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## A Pan-Pneumovirus vaccine based on immunodominant epitopes of the fusion protein

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Jarrod Mousa - ARNI0002

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### Background

Respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) are two leading causes of severe respiratory infections in children, the elderly, and immunocompromised patients. The fusion (F) protein is the major target of neutralizing antibodies, and recent developments in stabilizing the pre-fusion conformation of the F protein, and the identification of immunodominant epitopes that elicit potent neutralizing antibodies have led to testing of numerous pre-fusion RSV F-based vaccines in clinical trials.

### Method

We designed and tested the immunogenicity and protective efficacy of a chimeric fusion protein (RHMS-1) that contains immunodominant epitopes of RSV F and hMPV F (Figure 1). RHMS-1 has several advantages over vaccination with pre-fusion RSV F or hMPV F, including a focus on recalling B cells to the most important protective epitopes, and the ability to induce protection against two viruses with a single antigen. RHMS-1 was generated as a trimeric recombinant protein in HEK293F cells, and the protein conformation was assessed by negative-stain electron microscopy analysis. We probed the antigenicity of the vaccine candidate using a panel of human monoclonal antibodies and human serum. BALB/c mice were vaccinated with RHMS-1 and challenged with either RSV or hMPV.

### Result

RHMS-1 is pre-fusion-like based on a similar shape as pre-fusion RSV and hMPV F in electron microscopy images. RHMS-1 retains antigenic features of both viruses, including the pre-fusion site 0 epitope of RSV F, site II, and several epitopes of hMPV F. BALB/c mice immunized with RHMS-1 had serum binding and neutralizing antibodies to both viruses. RHMS-1 vaccinated mice challenged with RSV or hMPV had undetectable virus in lung homogenates for both viruses, in contrast to RSV F or hMPV F vaccinated mice, which had detectable virus for hMPV and RSV, respectively.

### Conclusion

Overall, this study demonstrates protection against two viruses with a single antigen, and our data supports further testing of RHMS-1 in additional pre-clinical animal models, which is currently underway.



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## Transmission of respiratory syncytial virus (RSV) from symptomatic and asymptomatic individuals in an urban and a rural community in South Africa, 2017-2018: results of the PHIRST cohort study

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### Background

Vaccination strategies targeting those more likely to transmit RSV may be helpful for mitigating transmission to vulnerable populations. We aimed to quantify transmission of RSV within South African households and describe factors associated with transmission.

### Method

We conducted a prospective cohort study in one rural and one urban community from 2017-2018. We aimed to enroll at least 50 households in each community (with over enrollment of 25% of the target to account for loss of follow up) annually and followed for 10 months each year. Nasopharyngeal swabs were collected twice-weekly from consenting household members of all ages irrespective of symptoms and tested for RSV using real-time reverse transcription polymerase chain reaction (rRT-PCR). Household cumulative infection risk (HCIR) was defined as the number of subsequent infections (<15 days apart in the chain of transmission) within a household following RSV introduction. The index case was defined as the first individual testing positive in the household. Socio-demographic factors associated with HCIR were evaluated using logistic regression. Households with co-primary index cases were excluded from the analysis.

### Result

We collected 81,991 samples from 1,116 participants in 225 households (follow-up rate 88%); 781 (1%) samples tested RSV positive. 359 (32%) of 1,116 individuals experienced  $\geq 1$  RSV infection episode (31 had 2 infections and 3 had 3 infections, total of 396 episodes). 75% of households (168/225) had  $\geq 1$  RSV-positive individual. Symptomatic infections were 33% (132/396). For analysis of HCIR we included 198 RSV introductions in 168 households (24 households experienced two distinct introductions, and three had three, separated by >2 weeks). The overall HCIR was 11%; 8% (47/561) from asymptomatic index cases, 8% (9/102) from those with one, and 21% (10/193) from those with  $\geq 2$  symptoms. Individuals  $\leq 12$  years old accounted for 60% (144/242) of index cases. On multivariable analysis, controlling for index case age, index cases with  $\geq 2$  symptoms (OR 2.3, 95% CI 1.1-5.0) vs no symptoms and whose duration of shedding was 4-10 days (OR 4.3, 95% CI 2.0-9.4) or >10 days (OR 9.7, 95% CI 3.4-27.9) vs <4 days were more likely to transmit infection; household contacts aged 1-4 years (OR 8.3, 95% CI 1.4-47.9) vs age  $\geq 65$  years were more likely to acquire infection. There were no statistically significant differences in the HCIR in rural vs urban households.

### Conclusion

Within a rural and an urban South Africa community RSV attack rate within households was high with most infections asymptomatic. Younger children were more likely to introduce RSV into the home, and more likely to



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be infected in the home suggesting that vaccines targeting young children could substantially reduce household transmission in these communities.



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## Resource-effective whole genome sequencing of RSV using hybridization capture

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### Background

Respiratory syncytial virus (RSV) is the leading cause of acute lower respiratory tract infections and hospitalizations in children worldwide. The development of new candidate vaccines and monoclonal antibodies highlights the need for a comprehensive molecular characterization of circulating RSV from either clinical samples or isolates. However, whole genome sequencing (WGS) of viral genomes from clinical samples is generally challenging due to low proportions of viral nucleic acids versus host RNA/DNA. Here, we describe an efficient and sensitive WGS method to enrich RSV A and B target sequences by using in solution hybridization capture followed by next generation sequencing.

### Method

RSV-positive tested specimens such as nasal/throat/nasopharyngeal swabs or corresponding cell culture isolates from patients with an acute respiratory infection and collected between 1999 and 2020 were selected for WGS. RNA was extracted and subjected to DNA digestion and cDNA synthesis. Viral loads were determined by an RSV-specific qPCR assay. Ds-cDNA was used for library preparation with NEBNext Ultra II (FS) library preparation kits (New England Biolabs). For hybridization capture, libraries were ranked by viral load to form pools of 5-20 libraries. Pools were hybridized with RNA baits (MYBaits®) designed to capture whole genomes of RSV A and B. The enriched libraries were amplified and sequenced in batches of 40-60 samples using Illumina MiSeq sequencing system.

### Result

A total of 110 swab samples and 139 isolates were sequenced with our newly established in-solution capture approach. Viral loads ranged between CT 18-32 for swab samples and between CT 11-30 for isolates. Of both specimen types, more than 95% (240/249) samples were sequenced successfully. In median, 99.8% on-target reads per sample resulting in >99% genome coverage with a mean sequencing depth of 4932 were generated.

All sequenced specimens were genotyped according to Goya et al., 2020. Phylogenetic analysis determined RSV A genotypes GA2.2, GA2.3.1-6, and GA3.0.3-4, and RSV B genotypes GB6 and GB5.0.0 - GB5.0.5, respectively.

### Conclusion

Hybridization capture-based target enrichment is a reliable approach for WGS of RSV from both cell culture isolates and clinical samples. By pooling multiple libraries per capture reaction, we developed a time- and cost-effective protocol to process a large number of samples in parallel. The custom-based design of target-specific baits enables sequencing of a wide range of ancient and recent RSV A and -B genotypes. In addition, the bait set could be easily extended to simultaneously detect further pathogens of interest, making this method suitable for a future molecular respiratory multi-pathogen surveillance.



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## Gap analyses to assess Canadian readiness for respiratory syncytial virus vaccines

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### Background

Respiratory syncytial virus (RSV) can cause severe disease in infants and older adults. Various vaccine candidates are in development and may become authorized for use in Canada within the next 2-5 years.

### Method

The Public Health Agency of Canada sought to enhance preparedness for RSV vaccine and passive immunization candidates by organizing an expert retreat to identify knowledge gaps in surveillance and research and development in the context of provincial and territorial RSV public health priorities.

### Result

We determined that RSV candidate vaccines in development directly address four out of five identified public health priorities, and identified remaining data gaps around vaccine efficacy and effectiveness. We determined that limited or sufficient surveillance data is available to support decision-making for four out of five RSV public health priorities and identified data gaps for several key populations: (i) for RSV cases under 17 years of age, gaps remain for denominator data to calculate incidence and data on medically attended outpatient visits; (ii) for RSV cases in Indigenous and remote communities, gaps remain for data on incidence, prevalence, specific risk factors, feasibility and acceptability; and (iii) for RSV cases in older adults, gaps remain for data on incidence.

### Conclusion

This process demonstrated the feasibility of, and stakeholder support for, gap analyses in surveillance data to support decisions about prospective vaccines and immune products.



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## New antivirals against human parainfluenza virus

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### Background

The human parainfluenzaviruses (hPIVs) pose a considerable burden on the respiratory health of the (very) young. This group of viruses consists of hPIV1, -2, -3 and -4 and represents the second main cause of hospitalization due to acute respiratory tract infection in children under five, only preceded by respiratory syncytial virus. These hospitalizations are mostly due to hPIV1 and -3, which cause 26% and 52%, respectively, of the clinical cases. There are no clinically approved antivirals or vaccines available against hPIVs to treat or prevent these infections.

### Method

Our aim was to develop potent hPIV3-neutralizing single-domain antibodies targeting either the hPIV3 fusion protein (F) or hemagglutinin-neuraminidase protein (HN). Hereto, an alpaca immunization protocol was set up based on intranasal immunization with live hPIV3 followed by a subcutaneous boost. A combination of bio-panning and a virus-neutralization screen was used to isolate hPIV3-neutralizing single-domain antibodies. Their neutralizing activity, target protein and mode of action were determined.

### Result

Two alpacas were immunized with a live hPIV3 immunogen. Analysis of serum samples showed an increase in hPIV3-neutralizing activity in both animals. Bio-panning followed by a virus-neutralization screen led to the isolation of five unique single-domain antibodies. All five could neutralize hPIV3, with varying potencies. Flow cytometry analysis showed that all selected single-domain antibodies bind the hPIV3 HN protein. Four of the five candidates can inhibit the neuraminidase activity of hPIV3 HN, suggesting that they bind near the sialic acid binding site of HN.

### Conclusion

Immunization of the alpacas with live hPIV3 led to the isolation of five hPIV3-neutralizing single-domain antibodies. Further research is warranted to determine their clinical potential as antiviral agents against hPIV3.



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## Evaluation of the respiratory syncytial virus G-directed neutralizing antibody response in the human airway epithelial cell model

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### Background

It was shown that the RSV G glycoprotein mediates attachment to cells using CX3CR1 as a receptor and that G-specific neutralizing antibodies can be detected using human airway epithelial (HAE) cell cultures. To investigate the contributions of G-specific antibodies to RSV neutralization, we performed HAE neutralization assays on sera from RSV G-immunized mice and RSV-infected infants.

### Method

Mice were immunized 3 times with either the pre-fusion form of RSV F (pre-F), RSV G from subgroup A (Ga), a mixture of RSV pre-F and Ga, or a mixture of RSV pre-F and RSV G from subgroup B (Gb). Serum was collected 1 day prior to the first immunization and 2 weeks after the third immunization.

Human adult and RSV-infected infant sera were commercially acquired. As selected infants were 6 to 22 months old, it is likely that maternal Ab levels would have waned and any detectable titers were due to a single infection.

To adsorb RSV pre-F, Ga or Gb specific Abs, sera were mixed with a slurry of Sepharose beads conjugated to pre-F, Ga or Gb, incubated and filtered. The flow-through was then tested for neutralizing activity.

Complement-dependent and independent neutralizing Ab titers against RSV subgroups A and B were determined on Vero cells using a standard PRNT60 assay.

To determine complement-independent neutralizing Ab titers against RSV subgroups A and B on HAE cells, serial dilutions of heat-inactivated serum were combined 1:1 with RSV viral stocks encoding fluorescent reporters and incubated for 1.5 hours at 37°C. The virus-serum mixture was then added to fully differentiated HAE cells and incubated for 1.5 hours at 37°C. The inoculum was then removed, the cells were washed to remove unbound virus and incubated a further 20 hours. Infection events were counted on a fluorescent microscope and the PRNT60 were determined.

### Result

We developed a neutralization assay that utilizes HAE cells to measure RSV G-specific neutralizing antibody titers and utilized F-specific Ab depletion from sera of mice co-administered with G and F and adult human sera to demonstrate G-specific activity. Sera from 6 RSV positive infants exhibited G-specific neutralizing activity against at least one RSV subgroup. Sequential depletion of F-, Ga-, and Gb-specific antibodies revealed that, after a single RSV infection, some infants exhibited cross neutralizing G-specific antibodies while other infants only had subgroup specific activity.

### Conclusion

Our results suggest that G is an important target for generating neutralizing antibodies and would be beneficial to include in an RSV vaccine. Inclusion of G antigens from both subgroups may enhance the vaccine cross protection potency.





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## New perspectives on respiratory syncytial virus (RSV) surveillance at the national level; lessons from the COVID-19 pandemic

Anne Teirlinck - ARNI0016

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### Background

Previously, we proposed a set of recommendations to develop feasible and sustainable respiratory syncytial virus (RSV) surveillance at a national level in European Union/European Economic Area (EU/EEA) countries. The coronavirus disease 2019 (COVID-19) pandemic, has impacted, and in several cases disrupted, existing respiratory surveillance systems, and this has led us to draw lessons from this pandemic and re-evaluate previous recommendations.

### Method

We updated the previous recommendations in light of the COVID-19 pandemic and its impact on RSV circulation and its seasonal epidemics in EU/EEA countries. We performed a revision of the recommendations for RSV surveillance during a virtual workshop in October 2021, with 40 participants from 16 EU/EEA countries, representing expertise within RSV epidemiology, virology, and public health.

### Result

We recommend moving towards a multi-pathogen respiratory surveillance, adaptive and flexible to accommodate the detection and reporting of novel pathogens while reinforcing the surveillance of existing respiratory viruses. Appropriately positioning RSV surveillance in such a system requires one to address the specific needs of RSV surveillance. RSV seasonality can potentially remain disrupted for the next few years, therefore year-round surveillance is highly recommended. We state the importance of using the acute respiratory infection (ARI) and severe acute respiratory infection (SARI) case definition considering the wide symptomatic spectrum of RSV. Collecting data on symptoms would enable the continued collection of more narrow case definitions such as





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influenza-like illness (ILI). Age-stratified data should be collected to cover the full range of mild and severe RSV cases. As high throughput rapid whole genome sequencing (WGS) proved feasible during the COVID-19 pandemic, sequencing of an increased number of RSV positive specimens should be considered. At the European level, collection of case-based data would also enable more detailed analyses. New data sources and surveillance systems that proved useful in the COVID-19 pandemic or in other situations, such as national health registries and syndrome surveillance (e.g. bronchiolitis), should be considered.

### **Conclusion**

The COVID-19 pandemic has highlighted the importance of robust, flexible, multi-respiratory pathogen surveillance and many initiatives for integrated surveillance are in development. It is important to assess how RSV surveillance strategies align with these broader initiatives to enhance their sustainability. At the same time, it is important to address the specific needs of RSV surveillance, such as year-round surveillance and collecting data in the community and hospital settings.



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## Annual RSV-associated hospitalization rates among children aged less than 5 years during 2021 and comparison to pre-pandemic rates, New Vaccine Surveillance Network (NVSN)

Meredith McMorrow - ARN0017

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### Background

Respiratory syncytial virus (RSV) is the leading cause of hospitalization in children aged <1 year in the United States. RSV typically circulates during late fall and winter in the U.S.; circulation declined abruptly in February 2020 and did not resume until late March 2021, likely due to pandemic-associated non-pharmaceutical interventions and community mitigation measures. Initial RSV infections, which often occur in early infancy, result in high rates of hospitalization; it is less clear whether children who experience their initial RSV infection in the second year of life have similar hospitalization rates.

### Method

We estimated annual RSV-associated hospitalization rates in children aged <5 years enrolled at 7 U.S. pediatric medical centers in the New Vaccine Surveillance Network during January - December 2021 and compared them to pre-pandemic annualized rates (from December 2016 to September 2020). We enrolled children with acute respiratory illness (ARI) and tested mid-turbinate nasal swabs (with or without throat swabs) for RSV by reverse transcription polymerase chain reaction. We adjusted population-based hospitalization estimates for days of surveillance per week, eligible children not enrolled, and the proportion of ARI hospitalizations captured by each hospital in their catchment area. 2020 county population estimates were denominators for rates.

### Result

Among 3705 hospitalized children aged <5 years enrolled and tested for RSV during 2021, 1162 (31%) were RSV-positive, comparable to the pre-pandemic mean. The highest proportion tested RSV-positive in August 2021. The RSV-associated hospitalization rate per 1000 children aged <5 years was 3.7 (95% CI: 3.4-3.9), not significantly different from the pre-pandemic average, 3.4 (95% CI: 3.2-3.5) per 1000. Likewise, in 2021, the highest RSV-associated hospitalization rates occurred among infants aged 0-2 months (21.4 (95% CI 19.0-23.8) per 1000) and declined with increasing age: 13.6 (95% CI: 11.9-15.5), 7.0 (95% CI: 6.0-7.9), 3.4 (95% CI: 3.0-4.0), and 1.0 (95% CI: 0.8-1.1) per 1000 children aged 3-5, 6-11, 12-23, and 24-59 months, respectively. Rates for all age groups were within the confidence intervals of pre-pandemic mean estimates.

### Conclusion

Despite the reported absence of RSV circulation for approximately 14 months of the SARS-CoV-2 pandemic and the unusual timing of the RSV season in the US in 2021, hospitalization rates in children aged <5 years in 2021 resembled prior seasons in terms of magnitude. Furthermore, relative to prior seasons, RSV-associated



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hospitalization rates were similar in 2021 among children aged 6-11 and 12-23 months who may have experienced their first RSV infection.



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## Estimating the Burden of Adult Hospitalized RSV Infection Including Special Populations

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**Balasubramani G.K.** - ARNI0018

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### Background

Numerous studies in the U.S. have made estimates of the RSV burden among adults. Estimates vary widely due to differences in methodology; reliance on influenza surveillance, which does not adequately capture all RSV clinical symptoms; and lack of diagnostic methods to identify RSV when viral loads are low. Nevertheless, accurate burden estimates can inform healthcare planning, resource allocation, and potentially, RSV vaccine policy.

### Method

We used a simple method combined with statewide and local hospitalization, medical record, and U.S. Census data to estimate population-based RSV hospitalization burden among adults ages 18-64 years and ≥65 years for Allegheny County, Pennsylvania between September 1, 2015, and August 31, 2018. We also estimated RSV burden among immunocompromised, pregnant, and immunocompetent individuals. Finally, the economic burden of hospitalization was estimated using state-provided average hospitalization charges for comparisons across patient groups.

### Result

Estimates showed that the overall adult RSV burden varied across the three study years, with the lowest rate of 236/100,000 in 2015-2016 and the highest rate of 382/100,000 in 2016-2017. The largest burden was borne by adults ≥65 years of age whose rate each year per 100,000 population age ≥65 years (mean = 939/100,000) was 7.0-9.0 times those of adults 18-64 years of age (mean = 118/100,000 adults age 18-64 years). Immunosuppressed patients bore the greatest relative burden of RSV hospitalizations (1,288-1,562/100,000 immunosuppressed individuals). RSV burden estimates ranged from 0-808/100,000 pregnant women, suggesting that severe RSV requiring hospitalization of pregnant women is relatively uncommon. Average total charges for RSV hospitalization in Allegheny County across all adults increased from \$36 million in 2015-2016 to \$57 million in 2016-2017 to \$89 million in 2017-2018, likely due to both increased average charges for acute respiratory hospitalization and increased RSV cases.

### Conclusion

RSV is a common cause of ARI and a significant cause of hospital admissions in adults, resulting in a substantial burden on healthcare services. These RSV burden estimates add to the body of knowledge to guide public health policymakers and offer a method for simply and easily producing population-based burden estimates. These age and risk subgroup-specific disease burden will help guide priorities for RSV vaccine policy.



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## Systematic Literature Review of the Signs and Symptoms of Respiratory Syncytial Virus in Young Children

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Jessica Costello - ARN0019

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### Background

Respiratory syncytial virus (RSV) is the most frequent cause of lower respiratory tract infection in young children and a leading cause of hospitalization. Very young children cannot reliably report their RSV symptoms, so clinicians rely on caregivers to identify signs and symptoms of RSV.

A systematic literature review (SLR) was conducted to document caregiver-reported RSV signs and symptoms (CG-RSV S&S) and clinician-reported RSV signs and symptoms (Clin-RSV S&S) in children of different age groups and treatment settings (hospital and community).

### Method

Two researchers independently reviewed published articles from 2011 to 2021 from electronic database searches (MEDLINE, MEDLINE In-Process, Embase, PsycINFO, and Cochrane Library), conference abstracts from the past 3 years, and bibliographies of relevant SLRs to identify CG- and Clin-RSV S&S in children <5 years old. Another outcome of interest included hospital length of stay (LOS).

### Result

In total, 37 studies were reviewed, including those presenting CG-RSV S&S (n=6), Clin-RSV S&S (n=25), and hospital LOS (n=13). Studies differed by age group, treatment setting, and geographical location.

The RSV S&S commonly reported (in ≥40% of children) by both caregivers and clinicians across age groups and treatment settings were nasal discharge/congestion, cough, shortness of breath, fever, and feeding abnormalities. Caregivers also commonly reported vomiting (hospital setting only) and clinicians also commonly reported wheezing. There were no clear patterns of CG-RSV S&S by age or setting. Clinicians reported fever more frequently with increased age in hospitalized children.

Median hospital LOS (9 studies) was 4-5 days regardless of age group but varied by country.

### Conclusion

The most commonly reported RSV S&S were consistent across caregivers and clinicians (nasal discharge/congestion, cough, shortness of breath, fever, feeding abnormalities). Clinicians also commonly reported wheezing; caregivers possibly reported this symptom differently (e.g., as breathing difficulty). No notable differences by age were found for CG- and Clin-reported RSV S&S in the community setting; for hospitalized patients, clinicians reported higher frequency of fever with increased age.



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## Systematic Literature Review of the Signs and Symptoms of Respiratory Syncytial Virus in High-Risk Adults and Immunocompromised Populations

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Jessica Costello<sup>1</sup> - ARNI0023

Jessica Costello<sup>2</sup>, Ann Colosia<sup>2</sup>, Kristi Bertzos<sup>1</sup>, Kelly McQuarrie<sup>1</sup>

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### Background

Adults aged ≥65 years, those with chronic lung or heart disease, and those with weakened immune systems are at risk for severe respiratory syncytial virus (RSV) illness, such as symptoms consistent with lower respiratory tract (LRT) infection, pneumonia, respiratory failure, and death. No treatments are approved specifically to treat RSV in adults, and the symptom burden in these at-risk adult populations is not well documented.

A systematic literature review (SLR) was conducted to document RSV signs and symptoms (RSV S&S) in adults at high risk for disease progression due to age or comorbidities and in immunocompromised adolescents and adults.

### Method

Two researchers independently reviewed published articles from 2011 to 2021 obtained from electronic database searches (MEDLINE, MEDLINE In-Process, Embase, PsycINFO, and Cochrane Library), conference abstracts from the past 3 years, and bibliographies of relevant SLRs to identify patient-reported RSV S&S in high-risk adults and immunocompromised patients. Other outcomes of interest included RSV S&S duration and length of stay (LOS) for hospitalized patients.

