typically required for computationally expensive phylogenetic distance calculations.

FluMut is another Python software, but it additionally features a PyQt5 graphical interface. The algorithm relies on a curated, literature-based SQLite database of mutations linked to host adaptation, virulence, and antiviral resistance.

Results

Both programs are available as command-line utilities, which makes them fast and versatile for various deployment scenarios. FluMut is also available with a graphical user interface, which increases accessibility for users with varying levels of computational expertise.

Genin2 (https://izsvenezie-virology.github.io/genin2) and FluMut (https://izsvenezie-virology.github.io/FluMut) generate tabular files that permit easy post-processing by the users and efficient pipelining with other tools. Genin2 produces a single table listing the predicted segment versions and the resulting detected genotype for each sample. FluMut creates tables listing the detected markers in each input sequence along with their associated biological effects, aminoacidic mutations, and relevant literature references. It can also merge all the outputs into an Excel file to make them easier to consult.

Conclusions

Genin2 and FluMut fill a critical gap in the real-time monitoring of the evolution and pandemic potential of HPAI H5N1, permitting quick and easy analyses of hundreds of H5 nucleotide sequences in a few seconds.

To keep these tools up to date with respect to the latest circulating viruses, Genin2 models are periodically retrained to include new sequence data. The FluMut database is frequently hand-curated to ensure it includes the latest molecular markers. Future updates will expand the ability of FluMut to encompass all influenza subtypes.

487

Epidemiological Trends of Respiratory Syncytial Virus-Associated Hospitalizations in Children Aged <15 Years in Southern Italy: A Comparative Analysis of the 2023/24 and 2024/25 Seasons

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Background:

Respiratory syncytial virus (RSV) is a leading cause of lower respiratory tract infections and hospitalizations among infants and young children. For the 2024/25 season, the Apulia Region implemented a universal immunization program with Nirsevimab, a monoclonal antibody with an extended half-life, for all newborns from 1st July 2024 to 31st March 2025. This measure is part of a preventive strategy to reduce severe RSV disease and RSV-related morbidity in infants during their first RSV season, a period of heightened vulnerability. This study aimed to analyze seasonal variations in RSV-associated hospitalizations in the last two seasons among children under 15 years of age in the province of Bari (Southern Italy), with a particular focus on infants under 1 year of age.

Methods:

A retrospective observational study was conducted by enrolling all pediatric patients (<15 years) hospitalized with acute respiratory infection (ARI) and a laboratory-confirmed RSV infection during the 2023/24 and 2024/25 winter seasons. Eligible patients were admitted to one of the hospitals in the province of Bari. Respiratory samples were collected and tested for RSV A/B infection by real-time PCR. Data regarding age, hospitalization timing, and test results were analyzed.

Results:

During the 2023/2024 season, 215 out of 969 (22.2%) children hospitalized with ARI and tested positive for RSV, with a peak of infections in January. Whereas during the 2024/2025 season, 155 out of 863 (18.0%) hospitalized children were RSV-positive (p=0.07), with a peak in February. During the 2023/24 season, children <1 year constituted 71.2% of RSV-associated hospitalizations, whereas in the 2024/25 season, this age group accounted for 36.8% of hospitalizations. In contrast, an increase in RSV-associated hospitalizations was observed in children >1 year of age by 34.4%. This shift in age distribution was statistically significant (p<0.001). During the 2024-2025 season, 31.6% of infants <1 year old hospitalized for RSV infection had received Nirsevimab before admission.

Conclusions:

The 2024/2025 RSV season in the Apulia region was characterized by a reduction in RSV-associated hospitalizations, particularly significant in children <1 year of age compared with the 2023/2024 season. These findings may reflect potential changes in RSV epidemiology and population immunity resulting from the potential impact of preventive interventions such as administration of Nirsevimab at birth. The shift in the age group of children hospitalized with RSV underscores the importance of ongoing surveillance, virologic characterization, and age-specific analyses. These efforts are crucial for evaluating the effectiveness of preventive measures and potentially implementing immunization strategies for older children.

488

Epidemiological Trends of Human Metapneumovirus Following the COVID-19 Pandemic: A Decade of Surveillance in Southern Italy

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Background:

Human metapneumovirus (hMPV) is a common cause of upper and lower respiratory tract infections across all age groups globally. Infants and young children are particularly vulnerable to hMPV infections, leading to increased morbidity and hospitalizations. hMPV circulates year-round with a characteristic epidemiological peak between December and February in temperate climates. The incidence of hMPV in hospitalized patients with respiratory illnesses increased from 1.45% during the COVID-19 pandemic to 6.76% in the post-pandemic period. This study describes the seasonal circulation of hMPV from 2016 to 2025, with a focus on changes observed following the COVID-19 pandemic (2016-2019 vs 2020-2021 vs 2022-2025) in the Apulia region.

Methods:

A retrospective study was conducted by analyzing nasopharyngeal swabs collected from 2016 to April 2025 from patients with acute respiratory infection (ARI) and tested positive for hMPV by **real-time** PCR at the Laboratory of Molecular Epidemiology and Public Health at the A.O.U.C. Policlinico of Bari. Patients were either hospitalized in the tertiary care hospital A.O.U.C. Policlinico of Bari or outpatients tested for respiratory viruses surveillance within the Apulia region (Southern Italy).

Results:

During the years considered, a total of 19,351 samples were tested for hMPV and 714 resulted positive, with a positivity rate of 3.7% (95% CI 3.4-3.9) ranging from 1.6% in 2021 to 8.2% in 2025. The median age of patients was 3 years (interquartile range [IQR]: 0-8 years) and 55.2% were male. The 0-5 years age group accounted for 67.6% of total cases, while the ≥65 age group averaged 11.8% of cases, peaking at 22.4% in 2025. Among the 15,167 hospitalized patients, 3.9% (95% CI, 3.6-4.2) tested positive for hMPV, compared to 3.0% (95% CI, 2.5-3.6) among the 4,184 outpatients (p=0.011). Among hospitalized cases, patients admitted to the Intensive Care Unit (ICU) were 5.4% (32/587) and 68.8% (22/32) were admitted in post-pandemic years (2022-2025). Specifically, 59.4% of patients in ICU were <5 years of age while 21.9% were >65 years.

Conclusions:

The epidemiology of hMPV infection across the Apulia region has changed over the last decade. Particularly in the post-COVID-19 period, an increased number of hMPV-positive cases was recorded with higher infection rates in young children and older adults. Higher hMPV-associated hospitalization rates were observed, and ICU admissions involved mainly individuals at extremes of age. The changes observed in the post-pandemic years may reflect the impact of the relaxation of COVID-19 restrictions, possible alterations in virus-specific characteristics and viral transmission and an increase in hMPV testing. Ongoing epidemiological surveillance is essential for more effectively targeting future research and public health interventions.

489

Visualising interactions between influenza viruses across length scales

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Background

The success of influenza A viruses (IAVs) depends on genetic exchange. Genome reassortment controls the generation of novel pandemic strains as well as the genetic diversity that can emerge from a single, mutation-prone virus as it spreads within its host. Reassortment requires coinfection of individual cells within the respiratory tract (RT), but it has typically been studied either within individual cells **in vitro** or across host populations. However, it is becoming increasingly clear that the spatial context of the RT matters. IAV does not spread homogeneously through the RT, and within a host individual IAV genotypes compete with each other in ways that cannot be explained either at the level of individual cells or of entire organisms. It is particularly challenging to study the interaction of IAVs within the RT due to the inaccessibility and convoluted structure of this organ system.

Methods

To address this, we developed light sheet microscopy methods which allowed us to visualise co-infections of fluorescently-tagged reporter IAVs in 3D throughout whole mouse lungs. We combined this confocal microscopy and spatial transcriptomics to visualise IAV infections across length scales within the lung without losing spatial context.

Results

We found that, on the scale of the whole lung, IAV co-infections preferentially occur at branchpoints in the airway, at sites of turbulent air flow. On closer inspection these seemingly co-infected lesions were revealed to be a network of discrete microdomains infected by the progeny of a single virus, with viral mechanisms of superinfection exclusion restricting genetic exchange between microdomains. We are now developing spatial transcriptomic methods to dissect the genetic exchange that occurs within a single microdomain.

Conclusions

We have established methods to study how IAV genetic exchange is controlled as the virus spreads from individual cells to form infected lesions within a host. Our multi-length scale approach enabled us to visualise the interaction between co-infecting IAVs, at scales from microscale intercellular processes to effects playing out over an entire lung. They show that opportunities for genetic exchange, and hence for population diversity and strain emergence, are much more restricted for IAV than **in vitro** studies of the virus would suggest.

490

Survey on attitudes, barriers, and facilitators to influenza vaccination among people living with HIV in Argentina.

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FUNDACION HUESPED, Argentine Republic

Background: People living with HIV (PLHIV) are included in the National Immunization Schedule due to their higher risk of developing severe illness, hospitalization, and death. However, vaccination coverage in this population remains suboptimal. This study examined attitudes, knowledge, and practices regarding influenza vaccination among adults living with HIV in Argentina.

Methods: A survey was designed based on the Vaccine Confidence and Access Index, adapted for people living with HIV (PLHIV). Between October 24 and January 25, it was distributed via social media and email to individuals who had participated in previous studies. The survey collected information on attitudes, practices, and sources of information related to influenza vaccination.

Results: A total of 829 PLHIV completed the survey. The median age was 41.5 years (IQR: 32–50). Of the participants, 50.5% identified as cisgender men, 27.7% as cisgender women, 1.8% as transgender women, 1.7% as non-binary people, and 0.5% as transgender men.

Over half lived in the City of Buenos Aires (61.3%), had a university or higher education (53.1%), were employed (62.1%), and had private or social health insurance (81.8%).

The primary sources of information on vaccination were infectious disease specialists (63.9%) and social media (40.8%).

Regarding clinical data, 98.1% of participants were receiving antiretroviral therapy, and 86.2% had an undetectable viral load. Among the 71.7% who received the influenza vaccine annually, 46.6% used public health services, 17% private services, and 7.7% pharmacies.

Although most participants considered the vaccine necessary (88.3%), safe (82.3%), and effective (75.4%), only 61.1% perceived influenza as a health risk. Among the 28.3% who reported not getting vaccinated annually, the most common reasons were lack of medical recommendation and distrust in the vaccine.

Conclusions: Nearly one-third of PLHIV did not receive the annual influenza vaccine, highlighting the need to implement strategies to increase vaccination rates in this population.

Communication gaps between healthcare professionals and PLHIV were evident, as some unvaccinated individuals reported never having received a recommendation for vaccination.

Although vaccination was generally viewed positively, some participants underestimated the risks associated with influenza.

These findings emphasize the need to strengthen education and improve communication between healthcare providers and PLHIV to boost vaccination coverage in this population.

491

Differential host immune responses of contemporary highly pathogenic avian influenza H5N1 virus and seasonal influenza virus in human airway organoids

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Background:

The historic highly pathogenic avian influenza (HPAI) H5N1 virus that was firstly identified 1996 caused sporadic dead-end transmissions to humans. However, a novel reassortant of HPAI H5N1 of clade 2.3.4.4b emerged in 2020 and have spread globally to multiple mammalian species, and, alarmingly, to dairy cattle since 2024. Sporadic mammal-to-human transmissions have occurred. There is a dire need to understand the infectivity, tropism, effects on host and pandemic potential of this novel H5N1 virus.

Methods:

Normal human nasal epithelial cells (NHNE) and bronchotracheal epithelial cells (NHBE) were obtained and differentiated at air-liquid interface to generate airway organoids for infection experiments. H5N1 influenza viruses, including a mink isolate, a bovine isolate of genotype B3.13 and a human isolate of bovine H5N1 (TX37) were generated by reverse genetics based on publicly available sequences. Seasonal H3N2 was isolated from a patient in 2022. Airway organoids were infected with the various viruses and replications kinetics were assessed. Immune responses were evaluated using multiplex assays and single cell RNA sequencing (scRNA-seq) analysis.

Results:

Despite our observation that the novel bovine influenza viruses possess low binding affinity for a2,6-sialic acid, both bovine B3.13 H5N1 and TX37 viruses replicated well in NHNE and NHBE organoids, while the mink isolate replicated less robustly. Seasonal H3N2 exhibited the least replication in airway organoids and yet, induced the strongest and fastest immune responses. The scRNA-seq data also indicated disparate transcriptomic responses upon infection with seasonal H3N2 and H5N1 viruses, and interestingly, TX37 induced a transcriptomic profile similar to that induced by seasonal H3N2. Remarkably, despite dissimilar cellular tropism, pseudotime analysis showed that TX37 infection induced a basal cell to Clara/multiciliated cell differentiation similarly to, albeit slower than, H3N2 infection.

Conclusions:

Existing ferret studies have suggested that the current HPAI H5N1 of clade 2.3.4.4b viruses possess limited airborne transmissibility, yet how they were transmitted to humans remains unknown. Here, we demonstrated that, given the circumstance, HPAI H5N1 viruses of B3.13 genotype could replicate robustly in human respiratory system. The host immune responses upon infection with H5N1 were somewhat delayed compared to that in seasonal H3N2 infection, suggesting potential immune-evasive capacity of these novel influenza viruses. In particular, infection of airway organoids with the human isolate TX37 induced a host transcriptomic profile resembling that with seasonal H3N2 infection, including ciliated cell loss and basal cell to Clara/multiciliated cell differentiation, suggesting that bovine H5N1 influenza virus maybe adapting to becoming a human-like virus.

492

The Influenza Genome Sequence Database – a tool for visualising influenza A virus sequence variation and host adaptation

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Background

Global influenza A virus (IAV) surveillance produces a vast amount of unsorted, publicly available sequence data. Concerns about IAV zoonosis have led to an extensive literature reporting mutations associated with IAV host adaptation. This wealth of information is itself a challenge: rigorous assessments of sequence variation and the identification of potentially concerning markers of adaptation in emerging IAV strains are now a time-consuming task which increasingly require specialist bioinformatic skills.