### Result

Limited evidence was identified assessing RSV S&S reported by adult patients at high risk for RSV-related disease progression (6 studies) or immunocompromised patients (1 study). No studies reported separate data for immunocompromised adolescents.

Across the 7 studies, high-risk and immunocompromised adults reported a variety of upper respiratory tract (URT), LRT, systemic, and behavioral symptoms. The most frequent commonly occurring (>40% of patients) RSV S&S were cough (7 studies), sputum, dyspnea, and fever/feverishness (5 studies each). There were no clear symptom trends by treatment setting, although a higher proportion of URT symptoms were seen in community versus hospital settings. LRT symptoms were common in both settings.

Duration of RSV S&S (2 studies) ranged from 17-19 days and did not vary substantially by region. Mean hospital LOS (5 studies) varied from 3.5-11 days in the Americas; the median varied from 6 (Americas) to 15 days (China).

### Conclusion

Based on the limited evidence published, the most frequent commonly reported RSV S&S across settings in high-risk and immunocompromised adults are primarily LRT symptoms, which may be relevant when considering assessment and treatment targets. RSV S&S in high-risk and immunocompromised adults can last weeks (17-19 days in this SLR). For hospitalized adults, LOS varied across countries, which may be due to different healthcare systems and discharge protocols. Further research is needed to assess RSV S&S in adult and adolescent populations, particularly immunocompromised patients and specifically adolescents.



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## Experimental and natural infection in calves with bovine respiratory disease associated viruses.

Louise Cosby - ARNI0025

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### Background

Bovine respiratory disease (BRD) is one of the most economically important diseases for the Agri-Food industry being the primary cause of mortality and morbidity in cattle of all ages. The initial insult is usually due to viral infection, in particular, bovine respiratory syncytial virus (BRSV), bovine herpes virus type 1 (BoHV-1), bovine coronavirus (BCoV), or bovine parainfluenza-3 virus, which often occur as co-infections and predispose calves to secondary bacterial infections. Experimental virus infection of cattle enables the examination of the immune response to individual viral pathogens. Investigation into natural infections allows better understanding of the type and longevity of immunity in BRD and may have relevance to human RSV, which closely parallels BRSV infection.

### Method

Holstein-Friesian calves were experimentally challenged (n=12) with either BRSV, BoHV-1 or PBS (mock). Respiratory virus loads and clinical/pathological parameters were examined. Calves were euthanised at the peak of infection (day (d)7 BRSV, d6 BoHV-1) and tissues collected for downstream analysis. Total RNA (including small RNAs) was extracted from bronchial lymph node (BLN), and cranial lung lobe (CLL) tissues. Sequence reads were aligned to either the UMD3.1 or ARS-UCD1.2 reference genomes. mRNA targets of differentially expressed (DE) miRNA were determined and context++ scores calculated using Targetscan and ViennaRNA. Target genes with a weighted context++ percentile rank of  $\geq 99$  were used for subsequent analysis. Pathway and functional enrichment analyses were performed on DE genes and miRNAs using the Database for Annotation, Visualisation and Integrated Discovery. Virus antibodies and T cell responses were examined by ELISA, VNT, and ELISPOT. A cohort of 40 calves exposed to natural BRD infection, some with overt disease, were similarly examined for response to BRSV, BoHV-1, and BCoV. T cell responses will be reported.

### Result

Multi-dimensional scaling using BLN mRNA data showed a clear separation between challenged and control calves based on gene expression. There were 934 and 337 DE genes (DEGs) identified in BLN tissue in response to BSRV and BoHV-1, respectively. A total of 334 and 67 DEGs were identified in healthy CLL and lesioned CLL, respectively, in response to BoHV-1. Small RNA-Seq analysis identified 119 and 62 DE miRNAs in BLN in response to BRSV and BoHV-1, respectively. The KEGG pathways enriched for DEGs across all tissues were 'Influenza A' (both BRSV and BoHV-1) and 'Herpes Simplex Infection' (BoHV-1). There were no sera converted by d6/7post challenge. IL17 and IL2 cytokines were decreased in BoHV-1 challenged animals. Of the 40 naturally infected animals, 39, 34, and 20 were sera-positive for BRSV, BoHV-1, and BCoV, respectively.

### Conclusion

We have identified DEGs and pathways involved in the immune response to primary BRD viruses. The findings from these experimental and natural infections, will inform diagnostic, vaccination, and therapeutic approaches to





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improve levels of mortality and morbidity due to BRD. Additionally, this information may aid the understanding of human RSV and the impact of co-infections with other pathogens.



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## A *Heligmosomoides polygyrus* induced serum-borne factor promotes monocyto-sis and protects against respiratory syncytial virus infection in the lung

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Matthew Burgess - ARNI0026

**Matthew Burgess<sup>2</sup>, Karla Berry<sup>2</sup>, Amanda McFarlane<sup>3</sup>, Henry McSorley<sup>1</sup>, Jurgen Schwarze<sup>2</sup>**

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### Background

Infant respiratory viral infections are a major cause of infant hospitalisation and a risk factor in the development of persistent wheeze, airway allergic responses and ultimately asthma. We have previously shown that ongoing infection in mice with the gut helminth *Heligmosomoides polygyrus* (*H. polygyrus*) protected against respiratory syncytial virus (RSV) infection through reduced viral load, associated immunological changes and airway impairment. This protective effect was independent of adaptive immune responses or helminth secretory/excretory products, and dependent upon the induction of type-I interferons in the gut and/or lung and the presence of normal gut microbiota. Here we present further investigation into the mechanisms of this anti-viral effect.

### Method

Mice were infected with *H. polygyrus* via oral gavage. Some mice were also infected with RSV via intranasal administration. Monocytes depleted by intraperitoneal injection of anti-CCR2 antibody (M. Mack). Lungs, blood and bone marrow were collected for analysis by flow cytometry, RNA extraction, serum isolation and colony forming assays. Bone marrow monocytes also administered to naïve mice via intravenous injection prior to RSV infection.

### Result

Ongoing *H. polygyrus* infection induces bone marrow monocytopoiesis, in turn driving an increase in both circulatory monocyte populations and recruited inflammatory macrophages in the lung. Treatment of *H. polygyrus* infected animals with an anti-CCR2 antibody depletes these expanded monocyte populations and ablates the enhanced anti-viral state in *H. polygyrus* infected animals. Elevating monocyte numbers through their IV administration can also replicate the anti-viral effect.

Intravenous serum transfer from mice 10 days after *H. polygyrus* infection to naïve mice reproduces the increase in interferon beta and interferon stimulated genes, increased bone marrow monocytopoiesis, elevated lung monocyte counts, and reduced peak viral load in subsequent RSV infection comparable to that seen with host *H. polygyrus* infection.

### Conclusion

These results show that during *H. polygyrus* infection serum borne factor(s) are released inducing an antiviral state in the lung. These factor(s) also drive systemic monocyto-sis leading to increased numbers of anti-viral monocyte-derived macrophages within the lung that are sufficient and essential for mounting an effective immune response to RSV infection.



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## Burden of Respiratory Syncytial Virus (RSV) in Adults in the United Kingdom (UK): A Systematic Literature Review (SLR) and Gap Analysis

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Annie Lied-Lied - ARNI0030

**Annie Lied-Lied<sup>1</sup>, Malcolm Macartney<sup>1</sup>, Steph Beaver<sup>2</sup>, Eve McArthur<sup>2</sup>, Faye Saville<sup>1</sup>, Evelyn Turner<sup>1</sup>, Vaishali Yadav<sup>3</sup>**

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### Background

RSV is commonly diagnosed in children, but due to limited routine diagnostic testing in adults, the burden of RSV in adults is poorly understood. Infection with RSV may lead to severe disease or complications in certain clinical risk groups. Given the number of prophylactic and therapeutic candidates in advanced development, understanding the burden of RSV disease in different risk groups will be critical to appraise these options. We conducted an SLR to characterise the disease burden of RSV in adults ( $\geq 15$  years) in the UK, including in high-risk subgroups, and to highlight where evidence gaps exist.

### Method

An SLR was conducted in October 2021 querying MEDLINE, Embase, the Cochrane library and York Centre for Reviews and Dissemination databases, relevant congresses, and economic and Health Technology Assessment websites. Records were reviewed by two individuals and eligible studies reported epidemiological, economic, and/or clinical burden outcomes for RSV infection in UK adults. High-risk subgroups of interest included elderly ( $\geq 65$  years;  $\geq 75$  years of age), immunocompromised and people with a certain underlying health condition. A gap analysis was conducted to identify subgroups/outcomes where little or no data exist in the literature.

### Result

Among 3,142 records reviewed; 22 studies fulfilled the SLR eligibility criteria. Most literature assessed a general (non-high risk) cohort ( $n=14$ ), followed by elderly ( $\geq 65$  years:  $n=6$ ;  $\geq 75$  years:  $n=2$ ) and immunocompromised individuals ( $n=5$ ). In these subgroups, the most frequent outcomes were RSV incidence, RSV-attributable mortality and direct resource use (including drug prescription and hospital/ICU admissions). RSV incidence rates ranged from 0.09-17.9% in general cohorts and 0.7-26.5% in high-risk cohorts; with highest results from 2009/10 (influenza pandemic) when high rates of diagnostic testing likely increased detection. Only studies of immunocompromised patients reported RSV clinical burden outcomes. A particularly limited evidence base for RSV burden in comorbid and elderly ( $\geq 75$  years) patients was highlighted, indicating these high-risk groups would benefit from future research notably on RSV clinical pathway.

### Conclusion

Evidence gathered in this comprehensive review suggests a substantial disease burden associated with RSV in the UK adult population. However, the paucity and heterogeneity of evidence captured in the SLR means it is challenging to fully describe the burden across different adult risk groups, particularly in comorbid and elderly patients. Further research on RSV clinical pathway including cost-of-illness evaluation would help quantify RSV burden, support economic evaluation for decision-making and inform policy decisions.



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## An unexpected encounter: RSV-NS1 interacts with a subunit of the mediator complex

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Tessa Van Royen - ARNI0031

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### Background

RSV encodes two non-structural (NS) proteins, NS1 and NS2. These proteins can counteract the type I and III interferon responses and do so independently as well as cooperatively.

### Method

To better understand how RSV-NS1 modulates host cell functions, we mapped the NS1 cellular interactome by three different protein-protein interaction methods: BioID, Mammalian Protein Protein Interaction Trap (MAPPIT) and Kinase Substrate Sensor (KISS).

For the BioID screen, we generated a recombinant RSV virus in which a promiscuous point mutant of the E. coli-derived BirA biotin ligase (BirA\*) is genetically fused to NS1. This was established through a BAC-based reverse genetics system. The first gene position in this BAC vector encodes the far-red fluorescent protein monomeric Katushka 2 (mKate2). We thus also generated a recombinant mKate2-BirA\* expressing RSV virus, which is an adequate control virus to perform the BioID screen. Human lung carcinoma A549 cells were infected with the recombinant BirA\*-bearing viruses in the presence of an excess of free biotin. As such, proteins in the proximity of the baits, hence comprising candidate interactors, were biotinylated. These biotinylated proteins were subsequently enriched with streptavidin conjugated beads and further identified and quantified by label-free quantification (LFQ) mass-spectrometry.

MAPPIT and KISS are binary methods based on cytokine signal transduction. We applied these methods to identify NS1-interacting proteins in HEK293T cells against a prey collection of 14817 proteins, selected from the human ORFeome collection version 8.1 and the ORFeome collaboration (OC) collection.

### Result

MED25 was identified by all three screening methods as an NS1 interactor. MED25 is a subunit of the Mediator complex and involved in the recruitment of transcription factors to promoters of their target genes, activating their transcription. The interaction between MED25 and NS1 was also confirmed by co-immunoprecipitation.

An important question is what the biological relevance of the NS1-MED25 interaction is for the host cell, RSV or both. To address this, we generated MED25 knockout A549 cell lines by CRISPR/Cas9. We observed that the replication of RSV B1 was enhanced in MED25 knockout A549 cells compared to wild type A549 cells, suggesting that MED25 has an antiviral role.

### Conclusion

RSV NS1 interacts with MED25 and thereby potentially hinders the binding of MED25 to transcription factors that regulate the expression of immune response genes.



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## COST-EFFECTIVENESS OF PALIVIZUMAB FOR THE PREVENTION OF SEVERE RESPIRATORY SYNCYTIAL VIRUS (RSV) INFECTION IN MODERATE-TO-LATE PRETERM INFANTS USING THE INTERNATIONAL RISK SCORING TOOL (IRST)

Barry Rodgers-Gray - ARN10032

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### Background

Palivizumab (PVZ) is the only licensed effective therapy for preventing RSV hospitalisation (RSVH) but reported cost-effectiveness varies in moderate-to-late preterm (32-35 weeks' gestational age [wGA]) infants. The 3-variable IRST can guide prophylaxis for infants at greatest risk of RSVH and may potentially improve cost-effectiveness of palivizumab.

### Method

The cost-effectiveness of IRST-guided prophylaxis was assessed using a new up-to-date cost-utility model. A systematic review of previous economic evaluations of PVZ in 32-35wGA infants and expert input informed the structure, inputs and costs. Prophylaxed/untreated infants followed a semi-Markov process having either an RSVH, emergency room/outpatient-attended RSV infection, or were uninfected/non-medically attended. The IMPact randomised trial was the primary source of PVZ efficacy (82% reduction in RSVH), with birth data and hospital outcomes derived from the pooled dataset of 7 Northern Hemisphere studies used to develop the IRST. The Canadian publicly funded healthcare system was used for the base case, with no vial sharing permitted (50mg: CAN\$752; 100mg: \$1,505), mortality applied only in intensive care (0.43%), and long-term respiratory morbidity modelled to 18 years across a lifetime horizon. Cost per quality-adjusted life year (QALY) was assessed in high- and moderate-risk (HM) infants as defined by the IRST.

### Result

PVZ was highly cost-effective (\$18,546/QALY) in HM infants (Table) and remained so when assessed only in moderate-risk infants (\$23,109). In deterministic sensitivity analyses ( $\pm 20\%$  on main variables) the model was most sensitive to utility scores, PVZ cost, and non-prophylaxed hospitalisation rate. Probabilistic sensitivity analyses (10,000 iterations) resulted in incremental costs of \$19,376/QALY, with a 92.4% probability of palivizumab being cost-effective at a \$50,000 willingness-to-pay threshold. Vial sharing (5% wastage) considerably improved cost-effectiveness in HM infants (\$12,553). Extending prophylaxis to cover 98% of potential RSVHs (base case: 85%) was equally cost-effective (\$21,498).

### Conclusion

PVZ demonstrated robust cost-effectiveness using the IRST in this new cost-utility model. With COVID placing increased pressures on healthcare systems, extending prophylaxis to cover nearly all potential RSVHs appears a cost-effective option.



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## Dose adjustment of rilematovir in combination with CYP3A4 inhibitors based on PBPK modeling and clinical data

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Tristan Baguet - ARNI0034

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<sup>1</sup>Janssen Research & Development

### Background

Rilematovir (RMV) is a small molecule in development for treatment of respiratory syncytial virus (RSV) infection. Antiviral activity and clinical benefit are being explored in phase 2 clinical studies in non-hospitalized RSV-infected adults and children.

RMV metabolism occurs predominantly via CYP3A4 (92-94%), with 6-8% metabolized via the UGT-mediated pathway. Posaconazole, a strong CYP3A4 inhibitor, and other azoles are frequently prescribed prophylactically in immunocompromised individuals. Therefore, a physiologically based pharmacokinetic (PBPK) model was developed to investigate the impact of drug-drug interactions (DDI) on RMV pharmacokinetics. Subsequently, a phase 1 study was conducted to evaluate RMV in combination with posaconazole.

### Method

A PBPK model was developed in SimCYP V19 to investigate the effect of strong and moderate CYP3A4 inhibitors (eg, itraconazole, clarithromycin) on the single dose and steady state (ss) maximum concentration (C<sub>max</sub>) and area under the curve (AUC) of RMV. Model inputs included physicochemical properties, in vitro metabolism data, data from existing phase 1 clinical studies including a DDI study with itraconazole, and a human mass balance study.

A phase 1, open-label study was conducted in healthy participants (n=15) to assess the effect of posaconazole on RMV exposure. Participants received RMV 125 mg daily (QD) on Days 1-3. Administration of posaconazole commenced on Day 6 with morning and evening (loading) doses of 300 mg. Subsequently, posaconazole 300 mg QD was administered on the mornings of Days 7-16 and coadministered with RMV 125 mg QD on Days 12-16. Venous blood samples were collected over 24 hours after study administration on Days 3 and 16. Least squares geometric mean ratios and 90% CIs were constructed for RMV AUC<sub>0-24hrs</sub> and C<sub>max</sub> comparing Day 16 to Day 3.

### Result

In PBPK analyses, moderate and strong CYP3A4 inhibitors increased the RMV C<sub>max,ss</sub> by 1.5-fold. RMV AUC<sub>24h,ss</sub> increased by 2.5-fold with itraconazole, 2.9-fold with clarithromycin, and up to 4.7-fold with posaconazole. In the phase 1 study, co-administration of posaconazole with RMV 125 mg QD resulted in a 2.0-fold increase in RMV C<sub>max,ss</sub> and a 4.3-fold increase in RMV AUC<sub>24hrs,ss</sub>, which confirmed the PBPK predictions. RMV was safe when administered alone and in combination with itraconazole and posaconazole; all adverse events were mild or moderate in severity.

### Conclusion

Based on PBPK simulations, the RMV dose was adjusted from 250 mg to 125 mg twice daily when co-administered with moderate/strong CYP3A4 inhibitors, with the exception of coadministration with posaconazole, for which the RMV dose was adjusted to 125 mg QD, as confirmed by the DDI study.



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## Optimization and characterization of a live attenuated respiratory syncytial virus vaccine candidate

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Judith Alamares-Sapuyay - ARNI0035

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### Background

Respiratory syncytial virus (RSV) is the leading cause of bronchiolitis and pneumonia in infants, but no vaccine is available. Our team at Sanofi has partnered with the National Institutes of Health (NIH) to generate a safe and effective live attenuated RSV vaccine. One of the candidates that is being evaluated in a phase 1b clinical trial is  $\Delta$ NS2/ $\Delta$ 1313/I1314L ( $\Delta$ NS2). This construct has a deletion of the IFN antagonist NS2 and a deletion in codon 1313 of the L polymerase gene that is stabilized by an I1314L mutation. The goal of this study is to further improve  $\Delta$ NS2 for ease of manufacture and enhanced immunogenicity.

### Method

To improve the stability and immunogenicity of  $\Delta$ NS2, four mutations in the F protein of RSV strain "line 19" (I79M, K191R, T357K, N371Y) that confer enhanced pre-fusion (pre-F) levels and thermal stability (Stobart et al. 2016) were incorporated into  $\Delta$ NS2 to generate the modified candidate  $\Delta$ NS2-L19F-4M. The growth of  $\Delta$ NS2-L19F-4M was compared to  $\Delta$ NS2 by infecting Vero cells at MOI of 0.1 and harvesting the virus at 1 to 10 days post-infection followed by viral titration. The pre-F/total F ratio on the virion was determined by ELISA using D25 (site 0, pre-F-specific antibody) and Synagis (antibody specific to the site II region common to pre-F and post-F). To evaluate thermal stability, the virus was incubated at 4°C, 25°C, 32°C and 37°C and viral titers at several time points were determined in Vero cells. Attenuation of  $\Delta$ NS2-L19F-4M was compared to  $\Delta$ NS2 by infecting human airway epithelial cells (HAE) at MOI of 5 and viral titration at 1,3,5,7 and 9 days post-infection. To evaluate genetic stability,  $\Delta$ NS2-L19F-4M was serially passaged 10 times in Vero cells. In vivo attenuation and immunogenicity were assessed in African green monkeys (AGM). Briefly, AGMs were immunized with the virus (intranasal +/- intratracheal administration) and viral titers in tracheal lavages and nasopharyngeal samples were determined to assess attenuation. Immunogenicity was assessed by PRNT60 assay using AGM sera collected at 21 and 28 days post-immunization.

### Result

In vitro studies demonstrated that  $\Delta$ NS2-L19F-4M reaches a peak titer similar to that of the parental  $\Delta$ NS2 and exhibits enhanced pre-F levels and thermal stability. The  $\Delta$ NS2-L19F-4M candidate is attenuated in HAE cells, similar to the parental strain. Serial passage in Vero cells showed that the four "line 19" F mutations are stable over 10 passages. In vivo studies demonstrated that  $\Delta$ NS2-L19F-4M and the parental strain have comparable attenuation and immunogenicity in African green monkeys.

### Conclusion

We successfully generated a modified  $\Delta$ NS2 construct containing four "line 19" F mutations ( $\Delta$ NS2-L19F-4M). Our results demonstrated that  $\Delta$ NS2-L19F-4M is a promising live attenuated vaccine candidate that merits further evaluation in a clinical trial in humans.





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## Preterm Infants Exhibit a Reduced Pro-inflammatory Immune Response to RSV

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Jeremy Anderson - ARNI0036

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### Background

Respiratory syncytial virus is the most common viral pathogen associated with acute lower respiratory tract infections in children. A highly vulnerable group to severe RSV disease is infants born preterm, being more likely to require hospitalisation, admission to the intensive care unit and ventilatory assistance. The reason for their increased susceptibility is multi-factorial, however, a major contributor is thought to be an immature innate immune system.

### Method

In this study, we collected cord blood from 25 moderate preterm infants (30-34 weeks gestational age) and 25 term infants and stimulated cord blood mononuclear cells (CBMCs) with RSVA and RSVB for 24h. Following stimulation, multiplex cytokine assays, flow cytometry and RNA-sequencing were used to determine differences in their innate immune response to RSV.

### Result

We found that preterm and term infants produced a similar inflammatory cytokine/chemokine profile to RSVA and RSVB that was associated with elevated IP-10, IFN- $\alpha$ , IL-6, IL-1 $\beta$  and TNF- $\alpha$  levels. A reduced frequency of monocytes and increased frequency of plasmacytoid dendritic cells were also similarly observed in preterm and term infants. RNA-sequencing revealed that term infants displayed a unique signature consisting of upregulated CSF2, CXCL1, CXCL2, GZMB, IL-10, MMP1 and XCL1 following RSVA stimulation, suggesting enhanced cytotoxicity and neutrophil chemotaxis compared to preterm infants. Weighted gene co-expression network analysis (WGCNA) analysis showed term infants expressed a unique inflammatory regulatory module that was not observed in preterm infants.

### Conclusion

Overall, these data support the idea that preterm infants differ to term infants in their capacity to regulate the inflammatory response to RSV, which may help to explain why preterm infants are at an increased susceptibility to severe RSV disease. Strategies to improve immune regulation in preterm infants may be key to reducing the burden of disease in this highly vulnerable group.



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## Epidemiology of respiratory syncytial virus in children aged less than 5 years during the COVID-19 pandemic in South Africa

Nicole Wolter - ARN0037

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### Background

The annual respiratory syncytial virus (RSV) season in South Africa typically occurs in the autumn months (March through May), with a peak preceding the winter influenza peak. Most infections occur in children aged <1 year. The first COVID-19 case was reported on 5th March 2020. Control measures implemented during the COVID-19 pandemic have impacted the circulation of other respiratory viruses. We aimed to describe the epidemiology of RSV in children aged <5 years in the pre-pandemic period (2018-2019) compared to during the COVID-19 pandemic (2020-2021).

### Method

We conducted syndromic surveillance for influenza-like illness (ILI) and severe respiratory illness (SRI) at sentinel public health clinics and hospitals located in five of the nine South African provinces. For children aged <5 years meeting the surveillance case definition, respiratory specimens were collected and tested by reverse transcription real-time PCR for RSV, influenza and SARS-CoV-2. Clinical and demographic information was collected through patient caregiver interview and review of clinical records. Categorical variables were compared using the chi-squared test.

### Result

The RSV detection rate was 27% (856/3181), 23% (873/3888), 25% (589/2383) and 17% (417/2398) in 2018, 2019, 2020 and 2021 respectively ( $P<0.001$ ). In 2018-2019, most RSV infections were detected in autumn (70%, 1216/1729) whereas in 2020-2021 infections were distributed throughout the year (autumn 25%; winter 25%, spring 32%, summer 18%) ( $P<0.001$ ). In the pandemic, the age distribution shifted to older children, with cases aged 1-4 years comprising 18% (152/856), 22% (191/873), 22% (130/589) and 27% (112/417) in 2018, 2019, 2020 and 2021 respectively ( $P=0.002$ ). Of 919 children with RSV infection in 2020-2021, 19 (2%) were co-infected with SARS-CoV-2. Among hospitalised children with SRI, a greater proportion of children in 2020-2021 required supplemental oxygen (75% (666/893) in 2020-2021 vs. 59% (914/1547) in 2018-2019,  $P<0.001$ ) and



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ventilation (8% (68/893) in 2020-2021 vs. 3% (52/1547) in 2018-2019,  $P<0.001$ ), although no difference was observed in in-hospital mortality (0.1% (1/886) in 2020-2021 vs. 0.4% (6/1547) in 2018-2019,  $P=0.223$ ).

### **Conclusion**

Since the COVID-19 pandemic, South Africa has experienced lower and year-round circulation of RSV, without typical seasonality, and a shift towards older children. More severe disease among hospitalised children may be due to reduced immunity resulting from decreased exposure to respiratory viruses during COVID-19, changed clinical practices, or reflect the change in healthcare seeking behaviour to more severe cases.



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## Disease burden of RSV infections in young children in primary care: preliminary results from the RSV ComNet study in five European countries

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Jojanneke Van Summeren - ARNI0038

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### Background

The objective of the RSV ComNet study is to measure the clinical burden and societal impact of RSV in young children in primary care. The study is being performed in Italy, Spain, England, Belgium and the Netherlands and will be the most comprehensive RSV burden of disease study in primary care. Data collection is still ongoing and preliminary results are shown.

### Method

RSV ComNet is a prospective cohort study using a standardized study protocol. In England data have been collected since October 2020 and in the other countries since October 2021. Paediatricians or general practitioners (GP) swabbed children aged <5 years with an acute respiratory infection. Parents of children with an RSV positive laboratory test result were asked to complete two questionnaires, after 14 and 30 days. The questionnaires included questions about healthcare use, days of illness, societal impact and current health status. The sample size calculation showed at least 100 RSV positive children were needed in each country.

### Result

From Oct 2020 until April 2022, 1087 children were swabbed and 409 tested RSV positive. This included 216, 87, 52, 46, and 8 RSV positive children in Italy, England, Belgium, Spain and the Netherlands, respectively. For Italy, questionnaire data from only 17 children were available at this stage. The median age of RSV positive children varied from country to country and ranged from 7 to 20 months. The median duration of illness, ranged by country from 7 to 14 days. 20-76% of the RSV positive children consulted their paediatrician or GP because of RSV related problems in the 30 days after the swab was taken. The percentage of hospitalized children was 35% (Belgium), 12% (Italy), 11% (Spain), 7% (England), and 0% (Netherlands). Aerosol therapy was the most prescribed medication, the percentage of prescriptions of aerosols was the smallest in England (9% of the children) and highest in Spain (53%). Fourteen and 30 days after taking the swab, at least 1 persisting symptom was reported in respectively 29%-57% and 29%-50% of the children.

### Conclusion



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Preliminary results indicate that RSV infections contribute to a considerable clinical burden and healthcare use of young children in primary care. The RSV ComNet study also collects data about the societal impact of an RSV infection. RSV ComNet data will be a baseline reference point to assess the impact of future immunisation programmes.



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## Defining the Burden of Disease Pyramid of RSV in the European Union: estimates of RSV-associated hospitalizations in children under 5 years of age

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Marco Del Riccio - ARNI0039

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### Background

Globally, respiratory syncytial virus (RSV) causes an estimated 22-50 million episodes of acute lower respiratory infections (ALRI) every year in children younger than 5 years, and 3.4 million cases require hospitalization. Future evaluations on new preventive strategies (i.e. immunisation) require the availability of robust age-specific burden of disease estimates. To date, no collective estimates on RSV-associated hospitalizations in children under 5 years have been published for the European Union (EU). Through statistical modeling, we estimated the RSV hospitalization burden in children under 5 years of age for the EU, by age group and country.

### Method

First, we collated national RSV-associated hospitalization estimates in children under 5 years via the RESCEU project for Denmark, England, Finland, Norway, the Netherlands, and Scotland during 2006-2018; the estimates for two additional countries (France and Spain) were added after conducting a literature review. Then, we used these estimates, plus two different indicator sets, to estimate the hospitalization burden in all EU countries (28 countries including the UK) with the multiple imputation and the nearest neighbor matching approaches. We present the results of the average of the 4 models.

### Result

We estimated that an average of 213,014 (95% CI:192,181-233,844) hospital admissions with respiratory infection were associated with RSV in the 28 EU countries per winter in children under the age of 5, with most cases occurring among children aged less than 1 year (64%) and those aged 1-2 years (28%). Infants aged less than 2 months represented the most affected group (71.3 per 1,000 children; 95%CI: 66.1-76.6), with the rates declining as the children got older: 38.7 per 1,000 in children aged 3-5 months, 17.6 (6-11m), 5.9 (1-2 years) and 1.5 (3-4 years). The hospitalization rates varied widely across the EU: rates in the first age group (0-2m) ranged from 46.3 (36.5-56.2) per 1,000 in the Netherlands to 99.2 (89.4-109.0) in France.

### Conclusion

To our knowledge, this is the first attempt to estimate the RSV hospitalization burden in children under the age of 5 years in the total EU. Our extrapolations would benefit from more countries with RSV-associated estimates to populate the statistical models (e.g. additional country estimates in southern and eastern Europe) and more research is needed to explain the variability in the observed hospitalization rates across the EU. Our estimates could help optimize public health responses and resource allocation, provide new evidence that can support



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prevention efforts such as future immunization programs, and represent a benchmark to understand the changes in RSV burden in the post-COVID-19 era.





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## Societal impact of RSV infections in young children in primary care in Italy

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Jojanneke Van Summeren - ARN10040

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### Background

RSV infections cause significant symptoms and distress among children and parents, but little is known about the societal impact of RSV in primary care. We initiated a study in Italy during the winter of 2019/20 to assess the clinical burden and societal impact of RSV infections in children (<5 years of age) in primary care.

### Method

The RSV ComNet study uses a prospective cohort study design and a standardized study protocol. Data were collected in two Italian regions: Lazio in the Centre of Italy and Puglia in the South. After obtaining informed consent, paediatricians swabbed children aged <5 years with an acute respiratory infection (ARI). Parents of children with an RSV positive laboratory test result completed a follow-up questionnaire after 14 days, with questions about healthcare use, days of illness and the societal impact. Data are presented for three age groups (0-12, 13-24 and 25-60 months).

### Result

In total, 119 children were included in the study between week 45/2019 and 12/2020 (data collection was then disrupted with the emergence of SARS-CoV-2). The median age of RSV positive children was 15 months (IQR: 7-30). The median duration of illness was 7 days (IQR 5-10) and did not vary by age group. In total, 53% of the parents missed at least 1 working day because of the child's RSV infection (Table 1). The mean number of days missed was 4.8, 5.04 and 5.2 days for the 3 age groups, respectively. The percentage of children who missed ≥1 day from day-care facilities was 8%, 68% and 82% for the 3 age groups, respectively, with a mean number of days missed per child of 1.8, 8.8 and 10.4 days.

### Conclusion

RSV infections in young children in primary care have a considerable societal impact, with the impact being higher after 12 months of age when parents are probably no longer on maternity/paternity leave and children go to day-care facilities. More knowledge is needed regarding the indirect costs associated with an RSV infection and total societal cost estimates (including direct health care costs) are necessary to make informed decisions on the optimal use of newly emerging immunization strategies. These cost estimations are currently under preparation and will be presented during the RSV Symposium.



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## Defining the burden of disease pyramid of RSV in the European Union: estimates of RSV-associated hospitalisations in adults

Richard Osei-Yeboah - ARNI0042

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### Background

Respiratory syncytial virus (RSV) is a major cause of lower respiratory infections in older adults, which could lead to hospitalisations and even death. Only the more severe cases, once hospitalised, are diagnosed. Severe forms of RSV infections are common in adults with cardiopulmonary and other immunosuppressing conditions. Studies have shown that the burden of RSV-attributable morbidity and mortality among the older adult population may be comparable to seasonal influenza. To effectively plan healthcare resource utilisation and adequately manage the increasing RSV-related healthcare needs of the older adult population across Europe, it is important to understand the disease burden.

### Method

We used a two-stage approach in this study. In Stage 1, we gathered the RSV Consortium in Europe (RESCEU) estimated RSV-associated hospitalisation rates for adults in Denmark, England, Finland, France, Norway, Netherlands, and Scotland from 2006 to 2018. We also conducted a scoping literature review to identify estimates using the same methodology and found 3 new estimates for England. In Stage 2, we extrapolated these estimates to the EU using two sets of 10 predictors and two different approaches (matching and imputation), which generated four sets of estimates and the averages of these results are reported.

### Result

Extrapolating data from 7 to 28 EU countries, we estimate that on average 158,185 (95% Confidence Interval (CI): 140,651-175,742) RSV-associated hospitalisations occur annually among adults (aged 18 years and older). The highest proportion of hospitalisations (47%) occurred among adults aged 75-84 years, with an annual average of 75,013 (95%CI: 70,466-79,580) at a rate of 2.25 (95% CI: 2.12-2.39) per 1000 adults. The estimated rate of hospitalisation was even higher for adults ≥85 years (3.09, 95%CI: 2.63-3.55 per 1000) with an average of 39,153 (95%CI: 33,368-44,939) hospitalisations per year. Persons aged 18-64 years had the lowest hospitalisation rate of 0.04 (95% CI: 0.03-0.05 per 1000) and annual average hospitalisation of 12,581 (95%CI: 10,578-14,585).

### Conclusion

Our extrapolation of RSV-associated hospitalisations in older adults is the first analysis integrating available data to provide empirical evidence of the disease burden in this population across the EU. Importantly, the average hospitalization estimate in adults (158,185 hospitalisations per annum) was relatively high compared to children aged 0-4 (213,014 hospitalisations per annum) [RESCEU EU estimate using the same methodology]. These data



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will significantly contribute to decision-making and policy formulation toward scaling up healthcare service delivery for this population.



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## IN VIVO EFFICACY OF EDP-323, A NOVEL L-PROTEIN INHIBITOR, FOR THE TREATMENT OF RESPIRATORY SYNCYTIAL VIRUS

Rachel Levene - ARNI0044

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<sup>1</sup>Enanta Pharmaceuticals

### Background

Almost every child is infected with respiratory syncytial virus (RSV) before age 2. In most, RSV presents as a common cold; however, for premature infants, the elderly, and the immunocompromised, RSV can result in substantial morbidity and mortality. Despite the availability of a prophylactic monoclonal antibody (Palivizumab) and aerosolized ribavirin, there is a high unmet medical need for RSV therapeutics. Here we describe the in vivo efficacy of EDP-323, a novel non-nucleoside, small molecule L-protein inhibitor of RSV.

### Method

The antiviral activity of EDP-323 against clinical and laboratory isolates of RSV was evaluated in HEP-2 cells and 3-dimensional primary human airway epithelial cells grown in an air-liquid interface system using cytopathic effect and RT-qPCR as readouts. The antiviral activity of EDP-323 was evaluated in vivo in BALB/c mice infected with RSV-A2 in two separate studies: a prophylactic study where mice were dosed prior to infection and a therapeutic study where mice were dosed 1 and 24 hours post infection. In both studies, animals were weighed daily and dosed for 5 days prior to euthanasia at which point, viral load was determined in lung homogenates. In the prophylactic study, additional lung histopathology, serum cytokine analysis, and next generation sequencing (NGS) of purified viral RNA for variant detection were performed after euthanasia.

### Result

EDP-323 displays potent anti-RSV activity with a half-maximal effective concentration (EC<sub>50</sub>) ranging from 44-360 pM against multiple laboratory and clinical isolates of RSV A and RSV B subtypes. In BALB/C mice infected with RSV-A2, EDP-323 significantly reduced viral replication when dosed prophylactically and therapeutically, as measured by both RT-qPCR and plaque reduction assay on lung lysates. Both prophylactically and therapeutically dosed EDP-323 protected animals from virus-induced changes to body and lung weight. In the prophylactic study, EDP-323 was associated with reduced lung immunopathology and dose-dependent decreases in pro-inflammatory cytokines including IFN $\gamma$ , TNF $\alpha$ , and IL1 $\beta$ . NGS of virus recovered at the end of the prophylactic study revealed many L protein variants in both EDP-323 treated and non-treated mice. However, no viruses were isolated in any group with variants at residues that were identified through in vitro drug resistance generation as conferring EDP-323 drug resistance.

### Conclusion

EDP-323 potently inhibited RSV replication both in vitro and in vivo. EDP-323 blocked virus-associated pathology in a mouse infection model without generating EDP-323 drug resistant viruses. These data support further clinical investigation of EDP-323 as a treatment for RSV infection.



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## RSV SEVERITY BEFORE AND AFTER THE FIRST WAVES OF THE SARS CoV 2 PANDEMIC: A COHORT STUDY IN MELGHAT INDIA

Rowena Crow - ARNI0046

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### Background

Melghat has a quasi-biennial RSV periodicity with peaks occurring every alternate year. During the COVID pandemic outbreak waves in 2020 respiratory infections were abrogated in India as worldwide primarily by mask usage and varying degrees of lockdowns. The question addressed in this analysis, is: Did the incidence of severe RSV lower respiratory tract infections (LRTI) in children under 2 change and what age groups did this affect?

### Method

Active surveillance of infants and children younger than 2 years of age, with weekly home visits for detection of acute lower respiratory tract infection, in all consented subjects was conducted in 93 villages of Melghat Maharashtra India from August 2016 to December 2020. All hospitals and primary health centers were surveyed daily for admissions of subjects from the study area. Surveillance in 33 villages was restarted in June of 2021. Nasopharyngeal swabs were collected from children with severe, or very severe LRTIs and all who died, using flocked swabs, stored in PrimStore MTM, and tested for RSV using nucleic acid tests at the ICMR, National Institute of Virology Pune. RSV + severe and very severe LRTI in the community and hospital were compared in the cohort of subjects from the 33 villages before (2016-2020) and after the major SARS CoV 2 outbreak in India. Population based rates, of severe and very severe RSV LRTI were measured over the RSV seasons, were compared during the 2 periods using incidence rate ratios (IRR) with 95% CI.

### Result

The incidence of severe and very severe RSV increased in all age groups in the post pandemic period. In the 0 to 90 days and 91 to 180 days age groups there was an almost 3-fold increase, with a IRR of 2.8 (1.4-5.2) and 2.8 (1.4-5.0) respectively. This was due to a dramatic increase in community cases- IRR of 26.6 (9.0-94.3) and 7.8 (3.3-18.0) in the 2 age groups respectively. In contrast, the incidence of hospital admissions for RSV LRTI dropped dramatically likely reflecting a change in the healthcare seeking behaviour.

### Conclusion

Following almost complete abrogation of circulation of RSV during COVID lockdown, RSV LRTI rebounded the community, in young children after restrictions were lifted, diametrically opposite to what happened with RSV hospitalization.



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## Lack of Temporal Prevalence and Geographic Distribution of Nirsevimab Escape Variants among Global RSV Strains Since 1956

Michael Abram - ARNI0047

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### Background

Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infection and hospitalization in infants. Nirsevimab is a long-acting neutralizing monoclonal antibody that binds the RSV prefusion (F) protein and is being developed to protect all infants for an entire RSV season. The nirsevimab binding site has been historically well conserved, however, the paucity of recent prospective genomic data limits investigation of temporal evolution and transmission patterns of potential escape variants. Here we report temporal prevalence, geographic diversity, and resistance profile of global RSV isolates containing nirsevimab binding site substitutions through 2021.

### Method

RSV-positive samples, primarily from infants, were collected and sequenced as part of ongoing INFORM-RSV (global) and OUTSMART-RSV (USA) molecular epidemiology studies (2015-2024). Additional RSV F protein sequences were obtained from NCBI GenBank (1956-2016). Identified RSV F protein substitutions in the nirsevimab binding site (AA 62-69 and AA 196-212) compared to 2013 NLD reference strains were evaluated in a recombinant RSV neutralization susceptibility assay.

### Result

Overall, 2,385 published RSV F sequences from 37 countries (RSV A: N=1,525; RSV B: N=860) and 5,675 prospective RSV F sequences from 17 countries (RSV A: N=2,875; RSV B: N=2,800) have been collected and analyzed. More than 98% of amino acids in the nirsevimab binding site have remained highly conserved at all 25



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positions in RSV A and at 23 of 25 positions in RSV B during nirsevimab clinical development from 2016 to 2021. In 2015, nirsevimab binding site polymorphisms I206M:Q209R that maintain susceptibility to nirsevimab neutralization emerged among circulating RSV B strains. RSV B F variants with reduced susceptibility to nirsevimab neutralization were periodically detected in several different countries at low frequencies (<1.0%), including: L203I (USA, 1993; 3005-fold), K65Q:K68N (KEN, 2012; 1239-fold), K68Q:S211N (NLD and TWN, 2005-2007; 35.7-fold), N201S (ZAF, 2017; 126.7-fold), K68Q:I206M:Q209R (JPN, 2018; 46.4-fold), N201T:I206M:Q209R (USA, 2018; >417.8-fold), and K68N (CAN, 2019; 29.9-fold). Nirsevimab neutralized all other RSV-A F and -B F protein variants containing binding site substitutions identified in both northern and southern hemispheres.

### **Conclusion**

Since 1956, the nirsevimab binding site has remained highly conserved among circulating RSV strains. Nirsevimab escape variants have been rare and have not increased in geotemporal frequency. However, as RSV continues to evolve, ongoing surveillance of circulating strains is necessary to characterize the impact of genetic variability on nirsevimab susceptibility.





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## Structural proteins of SARS-CoV-2 and their mutations to form basis of efficacy and longevity of vaccines.

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### Background

Immunological profiling of human response as generated by natural infection or vaccination of SARS-CoV-2 proteins/genes need to be addressed as an essential study for development of a dependable vaccine against COVID-19. Of four structural proteins viz; Envelope protein, Spike protein, Nucleocapsid and Membrane protein, the dynamic mutations in Physico-chemical properties of constituting amino acids in Spike and Envelope proteins could be crucial in internalization and subsequent rupturing of virion in the cytoplasm of the host cell.

### Method

A systematic search of published papers on structural protein of SARS-CoV-2 from different countries/population groups globally was attempted. Amino acid sequences of all strains of coronavirus were obtained from a software, Clustal Omega. The obtained amino acid sequences were compared with reference to the replacement patterns between two strains. The changing patterns of amino acids were observed to understand their corresponding role in the ultimate pathogenicity and severity of the particular viral strain.

### Result

In the travel of SARS-CoV-2 from Wuhan to Omicron strain, our analysis has shown that altered chemical properties of mutated amino acids in Spike protein could influence the internalization competence of the virus in favor of the host. However, prospective changes during time to come may bring virus supportive changes causing severe clinical conditions. Among patients the Spike, Membrane, Envelope and Nucleocapsid proteins are the principal antigenic proteins in SARS-CoV-2 but there will be localized initially within epithelial cells of the respiratory tract and their real antigenic competence to produce an antigenic specific clone of IgG antibodies will depend on how these proteins come in contact with immune cells circulating in blood.

### Conclusion

For an effective vaccine, study of how T cells generate phagocytic or sensitizing role by securing Antigenic Presenting Cells and how B cells will secure vaccine-induced spike proteins and generate specific antibodies. The study of chemical changes in the Spike protein or Membrane protein will be crucial for an effective vaccine as mutated proteins need to be supplemented as vaccine to neutralize mutated viruses.



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## Interleukin-1a evokes cachexia post respiratory viral infection

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**Ziyin Wang** - ARNI0050

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### Background

Respiratory syncytial virus (RSV) remains a global challenge of high morbidity and mortality due to the lack of available treatment and a protective vaccine. One of the most detrimental symptoms post RSV in infants and the elderly is cachexia, which is the systematic depletion of fat and muscle mass due to the loss of appetite and subsequent weight loss. Understanding the mechanisms of infection-associated cachexia could have important therapeutic implications. However, little is known regarding the initiation and regulation of cachexia in the context of respiratory viral infections.

### Method

In order to elucidate the inflammatory drivers of infection-associated cachexia, we utilised a model of reversible cachexia in mice with RSV where CD8<sup>+</sup> cell depletion rescues weight loss. We compared the blood RNA transcriptomic profile and cytokine readouts in bronchoalveolar lavage & lung supernatant in RSV-infected mice, with and without CD8 cell depletion.

### Result

Here, we identified 16 genes and 12 cytokines differentially expressed between groups with and without CD8 cell during RSV infection. Our ongoing depletion studies revealed an underappreciated role of the alarmin IL-1 alpha in respiratory viral infections. The IL-1 signalling pathway was systematically upregulated during RSV infection in our mouse model. In the absence of IL-1 alpha, RSV viral load was higher, but mice recovered faster with reduced symptoms of cachexia, including changes in appetite and clinical score. We also show that IL-1 alpha was critical at inducing weight loss in influenza H1N1 infection, suggesting a common mechanism linking systemic antiviral immune responses.

### Conclusion

As IL-1 alpha neutralisation has been shown critical in various cancer-associated cachexia models and parasitic models, dissecting the mechanism of IL-1 alpha-driven infection-associated cachexia could offer exciting therapeutic avenues for treatment against general cachexia.



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## A respiratory syncytial virus prefusion F protein (RSVPreF3) candidate vaccine elicits persistent immune responses when administered to older adults in a phase 1/2 randomized trial

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Veronica Hulstrøm - ARNI0051

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### Background

GSK's RSV candidate vaccine (RSVPreF3) showed the ability to boost humoral and cellular immune responses in older adults in a phase 1/2, placebo-controlled, observer-blind, multi-center trial (NCT03814590). Here we present immunogenicity results up to 12 months post-vaccination from the same trial.