Methods

We curated a phylogeny of all IAV sequences publicly available through GenBank, addressing different residue numbering conventions, errors in records and inconsistent host taxonomies. We reviewed the literature to assemble (to the best of our knowledge) the largest curated database of IAV adaptation mutations, containing hundreds of markers of adaptation. Finally, we combined these into an online tool, the Influenza Genome Database Mutation Explorer.

Results

Our web-based application (https://flu-gdb.cvr.gla.ac.uk/) provides interactive visualisations of influenza phylogenetic trees and tools to identify mammalian adaptations in IAV sequences. It enables researchers to explore evolutionary relationships among reported IAV strains while dynamically integrating user-provided sequence data. Our free, online application offers several key features:

1. Interactive, publication-quality phylogenetic tree visualisation for each segment with customisable metadata display, including host and subtype information and amino acid usage.

2. Identification and frequency analysis of amino acid substitutions associated with mammalian adaptations, with links to supporting literature.

3. Integration of user-uploaded query sequences into these analyses. Tools for community reporting of additional adaptation mutations.

Conclusions

This powerful and user-friendly tool allows the rapid exploration of influenza evolution, aids in strain characterisation, and supports the identification of potentially zoonotic variants. By combining phylogenetic analysis with dynamic sequence querying and adaptation marker detection, our application provides a comprehensive platform for influenza research and surveillance efforts.

EPIDEMIOLOGY, SURVEILLANCE

494

Comparative Burden of COVID-19 in Adults With and Without Underlying Health Conditions

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Background: In May 2025, the US FDA issued a revised COVID-19 Vaccine Framework limiting the indication of licensed COVID-19 vaccines to individuals aged ≥65 years and those ≥6 months considered high-risk. The framework calls for additional randomized controlled trials in adults 50-64 years without high-risk health conditions (HRHC). With this new framework, understanding the contemporary epidemiology of COVID-19 in adults with and without HRHC is critical. This study compares COVID-19 incidence, severity, and impact on productivity in adults with and without HRHC.

Methods: Data analyzed included adults aged ≥18 years from a prospective community cohort (PACC-ER, September 2024 – May 2025) and a medically attended (MA) surveillance study (inSPIRE, June 2024 – May 2025), supplemented by MA illness data from Marshfield Clinic Health System electronic health records (MCHS, June 2024 – May 2025). Participants were categorized by HRHC status and stratified by age (18–49 years, 50–64 years, and ≥65 years). Outcomes included incidence of symptomatic COVID-19, MA COVID-19, and severe COVID-19 outcomes (hospitalization, emergency department [ED]/urgent care [UC] visits, and mortality), and self-reported work absenteeism and productivity loss.

Results: Between September 2024 and May 2025, symptomatic COVID-19 incidence from PACC-ER were similar among adults aged 50–64 with and without HRHC: 52.1 per 1,000 with HRHC (95% CI: 20.7–83.5) and 80.6 per 1,000 without HRHC (95% CI: 12.9–148.4) (Table 1). Symptomatic rates were also similar in adults 18-49 with and without HRHC. Most adults aged ≥65 in PACC-ER had HRHC and symptomatic rates were similar to those aged 50–64 (Table 1). Among MCHS adults aged 50–64, those with HRHC had higher incidences of MA COVID-19 (9.4 [95% CI: 8.3–10.5] vs. 2.0 [95% CI: 1.4–2.6] per 1,000) and severe outcomes (6.8 [95% CI: 5.9–7.7] vs. 1.3 [95% CI: 0.8–1.8] per 1,000) than those without HRHC. This trend was consistent across age groups. Work hours missed and productivity impacts were similar in individuals with and without HRHC in inSPIRE and PACC-ER. In inSPIRE, mean work hours missed were 25.9 hours (HRHC) and 27.2 hours (no HRHC; p=0.59), with productivity impact scores also comparable (5.8 vs. 5.3; p=0.55). In PACC-ER, mean work hours missed were 10.3 (HRHC) and 1.5 (no HRHC; p=0.24), with similar productivity scores (4.3 vs. 3.4; p=0.87) (Table 2).

Conclusions: Although incidences of severe outcomes were higher in older adults and those with HRHC, symptomatic COVID-19 rates and productivity impacts were substantial and similar in adults with and without HRHC, highlighting COVID-19's broad implications beyond clinical outcomes. Incidences of MA COVID-19 and severe outcomes in this study may have been underestimated due to the lower testing probabilities in younger adults, those without HRHC, and those with less severe illnesses.

Table 1. Incidence of Symptomatic COVID-19 Cases, Medically Attended Cases, and Severe Outcomes by Age and High-Risk Health Condition (HRHC)

	Symptomatic COVID-19 cases per 1000 people (95% CI)[1]	Medically attended COVID-19 cases per 1000 people (95% CI)[2]	Severe COVID-19 cases (hospitalization, ED/UC visit, or death) per 1000 people (95% CI)[2]
Age 65+			
≥1 HRHC	66.8 (41.5, 92.2)	15.6 (14.6, 16.6)	12.3 (11.4, 13.2)
No HRHC	51.3 (0, 120.5)	3.4 (2.9, 4.4)	2.4 (1.8, 3.3)
Age 50-64			
≥1 HRHC	52.1 (20.7, 83.5)	9.4 (8.3, 10.5)	6.8 (5.9, 7.7)
No HRHC	80.6 (12.9, 148.4)	2.0 (1.4, 2.6)	1.3 (0.8, 1.8)
Age 18-49			
≥1 HRHC	76.0 (36.3, 115.7)	8.7 (7.8, 9.6)	7.2 (6.3, 8.0)
No HRHC	101.1 (38.5, 163.8)	1.8 (1.4, 2.3)	1.4 (1.1, 1.8)

[1] Based on data collected from 927 adult participants in the PACC-ER cohort.
[2] Based on data of Marshfield Clinic Health System source population of 271,786 adults, defined by ≥1 well visit or ≥2 problem visits in the 3 years prior to 7/1/2024.

Table 2. Missed Work Hours and Productivity Loss due to COVID-19 by High-Risk Health Condition (HRHC) in inSPIRE (Medically attended) and PACC-ER (Community cohort)[1]

	No.	Work Hours Missed		P-value	Work Productivity Impact (0: None to 10: High)		P-value
		Mean (SD)	Range		Mean (SD)	Range	
inSPIRE							
≥1 HRHC	54	25.9 (17.5)	0, 80	0.59	5.8 (2.6)	1, 10	0.55
No HRHC	26	27.2 (17.0)	0, 60		5.3 (2.9)	0, 10	
PACC-ER							
≥1 HRHC	9	10.3 (13.0)	0, 32	0.24	4.3 (3.4)	0, 10	0.87
No HRHC	8	1.5 (3.0)	0, 8		3.4 (2.0)	1, 6	

[1] Based on employed adult participants with COVID who were expected to work ≥ 20 hours from illness onset to first follow-up survey (approximately 2 weeks).

495

Immunogenicity of an mRNA-Based Seasonal Influenza Vaccine, mRNA-1010, in Adults ≥50 Years

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Background: Seasonal influenza leads to 650,000 deaths annually, with the majority occurring in older adults.[1] mRNA-1010, a novel investigational mRNA-based influenza vaccine, demonstrated superior immunogenicity compared with licensed standard-dose (SD) and high-dose (HD) comparators, as measured by hemagglutination inhibition (HAI) assay.[2] In line with updated WHO recommendations for trivalent influenza vaccines (TIVs), we report immunogenicity results of mRNA-1010 in adults from a subset of participants in the pivotal relative efficacy study (NCT06602024).

Methods: In this phase 3, double-blind, active-controlled study in adults ≥50 years across the Northern Hemisphere, participants were randomized (1:1) to receive a single 37.5-µg dose of mRNA-1010 (12.5 µg/strain) or a licensed SD influenza vaccine. The immunogenicity subset included ~2400 randomly selected participants who received mRNA-1010 or SD comparator and had baseline and Day 29 HAI data available. The primary immunogenicity objective was to evaluate antibody response to A/H1N1, A/H3N2, and B lineage strains following vaccination based on geometric mean titers (GMTs), Day 29 GMT ratios (GMRs), and seroconversion rates (SCRs; ≥4-fold rise).

Results: 2342 participants were included in the immunogenicity subset (mRNA-1010, n=1167; SD comparator, n=1175). Post-vaccination, HAI GMTs for mRNA-1010 increased above baseline levels, with geometric mean fold rises of 4.07 for A/H1N1, 4.70 for A/H3N2, and 3.14 for B/Victoria (**Table 1**). While descriptive, Day 29 responses were numerically higher for mRNA-1010 compared with the SD comparator. Day 29 GMRs for mRNA-1010 versus SD comparator exceeded 1 for all 3 influenza strains: 1.82 (95% CI: 1.69-1.97) for A/H1N1, 1.59 (1.48–1.71) for A/H3N2, and 1.67 (1.57–1.78) for B lineages. The lower bounds of the 95% CIs for the SCR differences (mRNA-1010 vs SD comparator) all exceeded 10%.

Conclusion: A single dose of mRNA-1010 elicited a robust antibody response in adults ≥50 years. Immune responses were higher for mRNA-1010 than SD comparator and aligned with previous results in adults ≥65 years, where mRNA-1010 demonstrated superior immunogenicity compared with HD influenza vaccination.[2] These results provide further evidence supporting mRNA-1010 as a potential enhanced influenza vaccine candidate in adults ≥50 years.

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Table 1. Summary of HAI Antibody Levels and Seroconversion for Vaccine-Matched Seasonal Influenza A and B Strains Measured by HAI Assay by Timepoint Per-Protocol Immunogenicity Subset by Visit up to Day 29

	Baseline (Day 1) GM Titer (95% CI*)	Day 29 GM Titer (95% CI*)	Day 29 GM Fold Rise (95% CI*)	Day 29 Seroconversion Difference (%) (mRNA-1010 vs Active Comparator)† (95% CI*)
Influenza A/H1N1 Antibody Titer				
mRNA-1010 37.5 µg (n=1167)	35.91 (34.07-37.84)	146.15 (136.43-156.56)	4.07 (3.84-4.31)	27.86 (24.09-31.75)
SD Active Comparator (n=1175)	36.80 (33.86-37.84)	80.08 (74.84-85.63)	2.24 (2.11-2.37)	
Influenza A/H3N2 Antibody Titer				
mRNA-1010 37.5 µg (n=1167)	31.83 (30.12-33.65)	149.48 (140.19-159.39)	4.70 (4.44-4.97)	18.89 (15.89-23.82)
SD Active Comparator (n=1175)	31.06 (29.37-32.84)	92.60 (86.85-98.75)	2.98 (2.81-3.16)	
Influenza B/Victoria Antibody Titer				
mRNA-1010 37.5 µg (n=1167)	79.79 (75.92-83.85)	250.92 (237.83-264.75)	3.14 (2.96-3.31)	26.33 (21.85-30.94)
SD Active Comparator (n=1175)	79.46 (75.70-83.41)	149.64 (142.08-157.61)	1.88 (1.80-1.97)	

Abbreviations: CI, confidence interval; GM, geometric mean; HAI = hemagglutination inhibition; LLOQ, lo-wer limit of quantification; ULOQ, upper limit of quantification.
GM Titers are shown for A/H1N1 (LLOQ: 10, ULOQ: 3620), A/H3N2 (LLOQ: 10, ULOQ: 2560), and B/Victoria (LLOQ: 10, ULOQ: 1358).
Antibody values reported as below the LLOQ are replaced by 0.5 × LLOQ. Values greater than the ULOQ are replaced by the ULOQ.
*95% CI was calculated based on the t-distribution of the log-transformed values or the difference in the log-transformed values for GM titer and GM fold rise, respectively, then back-transformed to the original scale for presentation.
†Rate of seroconversion was defined as the proportion of participants with either a baseline HAI titer <1:10 and a post-baseline titer ≥1:40 or a baseline HAI titer ≥1:10 and a minimum 4-fold rise in post-baseline HAI antibody titer.
‡95% CI was calculated using the Miettinen-Nurminen (score) method.

497

Determinants of Antiviral Activity of Influenza A Virus-Derived Defective Interfering Particles (DIPs)

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Background: Influenza A virus (IAV) remains a major public health threat, necessitating the development of novel antiviral strategies. Defective interfering particles (DIPs), which harbor truncated viral genomes (DI RNAs), represent promising antiviral agents. While their activity has been attributed to competition for replication resources and induction of interferon (IFN) responses, the precise mechanisms remain unclear.

Methods: We employed a virus-free mini-replicon system and a cell culture-based DIP production platform using reverse genetics in a PB2-complementing cell line. The DI RNA prototype, DI 244 (derived from segment 1), was analyzed alongside additional DI RNAs. Antiviral activity was assessed in MDCK and A549 cells. Interferon-stimulated gene (ISG) expression and IFN responses were characterized using RNA sequencing and qRT-PCR.

Results: DI RNA-mediated replication interference was inversely correlated with DI RNA length in the mini-replicon system. Consistently, antiviral activity of DIPs in MDCK cells followed the same trend. In contrast, DIP antiviral activity in A549 cells was independent of DI RNA length and partially STAT1-independent, suggesting a role for innate immune activation. RNAseq revealed that both IAV and DI 244 upregulated ISGs, yet only IAV strongly induced IFN gene expression. These findings suggest that ISG induction by DIPs occurs independently of robust IFN production.

Conclusions: Our data demonstrate that both replication interference and host response contribute to DIP-mediated antiviral activity, with their relative importance being cell type-dependent. The results highlight IFN-independent ISG induction as a key feature of DIP function, offering mechanistic insight for the development of host-directed antivirals against influenza.

498

ECaS

Validation of a high-throughput ELISA-based Microneutralization assay for human Metapneumovirus A1, A2, B1 and B2 subtypes

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Background: Human metapneumovirus (hMPV) is a major viral cause of respiratory diseases that primarily affect young children, elderly, and immunocompromised people. The two genetic lineages (A and B) spread throughout the world and can be classified into four genetic sublineages, including A1 (NL/1/00), A2 (NL/17/00), B1 (NL/1/99), and B2 (NL/1/94). Despite its global spread and clinical impact, no vaccine has been approved to date and there is a lack of standardized tests measuring neutralizing antibodies (nAb). In this study, we report the design and analytical validation of an ELISA-based microneutralization assay (EMN) for the detection of nAb against the four major hMPV subtypes: A1, A2, B1 and B2.