### Method

Older adults (60-80 years) were randomized in 10 equally sized groups to receive 2 intramuscular doses, 2 months apart, of 1 of 9 RSVPreF3 formulations (30 µg, 60 µg or 120 µg RSVPreF3 dosage; non-adjuvanted or with AS01E or AS01B adjuvant) or placebo. Humoral (RSVPreF3-specific IgG, RSV-A and RSV-B neutralizing antibodies [nAb]) and cellular immune responses were assessed on the day of and 1 month after each dose, and 6 and 12 months post-dose 2 (study months [M]8 and 14).

### Result

1005 older adults received dose 1, of whom 970 received dose 2 and 975 completed the M14 visit.

In the vaccinated groups, RSVPreF3 IgG geometric mean concentrations (GMCs) and RSV-A and RSV-B nAb geometric mean titers (GMTs) were highest post-dose 1 without further increase post-dose 2. Although GMCs and GMTs declined over time, they remained above baseline values until M14 (Figure). Geometric mean (GM) fold-changes between baseline and M14 ranged from 2.6 to 4.5 for RSVPreF3 IgG, from 2.7 to 4.4 for RSV-A nAb and from 1.5 to 2.9 for RSV-B nAb.

### Conclusion

All RSVPreF3 candidate vaccine formulations induced humoral and cellular immune responses in older adults. A second dose, given 2 months post-dose 1, did not further increase the induced immune responses, which remained above baseline up to 12 months post-dose 2.



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## RSV burden in the Middle Eastern countries among adults and elderly populations Systematic Review and Meta Analysis

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Eiman Gaid - ARN10052

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### Background

Respiratory syncytial virus (RSV) is the leading cause of acute lower respiratory infections (ALRI), especially among children and has recently been recognised as one of the most common causes of ALRTIs in adults. Excess death from RSV infection is often reported to increase with age. RSV infection studies in adults, including in older adults, are generally scarce in most countries and absent in the Middle Eastern Region (MER). Such evidence forms important baseline information to guide future research and policies.

### Method

The objective of this study is to conduct a systematic review and meta-analysis to estimate the burden of RSV infection associated with lower respiratory tract infections (LRTI) among adult populations in the MER including Gulf Cooperative Countries (GCC). The electronic literature databases: Ovid (MEDLINE, Embase, CAB abstract, Global health), Web of Science, Scopus, and Saudi Digital Libraries (SDL) were searched for studies that focused only on RSV and respiratory virus, where RSV was included among all ages and adults in the MER and GCC. Data on RSV prevalence and by seasonality (where available) were extracted. No date restrictions on publications were applied and the search was limited to English and Arabic studies. Meta-analysis was performed on RSV data and heterogeneity was assessed statistically using the I<sup>2</sup> test. Outcomes were expressed as percentages with 95% confidence intervals (95% CI) around the summary estimate. Age subgroup analyses were conducted wherever sufficient data were available. If statistical pooling was not possible, the findings were presented in a narrative form.

### Result

We retrieved 4,335 studies. After removing all duplicates and unrelated studies, 77 studies were included; 42 studies on the burden of RSV and 35 on burden by seasonality. The pooled prevalence of RSV of all age groups was 19% (95%CI 16-22), heterogeneity I<sup>2</sup> 98.99%. The RSV positive cases were mostly found in hospitalized patients rather than outpatients (22.16% vs 3.57%). The pooled prevalence of RSV in adults was 2.87% (95%CI 2.27-3.47), heterogeneity I<sup>2</sup> 91.39%. A higher prevalence was observed in adult patients above 50 years compared to those with less than 50 years (5% vs 2%, p value <0.001). All the included studies from GCC showed RSV seasons consistently last approximately 6 months or less during winter months between October and March, with peaks commonly occurring in December/January. The slight variation in peaks may be related to the period of data collection. An inverse correlation was observed between the monthly mean temperature and relative humidity.

### Conclusion

RSV in adulthood is more common in people aged 50+, especially during winter months. This study provides important epidemiological information about RSV as most countries in the MER and GCC had limited or no data about adults' infection with RSV. Such information is crucial in designing future public health measures, such as vaccination programs. Further studies and surveillance are needed to improve the understanding of the burden of RSV and close the gap in this field. Modelling studies might be another route to understand better the transmission dynamics and potential vaccine effect in the region.



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## Vaccine-induced antibody Fc-effector functions in humans immunized with a combination Ad26.RSV.preF/RSV preF protein vaccine

Galit Alter - ARNI0053

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### Background

Respiratory syncytial virus (RSV) causes serious lower respiratory tract disease (LRTD) in older adults. No licensed RSV vaccine exists. A single-dose Ad26.RSV.preF/RSV preF protein combination vaccine induces robust humoral and cellular responses in older adults and demonstrated a promising vaccine efficacy of 80.0% for prevention of RSV-LRTD in a proof-of-concept study (CYPRESS; NCT03982199). Antibody Fc-effector functions may protect against RSV infection; however, vaccine-induced antibody Fc-effector functions have not been reported for the Ad26.RSV.preF/RSV preF protein vaccine. The objective of this study was to evaluate vaccine-induced antibody Fc-effector functions in participants receiving Ad26.RSV.preF, RSV preF protein, or Ad26.RSV.preF/RSV preF protein combinations.

### Method

Data from a subset of participants enrolled in a Phase 1/2a study (NCT03502707) evaluating safety and immunogenicity of Ad26.RSV.preF, RSV preF protein, and Ad26.RSV.preF/RSV preF protein combination vaccines in adults aged  $\geq 60$  years were included. On Day 1, participants received Ad26.RSV.preF ( $1 \times 10^{11}$  viral particles [vp]; n=24), RSV preF protein (50  $\mu$ g or 150  $\mu$ g; n=8 per group), a combination of Ad26.RSV.preF/RSV preF protein ( $1 \times 10^{11}$  vp/150  $\mu$ g; n=42), or placebo (n=24). RSV-preF-specific antibody subclasses, isotypes, Fc $\gamma$ R receptor binding, and Fc-effector functions were evaluated pre-vaccination on Day 1 and on Days 15, 29, and 183 post-vaccination.

### Result

Both Ad26.RSV.preF alone and Ad26.RSV.preF/RSV preF protein elicited robust serum RSV-F-specific IgG1 and IgA1 antibody responses, with Ad26.RSV.preF/RSV preF protein inducing substantially higher responses than Ad26.RSV.preF alone both at peak (Day 15) and at 6 months following vaccination. Compared with Ad26.RSV.preF or RSV preF protein alone, the combination of Ad26.RSV.preF/RSV preF protein showed greater induction of Fc-effector functions including antibody-dependent neutrophil phagocytosis and antibody-dependent natural killer cell activation. Antibody functions not observed at baseline in RSV pre-exposed participants were induced following vaccination, with Ad26.RSV.preF/RSV preF protein showing the greatest induction compared with other active regimens tested. System serology profiling using multivariate logistic regression showed that all active vaccine combinations elicit unique and distinct antibody features, with Ad26.RSV.preF/RSV preF protein inducing a more polyfunctional antibody response compared with individual vaccine components tested.

### Conclusion

The Ad26.RSV.preF/RSV preF protein combination vaccine induced robust antibody responses and a more polyfunctional antibody response compared with Ad26.RSV.preF or RSV preF protein alone.



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## Clinical trial simulation predicts higher efficacy against RSV for MK-1654 than for maternal vaccination

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### Background

Passive immunization with neutralizing monoclonal antibodies, like MK-1654, is a promising approach for the prevention of RSV lower respiratory tract infection (LRTI) in infants. Active maternal vaccination to prevent RSV disease in infants is another approach currently under development. This work qualifies a model for the prediction of efficacy against RSV in infants following maternal vaccination. Model simulations then predict the fold-increase in serum neutralization antibody (SNA) titer needed from maternal vaccination to achieve similar protection to that of MK-1654 given to infants born just before and during the RSV season.

### Method

A published model was adapted to predict SNA titer profiles in infants born to mothers who have been vaccinated against RSV. Published efficacy data from a Phase 3 trial evaluating RSV maternal vaccination were used to qualify the model's ability to predict efficacy in infants. Next, a virtual clinical trial was designed wherein mothers expected to deliver just before or during the RSV season were administered either a hypothetical vaccine or placebo. The hypothetical vaccine was assumed to increase infant titers at birth by a factor of 15, 30, 45, and 60-fold. Titers from maternal vaccination were assumed to decay with a half-life of approximately 30 days. Clinical trial simulation was used to predict efficacy for the prevention of RSV LRTI 3 and 6 months after birth, comparing efficacy following maternal vaccination and efficacy of MK-1654 in preterm or full-term infants.

### Result

Model predictions accurately captured the efficacy for the published [2] Phase 3 trial (mean [95% CI] of 27% [21.6 - 32.5%] predicted vs 39% [5.3 - 61.2%] observed, for a duration of 3 months). A 60-fold increase in infant titers at birth was predicted to provide comparable efficacy to the clinical dose of MK-1654 for 6 months. The increase in infant titers at birth required to achieve similar protection to MK-1654 for 3 months was 30-fold. Larger increases in titers are required in mothers of preterm versus full-term infants to maintain a similar level of protection, an effect attributable to incomplete transfer of maternal antibody to the fetus.

### Conclusion

In order to provide comparable efficacy to MK-1654 (especially for a 6-month duration), these predictions suggest a maternal vaccine would need to increase titers to a higher range than that reported for existing candidates. Overall, MK-1654 is likely to provide greater efficacy for the prevention of RSV in infants than maternal vaccines under development.





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## Asthma dampens the human bronchial epithelial cell immune response to RSV infection

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### Background

Respiratory syncytial virus (RSV) can cause asthma exacerbations in adults. The airway epithelium serves as an immune barrier against allergens and pathogens. The airway epithelium of asthmatics has been reported to be more susceptible to respiratory viral infections compared to non-asthmatics. We aimed to delineate the difference in immune response between epithelial cells derived from asthmatic vs. non-asthmatic donors, after RSV infection in vitro.

### Method

Air-liquid interface (ALI) cultures were derived using human bronchial airway epithelial cells (hBECs) from 5 asthmatic and 4 non-asthmatic, age-matched women (asthma: 60.8 $\pm$ 3.1 vs. non-asthma: 61.8 $\pm$ 4.7 yr (mean $\pm$ SE),  $P>0.05$ ). ALI cultures were apically infected with RSV A2 at MOI=1. RNA was extracted at 0h (pre-infection), 4h, 8h and 24h post-RSV using Norgen RNA/DNA/Protein purification Plus micro kit. The resulting 36 samples (9 individuals x 4 time points) underwent targeted gene expression profiling of 600 immune-related genes using Nanostring nCounter. Data were normalized using positive controls and house-keeping genes and post-normalization, genes with counts greater than the average of the negative controls were retained. Linear mixed effects models were used to compare gene expression between asthmatic and non-asthmatic groups accounting for time. Gene set enrichment analysis was performed using EnrichR. All statistical analysis was performed using R (version 4.1.0).

### Result

Comparing the temporal change in gene expression between non-asthma and asthma groups, 17 genes were significant at a nominal  $P$ -value=0.05, with expression of Guanylate binding protein (GBP) 5, a mediator associated with innate immunity against microbial pathogens, significantly different after multiple testing correction (FDR<30%). Specifically, GBP5 expression increased in non-asthma hBECs after RSV infection over 24h, whereas this effect was dampened in asthma (Figure 1). We also identified genes whose expression levels differed ( $P<0.01$ ) between the two groups at each time point (in relation to baseline levels in uninfected cells). Gene set enrichment analysis identified five pathways (FDR<10%) associated with viral protein interaction, with cytokine and cytokine receptor as the top ranked pathways.

### Conclusion

Airway epithelial cells from asthmatics show differences in the expression of immune-related genes after RSV infection in vitro compared to non-asthmatic donors. Significant dampening of GBP5 expression observed post-RSV exposure over time may be relevant to the host response and mechanisms of RSV-associated exacerbations in asthmatics.





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## Respiratory syncytial virus burden in infants born before or during the season in England in their first year of life

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**Craig Davidson** - ARNI0060

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### Background

RSV is a leading cause of lower respiratory tract infections and hospitalisations in children(1), and in England cases follow a seasonal pattern with a peak between November and January each year(2).

This study aims to describe the burden of RSV hospitalisations in infants <1 year of age in England by timing of birth in relation to the RSV season, to further understand the burden of disease in this at-risk group.

### Method

Using Hospital Episode Statistics (HES) data of admissions at NHS hospitals, we extracted admissions numbers, bed days, costs and age at admission for ICD10 coded RSV in infants <1 yr between 01/10/2014 - 31/3/2019.

Age on admission was categorised into <28; 29-90; 91-181; 182-272 and 273-365 days.

For each age class and admission month, we used an algorithm to estimate the possible birth dates and classify these as in or out of season births (assuming RSV season from 1st October - 31st March and uniform birth rate).

The ICD-10 coded RSV admissions were described by the proportion of admissions in infants born in and out of season and that the admissions and associated bed days and costs are presented.

### Result

Overall, infants <1yr born before the season contributed an estimated 18,466 of 36,748 RSV admissions (50.2%; Figure 1) and 72,717 of 150,133 bed days (48.4%).

Before season births contributed £28,025,189 (50.2%) of RSV costs.

During the RSV season, between 33.0% and 69.7% of monthly admissions consisted of infants born before the season.

### Conclusion

While some literature has highlighted infants born around the beginning of RSV season are at high risk of RSV infection<sup>3</sup>, our study shows infants born before the English RSV season contribute a substantial clinical and economic burden, representing approximately half of all RSV-coded admissions, bed days and costs.

With a number of RSV immunisations in late-stage development, it is timely to consider how the deployment of these can be implemented to maximise the number of infants protected, and not just those born within the season.

Future analyses should consider the impact of including infants born before the season in immunisation programs on RSV hospitalisations and costs.



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## RISK FACTORS FOR THE DEVELOPMENT OF SEVERE OR VERY SEVERE RSV LRTI IN INDIAN INFANTS: A COHORT STUDY IN MELGHAT INDIA

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Rowena Crow - ARNI0062

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### Background

RSV undoubtedly is the single most important cause of severe lower respiratory tract infection (LRTI) globally. Prevention of RSV LRTI in young infants is likely many years away in developing countries, however nonspecific prevention measures are already available. Risk factors for the development of severe or very severe RSV LRTI in rural areas are likely to be different from those in urban areas. There are very few studies examining this association in impoverished rural areas in developing countries, especially from India.

### Method

Active surveillance of infants and children younger than 2 years of age, with weekly home visits for detection of acute lower respiratory tract infection in all consented subjects, was conducted in 93 villages of Melghat, Maharashtra, India from August 2016 to December 2020. All hospitals and primary health centers were surveyed daily for admissions of subjects from the study area. Nasopharyngeal swabs were collected from children with severe, or very severe LRTIs and all who died, using flocked swabs and transported in PrimStore MTM, for RSV testing using nucleic acid tests at ICMR, National Institute of Virology Pune. Univariate and multivariate logistic regression was used to identify significant risk factors for severe and very severe LRTI, both RSV and non RSV. Identified risk factors were used to populate a multivariate model on the complete cohort. The comparable wealth score index was derived from the household's durable assets and housing characteristics.

### Result

There were 487 RSV and 2839 all-cause LRTI infections in a cohort of 13411 children. Weight zscore <-2, the use of kerosene or wood for cooking, obtaining drinking water from a public tap and low gestational age at birth significantly increased the risk of an RSV. A higher wealth score index and water purification were protective. Comparison with all-cause LRTI showed male sex as an additional risk factors. The analysis highlighted, the risk of kerosene use [OR=17.7 (3.0-103.7)(p<=0.001)] and [ OR=4.3 (1.1-16.5))(p<=0.05)] for RSV and all-cause LRTIs respectively.

### Conclusion

Kerosene cooktops and unsafe drinking water are significant preventable risk factors for development of severe RSV LRTI



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## Isolation of wild-type BRSV in differentiated bovine tracheobronchial epithelial cells cultured at air-liquid interface

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Rik de Swart - ARNI0063

**Rik De Swart<sup>1</sup>, Nora Gerhards<sup>1</sup>, Manouk Vrieling<sup>1</sup>, Sophie Van Oort<sup>1</sup>, Romy Dresken<sup>1</sup>, Judith Bonsing<sup>1</sup>, Frank Harders<sup>1</sup>, Rineke De Jong<sup>1</sup>**

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### Background

Bovine respiratory syncytial virus (BRSV) is an important cause of respiratory tract disease in calves, and its pathogenesis closely mimics that of HRSV infection in infants. We have used experimental BRSV infections of calves as a natural host animal model for evaluation of vaccines or therapeutics against either BRSV or HRSV. Intranasal inoculation of calves with wild-type BRSV strain Odijk results in overt and reproducible clinical signs and virus shedding. For generating new challenge virus stocks, we rely on broncho-alveolar lavages of experimentally infected calves. Propagation of the wild-type virus in immortalized cell lines results in mutations and loss of virulence in vivo.

### Method

We harvested bovine lungs, dissected the primary bronchi and isolated epithelial cells by enzymatic digestion and cell scraping. After expansion in culture the cells were seeded in transwell filters. When monolayers were complete the apical medium was removed, and the cells were allowed to differentiate at air-liquid interface. Over a period of 4-6 weeks this resulted in the generation of a well-differentiated pseudostratified columnar epithelium that contained ciliated and mucus-forming cells. The epithelium was apically inoculated with wild-type BRSV strain Odijk ( $10^2$  TCID<sub>50</sub>) or cell-adapted BRSV strain RB94 ( $10^4$  TCID<sub>50</sub>). Readouts included virus titration, quantitative RT-PCR, immune-peroxidase staining of infected cells and RNA sequencing.

### Result

Whereas the cell-adapted BRSV strain replicated well in bovine Embryonic Bovine Trachea (EBTr) cells and poorly in well-differentiated bovine epithelial cells grown at air-liquid interface, we observed an inverse pattern for wild-type BRSV strain Odijk. The wild-type strain showed a progressive increase in viral genomes and infectious titers measured in apical washes collected between 3- and 10 days post inoculation (DPI). Plaques of wild-type BRSV-infected cells were detected by staining at 6 DPI, and had increased in size by 10 DPI, while RB94-infected cells were undetectable by staining. Genome sequences assessed in supernatants collected at 7-, 10- and 14 DPI showed no indications for tissue culture adaptation as compared to the inoculum.

### Conclusion

This study demonstrates that differentiated bovine tracheobronchial epithelial cells cultured at air-liquid interface can be used for isolation or propagation of field isolates of BRSV, with minimal risk of tissue culture adaptation. This avoids the need of growing virus stocks in calves, thus contributing to 3R principles in animal research. Moreover, these culture systems enable in vitro studies into virulence factors of BRSV strains.



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## AN IMPROVED TOOLKIT FOR IN VITRO HMPV CHARACTERIZATION

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Joyce Sweeney - ARN0064

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### Background

Human metapneumovirus (HMPV) is a leading cause of acute viral respiratory infections and is associated with considerable morbidity. Young, elderly, and immunocompromised individuals are most at risk for developing severe disease. Nucleotide analysis of HMPV's fusion (F) and glycoprotein (G) genes class HMPV into four distinct clades: A1, A2, B1, and B2. Due to the slow and inconsistent growth of HMPV in vitro, limited reagents and assays exist for its characterization. Herein, we describe several advances in virus detection, quantification, and growth methods for the generation of an improved toolkit for in vitro characterization of multiple HMPV strains across each of the four clades.

### Method

Complete genome next-generation sequencing of 22 HMPV strains was performed, followed by nucleotide alignment using ApE. A universal RT-qPCR primer-probe set with 100% sequence identity across all strains was designed to detect the phosphoprotein (P) gene. All 2D cell-based experiments were performed in rhesus monkey kidney epithelial cells (LLC-MK2s) at MOIs ranging from 0.005 to 0.014. Plaque assays were optimized with a microcrystalline cellulose (MCC) overlay using the TN/1501/A1, TN/94-49/A2, and TN/98-242/B1 strains. Virus growth conditions were optimized for the TN/94-49/A2 strain. Differentiated 3D tissues (EpiAirwayTM, MatTek Corporation; Ashland, MA) with an airway-liquid interface (pHAEC-ALI) were infected with the TN/94-49/A2 and TN/91-316/B2 strains. RT-qPCR was used to determine viral RNA amplification.

### Result

The universal P RT-qPCR primer-probe set detected all HMPV clinical isolates across all 4 genetic subgroups. Virus quantification for TN/1501/A1, TN/94-49/A2, and TN/98-242/B1 strains were successfully determined by utilizing a MCC overlay, yielding visible and countable plaques. Virus growth conditions and titers for the TN/94-49/A2 strain were improved, with optimization yielding a 32-fold increase in viral titers compared to prior growth conditions. 3D airway tissues were also successfully employed for HMPV infections with a considerable increase in viral amplification over initial viral load for both the A2 and B2 strains.

### Conclusion

We have successfully expanded the molecular virology toolkit for in vitro characterization of genetically distinct HMPV strains. These improvements will contribute to the advancement of HMPV virology and the development of direct-acting antivirals targeting this virus.



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## Streptococcus pneumoniae Nasal Carriage Patterns with and without Common Respiratory Virus Infections in Seattle, WA, USA

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### Background

Respiratory virus infections might influence *Streptococcus pneumoniae* nasal carriage and subsequent risk of pneumococcal disease. We describe community *S. pneumoniae* nasal carriage with and without common respiratory viral infections in a prospective household setting.

### Method

From November 2019-June 2021, participants were consented and enrolled in a prospective household surveillance study of respiratory pathogens. Participants self-monitored weekly online for symptoms of acute respiratory illness (ARI). Midturbinate or anterior nasal swabs were self-collected at enrollment, when ARI occurred, and, in the second year only, from household contacts after SARS-CoV-2 was detected in a household member. Specimens were mailed in universal transport media during year 1 and as dry swabs in year 2 and were tested using multiplex reverse-transcription polymerase chain reaction for respiratory pathogens, including *S. pneumoniae*, rhinovirus, adenovirus, seasonal human coronavirus, influenza A and B viruses, RSV, metapneumovirus, enterovirus, parainfluenza virus, bocavirus, and SARS-CoV-2. Wilcoxon rank-sum tests were used to test for differences in median *S. pneumoniae* relative cycle threshold (Crt) values.

### Result

We collected 211 swabs from 139 individuals in 96 households that tested positive for *S. pneumoniae* (n=109 swabs with and 102 without one or more viruses detected). Approximately half of swabs were from men and 83% were from White individuals. Most swabs with *S. pneumoniae* detected were in children (41% <5, 36% 5-17, and 23% 18-64 years) and half of swabs with a viral infection were among children <5 years. Rhinovirus (n=62) was the most frequently detected virus with *S. pneumoniae*, followed by adenovirus (n=15), coronavirus (n=14), influenza B (n=9), influenza A (n=8), RSV (n=7), metapneumovirus (n=6), enterovirus (n=4), parainfluenza (n=3), bocavirus (n=2), and SARS-CoV-2 (n=1). Of swabs with viral detection compared to swabs without viral detection, 79% and 41% were symptomatic, 10% and 7% sought medical care, and 29% and 16% were absent from work or school. Median *S. pneumoniae* Crt values were lower for episodes with viral detection (16.8, IQR: 14.0, 24.8) compared to no viral detection (24.8, IQR: 20.3, 26.0, P<0.001). *S. pneumoniae* Crt values were lower when detected with enterovirus, RSV, adenovirus, rhinovirus, coronavirus, influenza A, or influenza B viruses. No differences were detected for *S. pneumoniae* Crt values with or without bocavirus, parainfluenza, metapneumovirus, or SARS-CoV-2 (Figure 1).

### Conclusion



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Several common respiratory viruses were associated with greater concurrent *S. pneumoniae* semiquantitative nasal carriage density in a household setting, which might increase risk of pneumococcal disease.



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## Relative Contribution of Diagnostic Testing to RSV Diagnosis in Adults in United States

Evan Anderson - ARNI0067

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### Background

Respiratory Syncytial Virus (RSV) is an underappreciated cause of adult respiratory-tract related hospitalizations. Data are limited about the relative additional contribution of serology versus molecular testing of nasopharyngeal/oropharyngeal (NP/OP) swabs and whether the yield could be improved by testing sputum.

### Method

We conducted prospective RSV surveillance at two US hospitals from Oct 2018 - March 2020 for adults  $\geq 50$  years of age admitted with acute respiratory tract infections (ARI) and adults age  $\geq 18$  years admitted with COPD or CHF exacerbations. Adults were eligible if they resided in 1 of 8 counties around Atlanta, GA. Persons with symptoms  $>14$  days were excluded. A BioFire® FilmArray® respiratory panel was used to test NP/OP swabs for study testing (molecular testing results). Findings for those that had acute and convalescent (collected 21-60 days after enrollment) serology and molecular testing were compared. An RSV Lysate (A2 and B1) enzyme immunoassay (EIA) was used to detect a 4-fold IgG seroconversion. Sputum was also collected on a subset of participants in the 2019-2020 season and tested by BioFire® FilmArray® respiratory panel, and results compared to NP/OP swabs.

### Result

Of 574 persons with acute and convalescent serology and NP/OP specimens available, 31 had a 4-fold rise in antibody or a positive molecular test of NP/OP specimens. Sixteen (52%) had both a 4-fold rise and a positive molecular test; 10 (32%) had a positive molecular test without a 4-fold rise; and 5 (16%) had a 4-fold rise without a positive molecular test. Overall, RSV detections increased 19% (31 vs 26) with addition of paired serology testing. For 366 events with acute sera collected  $\leq 5$  days from symptom onset, adding sera to molecular testing increased diagnostic yield by 25% (15 vs 12). Among 208 events with acute sera collected  $>5$  days, sera increased case detection by 14% (16 vs 14).

Sputum was obtained in 159 participants: 6 (3.7%) were positive by sputum RVP testing. Four were also detected by standard NP/OP swabs using BioFire RVP, and 2 additional positives were detected in sputum only. RSV detection increased 50% (6/4) with addition of sputum specimens. One of these that was positive from sputum only was also detected using standard of care molecular testing.

### Conclusion

NP/OP swabs molecular testing diagnosed most RSV in hospitalized adults enrolled in this study, but serology increased case yield by 19%. Acute serum collection at  $\leq 5$  days may increase RSV detection. Sputum, while difficult to collect, may improve yield over standard NP/OP swabs. Future studies should assess whether other specimen types could further increase RSV detection.





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## Burden of Respiratory Syncytial Virus and Influenza Among Adults 50 Years of Age or Greater Hospitalized with Acute Respiratory Infection vs Healthy Controls in the Pre-SARS-CoV-2 Era in the United States

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### Background

The burden of Respiratory Syncytial Virus (RSV)-associated hospitalizations in adults  $\geq 50$  years of age hospitalized with acute respiratory infection (ARI) is underreported. We sought to compare the prevalence of RSV vs. influenza among ARI hospitalizations from two winter respiratory viral seasons (2018-2019, 2019-2020) compared to healthy controls.

### Method

We conducted an IRB-approved prospective surveillance at two hospitals in Atlanta, Georgia, USA during the winter respiratory viral seasons from Oct 2018-March 2020 for adults  $\geq 50$  years of age admitted to the hospital with ARIs. Adults were eligible if they had symptoms of  $\leq 14$  days and resided in 1 of 8 counties surrounding Atlanta, GA. Asymptomatic adults age  $\geq 50$  years were enrolled as controls. Combined nasopharyngeal and oral swabs from cases and controls were tested for RSV and influenza using BioFire® FilmArray® Respiratory Viral Panel (RVP), and acute and convalescent RSV serology was tested with an RSV-lysate EIA ( $\geq 4$ -fold rise considered positive). We abstracted demographic features, outcomes, and any standard of care molecular testing from RSV and influenza cases.

### Result

RSV infection was detected in 71/1426 (5.0%) hospitalized adults and in 6/483 (1.2%) controls. By comparison, influenza A was detected in 104 (7.3%) and influenza B in 8 (0.6%) hospitalized adults, and neither was detected in healthy controls. Median age of RSV+ cases was older than influenza cases: 67 years (interquartile range (IQR): 58, 73) versus 62 years (IQR: 56, 71;  $p=0.04$ ). Of cases with RSV, 13 (18.0%) were admitted to the ICU and two required mechanical ventilation which was similar to influenza (ICU admission in 25 (21.9%), mechanical ventilation in 5). The median length of hospitalization was 4 days (IQR: 2, 7). Overall, the comorbidities and outcomes of adults with RSV were similar to those observed with influenza.

### Conclusion

Over two respiratory viral seasons pre-SARS-CoV-2, RSV was detected in 5.0% of adults  $\geq 50$  years of age hospitalized with ARI. Demographics, comorbidities, and outcomes of adults hospitalized with RSV were largely similar to those with influenza.



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## Evaluation of a Human 3D Airway Tissue Culture Model for the Study of Respiratory Syncytial Virus infection and the Development of Antiviral Drugs

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### Background

Respiratory syncytial virus (RSV) is a leading cause of acute respiratory tract infections in children, the immunocompromised, and the elderly. Despite substantial morbidity and mortality, there are limited therapeutic options for infected patients. Animal models do not completely reproduce human RSV infection pathogenesis, manifesting the need for preclinical models to study RSV and evaluate novel antiviral agents. Although two-dimensional (2D) cell culture models are widely used in drug discovery, factors such as surface stiffness, forced polarity, and uncontrolled cell spreading may confound measures of antiviral activity. In contrast, 3D models provide a more representative environment and a unique advantage for respiratory virus studies by using different cell types and an air-liquid interface. Here we describe RSV studied in a 3D pseudostratified epithelium to simulate in vivo conditions.

### Method

A 3D tissue model (EpiAirway, MatTek Ashland, MA) was used to evaluate the growth dynamics of RSV, utilizing plaque-forming units (PFUs) ranging from  $4 \times 10^3$  to  $2 \times 10^5$  for apical dosing during a one-hour infection. Viral growth from the tissue and apical washes was quantified by RT-qPCR and plaque assay. EDP-323, a novel non-nucleoside RSV L-inhibitor, and EDP-938, a non-fusion RSV replication inhibitor, were evaluated against RSV-A and -B isolates to determine antiviral activity in the 3D model. Compound was delivered via the basolateral media using a six-point dilution curve. Compound antiviral activity was assessed in parallel in HEp-2 2D cell culture (5-day treatment). Tissues were immunostained to visualize cell types (ciliated and goblet) and infection (RSV N and F proteins).

### Result

RSV-A Long and RSV-B VR-955 infected the EpiAirway tissue, producing >1000-fold more virus over the initial infection by the end of 10 days. Increasing the PFUs used for apical dosing had a limited positive correlation with tissue viral load at 5 days. EDP-938 and EDP-323 displayed potent anti-RSV activity with EC<sub>50</sub>s below 20 nM and 1 nM, respectively. Importantly, viral load decreased in the tissue and apical washes. Fluorescent imaging of viral proteins demonstrated that RSV infection was isolated to the ciliated cells of the EpiAirway model, and a viral load reduction was observed in compound-treated tissues.

### Conclusion

The 3D EpiAirway tissue can be successfully used to study the dynamics of RSV infection and evaluate the antiviral activity of novel therapeutics. The 3D airway model generates reproducible viral replication kinetics and consistent compound-induced viral inhibition while surpassing the preclinical testing limits of traditional 2D culture models.



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## Improving virus neutralization assays for human respiratory syncytial virus using airway organoids

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Laurine Rijsbergen - ARNI0071

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### Background

High levels of neutralizing antibodies against human respiratory syncytial virus (HRSV) are associated with protection from severe disease. The HRSV fusion (F) protein and glycoprotein (G) are major targets of the humoral immune response and measuring F and G antibodies is crucial for vaccine development. Current virus neutralization assays (VNA) are performed on cell lines, in which G-specific neutralizing antibodies cannot be measured due to absence of relevant *in vivo* receptors, e.g. CX3CR1, which is an important cellular receptor for HRSV-G. Hence, the relative contribution of G-specific antibodies to prevention of HRSV-associated disease remains unknown. We aim to develop a high-throughput VNA on airway-organoids (AO) differentiated at air-liquid interface (ALI) to measure F- and G-specific antibodies in a well-characterized cohort of infants.

### Method

We selected sera from 125 infants (<3 years, 2014-2019) and measured neutralization of a recombinant (r) subgroup A or B clinical isolate-based rHRSV strain, expressing a reporter protein, on Vero cells. Additionally, we measured antibodies against G from rHRSV-A and rHRSV-B (Ga, Gb), pre-F, post-F and the nucleoprotein using a multiplex bead-based immunoassay (MIA). To measure functionality of G-specific antibodies, we performed VNA on AO. AO at ALI were cultured with a Notch inhibitor (DAPT) to allow for faster differentiation, and assessed for the percentage ciliated and CX3CR1+ cells after detachment with EDTA, to prevent disrupting the surface proteins. Lastly, the AO at ALIDAPT were seeded in suspension in a 96-well plate for a VNA.

### Result

The VNA on Vero cells correlated best with pre-F-specific antibodies in MIA:  $R^2=0.76$  for rHRSV-A and  $R^2=0.62$  for rHRSV-B. We selected sera that were rHRSV-negative ( $n=21$ ), rHRSV-A positive ( $n=14$ ) or rHRSV-B positive ( $n=12$ ) in both VNA on Vero cells and MIA. The AO at ALIDAPT had  $\pm 50\%$  acetylated  $\alpha$ -tubulin+ cells, compared to  $\pm 20\%$  without DAPT. About 33% of the cells was CX3CR1+ and 3% was also acetylated  $\alpha$ -tubulin+. The AO at ALIDAPT in suspension could be infected with HRSV-A and HRSV-B, and infected cells were quantified by flow cytometry. Results of the VNA on AO are pending.

### Conclusion

Both classical VNA and MIA measured HRSV-specific antibodies, however there were significant differences between both assays. MIA is mainly useful for multiplex detection of antibodies against several HRSV proteins. We have developed a VNA based on AO at ALI, which express CX3CR1, that has the potential to measure both F- and G-specific antibodies on clinically relevant cells. With this model the immune response after vaccination or infection can be evaluated, which will potentially result in an improved correlate of protection against HRSV disease.



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## New insights on RSV nucleoprotein structure and RNA encapsidation mechanism

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### Background

The negative-strand RNA genome of RSV is constantly encapsidated by the viral nucleoprotein N forming a helical nucleocapsid (NC), which is a template for the viral polymerase L for both replication and transcription. The L-NC interaction is mediated by an essential cofactor, the viral phosphoprotein P. The NC-P interaction is also required for the morphogenesis of cytoplasmic viral factories, called inclusion bodies. Furthermore, during genome replication, P plays the role of molecular chaperon by maintaining neosynthesized N protein in an RNA-free monomeric form, named N0, competent for the encapsidation of newly synthesized genomes and antigenomes. However, the mechanisms involved in the specificity of viral genome and antigenome encapsidation, and thus in the transition from N0-P to N-RNA-P interactions, and in the formation of inclusion bodies, are still poorly understood. It is noteworthy that detailed cryo-EM characterisation of the pneumoviral NC, which could allow to better understand the polymerase functioning, is still lacking.

### Method

We recently succeeded in obtaining a recombinant monomeric and RNA-free N protein, competent for in vitro RNA encapsidation. By combining biochemical and biophysical approaches with structural studies by electron microscopy, we assessed the impact of RNA sequence, length, and 5' end modification on the capacity of N to oligomerise. We also investigated the role of RNA and N oligomerisation in in vitro reconstitution of pseudo-inclusion bodies. Finally, in order to gain structural information on N-RNA oligomers, we performed cryo-electron microscopy study of purified recombinant NC produced in insect cells, and revealed a diversity of N complexes.

### Result

We observed that the helical RSV NCs is highly unconventional not only in comparison to the solved helical NC structures but also for helical biological polymers in general. In addition, we reveal that RSV NCs are remarkably polymorphic and present the structures of three other assemblies of RSV NCs.

### Conclusion

Altogether, our data allowed to address the molecular mechanisms of RSV assembly and replication in terms of the structural plasticity of its nucleocapsids.



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## Bacterial and viral interference affects the susceptibility of respiratory epithelial cells to human respiratory syncytial virus

Laura L.A. Van Dijk - ARNI0074

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### Background

Susceptibility to infection with human respiratory syncytial virus (HRSV) may be influenced by the immune status of epithelial cells in the respiratory tract. Several studies have indicated that interferons (IFN) play a critical role early in HRSV infection and affect disease severity. In addition, it has been described that the composition of the respiratory microbiome or viral interference can influence HRSV disease severity. We hypothesized that specific respiratory bacterial or viral species induce an IFN-dependent antiviral immune state in epithelial cells and assessed the impact of in vitro exposure to these on IFN production and induction of an antiviral state.

### Method

We generated A549 cells with IFN type I receptor (IFNAR1) or IFN type III receptor (IFNLR1) knockouts, which were infected with a recombinant (r) clinical isolate-based HRSV strain expressing enhanced green fluorescent protein (eGFP). Human wild-type (wt) A549 cells were pre-treated with type I and type III IFNs and subsequently infected. Read-out parameters were viral replication (TCID<sub>50</sub>/ml) and dissemination (% infected cells). Additionally, we optimized a co-culture model of A549 (wt) cells or airway organoids (AO) at air-liquid interface (ALI) with bacteria. Cells were infected in the absence or presence of bacteria, and viral replication, dissemination and cytokine responses were monitored. Finally, AO at ALI were infected with a recombinant clinical isolate-based human parainfluenza virus type 3 (rHPIV-3) expressing eGFP, and, after clearing of the virus, the cells were re-infected with rHRSV and viral dissemination, replication and cytokine responses were measured.

### Result

Type I and type III IFNs were found to have a synergistic effect on inhibiting HRSV infection in the knock-out cell lines, which was confirmed by pre-treating these cells with either or both type I and type III IFNs. Co-cultures of AO with respiratory bacteria demonstrated minimal upregulation of IFNs. Susceptibility of A549 cells to HRSV was altered, however, in AO at ALI there was no significant effect of the bacteria. Viral interference experiments are currently ongoing.

### Conclusion

In conclusion, type I and type III IFNs are needed for inhibition of HRSV in vitro. Although we found significant effects of several bacteria on HRSV infection in A549 cells we were unable to confirm this in AO at ALI. Therefore, we concluded that the bacteria tested are not responsible for an antiviral immune state of the respiratory epithelium. Unravelling risk factors for severe HRSV disease will have a major impact on predicting disease severity and designing prevention and treatment strategies.



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## Bovine Respiratory Syncytial Virus experimental challenge study: Whole blood transcriptome response in dairy calves

Stephanie O Donoghue · ARNI0076

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### Background

Bovine Respiratory Disease (BRD) is the leading cause of morbidity and mortality in cattle of all ages, affecting livestock industries both in Ireland and internationally. Both viral and bacterial agents can cause initial infection either solely or through co-infections. Bovine Respiratory Syncytial Virus (BRSV) is a primary viral pathogen involved in BRD development. Here we report, an experimental challenge with BRSV, coupled with RNA-Sequencing of whole blood, which enabled the examination of the host response, at the molecular level, to this single BRD causative agent.

### Method

On the day of the challenge (day (d) 0) Holstein-Friesian bull calves were either challenged with BRSV inoculum (n=12) or were mock challenged with sterile PBS (n=6). Clinical signs were recorded and whole blood was collected in tempus tubes daily, from challenge until animals were euthanised on d 7. Total RNA was extracted from whole blood and prepared libraries were sequenced on an Illumina NovaSeq 6000 (150 bp paired-end). Sequence reads were quality assessed, trimmed and aligned to the ARS UCD 1.2 bovine reference genome. Differential expression analysis was performed using the EdgeR package in R. Pathway and gene ontology analysis of differentially expressed genes (DEGs) was performed using the Database for Annotation, Visualization and integrated Discovery.

### Result

Multi-dimensional scaling based on global gene expression showed a clear separation between the treatments with a total of 306 DEGs identified between the BRSV challenged and control calves ( $P < 0.05$ , FDR  $< 0.1$ , fold change  $> 2$ ). Pathway analysis of the DEGs revealed six enriched pathways with the most statistically significant ( $p < 0.05$ , FDR  $< 0.05$ ) being 'Influenza A', 'Coronavirus disease', and 'NOD-like receptor signaling'. The 29 gene ontology 'biological process' terms identified for the DEGs were primarily associated with the immune response to viral infection including 'defence response to virus' and 'negative regulation of viral genome replication'.

### Conclusion

This study successfully identified genes and associated biological pathways involved in the immune response to BRSV infection. The detection of gene expression changes in whole blood may be useful for the diagnosis of sub-clinical BRSV infections. Furthermore, the identification of DNA variants in the identified DEGs, following validation, may provide information on BRD resistance or susceptibility for inclusion in future breeding programmes. The presented gene signatures may inform the development of prognostic biomarkers for human RSV.





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## In vitro and in vivo evaluation of a novel bivalent HMPV/RSV live-attenuated vaccine candidate

Julia Dubois - ARNI0078

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### Background

Respiratory syncytial virus (RSV) and human metapneumovirus (HMPV) are major pediatric respiratory pathogens with currently no approved vaccine. Over the years, different strategies have been evaluated to prevent these infections, including live-attenuated vaccines (LAV), with the challenge to balance attenuation and immunogenicity in the development of novel vaccine candidates against Pneumoviruses. In that regard, we previously engineered a specific HMPV strain (A1/C-85473) by molecular biology and generated by reverse genetics an efficient recombinant LAV candidate with deletion of the SH gene (i&#129;&#132;SH-C-85473, METAVAC®) which protects mice against lethal HMPV infection. This approach offered promising perspectives in the development of recombinant chimeric vectors, based on the versatile METAVAC® backbone and expressing additional viral antigens of interest, such as the RSV F fusion glycoprotein, to provide a bivalent HMPV/RSV vaccine candidate.

### Method

We inserted different constructions of RSV F genes into the HMPV i&#129;&#132;SH-C-85473 genomic plasmid and generated the corresponding recombinant chimeric viruses by reverse genetics. After viral rescue, we performed the visualization of viral particles by transmission electron microscopy and evaluated the viral replicative capacity and the expression of both RSV and HMPV F proteins in LLC-MK2 cells. We then infected 3D-reconstituted human airway epithelia (HAE) in order to confirm the infection of such chimeric viruses onto human ciliated cells and the expression of the RSV F antigen. Finally, we infected BALB/c mice to investigate the





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capacity of the chimeric HMPV/RSV viruses to replicate in vivo and to induce efficient neutralizing antibody responses.

### **Result**

Our reverse genetics approach succeeded in rescuing viable and replicative chimeric HMPV/RSV viruses expressing both HMPV and RSV F glycoproteins at the surface of infected LLC-MK2 cells but also, at the surface of viral particles. Compared to the recombinant HMPV wild type strain C-85473, chimeric HMPV/RSV viruses showed a mild attenuated in vitro phenotype in LLC-MK2 cell and HAE models, stably expressing the exogenous F RSV gene along the time-course of infection. Finally, we showed that, at the image of the monovalent METAVAC® LAV candidate, the chimeric HMPV/RSV viruses conferred protection against lethal HMPV challenge and also induced a strong neutralizing antibody response against RSV in mice.

### **Conclusion**

Our results show that a bivalent chimeric HMPV/RSV LAV, based on the METAVAC® viral vaccine platform, could be a very promising vaccine candidate against both RSV and HMPV infections.



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## Clinician Evaluations Show Increased Acute Respiratory Tract Infection Severity in Older Adults and Adults With Core Risk Factors - Data From the Global Hospitalized Acute Respiratory Tract Infection (HARTI) Study

Ann Falsey - ARNI0079

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### Background

Influenza, respiratory syncytial virus (RSV), and human metapneumovirus (hMPV) can cause severe acute respiratory tract infections (ARTIs) in older adults and those with certain chronic illnesses. Here, we report ARTI disease severity evolution based on clinical symptom scores (CSS) in adults hospitalized due to influenza, RSV, or hMPV.

### Method

HARTI was a prospective global epidemiological study in adults hospitalized due to confirmed influenza, RSV, or hMPV ARTI (12 countries, 40 centers). Clinicians rated patients' sign/symptom severity using a CSS with bothersomeness/activity interference (cough, sputum production, shortness of breath, malaise), upper respiratory (nasal discharge, pharyngitis, sinus tenderness), and lower respiratory (dyspnea, rales/rhonchi, wheezing) subdomains; each sign/symptom was rated on a scale of increasing (worsening) severity from 0 to 3. Total and subdomain CSS were calculated by summing sign/symptom scores. Assessments were performed at screening, 48 hours after screening/at early discharge, and approximately 2 days prior to discharge. CSS was analyzed by pathogen, age, length of hospital stay (LOS), and presence of core risk factors (CRFs; age ≥65 years, chronic heart disease, chronic obstructive pulmonary disease [COPD], chronic renal disease, or asthma).

### Result

Data from 706 participants were included; among these, 567 (80.3%) had CRFs. Lower respiratory subdomain scores (LRS) showed the greatest differences between pathogens at all time points; mean (SD) LRS for all pathogens decreased from screening to 2 days prior to discharge (influenza: 3.82 [2.7] to 1.7 [2.1]; RSV: 4.6 [2.7] to 2.3 [2.2]; hMPV: 5.0 [2.4] to 2.1 [2.2]). Mean upper respiratory subdomain scores were similar between pathogens at all timepoints. Mean LRS were lower for participants aged 18-39 years (n=55) compared with those aged 65-74 (n=174) and ≥75 years (n=219) at all time points; mean bothersomeness/activity interference scores and total CSS were lower at 48 hours after screening/early discharge for participants aged 18-39 years compared with all older age groups. Patients with CRFs had higher bothersomeness/activity interference scores, LRS, and total CSS at screening and 48 hours after screening/early discharge, and higher LRS 2 days prior to discharge than those without CRFs. At screening and 2 days prior to discharge, cough, malaise, and shortness of breath had the highest scores for all pathogens.

### Conclusion

LRS showed the greatest differences in disease severity between patients with influenza, RSV, or hMPV at all time points and were higher (indicating more severe disease) in patients with RSV or hMPV. LRS and total CSS were greater in older patients and patients with CRFs.