Methods:

The assay was developed using Vero E6 cells and validation was carried out according to ICH guidelines, evaluating critical parameters such as linearity, accuracy, precision, specificity and robustness. Since no WHO international reference material for hMPV was commercially available, a PCR-positive human serum was chosen as the positive control in the validation experiment. For all subtypes, a viral dose input of 2000 $TCID_{50}$/mL was used. After validation, the EMN assay was applied to a cohort of 20 human serum samples to evaluate the distribution of neutralizing antibody titres against hMPV-A1, A2, B1 and B2. In parallel, a larger panel of 105 human serum samples was analyzed via EMN and indirect ELISA assays. ELISA was performed to detect antibodies that bind to G glycoprotein (A1 and B1) and F0 glycoprotein (B1 only).

Results:

The assay met all predefined validation criteria for the four hMPV subtypes. Neutralizing titre results against A1, A2, B1 and B2 in human serum samples confirmed to be widespread, albeit moderate levels of immunity across the population, consistent with the epidemiology of hMPV. Indirect ELISA results revealed significantly higher titres observed against hMPV-B1 F0 glycoprotein, supporting its higher immunogenicity. A moderate correlation (Spearman's r = 0.5829) between ELISA and EMN results further supports F glycoprotein as a reliable marker of neutralizing activity. ELISA experiments for hMPV-A2 and B2 are currently ongoing to evaluate whether similar patterns and correlations are observed across all subtypes.

Conclusion:

We present a fully validated EMN assay for detecting nAbs against all major hMPV subtypes. The method is particularly suitable for large-scale serological studies with high-throughput and for the evaluation of current and future hMPV vaccine candidates. Our findings also support the F glycoprotein as a key target for vaccine design.

503

ECaS

Influenza A viruses exhibit distinct effects on transcription of host innate immune genes and proteases

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Background: Zoonotic events have been essential for the ability of Influenza A virus (IAV) to cause pandemics. The pandemic of 2009 was caused by transmission of an H1N1 IAV from pigs to humans, resulting in worldwide spread of the virus, and subsequent human-to-pig transmission. Studying the differences between pre-pandemic and pandemic viruses could reveal which viral characteristics are correlated with zoonotic events, aiding in early detection of high-risk clades. In this study, host innate immune response to infection with pre-pandemic swine-adapted IAV, pandemic human-adapted IAV and pandemic swine-adapted IAV is investigated **in vitro** over the course of infection.

Methods: Swine immortalized nasal epithelial cells (siNEC) were cultured at air-liquid interface (ALI) and infected with a swine-adapted (swH1N1pdm09), human-adapted (huH1N1pdm09), or pre-pandemic (mxH1N1pdm09) influenza strain at an MOI of 0.1. Wells were pre-washed with PBS before adding 500 µL of virus or control media for 1 hour. Post infection, wells were washed again and incubated at 37 °C. Cells were harvested at 0, 1, 2, 4, 8, 12, 24, and 48 hours post infection. RNA was extracted (Zymo), converted to cDNA, and analyzed by microfluidic qPCR (Standard Biotools, Fluidigm) targeting pattern recognition receptors (PRRs), interferons (IFNs), cytokines, interferon stimulated genes (ISGs), and host proteases.

Results: Infection with the selected IAV viruses resulted in distinct transcriptomic profiles, showing differences in both the identity of regulated genes and time of regulation. Regulation of gene transcription was observed in PRRs, IFNs, cytokines, ISGs and host proteases.

Conclusions: The results indicate that the host response to pre-pandemic and pandemic viruses differ at the transcriptional level for innate immune genes and host proteases. These findings may aid in linking genetic differences between viruses to effects on pathogenesis, furthering our understanding of viral characteristics associated with zoonotic potential.

505

Evolution of Influenza Incidence in Children and Adolescents over 9 Seasons in Spain: INFLUENZE Study

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Background

Our objective is to describe the incidence of seasonal influenza in children and adolescents by age group during 9 seasons (2016-2025).

Methods:

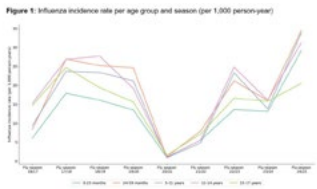
We present a descriptive analysis of data on influenza (flu syndrome) in children and adolescents (<18 years old), by age groups. The data were extracted from the Telotron ® database, which collects information on primary care, specialized care and social-health services in a population of 2.2 million subjects in 7 Spanish Autonomous Communities. The incidence rate (per 1,000 person-years) was stratified by age group and per season.

Results:

The age groups with the highest influenza incidence rates in most seasons were, by order, children aged 24-59 months, 5-11 years and 12-14 years (excluding the 2020/21 and 2021/22 seasons due to the COVID-19 pandemic). In the 2024/25 season, highest incidence rates were observed in most age groups, among children aged 24-59 months (34.37), 5-11 years (33.84) and 12-14 years (31.15). This season, 2.1% of children and adolescents experienced more than one episode of influenza, with the highest number of cases in the 5-11 years age group. At the same time, it was observed that influenza accounted for 47.24% of respiratory infections in this population, approaching pre-pandemic percentages.

Conclusions:

The analysis shows higher influenza incidence rates, by order, in the age groups of 24 to 59 months, 5–11 years, and 12–14 years throughout most seasons and a clear anomaly during 2020/21 and 2021/22 seasons due to the COVID-19 pandemic. These data indicate that the pediatric population, and especially these three age groups, are particularly susceptible to influenza, which could, in turn, facilitate community transmission. For this reason, it is recommended to prioritize influenza vaccination and epidemiological surveillance in this population, as well as to strengthen preventive education, to reduce the incidence and transmission of influenza in the community.



506

Diagnosis of RSV in Adults in Spain: A Multispecialty Survey on Testing Frequency, Barriers, and Opportunities for Improvement

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Background

Respiratory syncytial virus (RSV) is a significant yet underdiagnosed cause of respiratory infections in adults. This study assessed the frequency of testing, barriers, and opportunities for improving RSV diagnosis among Spanish specialists.

Methods

Nationwide cross-sectional study using an online survey—developed through expert focus groups on RSV management and prevention—and distributed to medical specialists in Spain.

Results

A total of 259 specialists participated (mean age 48 years, 18 years of experience): primary care (n=62), preventive medicine (n=30), internal medicine (n=32), microbiology (n=33), pulmonology (n=60), emergency medicine (n=42).

During the winter, 59-100% of hospital-based specialists always or frequently requested RSV tests in adults, versus 5% always and 17% frequently in primary care (**Figure 1**). Testing was most common in adults over 75 or institutionalised (51% always, 36% frequently) and in immunocompromised patients (54% always, 33% frequently). Access to multiplex PCR and COVID-influenza-RSV antigen tests was 93-100% and 27-41% among hospital specialists, and 27% and 41% in primary care, respectively.

Regarding testing perceptions, most specialists (88%) believed RSV is underdiagnosed in adults, though only 13% saw it as a major public health issue (9–10/10 rating). Microbiologists were least likely to perceive underdiagnosis (74%), while pulmonologists rated its public health relevance highest (20% scoring 9–10/10; mean rating: 7.53/10). The main perceived benefits of RSV diagnosis were optimising treatment (87%), reducing unnecessary antibiotics (65%), and patient isolation (52%).

Main barriers to RSV testing included time to results (63%), lack of specific treatment (50%), and limited test access (39%), particularly among pulmonologists (52%) and emergency physicians (51%). Absence of protocols was frequently noted in emergency medicine (34%) (**Figure 2**).

The most valued solutions were the development of specific clinical guidelines (mean usefulness score 8.7/10), improved access to rapid diagnostic tests (8.6/10), and training on adult RSV (8.6/10), with especially high ratings from preventive medicine and microbiology specialists.

Conclusions

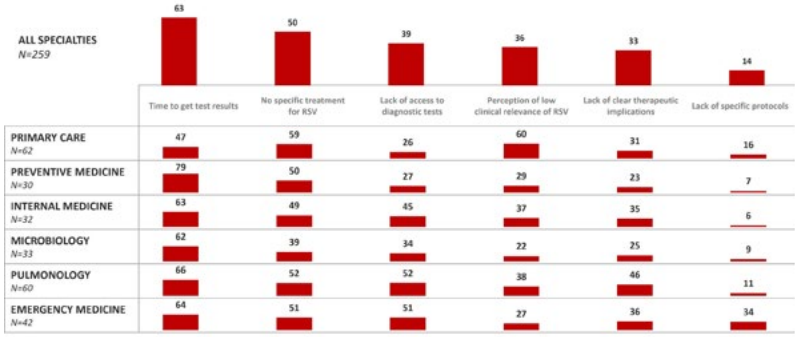
Many specialists, particularly in primary care, do not routinely request RSV tests in adults in Spain. While no single barrier predominated, coordinated interventions—including clinical guidelines, expanded access to rapid tests, and specialist training—are urgently needed to optimise adult RSV diagnosis.

Funded by: GSK

Figure 1. Frequency of requests for RSV diagnostic testing in adults with respiratory infection during the winter season.

Testing frequency by specialty (%)	ALWAYS	FREQUENTLY	OCCASIONALLY	NEVER
PRIMARY CARE N=62	5	17	47	32
PREVENTIVE MEDICINE N=30	39	20	35	7
INTERNAL MEDICINE N=32	59	41		
MICROBIOLOGY N=33	46	39	12	3
PULMONOLOGY N=60	59	33	9	
EMERGENCY MEDICINE N=42	27	57	16	

Figure 2. Factors that may hinder ordering a diagnostic test for RSV in adults.



509

SpikeID: Rapid and unbiased identification of SARS-CoV-2 variants by spike sequencing

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern (VOCs) are characterized by distinct mutation patterns in the S1 domain of the viral spike protein. This domain encompasses the N-terminal domain, the receptor-binding domain, and part of the cleavage site region. While mutations in other genomic regions of SARS-CoV-2 can impact VOC potential, the S1 domain holds particular importance for identifying variants and assessing antigenic evolution and immune escape potential. Here, we describe a rapid high-throughput sequencing-based assay, SpikeID, for the unbiased detection and identification of SARS-CoV-2 variants based on spike S1 amplicon sequencing. Benchmarking of the SpikeID assay against Illumina whole-genome sequencing across 621 clinical biospecimens, representing lineages globally circulating from October 2021 to January 2024, demonstrated that SpikeID could unambiguously detect 100% of WHO-designated VOCs and identify PANGO lineages circulating at ≥1% prevalence in the New York City (NYC) area with 93% accuracy in comparison to whole genome sequencing. The drop in accuracy occurred mainly due to lineages distinguished by mutations outside of the S1 domain. Additionally, we highlight the utility and scalability of the SpikeID assay through profiling of 3,358 nasopharyngeal and saliva specimens collected during multiple SARS-CoV-2 surges in NYC. Integration of SpikeID as part of our SARS-CoV-2 surveillance algorithm enabled the early detection of the Omicron variant in November 2021 and the JN.1 lineage in October 2023. As of mid-2025, SpikeID remains effective in detecting emerging variants, including XFG, LF.7, NB.1.8.1, LP.8.1, among other lineages. We therefore expect it to remain an unbiased and effective tool for detecting future SARS-CoV-2 lineages, and for estimating the relative abundance of co-circulating variants in ongoing surveillance efforts.

510

Sugar-binding characteristics of SARS-CoV-2 spike proteins

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[Background]Infectious disease caused by SARS-CoV-2 continues to be a major threat since the 2020 pandemic. Elucidating the molecular mechanisms of viral infection and replication are urgently needed for combating this virus. SARS-CoV-2 infection is triggered by interaction of its envelope spike (S) protein to angiotensin-converting enzyme 2 (ACE2) on the host cell surface. The S protein is known to interact with a variety of carbohydrates. The binding with carbohydrate, known as the glycan shield, protects the S protein from immune systems of host cells and supports its interaction with its receptor, ACE2. Therefore, we focused on the interaction between the S protein and carbohydrates and screened the carbohydrates and glycans that may interact with the S protein using a glycan array.

[Methods]First, we performed to screen the carbohydrates that bind to the S protein of Delta and Omicron strains of SARS-CoV-2 using a commercial glycan array kit. Next, we analyzed the molecular interaction of identified carbohydrates with the S protein of different strains in detail using various techniques, including surface plasmon resonance (SPR) binding analysis, circular dichroism (CD) titration analysis, nuclear magnetic resonance (NMR) analysis, and **in-silico** simulation. Finally, the antiviral activity of the identified carbohydrates was examined using human respiratory organoids derived from iPS cells.

[Results]Glycan arrays were performed using commercial biotinylated recombinant full-length S proteins. The results suggested that the aminoglycoside antibiotics, tobramycin and sisomicin, bind to the S protein. The SPR binding analysis and the CD titration analysis suggested that these antibiotics have stronger affinity for S protein of the Delta strain than for that of Omicron strain. While the S proteins were immobilized on a sensor tip of the SPR binding analysis, they were freely resolved in solution in the CD titration analysis. These results showed that the free state of the S protein exhibited stronger affinity with tobramycin and sisomicin. NMR analysis using recombinant proteins containing only the receptor binding domain (RBD) of the S protein revealed that tobramycin and sisomicin have low affinity for the RBD. **In-silico** binding simulations suggested that these carbohydrates exhibit stable binding affinity for the space between two RBDs, located in proximity to the S2 domain of the 1-up type S protein. Experiments using human respiratory organoids showed no antiviral activity of tobramycin and sisomicin.

[Conclusion]These results suggest that tobramycin and sisomicin can interact with the S protein and have stronger affinity for the S protein of the Delta strain than that of the Omicron strain. Furthermore, tobramycin and sisomicin were suggested to bind to the root region of the S protein for function as an interface forming trimer structure rather than the RBD, that is due to the lack of antiviral activity of these antibiotics.