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## Comparing the association between RSV and non-RSV lower respiratory tract illness and subsequent wheezing illness using causal models: a secondary analysis of a systematic review and meta-analysis

Samar Mehta<sup>1</sup> - ARNI0081

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### Background

Early childhood RSV lower respiratory tract illness (LRTI) is associated with an increased risk of subsequent wheeze and asthma, but it is unclear whether the association is causal or the result of shared genetic risk. In a previous meta-analysis comparing children with RSV LRTI to those without LRTI, we found that adjusting for genetic risk produced smaller estimates of the association between RSV LRTI and subsequent wheeze. Studies comparing children with RSV LRTI to those with LRTI caused by non-RSV respiratory pathogens often report no difference in association with subsequent wheezing illness. This could be because LRTI caused by RSV and non-RSV pathogens alike are causally associated with these outcomes or because LRTI, regardless of etiology, is a marker of underlying susceptibility to wheezing illness. We assessed the association between RSV LRTI and subsequent wheeze using non-RSV LRTI as a comparator, investigating genetic risk and age as potential effect modifiers.

### Method

We conducted a secondary analysis of the previous meta-analysis. Our objective was to evaluate the association between early childhood (<5 years of age) RSV LRTI and subsequent wheezing illness (occurring ≥30 days after the index RSV LRTI) among studies included in MEDLINE and Embase through August 2018. We evaluated the weighted mean difference in the odds of subsequent wheezing illness in RSV LRTI vs. non-RSV LRTI groups using robust variance estimation meta-analysis. Variations of wheezing illness (e.g., asthma and recurrent wheeze) were combined into a single outcome. We also performed stratified analyses according to whether studies adjusted for genetic risk or for age at exposure.

### Result

Our preliminary meta-analysis included 85 effect estimates from 28 studies comparing the odds of subsequent wheezing outcomes across groups with RSV LRTI vs. non-RSV LRTI. There was a trend toward reduced odds of subsequent wheeze for the comparison of RSV LRTI vs. non-RSV LRTI, though the comparison was not statistically significant (OR 0.77, 95%CI 0.56-1.07). The figure below further shows no statistically significant difference in the OR when comparing estimates that adjusted for genetic risk compared to those that did not (OR 0.48 95%CI 0.14-1.59 vs. OR 0.87, 95%CI 0.64-1.20). There was also no statistically significant difference in studies where the LRTI occurred in the first 12 months of life (OR 1.00, 95%CI 0.60-1.66) compared to later (OR 0.65, 95%CI 0.34-1.25).

### Conclusion



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Our study suggests that RSV LRTI is not more strongly associated with subsequent wheezing illness than non-RSV LRTI, and it may be less strongly associated. This relationship was unchanged after adjusting for genetic risk and for age at time of LRTI.



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## Ad26.RSV.preF completely protects calves from severe respiratory disease induced by bovine RSV challenge

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### Background

Adenoviral vector 26-based vaccine encoding human RSV fusion protein stabilized in its prefusion conformation (Ad26.RSV.preF) induces Th1-biased humoral and cellular responses in mice, and protection against RSV in the cotton rat challenge model. Human and bovine RSV (bRSV) share many aspects of pathogenesis, epidemiology, immune responses and clinical manifestations in their respective hosts. The viruses are relatively similar in gene function and sequence, e.g. the F protein is conserved to about 80%. Therefore, infection of calves with bRSV represents a relevant model to assess protective efficacy against distantly related RSV.

### Method

Calves without maternally derived bRSV antibodies were immunized at 6 and 10 weeks of age with Ad26.RSV.preF (n=8), or with buffer (n=8). Immune responses to the vaccine were assayed in blood samples collected during the study. Four weeks post 2nd immunization, calves were challenged by aerosol with in-vivo passaged bRSV. Post-challenge, calves were monitored for bRSV-related clinical signs, and upper and lower respiratory tract samples were examined for presence of bRSV. Calves were sacrificed 13 days post challenge, or earlier when reaching predefined humane endpoints, and lungs were examined for pathological lesions.

### Result

Immunization with Ad26.RSV.preF was well tolerated, and induced neutralizing antibodies against human and bovine RSV, as well as RSV F specific cellular responses.

Upon bRSV challenge, mock immunized calves developed fever and depression, accompanied by decrease in relative body weight. In addition, severe respiratory distress was observed corresponding to a pronounced drop in blood oxygen saturation and resulting in pre-termination of 7/8 calves between 6- and 9-days post-challenge. In contrast, all Ad26.RSV.preF immunized calves survived bRSV challenge with only mild clinical symptoms. bRSV could be detected in upper and lower respiratory tract of Ad26.RSV.preF immunized calves, albeit at significantly reduced levels compared to mock immunized calves. In mock immunized calves, moderate pathology was observed in post-mortem lung tissue from the 1/8 animal that survived bRSV challenge and extensive macroscopic and microscopic pathology in the 7/8 animals pre-terminated at peak infection. In contrast, in all Ad26.RSV.preF immunized bRSV challenged calves, pathology was absent or markedly lower.

### Conclusion

Ad26.RSV.preF is immunogenic in young calves, and provides protection in a stringent bRSV challenge model, thereby demonstrating breadth of protection against distantly related RSV strains. These results are in good agreement with the protective efficacy of Ad26.RSV.preF shown in a human challenge study (Sadoff, J Infect Dis 2021).



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## Exploring the burden of respiratory syncytial virus infections among adults in Denmark.

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Amanda Marie Cavling Clausen - ARNI0083

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### Background

Respiratory syncytial virus (RSV) is a viral pathogen acknowledged as a leading cause of respiratory tract infection (RTI) in young children worldwide. In recent years, studies have demonstrated that RSV also constitutes a significant burden among all adults and highlight a knowledge gap of high-quality data estimating the burden of RSV among adults in Europe. The Danish registers provide an opportunity to estimate the burden of RSV disease on an entire Danish population. We determined the age-specific burden of RSV infections among adults from week 40 2015 to week 40 2018. Linking the RTI-hospitalizations with information on RSV tests, and analyzing the trend of RSV laboratory testing, we assessed the burden of RSV infections among adult Danes >18 years old. In addition, the number of deaths attributable to RSV is presented.

### Method

This retrospective population-based study used national registries on hospitalizations and laboratory data (2015-2018). Information on deaths from the Danish Register of Causes of Death (2015-2016) was used to estimate the mortality burden in the adult population.

### Result

We identified an average of 147 RSV-diagnosed hospitalizations annually in adults (>18 years) during the 3-year period. The average incidence of RSV-diagnosed hospitalizations among all adults was estimated to be 3.2 per 100.000, and 9.4 per 100.000 for adults over 65 years during the period. Among the all conducted PCR tests, we identified 2530 positive RSV tests, of which 42.1% (1064) were patients hospitalized with a respiratory diagnosis. Among the RTI-diagnosed hospitalizations with a positive RSV test, 60.7% (645) were not hospitalized with a RSV diagnosis. In addition, 419 out of 440 (95.2%) patients with a RSV diagnosis had a positive RSV test.

We identified 4 deaths within 30 days after hospitalization with a RSV diagnosis in the period 2015/16 to 2016. We identified 32 deaths within 30 days after a positive RSV test during the same period. Of these 32 deaths identified with a positive RSV test, the most common cause of death was cancer (12), circulatory system disease (9), and respiratory disease (8), and 3 had been hospitalized with a registered RSV diagnosis.

### Conclusion

Our results demonstrate how a combination of routinely collected data can be applied to explore the burden of RSV disease. Our findings showed that many RTI-hospitalizations with a positive RSV test were not hospitalized with a registered RSV-diagnosis during their admission, indicating a large misclassification and underreporting of RSV-diagnosis among Danish adults. Based on these findings, more attention and awareness should be given to RSV infections among adults and high-risk groups and risk factors, especially among older adults.



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## NIRSEVIMAB AFFORDS HIGH LEVELS OF RESPIRATORY SYNCYTIAL VIRUS NEUTRALISING ANTIBODIES FOLLOWING A SINGLE DOSE

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Deidre Wilkins - ARNI0085

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### Background

Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract infection (LRTI) and hospitalisation in infants. Nirsevimab is a monoclonal antibody with an extended half-life that binds the prefusion conformation of the RSV fusion protein to block viral entry and spread. In two global, pivotal, placebo-controlled studies, a single injection of nirsevimab significantly reduced medically attended RSV LRTI versus placebo over the first RSV season (Phase III NCT03979313: MELODY, healthy term and late preterm infants, 74.5%; Phase IIb NCT02878330: healthy preterm infants, 70.1%). We measured RSV neutralising antibodies (RSV Nab) from these studies up to Day 361.

### Method

Infants were randomised 2:1 to receive one intramuscular injection of nirsevimab (Phase 2b: infants <5 kg at dosing who received the 50 mg dose; MELODY: infants <5 kg at dosing received 50 mg; infants ≥5 kg received 100 mg) or placebo, before their first RSV season. Serum samples collected pre-baseline and post-dose (Days 31 or 91, 151, and 361) were tested in a validated RSV neutralisation assay. Samples were preincubated with RSV expressing green fluorescent protein, and replication was measured in fluorescent foci units with an imaging reader. RSV neutralising antibody levels are reported in international units (IU)/mL.

### Result

Overall, data from 1402 infants from MELODY and 741 infants from Phase IIb were available for analysis. Baseline geometric mean RSV Nab levels were similar in both studies (MELODY, 134 IU/mL; Phase IIb, 87 IU/mL). At Day 151, nirsevimab recipients exhibited RSV Nab levels approximately 50-fold higher (MELODY, 6901 IU/mL; Phase IIb, 4799 IU/mL) than baseline, with the highest levels sampled at Day 31 in MELODY (19,737 IU/mL; Figure) and at Day 91 in Phase IIb (8479 IU/mL). RSV Nab levels remained >7-fold higher than baseline up to Day 361 (MELODY, 978 IU/mL; Phase IIb, 739 IU/mL). At Day 361, placebo recipients with no confirmed RSV infection during the studies had low RSV Nab levels of 38-48 IU/mL compared with 151-162 IU/mL in recipients with confirmed RSV infection; nirsevimab recipients without confirmed RSV infection had RSV Nab levels of 757-979 IU/mL, >19-fold higher than placebo recipients with no confirmed RSV infection and >3-fold higher than placebo recipients with a confirmed RSV infection.

### Conclusion

Following immunisation with nirsevimab, RSV Nab levels at Day 151 were approximately 50-fold higher than baseline levels. RSV Nab levels remained high up to Day 361, suggesting that protection could be achieved beyond Day 151.





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## POPULATION PHARMACOKINETICS AND EXPOSURE-RESPONSE OF NIRSEVIMAB FOR THE PREVENTION OF RSV DISEASE IN INFANTS: EXTRAPOLATION OF EFFICACY TO INFANTS WITH HEART OR LUNG DISEASE OR PREMATURITY

Ulrika Wählby Hamren - ARN10086

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### Background

Nirsevimab is a monoclonal antibody with extended half-life targeting the respiratory syncytial virus (RSV) fusion protein and is being developed for the prevention of RSV disease in all infants. In two global, pivotal, placebo-controlled studies, nirsevimab reduced RSV-confirmed medically attended (MA) lower respiratory tract infection (LRTI) versus placebo over the first RSV season (Phase III NCT03979313: MELODY, healthy term and late preterm infants, 74.5%; Phase IIb NCT02878330: healthy preterm infants, 70.1%). A third palivizumab-controlled study (Phase II/III NCT03959488: MEDLEY) evaluated nirsevimab in infants at risk for severe RSV LRTI, including extremely preterm infants (<29 weeks' gestational age [wGA]), and infants with chronic lung disease (CLD) of prematurity, and/or congenital heart disease (CHD). Extrapolation of efficacy based on pharmacokinetics (PK) is a common approach to bridge between populations and plays a major role in paediatric drug development. Extrapolation relies on the assumptions of similar exposure-response across populations; for nirsevimab, it is justified based on the mechanism of action (binding to RSV to prevent viral entry), there being no endogenous targets, and comparable viral aetiology. Here, we describe the analyses performed to support the extrapolation of efficacy from Phase IIb and MELODY trials to the MEDLEY trial population.

### Method

Nirsevimab was administered as a single intramuscular injection. MELODY and MEDLEY applied weight-banded dosing (<5 kg, 50 mg; ≥5 kg, 100 mg); in Phase IIb, all infants received 50 mg. PK data, pooled across studies, were analysed using a population PK approach. An efficacy exposure target was defined based on exposure-response analysis of the primary endpoint (RSV MA LRTI up to 150 days post dose), pooled from the Phase IIb and MELODY studies. Successful extrapolation would be confirmed if >80% of infants in MEDLEY achieved the target exposure.

### Result

The nirsevimab population PK model, including effects of body weight and age, adequately described the data. No difference in PK was found in infants with CLD or CHD. The target exposure for efficacy was determined to be area under the curve >12.8 mg•day/mL. In the overall MEDLEY population, 94.3% (558/592) of infants had exposures above the target exposure; this included 94.1% (128/136) infants with CLD of prematurity, 80.3% (53/66) infants with CHD, and 93.6% (44/47) extreme preterm infants <29 wGA without CLD or CHD.

### Conclusion

Nirsevimab has been shown to provide protection against RSV disease in healthy infants and, by PK extrapolation, to infants with CLD, infants with CHD, and those born extremely premature.



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## RSV and Influenza surveillance in school children and adolescents during 2 months of the second semester of 2021.

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**MARIA FLORENCIA LUCION** - ARNI0088

**MARIA FLORENCIA LUCION<sup>1</sup>**

<sup>1</sup>Ricardo Gutierrez Children's Hospital

### Background

During the COVID-19 pandemic, the number of other respiratory infections decreased in Argentina, with an almost absence of respiratory syncytial virus (RSV) and influenza virus during the winter season of 2020. Our aim was to estimate the prevalence of RSV and influenza in school subjects in a pediatric hospital during the return to face-to-face activities.

### Method

Cross-sectional study of COVID-19 suspected cases aged 3-18 years, negative test for SARS-CoV-2, between August-October 2021. Population was stratified by educational level. PCR was used to detect RSV and influenza.

### Result

A total of 619 subjects were included: 234 kinder, 224 primary and 161 secondary school level; from them 25.5% (158) resulted positive for RSV; the distribution of RSV cases by age group was variable, with higher incidence at kinder level: 36.3% vs 21% primary and 16% secondary level ( $p < 0.001$ ); among adolescents, RSV infection was associated with school contact with a case of respiratory symptoms ( $OR=2.5$  CI95%1-6.80;  $p=0.04$ ). No cases of influenza virus were detected.

### Conclusion

RSV was isolated in a quarter of the samples of school children and adolescents assisted in our center, with the kinder level group the most affected. Among adolescents, RSV infection was associated with a school contact with respiratory symptoms. No cases of FLU were detected during the study period. In the context of the return to face-to-face activities and the advancement of vaccination against SARS-CoV-2, it is necessary to maintain active surveillance of respiratory viruses.



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## Out-of-season epidemic of respiratory syncytial virus, starting summer 2021, Denmark

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**Ramona Trebbien** - ARNI0089

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### Background

Respiratory syncytial virus (RSV) is usually occurring as a seasonal infection in the winter period in Denmark from November to April with a peak during February/March. Incidence of RSV is usual highest in infants <1 year-of-age. During the COVID-19 societal restrictions RSV has been absent from April 2019 to May 2021. However, since May 2021 RSV began to circulate in Denmark, coinciding with the loosening of COVID-19 restrictions. This study investigate the characteristics of the out-of-season RSV epidemic in Denmark 2021.

### Method

Data on diagnostic testing for RSV and patient information was collected from the national microbiological database (MiBa). MiBa contains data on all microbiological diagnostic tests and test results in Denmark. Data was extracted weekly and is one of the standard methods to monitor the occurrence of RSV in the Danish surveillance program. The National Patient Register was used to identify individuals admitted to hospital following an RSV infection.

### Result

COVID-19 restrictions were gradually loosened starting 21st of April 2021. RSV began to circulate slowly from end of May and at the start of September 2021, the number of RSV cases in Denmark increased to a level, markedly higher than observed in the preceding five RSV seasons (figure 1), as was the number of admissions with RSV.

The distribution of laboratory confirmed RSV cases by age groups in the summer 2021 was compared to the seasons 2017/18, 2018/19, and 2019-20. The highest number of cases was observed in the age-group 0-3 months of age which is comparable to previous seasons, however, there was more children with RSV in the age groups 19-23 months and 2-3 years old during the 2021 summer epidemic when compared to previous seasons. This could indicate that the absence of RSV due to the COVID-19 societal restrictions had left a larger part of this slightly older group of children susceptible to the virus probably due to lack of buildup immunity.

### Conclusion

An unusual RSV epidemic in the summer 2021 was observed in Denmark, and the level of laboratory confirmed cases were higher than seen in the previous five RSV seasons. A relative higher proportion of children in the age groups 19 months-3 years were affected. The out-of-season RSV epidemic is anticipated to be connected to the loosening of the COVID-19 restrictions and the lack of immunity in the young children <3 years-of-age.



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## RSV A-based Ad26/preF protein vaccine induces broadly neutralizing antibodies in nonclinical models and humans, that are protective against both RSV A and RSV B subtypes

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Leslie Van der Fits - ARNI0090

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### Background

RSV is divided into two antigenic subtypes, RSV A and RSV B, that co-circulate in an alternating dominant, irregular seasonal pattern. While the G surface protein is highly variable between the subtypes, the fusion protein F is conserved and a target for antibody-mediated neutralization.

Our vaccine candidate currently in clinical development is based on prefusion stabilized F protein (preF) from RSV A2. It consists of a combination of preF subunit and adenoviral vector-based preF (Ad26/preF protein) that induces superior immune responses and efficacy against RSV A in preclinical models, when compared to the individual subunit and adenoviral vector components.

Here we evaluated the breadth of the protective immune responses across RSV A and RSV B subtypes induced by the RSV A-based preF vaccines.

### Method

Induction of virus neutralizing antibodies against various laboratory-adapted RSV A and RSV B strains, as well as against RSV A and RSV B clinical isolates (from the period of 2011 to 2018) was assessed after intramuscular immunization with RSV A-based preF vaccines in multiple animal models, as well as human subjects (clinical study NCT03502707). In addition, cross protection against RSV A and RSV B subtypes was assessed in the cotton rat challenge model after immunization, or after transfer of human serum from vaccinated subjects.

### Result

Immunization of naïve rodents with preF subunit, adeno-based preF or Ad26/preF protein resulted in induction of antibodies capable of neutralizing all RSV A and RSV B strains tested, as well as protective efficacy in cotton rats against challenge with RSV A strains (A2, Memphis 37, Long) and RSV B strains (B Wash, and B 17-058221, a recent RSV B clinical isolate). Immunization with Ad26/preF protein in RSV Memphis 37-pre-exposed African Green Monkeys induced serum antibodies that neutralized the 3 RSV A and 3 RSV B clinical isolates tested. In human subjects, Ad26/preF protein induced antibodies capable of neutralization all 12 strains tested (6 RSV A and 6 RSV B clinical isolates), without obvious differences in fold increase across the different strains. Transfer of serum of the immunized human subjects into cotton rats provided protection against challenge with both RSV A2 and RSV B 17-058221. Protective efficacy against RSV B 17-058221 was at least as high as against the vaccine homologous RSV A2 challenge strain, with complete protection against both strains observed in the upper respiratory tract.

### Conclusion

These results collectively show that immunization with our Ad26/preF protein vaccine induced neutralizing antibodies and protection in animals against both RSV A and RSV B subtypes, suggesting that clinical efficacy against both subtypes can be achieved.



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## Perturbations in Respiratory Syncytial Virus (RSV) Activity During the SARS-CoV-2 Pandemic

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**Ann R. Falsey** - ARNI0094

**Ann R. Falsey<sup>2</sup>, Andrew Cameron<sup>1</sup>, Angela Branche<sup>1</sup>, Edward Walsh<sup>1</sup>**

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### Background

The SARS-CoV-2 pandemic has been associated with a significant change in the circulation of seasonal respiratory viruses. The absence of RSV and influenza observed during the early months of the pandemic is presumed to be due to pandemic related public health measures. This period was followed by a surge of RSV activity in the late spring and summer of 2021 in temperate climates. We compared RSV activity among different age groups in pre- and pandemic periods for all tests performed in our clinical laboratory in Rochester, NY.

### Method

From 2017 to 2022, nucleic acid testing for RSV was performed on nasopharyngeal swab specimens using the Panther Fusion Flu A/B/RSV assay or successive generations of the Cepheid Xpert Xpress CoV-2/Flu/RSV assay. Anonymized data of the number of tests performed, RSV results, patient age and sample date were analyzed. The number of RSV infections and test % positivity were compared for subjects stratified by age in pre- and pandemic periods. Statistical significance was determined by t-test and Chi square where appropriate.

### Result

From Fall 2017 to Spring 2022, 4,850 of 39,941 (12.1%) and 2,409 of 111,441 (2.2%) of samples were positive for RSV in children and adults, respectively. The 3 pre-pandemic RSV seasons varied in magnitude with the peak in adult cases lagging ~3 weeks behind pediatric cases, and the adult to pediatric case ratio ranging from 61 to 154% with a mean of 73% (Figure A). During the 2021 summer RSV surge, the adult to pediatric case ratio was significantly lower at 35% compared to each prior season ( $p < .0001$ ) and atypically, incidence of adult cases was greatest in patients ages 18-50 years compared to older groups. RSV testing gradually increased over time, with a large increase in adult testing beginning in fall of 2020 and a similar increase in children starting in summer of 2021. Notably, following the onset of the pandemic, RSV test positivity fell by 70.7% in adults > 50 years compared to 6.4% in adults < 50 years and 21% in children,  $p < 0.0001$  for each comparison. (Figure B). The largest decrease in test positivity occurred in those over age 70 years (83%).

### Conclusion

The SARS-CoV-2 pandemic brought about substantial changes in viral testing practices, but also in test positivity rates among certain age groups, specifically those over age 50. Public health measures and possibly the impact of fear of infection from young children on older adult behavior may account for these differences. Our findings also highlight that RSV disease burden may be underestimated in younger adults. The altered RSV epidemiology during and following the pandemic will make planning adult RSV therapeutic and vaccine trials in the near future challenging.



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## Epithelial composition of human nasal organoids after infection with RSV

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**Gina Aloisio** - ARNI0095

**Gina Aloisio<sup>1</sup>, Anubama Rajan<sup>1</sup>, Ashley Weaver<sup>1</sup>, Hannah Johnson<sup>1</sup>, Trevor McBride<sup>1</sup>, Letisha Aideyan<sup>1</sup>, Vasanthi Avadhanula<sup>1</sup>, Pedro Piedra<sup>1</sup>**

<sup>1</sup>Baylor College of Medicine

### Background

The respiratory epithelium is a dynamic, first-line defense against viral respiratory pathogens. Traditional models of RSV upper respiratory tract infection (URTIs) include in vitro cell culture systems, primarily derived from immortalized and monomorphic cell types, and animal models, which do not accurately represent the pathogenesis or heterogeneity of the human population. We have pioneered the adult human nasal organoid-air liquid interface (HNO-ALI) platform a non-invasive method of deriving human airway epithelium to study viral pathogenesis. HNO-ALIs can be readily infected with RSV, and mimic cytokine responses similar to those seen in human RSV URTIs (Rajan et. al 2022). HNOs comprise of several distinct cell types as seen in the human respiratory epithelium. The mucous-producing goblet cells create a physical barrier against pathogens, ciliated cells mobilize secretions, club cells produce surfactants and basal cells serve as stem cells by renewing and differentiating into new cell types. We studied the effect of RSV A and RSV B infection on epithelial architecture and composition of HNO-ALIs.