512

State and Age-Specific Annual Estimates of Influenza-associated Deaths in the United States: Applying a Linear Time Series Model In the Post-COVID-19 Pandemic Period

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Background:

Causes of death are reported on death certificates to the National Vital Statistics System (NVSS), but deaths due to influenza are not always recognized and reported on death certificates because of care seeking behaviors, testing practices, sensitivity of diagnostic assays, time between illness onset and test, or exacerbation of chronic health conditions listed as the cause of death. To address limitations of cause of death data, statistical models have been developed to estimate influenza-attributable deaths. Previous models have relied on several years of historic data, have not estimated by finer geographic areas such as state, or have not allowed the models to vary by age group. The emergence of SARS-CoV-2 changed clinical, testing, and death coding practices for deaths. We aimed to estimate influenza-attributable deaths in the post-pandemic period adjusting for SARS-CoV-2 and influenza virus circulation by state and age group.

Methods:

We estimated unrecognized influenza-attributable deaths for the 2022/23 and 2023/24 seasons, using all–cause deaths reported to NVSS by week and five age groups (0–17, 18–64, 65–74, 75–84, and ≥85 years) for 50 states and the District of Columbia using a linear time series regression model. To account for the changing patterns in reported deaths from the COVID-19 pandemic, SARS-CoV-2, reported COVID–19 and influenza-coded deaths were subtracted from all–cause deaths first. Weekly expected deaths, assuming no SARS–CoV–2 or influenza virus circulation, and predicted all–cause deaths using the SARS–CoV–2, influenza A virus, and influenza B virus weekly percent positive as covariates were modeled by age group and included the jurisdiction as a random intercept. Influenza–attributable unrecognized deaths were calculated for state and age group by subtracting the expected all–cause deaths from the expected deaths and added to reported deaths to calculate total estimated influenza-attributable deaths. Models will be updated for the 2024/25 season prior to the conference.

Results:

There were 9,756 influenza-coded deaths during the 2022/23 season and 10,374 influenza-coded deaths during the 2023/24 season. We estimated that 43,399 additional influenza-attributable deaths occurred during the 2022/23 season and 42,343 additional influenza-attributable deaths occurred during the 2023/24 season in the United States. The number of deaths varied by state and age group, with more deaths occurring in older age groups and larger states. During both seasons, over 50,000 estimated deaths were related to influenza illnesses, but <20% of these deaths included influenza as a contributing cause of death on the death certificate, indicating a continued need to estimate the influenza death burden.

Conclusions:

Estimating influenza–attributable unrecognized deaths in the context of SARS-CoV-2 and influenza virus circulation at both the national and state-level provides a better understanding of the mortality burden and population subgroups that might be at increased risk of death.

513

Estimating the Annual Influenza-associated Hospitalization Burden by State and Age in the United States Using the 2022-23 and 2023-24 Seasons

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Background:

Historically, estimating influenza hospitalization burden has been done at a national level, but during the SARS-Cov-2 pandemic, it became evident that many states/jurisdictions could benefit from models that estimate the impact of influenza within their populations. Building on work that had been done globally to estimate influenza disease burden, we estimated influenza hospitalizations and deaths by state and age using surveillance data.

Methods:

We extrapolated monthly Influenza Hospitalization Surveillance Network (FluSurv-NET) hospitalization rates to non-FluSurv-NET states after adjusting FluSurv-NET data for clinical testing practices and diagnostic test sensitivities. We used a Poisson zero-inflated model with an over-dispersion parameter within the Bayesian hierarchical framework and accounted for uncertainty and variability between states and across time. We calculated estimates for the 2022-23 and 2023-24 season and present those findings in this abstract. Data for the 2024-25 season, which was a high severity season in the United States are currently being collected and we will re-run our model to include 2024-25 data.

Results:

We estimated 379,300 (90% Credible Interval: 305,400 – 479,300) influenza-associated hospitalizations in the U.S. for the 2022-23 season, and 400,700 (90% Credible Interval: 362,100 – 443,131) influenza-associated hospitalizations for the 2023-24 season. Median cumulative state rates ranged widely in both seasons (from 23.2 to 249.0 per 100,000 people in the 2022-2023 season and from 49.2 to 277.6 per 100,000 people in the 2023-2024 season). Estimates varied by state/jurisdiction depending on population size and other demographics.

Discussion:

Our estimates were comparable to U.S. national burden estimates using other approaches while accounting for variation in timing and geography of disease activity and changes in detection and reporting. These estimates are available by state, month, and age group, and this provides a complementary framework to calculate estimates at finer geographic scale, which can help inform understanding of burden at the state level.

514

VAXY: From Concept to Impact Development and First Insights into a Vaccination App for Registration, Real-Time Monitoring, and Tailored Interventions

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BackgroundIn many countries there is no central vaccination registry. The timing and type of vaccines administered are often unclear or only recorded in hardcopy vaccination booklets. This creates challenges for individuals who may not know their vaccination status when they fall ill either domestically or abroad. It also limits insight for public health officials. With the rise of new platforms such as mRNA and combination vaccines, accurate and accessible vaccine records are becoming increasingly important. At the same time, vaccination rates are declining. Although the causes of vaccine hesitancy have been studied extensively, effective interventions such as education and behavioral nudges must evolve from traditional paper-based systems toward digital solutions.

MethodsTo address this gap, we developed VAXY, a mobile application that allows users to create a personal digital vaccination record. After receiving a vaccine, users can manually register this in the app. VAXY also offers optional educational content and delivers reminders through push notifications. A live dashboard visualizes vaccine uptake trends at population level.

ResultsVAXY currently has over 175 thousand users. The most frequently registered vaccines are COVID-19 vaccines by Pfizer, Moderna, and AstraZeneca, showing that the platform was initially adopted largely due to the pandemic. These are followed by DTP and Hepatitis A. The average user age, excluding children, is 44. Over the past year, routine vaccinations such as influenza and COVID-19 were registered way more more frequently than travel-related vaccines, with 7,169 and 3,718 entries respectively. This suggests a discrepancy in user behavior. Furthermore, many users consistently log routine vaccines, but those registering travel vaccinations often do not include relevant respiratory vaccines. Additionally, 8.9 percent of users use the app to manage vaccinations for family members. So far, 15 percent have opted to receive reminders for booster doses, showing openness to long-term planning and digital nudging.

ConclusionVAXY is emerging as a widely accepted digital alternative to paper-based vaccination documentation. Its features for nudging and education appear promising, although further refinement is needed. Dashboard data reveal two distinct user patterns. One group registers routine vaccinations recommended by the national immunization program, such as influenza, and pneumococcal vaccines, but rarely logs travel-related vaccines. Another group registers travel vaccines such as hepatitis A, yellow fever, and typhoid with great consistency, yet shows little to no registration of respiratory vaccines. This suggests a conceptual separation in how users perceive travel versus routine immunizations, despite the fact that respiratory pathogens like SARS-CoV-2 and influenza are more frequently encountered during travel than diseases such as polio or yellow fever. This raises the question whether the traditional divide between travel medicine and national immunization remains appropriate. VAXY provides a scalable platform to bridge this gap and to support more integrated, risk-based vaccination approaches across populations.

515

ECaS

RSV Vaccination in the Netherlands: High Acceptance Among Elderly, High-Risk Patients, and Healthcare Providers

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Background

Respiratory Syncytial Virus (RSV) infection has a high burden of disease, particularly in children, elderly and immunocompromised. In the Netherlands, RSV vaccination has not yet been included in the national vaccination program for elderly and high-risk groups. Moreover, it is unknown what the acceptance or uptake of these groups are for theses vaccines, and how healthcare providers perceive and recommend it.

Methods

We conducted a cross-sectional survey in the Netherlands among participants with an indication for influenza vaccination (60 years and older, or younger with co-morbidities) and healthcare professionals. Participants 60 years and older were mainly recruited via a 50-plus fair; patients with a co-morbidity were mainly recruited via flyers in the hospital and via email. Healthcare providers (working in- and outside the hospital) were recruited via email, flyers and newsletters. The survey assessed participants willingness and motivation to receive a RSV vaccine and healthcare providers willingness and motivation to recommend it to high risk populations.

Results

A total of 1159 participants with a median age of 62 years (IQR 15) completed the survey, most of them were women (61.9%). 490 (42.3%, %) reported at least one co-morbidity and 190 (16.4%) reported 2 or more. Most reported were diabetes, low immune system, lung disease and heart disease. Among participants 71.5% reported routinely receiving influenza vaccination, 61.5% COVID-19 vaccination and 70.4% received a pneumococcal vaccination. The overall acceptability of RSV vaccination was high, with 78.7 % of respondents indicating they would (likely) accept a RSV vaccination. After adjusting for confounders, several participant characteristics were found to be associated with attitudes toward RSV vaccination. Factors such as sex, age, prior vaccination behaviour (e.g., influenza vaccination), presence of co-morbidities, and familiarity with RSV appeared to influence individuals' views regarding RSV vaccination. A substantial proportion (47.7%) had no or very little knowledge regarding RSV. Most participants (71%) preferred a combination vaccine, such as one including influenza, COVID-19, and RSV, over separate vaccines.

In addition, 201 healthcare providers completed the questionnaire. Of these, 97% indicated they would likely recommend the RSV vaccine to specific risk groups; 81% would likely receive it themselves.

Conclusions

Most patients and healthcare providers have a positive attitude toward RSV vaccination. However, many patients lack knowledge about RSV. Increasing awareness and understanding of the virus may further enhance vaccine acceptance.

517

Use of COVID-19 antivirals among older adults, by age group and season — Truveta, United States, June 1, 2023–May 31, 2025

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Background: SARS-CoV-2 circulates year-round and typically causes surges in the summer and fall/winter in the U.S. Older adults aged ≥65 years have the highest rates of COVID-19–related hospitalization and death. Despite the proven benefit of COVID-19 antivirals, data suggest that treatment uptake is low, likely due low perception of risk. To date, there have been no studies reporting the uptake of COVID-19 treatment by season. We examined antiviral use among older adults by season to understand variations in treatment uptake.

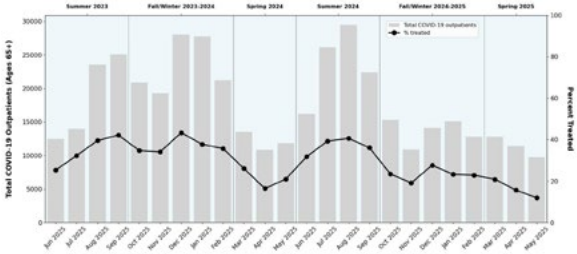
Methods: Outpatients aged ≥65 years with COVID-19 during June 1,2023–May 31,2025 were identified in the Truveta dataset, which covers >120 million patients receiving care at >20,000 clinics and 900 hospitals in the U.S. The main outcome was receipt of COVID-19 antivirals (prescription of nirmatrelvir/ritonavir, remdesivir, or molnupiravir dispensed) within 5–7 days of COVID-19 diagnosis or SARS-CoV-2 positive test. Characteristics of COVID-19 cases were described by age (65-69;70-74;75-79;80-84;85-89;90+), sex, race, vaccination status, co-morbidities, and COVID-19 antiviral use for each season: summer 2023 (June 1–Sept 30, 2023), fall/winter 2023–2024 (Oct 1, 2023–February 29, 2024), spring 2024 (March 1–May 31, 2024), summer 2024 (June 1–Sept 30, 2024), fall/winter 2024–2025 (October 1, 2024–February 28, 2025), and spring 2025 (March1–May 31, 2025). Percent of cases treated was assessed by season. Multivariable logistic regression was used to estimate the association between characteristics and receipt of antiviral treatment. All analyses were conducted using R software (version 4.2.1; The R Foundation).

Results: Of older adults with COVID-19, 19–35% were treated with an antiviral across seasons. We observed lower rates of treatment in seasons with relatively lower COVID-19 case counts compared with seasons with higher COVID-19 case counts (Figure). Among persons who received treatment, >99% received treatment within 7 days of COVID-19 diagnosis and 80% received nirmatrelvir/ritonavir. In adjusted analyses, there was no difference in receipt of treatment by age group. Native Hawaiians/Pacific Islanders (odds ratio [OR]=0.78; 95% confidence interval [CI]: 0.64–0.95), persons in rural communities (0.81 [0.78–0.83]), and with co-morbidities (one: 0.78 [0.76–0.8]; two: 0.73 [0.71–0.75]; three 0.71 [0.74–0.78]) had lower odds of receiving treatment. Persons who received one or two COVID-19 vaccine doses had higher odds of receiving treatment (1.57 [1.54–1.61]).

Conclusions: Antivirals are underutilized among adults ≥65 years and varies by season. Increased perception of risk especially during periods of lower SARS CoV-2 circulation might increase use antivirals and prevent significant morbidity.

Figure. Proportion of outpatient adults aged 65 years and older with COVID-19 who received treatment, by season — Truveta, United States June 1, 2023–May 31, 2025

Note: Seasons are defined as: summer 2023 (June 1–Sept 30, 2023), fall/winter 2023–2024 (Oct 1, 2023–February 29, 2024), spring 2024 (March 1–May 31, 2024), summer 2024 (June 1–Sept 30, 2024), fall/winter 2024–2025 (October 1, 2024–February 28, 2025), and spring 2025 (March1–May 31, 2025).



518

Delayed access to COVID-19 vaccination among migrants in Spain during 2021: an electronic health-records-based study

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Background: Structural factors may pose barriers to access vaccination for migrant populations. In Spain, COVID-19 vaccination started on 27 December 2020 prioritizing people in long-term care facilities (LTCF), the elderly and healthcare and other essential workers (HC&OEW). The campaign was subsequently rolled out by descending age. We assessed whether time to first COVID-19 vaccination varied by country of birth in Spain.

Methods: We studied individuals ≥12 years-old registered within the Spanish public healthcare administrative database between 27-December-2020 and 31-December-2021, not living in LTCF, with information on country of birth, and at least one COVID-19 vaccine dose in the vaccination registry. Using Weibull regression parametric survival, we compared time to first vaccination between Spanish-born and people born in: Western Europe and other Western Countries (WC), Eastern Europe (EE), North Africa and the Middle East (NAME), Sub-Saharan Africa (SSA), Southeast Asia (SEA), Central America & Caribbean (CAC) or South America (SA). We estimated Event Time Ratio (ETR) and Hazard Ratios (HR) adjusting for region, sex, age, autonomous region and population group (severe comorbidities, HC&OEW, or general population).

Results: We included 26,517,009 individuals (Spanish-born: 86.3%; WC: 3.4%; EE: 0.4%; SSA: 0.5%; NAME: 2.1%; SEA: 0.6%; CAC: 1.4%; SA:5.3%), 51.8% were females and 6.9%, 45.6%, 17.4%, 13.4%, 10% and 6.8% were aged 12-17, 18-49, 50-59, 60-69, 70-79 and ≥80 years, respectively. The ETR and HR are presented without 95% confidence intervals, as entire population was analyzed and the differences between the intervals were less than one percentage point. Adjusted ETR for vaccination was higher for migrant groups compared to Spanish-born. ETR by regions was for SA: 1.07; WC, SEA and CAC: 1.10; NAME: 1.14; SSA: 1.18; EE: 1.21. Corresponding median time to vaccination was higher among migrant groups, SA: 156 days, WC: 160 days, SEA: 160 days, CAC: 161 days, NAME: 165 days, SSA: 172 days and EE: 175 days compared to Spanish-born: 145 days. Probability of accessing the first vaccination was consistently lower for all migrant groups with maximum delay of up to 30 days (EE) in vaccination timing. HR, compared to Spanish-born, was for SA: 0.78, WC: 0.71, SEA: 0.71, CAC: 0.70, NAME: 0.63, SSA: 0.55, EE: 0.51. Slight variations in these estimates were found across age groups. Classifying individuals with unknown country of birth (n= 26,358,420) as Spanish-born did not significantly change our results.

Conclusions: Migrants of any origin living in Spain accessed COVID-19 vaccination later compared to Spanish-born population. Previous studies had shown that uptake of the vaccine is lower among migrants However, this delayed vaccination highlights existing barriers that may have negatively impacted migrants health and calls for additional efforts to ensure equitable access, as well as for future research aimed at understanding these barriers.

519

Effectiveness of nirsevimab reimmunization in the second respiratory season in high-risk children, Spain, October 2024-March 2025

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Background

Nirsevimab is an extended half-life monoclonal antibody against respiratory syncytial virus (RSV) first used in Spain during the 2023-24 season for immunization of children aged <6 months. We estimated real-life effectiveness against RSV hospitalization of reimmunization with nirsevimab in the second RSV season for children previously immunized during their first season who continued to be at risk due to comorbidities or prematurity.

Methods

A previously conducted population-based case-control study in 16 Spanish regions matched cases born April 2023 through March 2024, first hospitalized for severe acute respiratory infection and RSV PCR-positive, with four controls born on the same date (±2 days) and province. For the current analysis, we further selected cases and controls who received nirsevimab during 2023-24 and were eligible for reimmunization at the start of the 2024-25 campaign (mostly October 1, 2024), either because they had prevalent comorbidities or were early pre-term infants (<35 gestation weeks) aged under 12 months. We ignored the original matched-sets and re-matched cases with up to four controls born within one month and whose original matching date was later and closest to the case date. Causal per-protocol effectiveness of nirsevimab reimmunization within the first 30 days of the 2024-25 immunization campaign onset was estimated using target trial emulation. We assigned clones of cases and controls to either reimmunization or no reimmunization and censored them when they deviated from the assigned intervention. A conditional logistic regression was fit on the reimmunization assigned to uncensored clones of cases and controls, using inverse probability weighting to account for sex, birthweight (< or ≥2,500 g) and baseline comorbidities. Effectiveness was (1–Odds Ratio)x100.

Results

Of 560 cases and 2,240 controls matched during the 2024-25 season in the original study, 26 cases (4.6%) and 63 controls (2.8%) were eligible for the current analysis. We were able to re-match 22 cases with 72 controls (37 unique individuals) born within one month and whose original matching date was closest after the case date (median [IQR] of 12 [4–26] days). Seven cases (32%) and 42 controls (58%) were reimmunized. Estimated causal per-protocol effectiveness was 42.2% (conservative 95% confidence interval: -24.9% to 73.3%). The point estimate was similar in a pragmatic approach that classified reimmunization as observed at the matching date, with standard adjustment of a conditional logistic regression [effectiveness 42.0% (95%CI: - 1.06% to 83.6%)].

Conclusions

Despite wide confidence intervals and potential unmeasured or residual confounding, particularly by region in Spain, our results suggest that a second dose of nirsevimab at the onset of the second RSV season may be moderately effective in preventing severe RSV infection in high-risk children who have been previously immunized during their first season.

520

Strengthening Influenza Vaccination Strategies through Gamified Modelling: Let's Control Flu tool expansion to Germany and Czech Republic

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Background: Seasonal influenza represents a significant worldwide public health challenge, particularly among vulnerable populations. Despite recommendations, vaccination coverage rates (VCR) across Europe remain below the 75% target established by WHO and reiterated by ECDC. The "Let's Control Flu" (LCF) project developed an interactive, gamified modelling tool to support decision-making on public health policies (PHPs) for influenza vaccination. Following a successful pilot in Sweden, the LCF tool has now been adapted and validated for two additional European countries: Germany and Czechia.

Methods: The LCF tool allows users to simulate the implementation of 13 PHPs over a ten-year period. For Germany and Czechia, national demographic and epidemiological data were integrated to allow simulations between 2026 and 2035, enabling projections of outcomes across five target populations: older adults, pregnant women, children, healthcare professionals, and individuals aged <65 years with at least one chronic condition. Model outputs include key influenza related indicators, such as VCR, infection cases, hospital admissions, general practitioner (GP) consultations, deaths, productivity losses, and cardiovascular events. Users can test policy combinations and adjust their intensity to simulate different real-world scenarios.

Results: The LCF model application to these new countries, similarly to Sweden, indicate that the full implementation of all 13 policies could lead to significant increases in influenza VCR and substantially reduce the burden of disease. The models were validated by the countries' National Advisory Boards, to ensure the applicability to the national reality based on available data and experience. These results reinforce the tool's flexibility and applicability to different national contexts, supporting its relevance for national planning to optimize influenza vaccination planning.

Conclusions: The expansion of the LCF model to other countries, having distinct health landscapes, highlights its value as a scalable tool with real-world applicability. By combining evidence-based modelling with a gamified, user-friendly design, the tool makes complex policy planning more accessible for all - from policymakers and public health authorities to patient advocates and other stakeholders. This approach can contribute to improving seasonal vaccination uptake, guiding VCR strategies, preparedness planning, and addressing persistent challenges such as vaccine hesitancy and unequal policy implementation.

521

Burden of severe disease due to influenza, SARS-CoV-2 and RSV in Spain during the 2024-25 winter season

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Background

Estimating the burden of influenza, SARS-CoV-2 and RSV is essential for prioritization of resources. We estimated age-specific rates and total number of hospital admissions, intensive care unit admissions (ICU) and in-hospital mortality due to each virus in Spain between weeks 40/2024 and 20/2025.

Methods

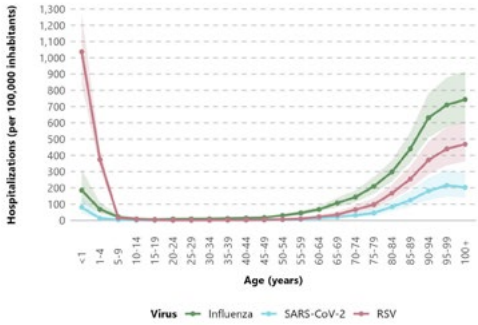
Patients hospitalized due to severe acute respiratory infection (SARI) and systematically tested for influenza, SARS-CoV-2 and RSV in 48 sentinel hospitals within the Spanish SARI surveillance system (SiVIRA) were included. Generalized additive models were fit to the pathogen-specific test positivity by week, age, sex and region for a more stable prediction of stratum specific positivity and 95% confidence intervals (95%CI). Estimated positivity was applied to stratum-specific SARI rates to estimate pathogen-specific hospitalization rate, and then adjusted to the distribution of the Spanish population by age, sex and region. Proportion with ICU or death for each pathogen and strata were further applied to derive number and rate of ICU admissions and mortality.

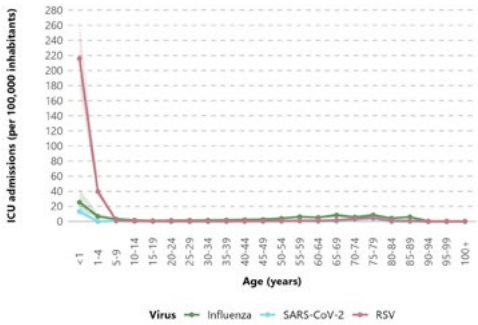
Results

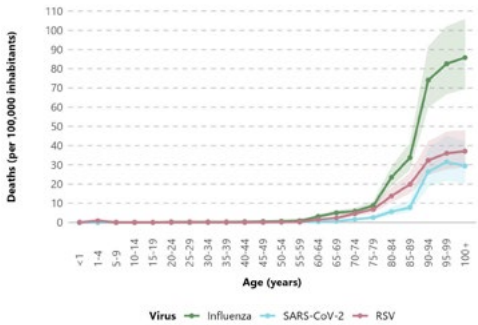
We estimated 33,131 (95%CI: 25,743-42,848) hospitalizations due to influenza, 7,732 (4,986-12,057) to SARS-CoV-2 and 22,885 (17,398-30,510) to RSV, corresponding to rates of 67.5 (52.5-87.3), 15.8 (10.2-24.6) and 46.6 (35.4-62.2) per 100,000 inhabitants, respectively for each virus. Hospitalization was highest for individuals aged ≥60 years, particularly ≥70 years, and children <5 years, particularly <1 year, while ICU admissions concentrated in younger age-groups and mortality in the elderly. Individuals aged ≥60 years represented 77%, 81% and 55% of all hospitalizations, 46%, 53% and 16% of ICU admissions and 94%, 96% and 95% of deaths, for influenza, SARS-CoV-2 and RSV, respectively.

Conclusion

The estimated burden of sever disease was highest for influenza, followed by RSV and SARS-CoV-2. The burden was concentrated in children and the elderly, supporting the current immunization programs and providing additional information that can help establish the optimal age cut-off for vaccine recommendations.


Hospitalizations (per 100,000 inhabitants)
1,300
1,200
1,100
1,000
900
800
700
600
500
400
300
200
100
0
<1
1-4
5-9
10-14
15-19
20-24
25-29
30-34
35-39
40-44
45-49
50-54
55-59
60-64
65-69
70-74
75-79
80-84
85-89
90-94
95-99
100+
Age (years)
Virus
Influenza
SARS-CoV-2
RSV


ICU admissions (per 100,000 inhabitants)
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
<1
1-4
5-9
10-14
15-19
20-24
25-29
30-34
35-39
40-44
45-49
50-54
55-59
60-64
65-69
70-74
75-79
80-84
85-89
90-94
95-99
100+
Age (years)
Virus
Influenza
SARS-CoV-2
RSV


Deaths (per 100,000 inhabitants)
110
100
90
80
70
60
50
40
30
20
10
0
<1
1-4
5-9
10-14
15-19
20-24
25-29
30-34
35-39
40-44
45-49
50-54
55-59
60-64
65-69
70-74
75-79
80-84
85-89
90-94
95-99
100+
Age (years)
Virus
Influenza
SARS-CoV-2
RSV

523

Comparative Evaluation of Airborne Virus Shedding in Ferrets Infected with Diverse Influenza Viruses

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Centers for Disease Control and Prevention, United States of America

Background: Recent cases of highly pathogenic avian influenza A(H5N1) viruses in North America, particularly those associated with poultry and cattle, have highlighted the urgent need to evaluate the pandemic potential of emerging clade 2.3.4.4b strains. The ferret transmission model is routinely used for risk assessments. However, concurrent measurement of viral load in the air is typically not included as a component of such studies but is a critical readout in understanding the potential for airborne transmission.

Methods: To address this knowledge gap and gain insights into the relationship between viral replication and transmission potential, we conducted aerosol sampling using two air collection platforms (BC251 and SPOT) from ferrets inoculated with nine influenza A viruses, representative of A(H5N1), A(H9N2), A(H7N9), and A(H1N1)pdm09 subtypes. Subsequently, we performed comparative statistical and logistic regression analyses.

Results: Through direct comparison of two air sampling platforms, we found that each sampler offers distinct advantages. The SPOT sampler, which uses water-based condensation capture, exhibited better retention of infectious virus, while the BC251 sampler, which collects particles onto dry surfaces, showed lower viability retention but achieved greater overall detection sensitivity, especially at low airborne virus concentrations. Our quantitative analysis of nasal wash and air samples post-inoculation revealed a strong correlation between viral loads in the respiratory tract and airborne virus shedding, especially for viruses known to transmit efficiently among ferrets. Interestingly, among non-transmissible A(H5N1) viruses, B3.13 genotype strains were shed into the air at higher levels, suggesting a higher overall airborne transmission potential compared to D1.1 genotype strains.

Conclusions: These data underscore the importance of integrating quantitative measurements of airborne virus shedding with observed transmission outcomes to enhance pandemic risk assessments. This methodology can aid in identifying zoonotic influenza viruses that may be adapting and evolving toward mammalian transmissibility, even in the absence of detectable airborne spread.

524

A Safety and Immunogenicity Study of Novavax's COVID-Influenza Combination and Stand-alone Influenza Vaccines in Adults 65 years and Older

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Novavax, Inc, United States of America

Using its recombinant protein nanoparticle and Matrix-M® adjuvant technology platform, Novavax has developed both a COVID-19 Influenza Combination vaccine (CIC) and a trivalent nanoparticle influenza hemagglutinin vaccine (tNIV). Here, we report on an ongoing, randomized, observer-blinded trial (CIC-E-301) to evaluate the safety and immunogenicity of these vaccines in adults ≥65 years.