### Method

Epithelial cell architecture and composition was analyzed after infection with two contemporaneous strains of RSV (RSV/A/Ontario and RSV/B/Buenos Aires) over time using 4 different adult HNO-ALIs lines. The amount of apoptosis and proliferation as well as the each major cell type (ciliated apical cells, goblet cells, & basal cells,) was analyzed utilizing a high-throughput immunofluorescence cell counting program. Finally, the RSV infection experiments were replicated using several pediatric-derived HNO lines, and observe differences in cellular kinetics compared to the adult HNO lines.

### Result

Mucous-producing goblet cells increase after RSV infection, which is a hallmark of many URTIs. Moreover, there are RSV subtype-specific differences in the degree of goblet cell proliferation. While ciliary damage is universal after RSV infection, different lines of HNOs vary in the degree of damage. Basal cells remain constant after infection with RSV, and interestingly the amount of proliferation after infection with RSV varies greatly by HNO-ALI.

### Conclusion

HNO-ALIs are an exciting novel method for studying how cellular composition of the respiratory epithelium changes during infection with RSV. The innovation of this study lies in the varied lines, as each HNO represents a single individual with a unique set of demographic information.



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## Molecular surveillance for respiratory viruses and genotyping of hRSV from a longitudinal prospective surveillance study of children under 2 in Melghat, India.

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Varsha Potdar - ARNI0097

**Varsha Potdar<sup>1</sup>**

<sup>1</sup>National Institute of Virology Pune

### Background

Respiratory syncytial virus (RSV) lower respiratory tract infections are a major cause of morbidity and mortality in developing countries, and India is the largest contributor to these deaths in infants globally. Globally the rapid evolution and spread of certain genotypes of RSV has highlighted the need to understand genetic diversity of RSV in India.

### Method

An active surveillance of children younger than 2 years of age in 93 villages of Melghat Maharashtra India from August 2016 to April 2022 was conducted. Nasopharyngeal swabs from Children with severe, or very severe LRTIs and all who died, were collected using flocked swabs and transported in PrimStore MTM, (Longhorn Vaccines & Diagnostics, and Bethesda, MD) to MAHAN hospital where they were stored at 4-80°C. Batches were transported to ICMR, National Institute of Virology Pune, for RSV and other respiratory viruses by real-time polymerase chain reaction using standardized protocols. Representative RSV A and B positive samples were sequenced for complete G gene.

### Result

In the 12 134 subjects, there were 2064 episodes of severe LRTIs and 1732 of very severe LRTIs. 4831 NP swabs were collected and tested by Real time RT-PCR for 16 respiratory viruses including RSV subtyping. During 2021 and 2022 additionally the samples were tested for SARS CoV 2. Out of 4831 almost 55% samples i.e., 2637 were positive for one or more respiratory viruses. RSV was detected in 740 cases (360 RSV A and 380 RSV B) followed by rhinovirus in 722 cases, PIV was detected in 345, Influenza 332, hMPV in 251, Adenovirus detected in 136. SARS CoV 2 was detected in 19 cases. RSV was detected in alternate year outbreaks with a dominance of RSV A or RSV B subtypes. In 2016 RSV A was predominantly in circulation where as in 2017 and 2018 RSV B was exclusively in circulation. In 2021 RSV A predominated.

Phylogenetic analysis revealed a wide diversity among RSV A viruses. In 2016 multiple genotypes such as ON1.1 and ON1.3 were in circulation, in 2020 ON1.1 and ON1.2 genotypes were in circulation whereas in 2021 strains belonged to ON1.2 genotype. BA9 genotype of RSV B was predominantly in circulation during 2018.

### Conclusion

Our study showed that multiple respiratory viruses were in co circulation Most of the years RSV A was predominantly in circulation with multiple genotypes. In the year 2017-18 RSV B was in circulation.

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## Epidemiology of RSV-associated hospitalizations in U.S. children aged <5 years before and during the COVID-19 pandemic, RSV-NET October 2018-2022

Fiona Havers - ARNI0098

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### Background

Respiratory syncytial virus (RSV) is a leading cause of hospitalizations in infants and young children, particularly from October-April in the United States; most children are exposed to RSV in their first two years of life. The COVID-19 pandemic and implementation of nonpharmaceutical interventions (NPI) such as daycare and school closures altered RSV circulation. To explore the impact of the pandemic on seasonality and age distribution of RSV-associated hospitalizations in children aged <5 years, we analyzed laboratory-confirmed RSV-associated hospitalizations through the RSV Hospitalizations Surveillance Network (RSV-NET).

### Method

RSV-NET is a population-based U.S. surveillance system that collects data on RSV-associated hospitalizations in acute care hospitals across 75 counties in 12 states. An RSV-NET case resides in a defined catchment area who tests positive for RSV through a clinician-ordered test within 14 days before or during hospitalization. Surveillance was conducted from October-April for the 2018-19 and 2019-20 pre-pandemic seasons and October 2020-September 2021 (2020-21 season). Available data from October 2021-February 2022 (ongoing 2021-22 season) are also presented. Chi-square and Wilcoxon rank-sum tests are used to compare age distribution and median age between seasons, respectively.

### Result

15,474 hospitalized cases in children aged <5 years were identified in RSV-NET: 4,291, 5,458, 2,936 and 2,789 cases in 2018-19, 2019-2020, 2020-21, and 2021-22, respectively (Figure). For 2018-19 and 2019-20, 2,173 (50%) and 3,128 (57%) cases were admitted in December-January respectively. During the 2020-21 season, 86 (3%) were identified from October 2020-April 2021; 2,850 (97%) cases were identified during May-September 2021.

Age distribution was similar between pre-pandemic seasons ( $p=0.2$ ). In 2020-21, a higher proportion (22.7%) of children hospitalized were aged 12-23 months compared with all other seasons (20.5%, 20.4%, and 19%



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respectively for 2018-19, 2019-20, and 2021-2022 seasons, all  $p < 0.01$ ). Median age in months was significantly higher in the 2020-21 season (8.4) compared with the 2018-19 (6.4), 2019-20 (6.8), and 2021-22 seasons (6.4); all  $p < 0.01$ .

### **Conclusion**

The COVID-19 pandemic markedly changed seasonal patterns of RSV circulation and the mean age of RSV-associated hospitalizations in RSV-NET among children aged  $<5$  years increased during the 2020-21 season. This may reflect changes in age of first RSV exposure in the setting of COVID-19 NPI measures or changes to testing practices during the pandemic. Continued monitoring of RSV-associated hospitalizations is critical to understand the ongoing impact of the COVID-19 pandemic on RSV circulation.



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## LOW LEVELS OF RSV TESTING AMONG ADULTS HOSPITALIZED FOR LOWER RESPIRATORY TRACT INFECTION IN THE UNITED STATES: IMPLICATIONS FOR THE ACCURACY OF INCIDENCE STUDIES

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### Background

Lower respiratory tract infections (LRTI) are a leading cause of hospitalizations worldwide, especially among older adults, and respiratory syncytial virus (RSV) is a leading cause of LRTI. RSV symptoms cannot be easily distinguished from other pathogens causing LRTI without confirmatory laboratory tests. However, because there is no targeted treatment for RSV, confirmatory testing in adults is not generally performed. Therefore, the accuracy of studies estimating RSV incidence will depend on the amount of RSV testing, particularly studies based on pathogen-specific ICD codes which generally would not be assigned without a standard-of-care RSV diagnosis. Here, we attempt to quantify the level of RSV testing in adults aged  $\geq 65$  years who are hospitalized for LRTI to inform interpretation of incidence estimates.

### Method

Nationally representative US hospital administrative and billing data from the all-payer Premier Healthcare Database (PHD) was used to extract patient-level data for inpatients with an LRTI diagnosis aged  $\geq 65$  years during the RSV seasons (Oct-April) 2016-2019. The following data were extracted: patient demographics (based on index visit), clinical details (ICD-10 diagnosis codes), standardized billing, and hospital characteristics (bed size, teaching status, and population served (urban/rural)). Billing codes, which were extracted from each hospital's chargemaster and mapped to Premier's standardized language, were analysed to calculate the percentage of LRTI inpatients tested for RSV during a hospitalization.

### Result

From 937 hospitals 2,018,434 LRTI-related inpatient visits during 3 consecutive RSV seasons were extracted. Of these visits, 306,618 (15.2%) had a billing code for RSV testing. The percentage with an RSV test varied by hospital and was highly skewed (mean (SD): 12.4% (15.8); median (IQR): 4.3%, (0.2, 20.7); skewness: 1.29) with 78.5% of hospitals testing less than 25% of LRTI patients (Figure 1). The median percentage tested was higher for hospitals with a larger bed size (6.9% versus 3.1%, for hospitals with a bed size  $\geq 200$  and  $< 200$ , respectively), for teaching versus non-teaching hospitals (11.0% versus 2.5%), and in urban hospitals versus rural hospitals (7.4% vs 0.7%). A modest increase in the mean percentage of RSV testing overall was observed over time: 11.0%, 15.7%, and 19.0% for 2016/17, 2017/18, 2018/19, respectively.

### Conclusion

In this large nationally representative network, the majority of the hospitals test  $< 25\%$  of LRTI inpatients for RSV, with large variability in the percentage tested by hospital. Using database analyses to estimate RSV-related hospitalization incidence will result in a substantial underestimation of the true incidence.



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## GENOMICS ANALYSIS OF RSV IN ARGENTINA BETWEEN 2018-2021

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**Dolores Acuña** - ARN10101

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### Background

The community mitigation measures taken because of the COVID-19 pandemic had a significant impact on the circulation of the most frequent respiratory viruses during 2020. In the case of RSV, a decrease in the number of hospitalizations and delayed outbreaks was described. We perform a genomic analysis of the RSV circulating strains before and during the COVID-19 pandemic in Buenos Aires, Argentina.

### Method

RSV (+) Nasopharyngeal samples collected from hospitalized pediatric patients with lower respiratory tract infections were used for sequencing the G gene and complete genomes by Sanger and NGS methodologies. Viral assembly and annotation were performed using a well-established bioinformatic pipeline. The G-gene genotyping by the ReSVidex online tool and complete genomes phylogenetic analyses by Maximum likelihood inference were performed. The amino acid changes of the F and G proteins and their potential N-glycosylation sites were analyzed. A FEL selection pressure analysis was also performed.

### Result

A total of 253 RSV (+) cases were detected in 2018, 215 in 2019 and 116 in 2021. In 2020 no hospitalizations due to RSV were registered. The RSV reemerged in 2021 with a delayed outbreak, starting in April and peaking in July-August. In addition, the total number of hospitalizations was lower than previous outbreaks. The patients' age distribution analysis showed that each year the highest percentage of hospitalizations occurred among the ages 0-12 months (>60% of cases).

A total of 147 G-gene sequences (57 from 2018, 44 from 2019 and 46 from 2021) were genotyped. RSV-A predominated in 2019 whereas RSV-B in 2018 and 2021. All the RSV-A strains were ON1-like (genetic lineages GA2.3.5 and GA2.3.6b). The RSV-B strains were BA-like (genetic lineage GB5.0.5a). From these, a total of 50 samples were selected to obtain complete genomes.

Amino acid changes on the F protein for both subgroups were analyzed against the prototype sequence of the A2 reference strain (GenBank Accession M74568). Three and 18 nonsynonymous substitutions were detected for RSV-A and RSV-B in antigenic sites respectively (7 in Ø, 4 in I, 2 in II, 1 in III, 3 in IV and 4 in V).

The F and G protein sequences presented 6 and 7 N-glycosylation sites respectively, and the selection pressure analysis showed purifying selection by detecting negative selected sites in both subgroups.

### Conclusion

The complete genome phylogenetic analyses for both subgroups suggested that the same three genetic lineages co-circulated during the three-year period, but the evolutionary evidence together with the epidemiological data support the idea that the lineages that spread in 2021 might be new introductions to our country, rather than cryptic circulation.



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## Hospitalizations Associated with Respiratory Syncytial Virus Among U.S. Children Less Than 5 Years of Age, New Vaccine Surveillance Network (NVSN), December 2016 - September 2020

Aaron Curns - ARN0104

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### Background

Respiratory syncytial virus (RSV) causes significant morbidity among young children. Accurate estimates of RSV-associated hospitalization rates are essential to identify disease burden and inform cost effectiveness analyses for RSV prevention tools including vaccines and monoclonal antibodies that are currently in late stages of clinical development.

### Method

We conducted active, prospective inpatient surveillance for children with acute respiratory infections from December 01, 2016, to September 14, 2020 in counties surrounding Nashville TN, Rochester NY, Cincinnati OH, Seattle WA, Houston TX, Kansas City MO, and Pittsburgh PA. Surveillance included parent/guardian interviews, medical chart review, and collection of midturbinate nasal +/- throat swabs for testing by reverse transcription polymerase chain reaction. Population-based hospitalization estimates were adjusted for days of surveillance per week, the proportion of eligible children not enrolled, and the proportion of ARI hospitalizations captured by each hospital in their catchment area. Population denominator data were from the 2020 US bridged-race population estimates.

### Result

Among 13,259 enrolled children <5 years of age hospitalized for ARI, almost a third (31%) were RSV positive. The percent RSV positive ranged annually from 26% to 39% by season among children hospitalized for ARI. Peak RSV activity was observed during December or January each season and the seasonal patterns were consistent across age groups. The RSV-associated hospitalization rate among children aged <5 years was 3.4 (95% CI: 3.2-3.5) per 1000 children and ranged between 2.9 (95% CI: 2.7-3.1) and 3.8 (95% CI: 3.6-4.1) per 1000 by season during the study period. The rate among children age <1 year was 11.1 (95% CI: 10.7-11.6) per 1000. Rates were highest among children age 0-2 months (20.3 [95% CI: 19.2-21.6] per 1000) and decreased sharply by increasing age group with children age 24-59 months having a rate of 0.9 [0.8-0.9] per 1000. Among children age <2 years, rates per 1000 by weeks gestational age (WGA) groups were 21.0 (95% CI: 16.6-25.8), 15.9 (95% CI: 12.2-19.6), 15.2 (95% CI: 13.1-17.5), 9.3 (95% CI: 8.1-10.4), and 6.4 (95% CI: 6.2-6.7) among <29, 29-31, 32-34, 35-36, and ≥37 WGA children, respectively.

### Conclusion

Nearly one-third of ARI hospitalizations in children age <5 years were associated with RSV during the study period. Younger infants and infants born prematurely experienced the highest rates of RSV-associated



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hospitalization. These groups should be considered for RSV prevention through immunoprophylaxis or in the future via maternal immunization.



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## Respiratory syncytial virus (RSV) and influenza infections in children under 2 years of age in Ulaanbaatar, Mongolia

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### Background

The 13-valent pneumococcal conjugate vaccine (PCV13) was introduced in Mongolia in a phased manner, from June 2016. A surveillance project was conducted to evaluate the impact of PCV13 introduction on the incidence of lower respiratory tract infections (LRTIs) among hospitalised children under 5 years of age in 4 districts of Ulaanbaatar for the period April 2015-June 2021. Here, we report the seasonality and prevalence of RSV and influenza infections, as well as the clinical outcomes in a sub-study population of children 2- 24months with arterial O<sub>2</sub> saturation (SaO<sub>2</sub>)<93% and children with radiologically confirmed pneumonia.

### Method

Very severe disease was defined by extremely low oxygen saturation (SaO<sub>2</sub><90%). We tested nasopharyngeal swabs for RSV and influenza using qRT-PCR. For comparisons between groups for binary outcomes, Fisher's exact test was utilised.

### Result

In total 5,705 children were enrolled, 1,528 (26.7%) were 2-6 months of age, 3,198 (56.1%) were male. The median age was 10 months (IQR 6-16 months); the median hospitalisation duration was 6 days (IQR 5-8 days). There were 1,961/5,705 (34.3%) patients with SaO<sub>2</sub><90%, 1,947 (34.1%) and 359 (6.3%) with RSV and influenza infections, respectively. Among RSV positive cases, 56.9% (1,117/1,961) were RSV A and 42.1% (827/1,961) were RSV B, 1.6% (33/1,961) had both RSV A and B. For influenza infection, 67.7% (243/359) were influenza A, 32.3% (116/359) were influenza B and 0.5% (2/359) had both influenza A and influenza B. The RSV and influenza peaks coincided with the winter peak of LRTIs cases from the surveillance project. RSV patients had a median age 9 months (IQR 5- 15 months), influenza patients had a median age 12 months (IQR 8-18 months). Children 2-6 months had a higher proportion of very severe RSV infection compared to those older than 6 months (42.2% versus 31.4%, p-value Fisher's exact=0.001). No association was observed between young age and very severe influenza infection, or between RSV or influenza infections or their subgroups and the severity of LRTIs.

### Conclusion

In Mongolia, we demonstrated that RSV is an important pathogen contributing to LRTIs in children under 2 years of age. Young infants less than 6 months were vulnerable to severe RSV infection. Sequencing RSV and influenza have been planned and this data will be helpful to understand the molecular characteristics of the two viruses during the study period and their relations to global RSV and influenza epidemiology.





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## Estimating the economic burden of respiratory syncytial virus infections in infants in Vietnam: a cohort study

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### Background

Worldwide Respiratory syncytial virus (RSV) is the leading cause of acute lower respiratory tract infections (LRTIs) in children under two years of age with 99% mortality occurring in developing countries. No information is available on the costs of RSV in Vietnam or other LMICs in Southeast Asia. The goal of this study is to estimate the costs of LRTIs associated with RSV infection among children in southern Vietnam.

### Method

The study will evaluate costs associated with LRTIs stratified by RSV status among children < 2 years who met WHO LRTIs case definition and seek care at Children Hospital 1, Ho Chi Minh, Vietnam - a major referral hospital for South of Vietnam. Nasopharyngeal swabs collected from these children were tested for RSV using a validated, peer-review published qRT-PCR. The study period was Sep 2019 - Dec 2021. COVID cases were not enrolled in the study.

### Result

In total, 537 children were enrolled in the study, median age was 8 months (IQR 5-14). There were 215/537(40%) from Outpatient Department, 8/537(1.5%) were from ICU and 314/537 (58.4%) were from Respiratory Department. Of these, 103/537 (19.2%) were RSV positive, 20 from Outpatient Department and 83 from Respiratory Department. RSV was not detected among eight ICU cases. The median of the total cost per LRTI period per patient was US\$53 (IQR 32 - 90) and US\$184 (IQR 109 -287) for outpatients and inpatients respectively. The total cost per ICU admission was 11-times of the total cost per non-ICU admission. For RSV-associated LRTIs cases, the median of the total cost per infection period per patient was US\$52 (IQR 32-88) and US\$165 (IQR 95 -246) for outpatient and inpatients respectively. Among RSV outpatients, the direct medical cost contributed ~40% while the direct non-medical cost (e.g., transportation, accommodation etc.) and the indirect costs (e.g., opportunity costs of missed work) contributed ~24% and ~35% of the total cost. Among RSV inpatients, the direct medical cost contributed ~ 55%, while the direct non-medical cost and the indirect cost contributed ~24% and 21% of the total cost. For non-RSV LRTIs cases, the median of the total cost was similar to the total cost of RSV-associated LRTIs for both outpatients and inpatients. The total cost of one none-ICU admission of RSV-associated LRTIs cases was 87% of the monthly minimum way per person in Ho Chi Minh City.

### Conclusion

This is the first data reporting the substantial economic burden on health systems, governments, and the society imposed by RSV infection in Vietnam. The results will be helpful for policy makers in planning health care resources and highlights the urgency of finding specific treatment and prevention for this disease.



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## The burden of respiratory syncytial virus on healthcare systems in Europe: results from a cross-sectional survey

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### Background

Respiratory syncytial virus (RSV) is a leading cause of hospitalization for all infants in their first RSV season globally and is responsible for a significant burden on healthcare systems. The evaluation of new vaccines and mAbs warrants consideration of the unmet medical need to prevent RSV disease in all infants. The aim of this current study was to estimate the burden of pediatric RSV infections on healthcare systems across Europe, particularly on healthcare system performance and resource use, over the last three RSV seasons (2018/19 - 2020/21) and to document what, if any potential actions are required to mitigate the effects of RSV infection on healthcare system disruption.

### Method

The study was based on a cross-sectional survey with a web-based 56-item questionnaire targeting healthcare professionals (HCPs) in 20 European countries, conducted between August 2021 and January 2022. Each of the 4 questionnaires were for different healthcare settings including primary care, emergency departments (ED), hospital pediatric general wards, and hospital pediatric intensive care units (PICU), respectively, and included questions on experience and perception of HCPs.

### Result

Out of 380 completed responses, almost 50% respondents considered RSV's effects on health system performance in peak RSV season to be very to extremely disruptive. The survey respondents reported increased workload, stress and exhaustion, and burnout in: 84, 76, and 59% in primary care, 90, 88, and 63% in the ED, 88, 85, and 58% in general wards, and 88, 75, and 61% in PICU, respectively. In primary care, 76% of respondents reported an increase in pediatric consultations for respiratory infections. In the ED, 81% and 77% of the respondents reported increased emergency admissions for respiratory illness, and increased emergency attendances for respiratory illness from urgent GP referrals, respectively. In general pediatrics, 84% of respondents agreed/strongly agreed that the increase in pediatric admissions was for respiratory illness. The increased volume of patients requiring PICU admissions for severe respiratory illness were reported by 76% of respondents. Respondents reported a median increase of 52.5% in ED daily visits vs. 30% in primary care visits for respiratory illnesses in the peak RSV season. The reported hospital pediatric bed occupancy for respiratory and RSV patients is shown in Figure 1.

### Conclusion

The results show that the burden of pediatric RSV is system-wide and affects all care settings. The survey has aided in identifying 5 evidence-supported, actionable recommendations geared towards building an RSV-resilient health system.



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## GP consultations before hospital admission for RSV respiratory tract infections in children

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### Background

Respiratory syncytial virus (RSV) is estimated to cause hospitalizations in 1%-2% of children <5 years. Ecological studies report a large RSV burden in primary care with 10%-20% of children <5 years consulting for RSV annually. However, little is known about the contribution of general practitioners (GP) to referring children with RSV-associated respiratory tract infections (RTI) to hospital.

### Method

The objective was to evaluate GP consultations before hospital admissions in children with RSV RTI, <5 years of age and born between 2013 and 2016 in the Netherlands. GP RTI episodes were extracted from the Nivel primary care database and defined as an episode of care by the GP with a respiratory ICPC code. RSV-associated episodes were approximated with bronchiolitis episodes as ICPC codes are not RSV-specific. All RTI admissions and RSV-coded admissions were extracted from the Dutch national hospital discharge data (ICD-10 codes). The data were linked at individual level. For each individual from 2013 to 2017, hospitalizations were matched to episodes if the admission occurred during the episode. We evaluated (1) the admission rates for RTI during GP bronchiolitis episodes and (2) the rates of GP RTI episodes before RSV-coded admissions. Children who had at least one GP RTI episode during the observation period were included in the analyses.