1985 participants in Australia and New Zealand received a single dose of either CIC, Novavax COVID-19 vaccine Nuvaxovid™ (JN.1), tNIV, or Fluzone® High-Dose in a 3:2:1:3 ratio, respectively. The tolerability and safety of CIC and tNIV were evaluated by assessing reactogenicity for 7 days post-dose and unsolicited adverse events (AEs) through Day 28. Immunogenicity was also assessed, with sera collected 28 days post-dose and analyzed for vaccine-homologous influenza A and B strain neutralizing antibody (NAb) responses and SARS-CoV-2 NAb responses to the JN.1 strain of SARS-CoV-2. CIC immune responses were compared to Nuvaxovid and Fluzone High-Dose; tNIV was compared to Fluzone High-Dose. The analyses were descriptive, with no prespecified statistical hypotheses.

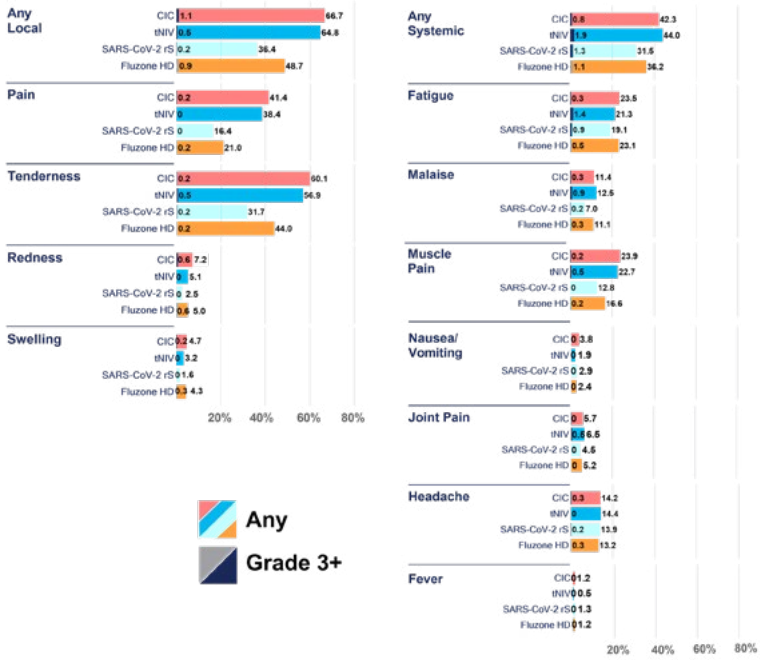
Demographic and baseline characteristics were balanced across vaccine groups. Overall, the median age of participants was 71 years, 54.5% male, 91.2% White, and median time since last COVID-19 vaccination of 46.7 weeks.

A single dose of CIC or tNIV has an acceptable safety and tolerability profile. Solicited local and systemic reactogenicity AEs, respectively, were reported more frequently following CIC (66.7%, 42.3%) and tNIV (64.8%, 44.0%) than Nuvaxovid (36.4%, 31.5%) and Fluzone High-Dose (48.7%, 36.2%) (Figure 1). Most (>98%) reactogenicity events were mild/moderate (~1% Grade 3), including local tenderness and pain, muscle pain, and fatigue. Unsolicited TEAEs, including related and/or severe TEAEs, MAAEs, and SAEs (reported for 0.5–1.4%) were comparable across vaccine groups. No AESIs were reported for CIC or Nuvaxovid, 1 (0.5%) for tNIV, and 3 (0.5%) for Fluzone High-Dose. There were no events of myocarditis/pericarditis or death.

Both CIC and tNIV induced robust humoral and cellular immune responses across all antigens tested. Neutralizing antibody responses increased 2.4–5.7-fold over baseline (Figure 2) and were similar to those seen with Fluzone High-Dose and Nuvaxovid (JN.1).

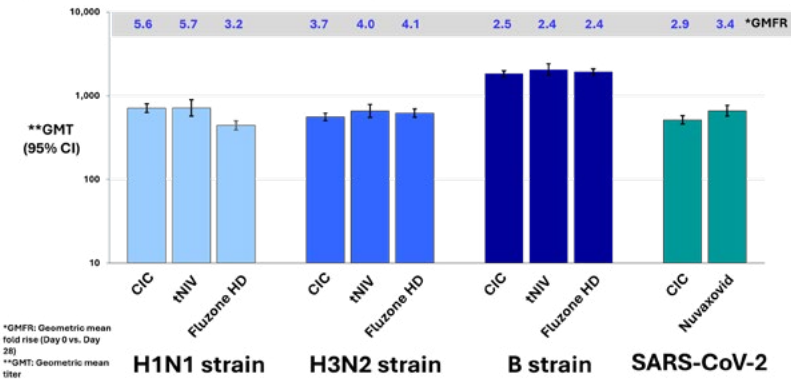
Both CIC and tNIV induced robust immune responses and were well tolerated.

Figure 1. Solicited local and systemic reactogenic events: within 7 days post-vaccination



Note: Data are presented as the percent of participants reporting solicited reactogenic events.

Figure 2. Day 28 neutralizing antibody responses following administration of CIC, tNIV, Fluzone HD, and Nuvaxovid vaccines



526

Incidence of laboratory-confirmed RSV infections in adults ≥60 years old attended in primary care. A three-seasons study.

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Background

The impact of Respiratory Syncytial Virus (RSV) in older adults is not well known. This study examines the incidence of lab-confirmed RSV in ≥60 years old (yo) managed in primary care over three consecutive seasons.

Methods

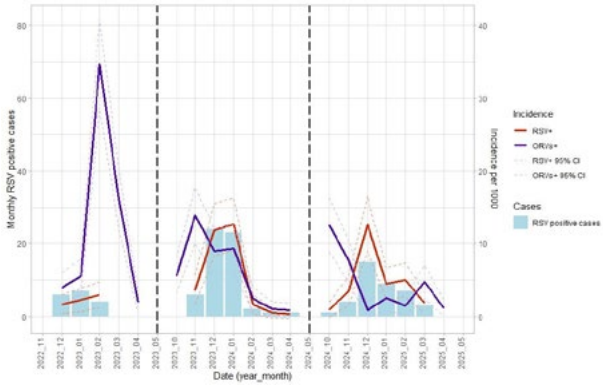
We conducted a prospective study analyzing the proportion of RSV and Other Respiratory Viruses (ORVs)detections among Acute Respiratory Infections (ARI) and the seasonal and monthly incidence of lab-confirmed RSV infections in three primary care settings of Valladolid (Spain) between seasons 2022-23 and 2024-25. These primary care sites covered 3,587 patients ≥60 yo respectively in 2022, 3,544 patients in 2023, and 3,477 patients in 2024. Patients with an Acute Respiratory Infection (ARI) who were attended by the clinicians participating in the study were sampled, and a multiplex Point-of-Care RT-PCR (FilmArray, Biomerieux) against multiple respiratory viruses was performed. The monthly incidence was calculated for RSV and non-RSV detections (ORVs) following the following formula: [Total ARI positive for RSV/(Total ARI enrolled/Total ARI eligible)]/number of persons covered by the three clinical sites (X 1,000).

Results

During the three seasons analyzed, a total of 973 patients with an ARI were screened (346 in 2022-23, 372 in 2023-24, and 255 in 2024-25). Of these, 477 agreed to participate in the study and consented to RT-PCR testing. Then, 113 (23.9%) had a lab-confirmed RSV infection, 245 (51.6%) had ORVs, and 116 (24.5%) were negative for any virus. RSV represents 31.6% (113 out of 358) of the total viral detections in the patients who tested positive for any respiratory virus. The incidence for RSV infection was 8.4 cases/1,000 inhabitants in season 2022-2023, 34.9 cases/1,000 inhabitants in 2023-2024, and 29.2 cases/1,000 inhabitants in 2024-2025. The incidence of ORVs was 59.4 cases/1,000 inhabitants in season 2022-2023, 50.3 cases/1,000 inhabitants in 2023-2024, and 32.2 cases/1,000 inhabitants in 2024-2025. RSV detections peaked in the month of February in the 2022-23 season (2.9 cases/1,000 inhabitants), in January in 2023-24 (12.6 cases/1,000 inhabitants) and in December of 2024-25 seasons (12.7 cases/1,000 inhabitants)(Figure 1).

Conclusion

Nearly three out of ten of the viral infections managed in primary care during the winter months in the ≥60-year-old group are caused by RSV. The incidence of RSV in primary care peaks during December and February. Our results show a substantial burden of RSV and ORVs on primary care.


Monthly RSV positive cases
Incidence per 1000
Date (year_month)
Incidence
RSI/+
OR/s+
RSI/+ 95% CI
OR/s+ 95% CI
Cases
RSV positive cases

527

Effectiveness of High-Dose vs Standard-Dose Influenza Vaccine in Adults Aged 65–79 Years: Primary and Bayesian Results from the GALFLU Pragmatic Individually Randomized Trial in Galicia, Spain

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Background: High-dose inactivated influenza vaccine (HD-IIV) has shown superior immunogenicity and efficacy over standard-dose (SD-IIV) in older adults. The GALFLU trial, a pragmatic randomized controlled trial embedded within the public health system, compared HD-IIV to SD-IIV in adults aged 65–79 years across two influenza seasons in Galicia, Spain. Here we present both the primary effectiveness results and a pre-specified Bayesian reanalysis to quantify the probability of clinically meaningful benefits.

Methods: This registry-based, open-label, 1:1 randomized trial enrolled 134,476 participants during the 2023/24 and 2024/25 influenza seasons. The primary outcome was hospitalization for influenza or pneumonia. Secondary endpoints included hospitalization for cardiorespiratory disease, all-cause hospitalization, mortality, and exploratory endpoint, laboratory-confirmed influenza (LCI) hospitalization. Bayesian log-binomial regression using non-informative, evidence-based, and skeptical priors estimated posterior probabilities of benefit.

Results: A total of 134,376 older adults were individually randomized to HD-IIV (67,093) or SD-IIV (66,789); baseline characteristics were balanced across both groups. Hospitalization rate for influenza or pneumonia was lower in the HD-IIV arm (N=174, 0.26%) vs in the SD-IIV arm (N=227, 0.34%) (rVE 23.7%, 95% CI: 6.6–37.7, Table 1). HD-IIV was also associated with reduced hospitalization for cardiorespiratory disease (rVE 8.4%, 95% CI: 0.1-16.1) and LCI (rVE 2%, 95% CI: 6.6–37.7). Bayesian analyses confirmed >99% probability of any benefit (i.e. rVE >0%) and >92% probability that rVE exceeded 10%. For cardiorespiratory hospitalization, rates were 1.47% (HD-IIV) vs 1.60% (SD-IIV) (rVE 8.4%, 95% CI: 0.1–16.1), with >97% Bayesian probability of benefit. Reductions in all-cause hospitalization, all-cause mortality and lab-confirmed influenza also favored HD-IIV (Table 1). No safety signals emerged.

Conclusions: HD-IIV reduced the risk of hospitalization for influenza or pneumonia, and cardio-respiratory disease compared to SD-IIV, with consistent evidence across both classical and further reinforced by high probabilities of clinically meaningful benefit in Bayesian analyses. These findings support the clinical benefit of HD-IIV, highlighting its potential for improved protection among older adults and value in public health programs.

Funding: The GALFLU study (EU CT number: 2023-506977-36-00) was an externally sponsored collaboration funded by Sanofi through a research grant to the Healthcare Research Institute of Santiago.

Trial registration
Clinicaltrials.gov: NCT06141655, submitted Oct 23, 2023. https://clinicaltrials.gov/study/NCT06141655.

Table 1: rVE of HD-IIV vs SD-IIV against severe outcomes in older adults

Outcome	HD-IIV (N=67,093 n (rate)	SD-IIV (N=66,789) n (rate)	rVE (95% CIs)	Bayesian probability any benefit* (rVE>0%)	Bayesian probability rVE>5%*	Bayesian probability rVE>10%*
Influenza or pneumonia hospitalizations (primary)	174 (0.26%)	227 (0.34%)	23.7% (6.6; 37.7)	99.6-100%	97.8-100%	92.7-99.8%
Cardiorespiratory hospitalization (Secondary)	985 (1.47%)	1071 (1.60)	8.4% (0.1; 16.1)	97.7-100%	77.6-97.4%	33.2-69.2%
All-cause hospitalization (Secondary	4336 (6.46)	4427 (6.63%)	2.5% (-1.7; 6.5)	89-100%	10.2-67.7%	0%
All-cause mortality (Secondary)	305 (0.45%)	348 (0.52%)	12.8% (-2.0; 25.4)	88.9-95.9%	4.8-86.4	0-64.8%
Laboratory-confirmed influenza hospitalization (Exploratory)	72 (0.11%)	89 (0.13%)	19.5% (-11.1; 41.8)	88.5-100%	79.7-99.8%	67.4-80.3%

* Range across the 3 priors (non-informative, evidence-based, skeptical). The non-informative prior was centered at rVE = 0 with a standard deviation of 100 on the log relative risk scale. The skeptical prior was also centered at 0 with a standard deviation of 0.0312, corresponding to a 10% prior probability of rVE ≥ 5%. Evidence-based priors were informed by published studies and specified per outcome: hospitalization for pneumonia/influenza (rVE 23.5%, 95% CI: 12.3–33.2%), cardiorespiratory hospitalization (18.2%, 95% CI: 6.8–28.1%), laboratory-confirmed influenza hospitalization (11.7%, 95% CI: 7.0–16.1%), all-cause hospitalization (7.3%, 95% CI: 4.5–10.0%), and all-cause mortality (1.6%, 95% CI: −2.0 to 5.0%).

529

ECaS

Clinical Impact of Acute Respiratory Virus Infections in Dutch Nursing Homes

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Background:

Nursing home (NH) residents are at risk for severe acute respiratory virus infections (ARVIs). Whereas increased morbidity and mortality has been reported for influenza viruses and SARS-CoV-2, the impact of other respiratory viruses remains unclear. This study investigates the relative clinical impact of respiratory viruses in Dutch NH residents.

Methods: Fifteen NHs in the Amsterdam region participated in a prospective cohort study (March 2023-June 2025). Residents with suspected ARVIs were enrolled based on clinical judgement of the physician. Clinical data on disease severity, recovery, mortality, and complications were collected on days 0, 14, and 28. Residual material from routinely collected combined nasopharyngeal and oropharyngeal swabs was tested for a broad range of respiratory viruses using multiplex RT-PCR. A Bayesian hierarchical model including seasonal and NH level random effects was used to estimate suspected ARVI risk. Fisher's exact tests and odds ratios were used to compare the relative clinical outcomes of each virus.

Results:

Among 3,195 residents, 526 suspected ARVI episodes were reported in 456 individuals (**Figure 1**). The estimated posterior suspected ARVI risk is 10.6% (95% credible interval: 2.2%-47.2%). NHs with fewer psychogeriatric residents had significantly lower risk levels ($p<0.001$). RT-PCR testing was performed in 420 episodes (79.8%) of which 291 (69.3%) tested positive for a respiratory virus. Although SARS-CoV-2 and influenza A virus were detected most frequently (89/291; 30.6% and 71/291; 24.4%, respectively), other respiratory viruses were identified in almost half of the episodes, including rhino/enterovirus (51/291; 17.5%), RSV (32/291; 11.0%), hMPV (19/291; 6.5%), PIV (15/291; 5.2%), and hCoV (14/291; 4.8%). The odds of increased bed hours during an ARVI episode among residents infected with hMPV was 3.93 (95% CI 1.45-11.85) times larger relative to residents infected by other viruses (**Table 1**). Additionally, hMPV-cases had the highest odds of moderate or severe disease (OR 2.15; 95% CI 0.83-6.04) and showed a trend towards delayed recovery between days 14 and 28 (OR 0.49; 95% CI 0.18-1.49). Residents with influenza A were half as likely to recover on day 14 (OR 0.52; 95% CI 0.28-0.97) and three times more likely to develop secondary bacterial pneumonia (OR 3.01; 95% CI 1.13-7.95). SARS-CoV-2 cases recovered more often on day 14 (OR 1.77; 95% CI (0.93-3.54) and tended to spend fewer additional hours in bed during their ARVI episode (OR 0.56; 95% CI 0.31-0.99).

Conclusions:

NH ARVI risks were higher in psychogeriatric wards, warranting further investigation into contributing factors. Influenza A virus remained as a major contributor to the impact of ARVIs in this setting, while the burden of SARS-CoV-2 in this largely vaccinated post-pandemic cohort appeared limited. In addition to influenza, our data suggests a substantial clinical impact of hMPV, indicating the need to consider diagnostic, preventive, and therapeutic strategies against hMPV within this population.

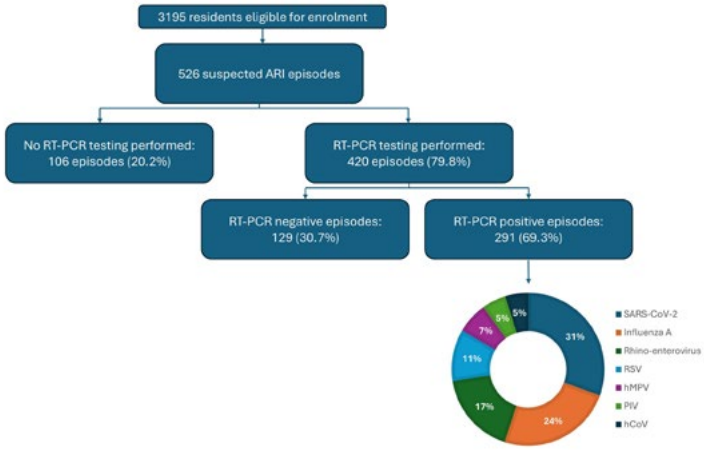

Table 1: clinical impact outcome measures in PCR-positive residents (n=291)

	14-day recovery			28-day recovery			14-day mortality			28-day mortality			Moderate/severe disease[a]		
	no./total no. (%)	*OR (95% CI)*	*p-value*	*no./total no. (%)*	*OR (95% CI)*	*p-value*	*no./total no. (%)*	*OR (95% CI)*	*p-value*	*no./total no. (%)*	*OR (95% CI)*	*p-value*	*no./total no. (%)*	*OR (95% CI)*	*p-value*
Influenza A	45/67 (67.2)	0.52 (0.28-0.97)	0.05	51/64 (79.7)	0.93 (0.47-1.95)	0.86	9/67 (13.4)	1.92 (0.77-4.53)	0.15	11/64 (17.2)	1.41 (0.63-2.99)	0.41	29/68 (42.7)	0.87 (0.50-1.50)	0.68
SARS-CoV-2	70/84 (83.3)	1.77 (0.93-3.54)	0.09	67/82 (81.7)	1.12 (0.58-2.22)	0.87	6/84 (7.1)	0.73 (0.25-1.82)	0.65	10/83 (12.1)	0.81 (0.35-1.70)	0.70	34/85 (40.0)	0.73 (0.43-1.23)	0.24
RSV	21/31 (67.7)	0.60 (0.27-1.41)	0.26	24/31 (77.4)	0.80 (0.33-2.15)	0.63	1/31 (3.2)	0.35 (0.01-1.78)	0.33	3/31 (9.7)	0.66 (0.15-2.03)	0.59	18/31 (58.1)	1.77 (0.83-3.87)	0.18
Rhino/enterovirus	37/46 (80.4)	1.29 (0.61-3.03)	0.57	39/47 (83.0)	1.21 (0.55-2.98)	0.69	4/50 (8.0)	0.89 (0.24-2.50)	1	7/49 (14.3)	1.06 (0.40-2.46)	1	24/49 (49.0)	1.19 (0.64-2.22)	0.64
hMPV	14/17 (82.4)	1.40 (0.42-6.49)	0.77	13/19 (68.4)	0.49 (0.18-1.49)	0.22	2/19 (10.5)	1.29 (0.18-4.97)	0.68	3/19 (15.8)	1.22 (0.26-3.96)	0.73	12/19 (63.2)	2.15 (0.83-6.04)	0.15
PIV 1-4	11/15 (73.3)	0.81 (0.26-3.11)	0.76	12/15 (80.0)	0.94 (0.28-4.43)	1	2/15 (13.3)	1.71 (0.23-6.83)	0.63	3/15 (20.0)	1.65 (0.35-5.62)	0.45	5/15 (33.3)	0.60 (0.18-1.75)	0.43
hCoV	12/14 (85.7)	1.77 (0.46-12.64)	0.53	12/13 (92.3)	2.68 (0.50-66.63)	0.47	1/14 (7.1)	0.87 (0.03-4.74)	1	1/13 (7.7)	0.57 (0.02-3.06)	1	5/13 (38.5)	0.75 (0.22-2.36)	0.78

	Increased bed hours[b]			Exacerbation underlying diseases[c]			Secondary bacterial pneumonia[d]			Oxygen requirement days 0-28[e]		
	no./total no. (%)	*OR (95% CI)*	*p-value*	*no./total no. (%)*	*OR (95% CI)*	*p-value*	*no./total no. (%)*	*OR (95% CI)*	*p-value*	*no./total no. (%)*	*OR (95% CI)*	*p-value*
Influenza A	28/67 (41.8)	1.42 (0.80-2.52)	0.24	5/61 (8.2)	2.00 (0.56-6.38)	0.32	9/62 (14.5)	3.01 (1.13-7.95)	0.03	17/59 (28.8)	1.75 (0.87-3.49)	0.14
SARS-CoV-2	21/79 (26.6)	0.56 (0.31-0.99)	0.05	3/76 (4.0)	0.69 (0.14-2.36)	0.76	4/76 (5.3)	0.61 (0.16-1.76)	0.44	13/77 (16.9)	0.68 (0.33-1.33)	0.31
RSV	11/29 (37.9)	1.12 (0.49-2.48)	0.84	1/30 (3.3)	0.66 (0.03-3.61)	1	2/31 (6.5)	0.87 (0.12-3.30)	1	7/27 (25.9)	1.36 (0.50-3.31)	0.62
Rhino/enterovirus	15/43 (34.9)	0.97 (0.48-1.90)	1	2/41 (4.9)	0.96 (0.13-3.84)	1	2/41 (4.9)	0.62 (0.09-2.30)	0.75	7/41 (17.1)	0.74 (0.28-1.70)	0.54
hMPV	12/18 (66.7)	3.93 (1.45-11.85)	0.01	2/15 (13.3)	3.23 (0.42-14.10)	0.18	1/15 (6.7)	0.97 (0.04-5.33)	1	3/14 (21.4)	1.05 (0.22-3.58)	1
PIV 1-4	5/15 (33.3)	0.91 (0.27-2.70)	1	0/12 (0.0)	-	-	1/13 (7.7)	1.14 (0.04-6.40)	1	3/15 (20.0)	0.96 (0.20-3.23)	1
hCoV	2/13 (15.4)	0.33 (0.05-1.30)	0.15	0/12 (0.0)	-	-	0/12 (0.0)	-	-	2/12 (16.7)	0.78 (0.11-3.13)	1

[a] *either a clinical assessment of moderate or severe illness at day 0, 14, or 28 by the attending physician, or the occurrence of hospitalisation or death.*
[b] *based on physician-reported time spent in bed during the ARI episode compared with baseline (<8, 8-12, 12-20, >20 hours).*
[c] *exacerbation of underlying cardiovascular, pulmonary, or metabolic disease.*
[d] *clinical diagnosis by attending physician.*
[e] *need for supplemental oxygen between day 0 and day 28.*

SARS-CoV-2 = Severe acute respiratory syndrome coronavirus 2; RSV = respiratory syncytial virus; hMPV = human metapneumovirus; PIV = parainfluenza virus; hCoV = human coronavirus; OR = odds ratio; CI = confidence interval

530

The Coronavirus Vaccine Landscape: recent advances and novel approaches to development of broadly protective human coronavirus vaccines

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Background: Over the last 25 years, three novel coronaviruses have emerged with vast implications for human health, highlighting the critical need for vaccines protective against viruses within the Coronaviridae family. The development and deployment of several SARS-CoV-2 vaccines in recent years has also elucidated preferred product characteristics, including thermostable vaccine platforms and needle-free routes of administration. Therefore, in September 2025, the Center for Infectious Disease Research and Policy (CIDRAP) at the University of Minnesota, in partnership with the Coalition for Epidemic Preparedness Innovations (CEPI), launched the Coronavirus Vaccine Landscape, an open-access, web-based platform that tracks development of vaccines protective against coronaviruses that threaten human health. This represents the first release of comprehensive data collected on coronavirus vaccines through 2025, and provides a unique and timely overview of the current vaccine pipeline.

Methods: Vaccine candidates were monitored from preclinical development through the different phases of clinical trials in real time. Information was curated from research published in the peer-reviewed scientific literature through extensive searches of databases such as PubMed, Google Scholar, and clinicaltrials.gov.

Results: At the time of launch, the landscape featured over 140 vaccine candidates, classified according to breadth of protection: SARS-CoV-2, MERS, or broadly protective coronavirus vaccines (BPCV). BPCV classification indicates the candidate had demonstrated a response against multiple viruses in the Coronaviridae family. The candidates comprised seven different vaccine platforms: protein subunit, nucleic acid, viral vector, live-attenuated, nanoparticle, virus-like particle, and inactivated vaccines. Different routes of administration for the candidates included aerosol, intradermal, intranasal, intramuscular, intra-lymph node, microneedle patch, oral, and subcutaneous. Multiple animal models were used in preclinical development for the vaccine candidates, some of the most common being Syrian hamsters and hACE2 transgenic mice. The most commonly used adjuvants were aluminum-based, but approximately half the candidates did not utilize adjuvants, likely due to use of nucleic acid and viral-vector vaccine platforms.

Conclusions: The Coronavirus Vaccine Landscape website is open-access and user-friendly. Data on all vaccine candidates can be downloaded locally for further use anyone interested in coronavirus vaccine development. The current landscape reflects a large number of candidates using diverse approaches, but few candidates have progressed to Phase III clinical trials. Continued investment will be essential to advance these candidates along the development pathway to combat current and future coronavirus threats.

532

ECaS

Changes in excess mortality attributable to influenza and RSV in the post-COVID-19 period, immunity debt, and viral interference

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Background:

The COVID-19 pandemic caused disruptions in endemic virus circulation, leading to speculation that the tripledemics in 2022-23 and atypical flu season in 2024-25 may be resulting from pandemic "immunity debt". At the same time, new immunizations for RSV targeting infants and older adults were introduced in 2023. Understanding how new immunizations for respiratory viruses and potential pandemic-related perturbations drive population-level mortality patterns has important implications for public health planning and future pandemic preparedness.

Methods:

Aggregating estimates from multiple models has gained popularity as a method to manage uncertainty when applying modelling to public health decision making, but these approaches have not been widely applied to burden of disease studies. We used as ensemble approach that integrates predictions from three time series models to improve the robustness of mortality estimates for influenza and RSV in the United States from July 2010 to June 2025.

Results:

In the decade prior to the COVID-19 pandemic, we estimate an annual average of 3.6 (2.8-4.4) and 2.1 (1.1-3.3) respiratory deaths per 100,000 attributable to influenza and RSV, respectively. For both viruses the highest mortality rate was in adults ≥65 years (18.9 [14.8-23.1] for influenza and 12.5 [7.1-18.1] for RSV). Infants were the only age group with consistently higher mortality from RSV than influenza. During the 2020-21 winter season there was no excess deaths attributable to either virus. RSV mortality was slightly above average during the 2021-22 and 2022-23 seasons and returned to normal in the 2023-24 and 2024-25 seasons with a substantial drop among infants and older adults. Influenza on the other hand remained low to average until the 2024-25 season, which was severe for all age groups (mortality rate = 6.2 [5.1 – 7.2]).

Conclusions:

The high influenza mortality rate during the 2024-25 season suggests the possibility of a lingering impact of the COVID-19 pandemic on population immunity. While severe influenza seasons occur periodically in inter-pandemic periods, these are often more pronounced in specific age groups depending on the predominant influenza A subtype. The high mortality of the 2024-25 season across all age groups may indicate the settling of a pandemic-related immunity debt. It is worth noting that RSV-attributable mortality appears to return shortly after pandemic-era interventions relax, and two years after the introduction of new RSV immunizations there is already evidence of a modest reduction in population-level respiratory mortality among infants and older adults. While the evidence regarding viral interference between RSV and influenza is mixed, one possibility is that the early seasonal onset of RSV from 2021 to 2023, dampened the impact of influenza epidemics, delaying repayment of the immunity debt until the 2024-25 season. Disease burden studies provide an opportunity to explore how respiratory virus immunity debt and virus interactions manifest on a population level.

534

High-Dose vs Standard-Dose Influenza Vaccine Against Severe Clinical Outcomes in Older Adults: An Updated Systematic Literature Review & Meta-Analysis of Randomized Trials

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Background:

Older adults remain at elevated risk of serious complications from influenza, including hospitalization and death, despite seasonal vaccination with standard-dose inactivated influenza vaccine (SD-IIV). High-dose inactivated influenza vaccine (HD-IIV) was developed to enhance immunogenicity in the elderly and has demonstrated superior protection against laboratory-confirmed influenza (LCI) infection vs. SD-IIV among older adults in a double-blind randomized-controlled trial. A prior meta-analysis of randomized trials found that HD-IIV reduced the risk of hospitalization for pneumonia or influenza as well as all-cause hospitalization, though without an apparent effect on mortality. Since this prior meta-analysis, several large-scale individually randomized trials powered to compare the relative vaccine effectiveness (rVE) of HD-IIV vs. SD-IIV against severe clinical outcomes in older adults have been completed. This meta-analysis aims to synthesize the totality of randomized evidence on HD-IIV vs. SD-IIV for the prevention of hospitalization and death.

Methods:

This prespecified meta-analysis (PROSPERO-ID: 1040175) will synthesize all randomized trials comparing HD-IIV vs. SD-IIV across at least one influenza season. Outcomes evaluated include hospitalization for: (1) influenza-related illness, (2) LCI, (3) pneumonia or influenza, (4) cardio-respiratory disease, (5) any cause, and (6) all-cause mortality. The primary analysis will focus on adults aged ≥65 years, with secondary analyses across subgroups. These include all adults, those aged 65-79 years and ≥80 years, individuals with and without cardiovascular disease, exclusion of trials conducted exclusively in high-risk populations, and exclusion of data collected during the early phase of the COVID-19 pandemic. Unpublished aggregate data will be included to allow comprehensive analyses of outcomes and subgroups. A systematic review of PubMed and Embase will be conducted in September 2025.

Results:

A total of eight randomized trials enrolling more than 600,000 older adults randomized to HD-IIV vs. SD-IIV are expected to be included in this updated meta-analysis. The addition of recent large-scale trials, accounting for more than 466,000 individually randomized older adults, will substantially increase statistical power to assess hospitalization outcomes and improve the evaluation of mortality and subgroup analyses. The combined study population will be approximately six times larger than in previous meta-analyses, enabling the most comprehensive estimates to date of the rVE of HD-IIV vs. SD-IIV against severe influenza outcomes in older adults and across key risk groups.

Conclusion:

This meta-analysis will provide the most comprehensive synthesis of evidence from randomized trials assessing the rVE of HD-IIV vs. SD-IIV against severe clinical outcomes in older adults. By incorporating recent large-scale pragmatic trials, this analysis is expected to clarify the effectiveness of HD-IIV in reducing severe outcomes, including hospitalization and death, providing crucial evidence to inform vaccination strategies and policy globally.

535

A Multidisciplinary Framework for Predictive Selection of High-Affinity Anti-Neuraminidase Monoclonal Antibody Ligands for Influenza Potency Assays

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Background:

To enhance influenza vaccines effectiveness, the inclusion of the neuraminidase (NA) antigen is being considered. This requires the development of a dedicated NA potency assay that meets the same regulatory standards and time-to-market constraints as the established hemagglutinin (HA) assay. Indeed, the antigenic variability of influenza viruses remains a major challenge for vaccine formulation and potency testing.

Methods:

To gain adaptability for neuraminidase (NA)-targeted potency assays, we are developing a multidisciplinary strategy for the rapid selection of high-affinity and high-specificity anti-NA monoclonal antibody ligands, either immunoglobulins (Ig) or fragment of Ig, tailored to the NA variants that could potentially be included in seasonal influenza vaccines.

Results:

Our approach combines ELISA-based binding assays, cryo-electron microscopy (Cryo-EM), and bioinformatic analyses of NA sequences to characterize ligand-NA interactions. A central component is an **in silico** prediction pipeline that evaluates ligand binding potential across circulating NA variants. These computational predictions guide the prioritization of wet-lab experiments, to enable efficient validation and refinement of the most promising candidates.

Conclusion:

By integrating structural, biochemical, and computational data, this framework is aimed at accelerating the selection of optimal ligands for inclusion in potency assays, ensuring their relevance to evolving viral strains.

Ultimately, this strategy supports the development of more robust and responsive influenza vaccine quality control tools, contributing to enhanced pandemic preparedness.

536

RSV Phylogenetic and Antigenic Surveillance from a Phase 3 Trial Supports the Breadth of mRNA-1345-Induced Immunity

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Background: Recent surges in RSV B incidence have raised concerns about the emergence of potentially new immune-evasive variants. We assessed the genetic and antigenic properties of circulating RSV strains in the context of the ongoing Phase 3 mRNA-1345 trial (P301) and evaluated the immunogenicity of mRNA-1345.

Methods: We analyzed 278 RSV-A and 406 RSV-B F protein sequences from RSV-positive P301 participants (2022–2024), alongside 13,686 public sequences spanning 1956–2024. Codon-correct phylogenies were constructed using the Nextstrain Augur pipeline. Antigenic site analysis focused on sites 0 and II–V, relative to the mRNA-1345 sequence. Separately, we measured serum neutralization titers in BALB/c mice immunized with monovalent or bivalent mRNA vaccines encoding RSV A and/or B preF proteins.

Results: Contemporary RSV-A strains formed multiple co-circulating clades with pre-pandemic origins. Most RSV-B sequences clustered into a monophyletic clade (B.D.E.1) characterized by S190N, S211N, R42K, and S389P amino acid substitutions relative to the mRNA-1345 A2 strain. The B.D.E.1 clade likely emerged prior to the COVID-19 pandemic, with an estimated tMRCA of 2018. No phylogenetic segregation was observed between mRNA-1345 and placebo breakthrough infections, suggesting that vaccinated individuals were not infected by antigenically distinct variants.

F gene sequence analysis showed only two fixed mutations in RSV-A (V152I and N276S). Fourteen RSV F mutations were fixed in in RSV-B (including S190N, S211N, R42K and S389P), indicating a level of divergence from the mRNA-1345 sequence in the major antigenic sites 0 and II-V. Most of the observed mutations in both RSV A and RSV B had historical origins, having emerged decades ago. Only two mutations S190N and S211N in RSV B started to increase in frequency beginning 2021. These results were consistent across different geographic sites and aligned with public sequence data.

In mice, immunization with mRNA formulations encoding RSV A preF (mRNA-1345) alone or in combination with a contemporary B preF elicited robust neutralization titers against both RSV-A2, RSV-B18537 and contemporary B strains. The bivalent RSV A+B preF mRNA vaccine showed similar neutralization activity compared to monovalent mRNA-1345, suggesting that mRNA-1345 alone induces antibodies capable of neutralizing contemporary RSV-B strains. Adding a second mRNA encoding RSV B preF did not further enhance RSV-B neutralizing titers.

Conclusion:

Phylogenetic and antigenic analyses of RSV strains from the P301 trial demonstrated that breakthrough infections in vaccinated individuals were not caused by antigenically divergent variants, providing no evidence of immune escape. Preclinical data support the cross-neutralizing breadth of mRNA-1345 against a contemporary RSV-B strain. Although direct comparisons of RSV-A and RSV-B titers are limited by assay differences, the data indicate that mRNA-1345 elicits strong immunity against both subtypes. These findings suggest that adding an RSV-B F sequence may not confer additional benefit beyond mRNA-1345 alone.

537

Novel Drug-Antigen Conjugate EV25: Results from a First-in-Human Phase 1/2a Trial in Healthy Volunteers and H3N2 Influenza Challenge Participants - Safety, Pharmacokinetics and Antiviral Activity

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BackgroundInfluenza remains a significant source of morbidity and mortality globally. There is a need for novel therapeutics with broader clinical efficacy. EV25 is a novel, single-dose dual mechanism, immunomodulatory small molecule drug conjugate being developed to treat influenza infections. EV25 has an influenza-specific targeting moiety, zanamivir to inhibit viral neuraminidase and rhamnose along with dinitrophenyl (DNP)-antigens to stimulate rapid immune response.MethodsEV-FLU-CL-01 is a first in human, Ph1 single dose escalation with Ph2A H3N2 human challenge study - evaluating safety, tolerability, pharmacokinetics and antiviral activity. Ph1 - total of n=24 randomized/treated with single doses intranasal EV25 (n=6/each; 30 mg, 100 mg, 300 mg EV25, placebo). Ph2A - total of n=57 randomized/treated/received challenge 1.0×10^6 TCID50/mL Influenza A Virus (Belgium/4217/2015 H3N2), followed by treatment 24 hrs later (n=20 given 30 mg EV25, n=18 given 300 mg EV25, n=19 given placebo).ResultsThe mean (SE) log viral load AUC decreased with increasing EV25 doses. Mean (SE) log viral load AUC was 177.224 (42.105), 129.217 (38.371), and 95.300 (41.382) log10 TCID50/mL×h for the placebo group, in Cohort 1, and Cohort 2, respectively. ANCOVA on the log viral load AUC showed statistical differences between cohorts (p-value: 0.0247). Observed decrease in Least Squares means vs. placebo (-59.798 log10 TCID50/mL×h after receiving 30 mg EV25 [Cohort 1] and -150.987 log10 TCID50/mL×h after receiving 300 mg EV25 [Cohort 2]). No deaths, other SAEs, or treatment-emergent AE's (TEAEs) leading to discontinuation. TEAEs related to EV25 were similar in frequency / severity to placebo.Conclusion

Intranasal administration of EV25 resulted in dose dependent, significant reduction of viral load in H3N2 challenge model. EV25 was generally safe, well tolerated in healthy volunteers and in participants inoculated with 1.0×10^6 TCID50/mL Influenza Type A Virus.

539

Differential dependency on IFN-I signaling between parenteral and mucosal immunization with the same adjuvant provides insights into overcoming deficits in IFN-Is through strategic vaccination regimen design

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Background:

While the intramuscular (IM) SARS-CoV-2 mRNA vaccines impart significant protection from severe disease, they provide suboptimal blockage of infection and transmission in due to poor induction of mucosal immunity. Mucosal vaccines, including intranasal (IN) vaccines, and heterologous vaccination regimens primed systemically and boosted mucosally, have significant potential for establishing both robust systemic and mucosal protective immunity. However, much remains to be defined regarding how route of administration impacts the mechanisms of adjuvanticity. We have developed an IN nanoemulsion (NE) adjuvant and a combination adjuvant (NE-IVT) consisting of the NE with an RNA-based RIG-I agonist (IVT). As NE activates TLRs2,4 and NLRP3, and IVT activates RIG-I, NE-IVT activates innate immune responses closely emulating those triggered during natural viral infection. We have shown the NE and NE-IVT adjuvants are effective in inducing robust protective immunity as standalone subunit vaccines with the SARS-CoV-2 spike (S) protein, and as heterologous IN boosters for IM mRNA vaccines. Given the strong synergistic induction IFN-Is by NE-IVT, we herein sought to determine the role of the IFN-I response to vaccine-mediated protective immunity. An advantage of NE-IVT is the ability to induce robust responses through either IM or IN routes, allowing us to investigate whether route of administration altered the immune pathways employed by the same adjuvant. Finally, we examined how heterologous vaccination regimens could be strategically used to modulate IFN-I responses.

Methods:

WT and IFNAR$^{-/-}$ KO C57Bl/6 mice were immunized with a prime/boost/boost schedule at 3wk intervals. Groups included IN or IM NE/IVT/S, IM SARS-CoV-2 mRNA (BNT162b2), and heterologous IM mRNA prime;IN NE/IVT/S boost. Comparators included IN NE/S and IM Addavax/S. Serum antigen-specific IgG and cross-variant neutralizing antibodies (nAbs) were assessed, along with BALF IgA. T cell responses were profiled by flow cytometry and multiplex immunoassay in spleen, cervical lymph nodes, and lungs 2wks post-final immunization.

Results: IM vaccines, especially IM NE/IVT/S, as well as IM mRNA and IM Addavax/S were notably impacted by the lack of IFNAR signaling, showing severe reduction in TH1-associated IgG subclasses, reduced nAb breadth and diminished TH1-polarized CD4/CD8 responses in spleen and mucosa. In contrast, both humoral and T cell response magnitude and polarization were maintained with IN NE/IVT/S without IFNAR signaling and were unaffected for IN NE/S singly-adjuvanted KO mice.

Conclusions: We demonstrate differential usage of innate pathways through parenteral vs. mucosal routes of vaccine delivery even with the same adjuvant. Our results suggest mucosal adjuvant delivery relies less upon IFN-I signaling than parenteral routes. Notably, heterologous IN boosting of IM mRNA primed responses restored many deficiencies in vaccine responses observed in KO mice, suggesting a potential strategy for overcoming deficits in IFN-Is, such as in the elderly or those with various interferonopathies through adjuvanted mucosal vaccination.

Supporting Partners

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