### Result

In total, 41,669 GP RTI episodes were identified, including 3,654 bronchiolitis episodes. In the study population over the same period, there were 6,994 RTI admissions to hospital, including 1,350 RSV-coded admissions. Overall, the admission rate during a GP bronchiolitis episode was 12%. The rate of admission was highest in children <3 months (40%) and in those who had a RTI admissions in the last 3 months (34%). The admission rate in preterm-born children (<37 weeks) was similar to the admission rate in term-born children (13% vs 12%). A RTI GP episode was registered before the RSV-coded admissions in 55% of cases. Children aged 3-5 months more frequently consulted a GP before being admitted (70%) than the younger (38% for <1 months) and older age groups (37% for 2-5 years old). First-time admissions were more likely to be attended by a GP first (58%) than those with two or more previous admissions (43%).

### Conclusion

Overall, the admission rate during bronchiolitis GP episodes was 12% which is consistent with the observations from ecological studies. Conversely, the proportion of admissions that were previously seen by a GP was low considering the gate-keeping role of Dutch GPs (55%). However, consultations to GP out-of-hours services were not taken into account and may be an important pathway to hospital for children with acute respiratory infections.



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## Mathematical modelling of RSV transmission and estimating the impact of novel pharmaceutical approaches in Tokyo, Japan

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**Ayaka Monoi** - ARN0110

**Ayaka Monoi<sup>1</sup>, David Hodgson<sup>1</sup>**

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### Background

Respiratory syncytial virus (RSV) is a common seasonal virus which causes lower respiratory tract infections. The current prevention strategy involves the monthly administration of an expensive monoclonal antibody, Palivizumab, meaning it is only available to a limited population. This results in a high risk of severe disease among vulnerable populations. Consequently, long-acting monoclonal antibodies (LAMA), such as Nirsevimab, are current in clinical trials and are likely to be licensed in the upcoming years. To make the most use of these emerging prophylactics, it is important to understand quantitatively the disease burden of RSV so the impact of the potential intervention approaches can be accurately evaluated. Specifically, identification of optimum timing of interventions against RSV disease among different strategies is essential in Japan, where the recent seasonal shift of RSV epidemiology may require a review of the current seasonal administration strategy of Palivizumab. Mathematical modelling can be used to obtain current seasonal trends by identifying the drivers of patterns and to forecast future trends under potential interventions. In this study, we developed a mathematical modelling of RSV transmission to assess the potential impacts of public health interventions against RSV disease in Tokyo.

### Method

This study includes; (a) construction of a Tokyo-specific RSV transmission model to obtain the current disease trend and (b) estimating potential impacts of new pharmaceutical approaches on the disease trends. The developed model is an age-structured Susceptible-Exposed-Infectious-Recovered-Susceptible (SEIRS) mathematical model. The data calibrated to the model is RSV surveillance data in Tokyo reported by age group. The scenarios assessed are different public health strategies such as seasonal administrations or year-round administration of prophylactic.

### Result

We developed an age-structured compartmental SEIRS model, fitted to RSV surveillance data in the prefecture, which has the largest population in Japan. Comparison of the alternative policy scenarios was followed to identify the strategy which has a greatest impact on reducing RSV incidence.

### Conclusion

To the best of our knowledge, this is the first study to obtain an epidemiological understanding of RSV disease in Japan and estimate potential impacts of upcoming prophylaxes, such as LAMA, by mathematical modelling methods. Findings from this study adds novel insights into RSV epidemiology in Japan, and helps inform Japanese policy-makers of the effectiveness of current strategy, and of the scope of new public health interventions.



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## Burden of respiratory syncytial virus-associated acute respiratory illness and severe acute respiratory illness South African Children

Jocelyn Moyes - ARNI0111

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### Background

Respiratory syncytial virus (RSV)-associated illness burden varies by age and new interventions such as maternal vaccination or monoclonal antibodies, are effective for limited time periods. We aimed to estimate age-specific burden to guide implementation strategies and cost effectiveness analyses.

### Method

We combined case-based surveillance and ecological data to generate a national estimate of the burden of RSV-associated outpatient acute respiratory illness (ARI) and hospitalised severe acute respiratory illness (SARI) in children aged <5 years, including adjustment for attributable fraction. We estimated the proportion of cases of RSV-associated ARI and SARI that did not access healthcare from a healthcare utilisation survey. We estimated the RSV burden by month of life in the <1-year age group, by 3-month age groups in 1-year-olds, and by year of life in 2-4-year-olds. Observed case fatality ratios were applied to burden estimates for national annual death estimates and published data was used to estimate out of hospital deaths.

### Result

We estimated a mean annual number of 264,112 (95% CI 134,357-437,187) cases of RSV-associated ARI and 96,220 (95% CI 66,470-132,844) cases of RSV-associated SARI including both medically and non-medically attended illness (4.7% and 1.7% of the population aged <5 years). The highest incidence of RSV-associated ARI was in 2-month-old infants (18,360/100,000 population, 95% CI 9,336-28,466) decreasing to 4,873/100,000 population (95% CI 1,976-8,259) by 11 months. The incidence in children aged 1-4 years varied with peaks in the 12-14 and 21-23-month age groups (11,935/100,000 (95% CI 6,952-18,025) and 10,260/100,000 (95% CI 4,305-17,507), respectively). Non-medically attended RSV-associated ARI burden was higher than medically attended for all age groups. The highest incidence of RSV-associated SARI was in <1-month-olds (14,674/100,000, 95%



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CI 11,175-19,644), decreasing to 2,156/100,000 (95 % CI 1,191-3,438) at 11 months and to 127/100,000 (95% CI 59-214) in 4-year-olds. Medically attended RSV-associated SARI burden was higher than non-medically attended burden across all age groups. RSV-associated deaths were highest in the first and second months of life (8,7 deaths/100,000, 95% CI 6,3-11,2 and 8,7 deaths/100,000, 95% CI 6,4-11,4, respectively).

### **Conclusion**

RSV-associated ARI and SARI burden peaks in infants aged <1-month and 2-months, respectively; this supports the use of maternal vaccines and monoclonal antibodies given at birth to protect the young infant.





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## The economic burden of RSV-associated illness in children aged

(Abstract title incomplete - poster title changed)

Jocelyn Moyes - ARNI0112

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### Background

Data on the economic burden of RSV-associated illness will inform decisions on the programmatic implementation of maternal vaccines and monoclonal antibodies. We estimated the cost of RSV associated outpatient acute respiratory illness (ARI) and hospitalised severe acute respiratory illness (SARI) in fine age bands to allow more accurate cost-effectiveness models to account for limited duration of protection conferred by short or long acting interventions.

### Method

We conducted a costing study at sentinel sites in 4 of the seven provinces across South Africa to estimate out-of-pocket and indirect costs for RSV-associated mild and severe illness. We collected facility-specific costs for staffing, equipment, services, diagnostic tests and treatment. Using case-based data we calculated a patient day equivalent (PDE) for RSV-associated hospitalisations or clinic visits; the PDE was multiplied by the number of days of hospitalisation to provide a case-cost to the healthcare system. We estimated the costs in 3-month age intervals in children aged <1 years and as a single group for children aged 1-4 years. We then applied our data to a modified version of the World Health Organization tool for estimating mean annual national cost burden, including medically and non-medically attended RSV-associated mild and severe illness.

### Result

The estimated mean annual cost of RSV-associated illness in children aged <5 years was United States dollars (\$)137 204 393, of which 81% (\$111 742 713) were healthcare system incurred, 6% (\$8 881 612) were out of pocket expenses and 13% (\$28 225 801) were indirect costs. Thirty-three percent (\$45 652 677/\$137 204 393) of the total cost in children aged <5 years was in the <3-month age group, of which 52% (\$71 654 002) were





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healthcare system incurred. The costs of non-medically attended cases increased with age, from \$3 307 218 in the <3-month age group to \$8 603 377 in the 9-11-month age group. The years of life lost due to mortality in children < 5years with RSV-associated illness was 39 625 years of which 45% (17 812 years) occurred in the 0-2 months of life.

### **Conclusion**

Among children <5 years of age with RSV in South Africa, the highest cost burden was in young infants; therefore, interventions against RSV targeting this age group are important to reduce the health and cost burden of RSV-associated illness.



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## Validation of a Global Respiratory Severity Score in Infants with Primary Respiratory Syncytial Virus (RSV) Infection

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Mary Caserta - ARNI0113

Mary Caserta<sup>2</sup>, Xing Qiu<sup>2</sup>, Amy Murphy<sup>1</sup>, Lu Wang<sup>2</sup>, Anthony Corbett<sup>2</sup>, Thomas Mariani<sup>2</sup>, Edward Walsh<sup>2</sup>

<sup>1</sup>Rochester Regional Health, <sup>2</sup>University of Rochester Medical Center

### Background

We developed a global respiratory severity score (GRSS) as a research tool from 139 infants with primary RSV infection enrolled prospectively in the Assessing Predictors of Infant RSV Effects and Severity (AsPIRES) study (2012-2015). Nine clinical variables were identified and weighted to create the GRSS with an area under the receiver operator curve (AUC) of 0.961 for hospitalization. The objective of the present study was to validate that the GRSS correlates well with hospitalization and length of stay (LOS) in an independent cohort.

### Method

Infants with primary RSV infection (2015-2017) were identified from Clinical Microbiology reports and the electronic medical record. Clinical and demographic data were abstracted onto standardized collection forms. First, the original training data formula was applied to the validation set. Next, multivariate logistic regression was used to develop new models associating the 9 variables with hospitalization in the combined data set (N=323). Leave one out cross validation was performed and results were evaluated with the AUC statistic.

### Result

184 (98 hospitalized and 86 non-hospitalized) subjects were enrolled in the validation cohort. The hospitalized and non-hospitalized infants were different in general appearance, percentage with rales, retractions, lethargy, respiratory rate and oxygen saturation. The hospitalized group had a significantly ( $t=9.33$ ,  $p<0.0001$ ) higher GRSS ( $4.20\pm2.10$ ) than the non-hospitalized group ( $1.76\pm1.41$ ). Using GRSS  $\leq 3.5$  as a classifier, the original GRSS formula correctly predicted hospitalization of 131 (71.2%) subjects in the validation set. The AUC of the GRSS as a classifier was 0.827 ( $p<0.0001$ ). Pearson correlation between the GRSS and LOS was  $r=0.397$  ( $p<0.0001$ ). Using multivariate logistic regression with stepwise model selection based on AIC, we trained an improved GRSS (iGRSS) formula with the new combined dataset. iGRSS is a composite score which contains 7 of the original variables with a cross-validated AUC= 0.92. A cut-off value of 2.1 on the iGRSS correctly classified 85% of subjects in leave-one-out cross-validation.

### Conclusion

We validated the GRSS on a new cohort of infants with primary RSV infection confirming a significantly higher GRSS in hospitalized than non-hospitalized subjects. The GRSS is also strongly associated with LOS. Further refinement with additional data led to the development of an improved GRSS (iGRSS) with excellent performance and a smaller number of clinical variables.



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## RSV Pathogen Mutations: An Open Source Browser to Visualize and Track RSV F Protein Variants

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David Tabor - ARNI0114

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<sup>1</sup>AstraZeneca, <sup>2</sup>Broad Institute of MIT and Harvard

### Background

As novel Respiratory Syncytial Virus (RSV) immunization strategies reach the final stages of development, real-time genomic RSV surveillance is critical to detecting emerging variants that may spread more quickly, alter disease severity, escape mAbs or vaccine-induced immunity. Here we launch RSV Pathogen Mutations ([rsv.pathmut.org](https://rsv.pathmut.org)) as an open portal that allows users to visualize lineages and mutations while filtering by location, date, gene, and mutation of interest based on available sequences deposited in the NCBI GenBank database.

### Method

Global RSV G-F gene sequences collected between 1956 and 2021 from 6 geographical regions and 31 countries were ingested into RSV Pathogen Mutations from GenBank (N=21,617 sequences as of April 11, 2022) and are automatically updated daily with newly available sequences. Each sequence is aligned to selectable human RSV subtype A or B reference genomes using Minimap2 to determine nucleotide mutations, indels, and amino acid substitutions. Spurious mutations and probable sequencing errors (defined as less than 3 global occurrences) are filtered out prior to downstream analysis. Ambiguous base calls, indels resulting in frameshifts, and mutations in non-protein-coding regions are ignored when determining amino acid substitutions. All code and relevant documentation are hosted on an open-source, publicly available GitHub repository (<https://github.com/vector-engineering/RSV-CG>).

### Result

Main functionalities include the visualization of the prevalence and incidence of RSV subtypes and F protein sequence variants within countries and time periods of interest. Both single and co-occurring nucleotide or amino acid polymorphisms can be visualized by geography and time. These functions can be tuned by the user to visualize data derived from specific submission date ranges, geographic location of sample collection, and/or data specific to chosen publications. Additionally, users may visualize select amino acid polymorphisms on 3D structures of RSV F, track global sequencing efforts, and download sequences that underlie the visualizations for downstream analysis.

### Conclusion

RSV Pathogen Mutations is a sustainable, open-source portal that harnesses the scientific and public health potential of publicly available genomic database(s) to permit community monitoring of the emergence and distribution of global RSV F protein variants. As real-time sequencing and data dissemination efforts increase, the visualization tools provided by RSV Pathogen Mutations will aid molecular epidemiological understanding and improve outbreak response.



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## The multifunctional role of RSV inclusion bodies; from viral RNA replication to the rerouting of innate immune signalling.

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**Fatoumatta Jobe** - ARNI0115

**Fatoumatta Jobe<sup>1</sup>, Jennifer Simpson<sup>1</sup>, Pippa Hawes<sup>1</sup>, Dalan Bailey<sup>1</sup>**

<sup>1</sup>The Pirbright Institute

### Background

To prevent the activation of innate immune signalling, respiratory syncytial viruses of humans (hRSV) and animals (bovine, bRSV) encode two accessory proteins (NS1 and NS2) which are well established to block interferon signalling. However, RSV-encoded mechanisms for inhibiting NF- $\kappa$ B signalling are less well characterised. In this study we identified RSV-mediated antagonism of this pathway, via a mechanism entirely distinct from the NS1 and NS2 proteins.

### Method

In both hRSV and bRSV infected cells we demonstrated, by immunofluorescence, that the p65 subunit of NF- $\kappa$ B is rerouted to perinuclear puncta in the cytoplasm. These puncta are synonymous with viral inclusion bodies (IBs) - liquid organelles that form in the cytoplasm of infected cells and are characteristic of RSV infection. Separately, we confirmed bRSV IBs as the likely sites of nascent viral RNA replication, with imaging of translation suggesting viral protein synthesis occurs elsewhere in the cytoplasm. We also observed that captured p65 is unable to translocate to the nucleus following TNF- $\alpha$  stimulation, highlighting the antagonistic nature of this sequestration. To confirm the authenticity of p65 re-localisation to the RSV IBs we used correlative light electron microscopy (CLEM) to colocalise RSV N protein and p65 within these liquid organelles, to our knowledge the first time this approach has been applied to RSV. We are now using a series of truncated p65 mutants to interrogate the underlying mechanism behind this protein's sequestration to RSV IBs.

### Result

### Conclusion

In summary, RSV IBs act like 'viral command centres' within the cytoplasm of infected cells; performing multiple roles in the life cycle of the virus, from RNA synthesis to innate immune antagonism.



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## Seasonality of Respiratory Syncytial Virus and Pediatric RSV-Associated Hospitalizations in the United States, 2017-2020

Mila Prill - ARN0116

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### Background

Respiratory syncytial virus (RSV) is a leading cause of severe respiratory illness among infants in the United States, and pediatric RSV-associated hospitalizations are an important indicator of RSV burden. Laboratory surveillance data are commonly used to monitor and describe RSV seasonality, informing the timing of prevention strategies. We compared the timing of hospitalization data from 7 New Vaccine Surveillance Network (NVSN) sites to laboratory data from 224 facilities in 43 states in the National Respiratory and Enteric Virus Surveillance System (NREVSS) to determine how well laboratory data correspond to prevalence of admissions.

### Method

To compare RSV season timing between NVSN and NREVSS, we applied a threshold of a 10-fold increase over baseline (as described in Midgley et al, 2017) to calculate median seasonal onset, peak, and offset/end weeks over 3 seasons from July 2017-June 2020. We set the 4-week moving average for detections/hospitalizations in the third week of July as the pre-season baseline. Data included NVSN RSV-associated hospitalizations among children <2 years of age and RSV PCR tests reported to NREVSS from all ages and levels of care. Florida, Hawaii, and Alaska were excluded due to their distinct circulation patterns.

### Result

Based on U.S. NREVSS data, the median seasonal onset, peak, and offset occurred in the weeks ending on October 27, January 4, and April 28, respectively. Seasonal periods included a mean of 95.3% of all detections over the 3 years; the mean 3-week moving average percent positivity was 5.2% at onset and 2.9% at offset. By U.S. Census Region, season onset was earliest in the South (October 20) and latest in the West (November 9); peaks and offsets followed similar temporal patterns regionally. Compared to NREVSS for the U.S., seasons based on NVSN data for RSV-associated hospitalizations began 1 week later, peaked 2 weeks earlier, and ended 3 weeks earlier.

### Conclusion

Although local variation exists, considerable consistency in RSV seasonality was seen across U.S. regions. RSV circulation patterns based on laboratory detections closely mirrored those from pediatric hospitalizations. Descriptions of national and regional RSV seasonality may inform the timing of future vaccines and monoclonal antibodies.



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## A two-component VLP vaccine platform for the development of a bivalent vaccine against RSV and hMPV

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Andrew Feldhaus - ARNI0118

Andrew Feldhaus<sup>1</sup>, Clancey Wolf<sup>1</sup>, Brooke Fiala<sup>1</sup>, Doug Holtzman<sup>1</sup>

<sup>1</sup>Icosavax, Inc

### Background

Icosavax is developing IVX-A12, a candidate vaccine for active immunization for protection against respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) infection and associated disease in older adults. IVX-A12 is based on I53-50, a computationally designed, protein-based, two-component virus-like particle (VLP), developed by the Institute for Protein Design (IPD) at the University of Washington. IVX-A12 is composed of a mixture of two VLPs: IVX-121, a RSV-targeted VLP displaying 20 copies of DS-Cav1, a clinically validated stabilized prefusion RSV F antigen, and IVX-241, a hMPV-targeted VLP displaying 20 copies of a stabilized prefusion hMPV F antigen. IVX-121 is currently in a Ph1 clinical trial in Belgium, with results expected in Q2 2022. IVX-A12 is anticipated to enter Ph1 testing in H2 2022.

### Method

Several nonclinical studies in rodent models were completed to assess IVX-A12 safety, immunogenicity and efficacy. In naïve animals an oil-in-water adjuvanted formulation was used while in primed animals both adjuvanted and non-adjuvanted formulations were assessed. Older adults, the target patient population for IVX-A12 have already been exposed to both RSV and hMPV, however, there is no good rodent model for hMPV pre-exposure. Therefore a RSV-primed mouse model was used to assess impact of IVX-121 monovalent VLP. Naïve mice and cotton rats were used for assessment of IVX-A12 immunogenicity. Cotton rats were infected with both RSV and hMPV and used for assessment of IVX-A12 efficacy. Rat toxicological testing of IVX-A12 is ongoing.

### Result

In an RSV-primed mouse, a single administration of IVX-121 resulted in high titers with both adjuvanted and non-adjuvanted formulations. In naïve mice and cotton rats, two administrations of IVX-A12 induced robust titers against both A and B strains of RSV and hMPV with no evidence of immune interference across the two antigens when tested in cotton rats. Vaccination with IVX-A12 protected against subsequent RSV or hMPV challenge in a cotton rat efficacy model, both in lung tissue and nasal turbinates. Lastly, IVX-241 has been shown to induce long lasting immunity and bone marrow derived long lived plasma cells (LLPC).

### Conclusion

IVX-A12 is a promising vaccine candidate for protection against RSV and hMPV infection and associated disease.



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## Burden and management of RSV in a pediatric primary care center during the second Covid-19 winter season

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Julian Voithl - ARN0121

**Julian Voithl<sup>1</sup>**

<sup>1</sup>First Vienna Pediatric Medical Center

### Background

RSV is a major cause of respiratory infections within the first years of life. Preterm or chronically ill children are particularly susceptible to acute infections and hospitalization. As Covid-19 preventive measures suppressed the RSV circulation in the first winter season, experts speculate that future outbreaks might be unusually intense in the absence of equally strict precautions. This prospective trial was conducted in an outpatient setting to investigate the burden of RSV in comparison to other pathogens among children with acute respiratory infection during the second Covid-19 winter season.

### Method

Children (0-36 months) admitted to Vienna's largest pediatric primary care center with acute respiratory symptoms (09/2021-04/2022) were included. Nasal swabs for 23 respiratory pathogens were tested with Biofire RP2.1 PCR. Risk factors, clinical features, and treatments were documented.

### Result

820 swab samples were collected from 55% male and 45% female patients (age 16.25 months (1-35)). The most common pathogens were rhinovirus (38.5%) and RSV (26.7%). 216 samples were RSV positive in PCR, but only 151 also gave positive rapid testing results. After a year without infections, RSV activity occurred earlier than expected and peaked in September, followed by a rapid decrease throughout the season. The average age of affected patients was 17.51 months (SD 0.722). The most common RSV symptoms were cough (95.7%), rhinitis (76.2%), and fever (56.7%). A significant correlation emerged between coughing and rhinovirus ( $p=.012$ ), RSV, adenovirus, metapneumovirus (all  $p<.001$ ), and parainfluenzavirus ( $p=.002$ ). RSV patients were treated with analgesics (64.7%), followed by inhaled salbutamol (22.9%), fluticasone (0.5%), 0.9% sodium chloride (0.5%), or systemic corticosteroids (9.6%). Salbutamol inhalation correlated significantly with RSV (Cramér V 0.178,  $p<.001$ ). Four percent of RSV-positive patients needed hospitalization due to severe symptoms (Cramér V 0.112,  $p<.001$ ).

### Conclusion

RSV was one of the leading causes of acute respiratory symptoms in infants and toddlers in a pediatric primary care center during the second Covid-19 winter. After a year without cases, the RSV epidemic started and peaked earlier than expected in Austria. The hospitalization rate was significantly higher in RSV patients than in other subjects.





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## Impact of Respiratory Syncytial Virus infection in infants on the quality of life of affected families

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**Christina Tischer** - ARNI0123

**Christina Tischer<sup>2</sup>, Johanna Kostenzer<sup>1</sup>, Ilona Trautmannsberger<sup>1</sup>, Luc Zimmermann<sup>3</sup>, Silke Mader<sup>1</sup>**

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### Background

Respiratory Syncytial Virus (RSV) is a leading cause of lower respiratory tract infection (LRTI) in all infants. RSV LRTI is often severe and associated with hospitalisation, increased need for intensive care unit admission and caregiver stress. The aim of the ongoing ResQ Family study (ResQ Family: Impact of Respiratory Syncytial Virus Hospitalisation on Quality of Life of Families - A Multi-Country Study) is to provide scientific evidence on how infection and hospitalisation due to RSV in infants up to 24 months impacts the quality of life of affected families in a holistic manner.

### Method

For the ResQ Family study, an online survey in four European countries (Germany, France, Italy and Sweden) will be conducted during the RSV season 2022/2023, starting in September 2022 until June 2023. The survey will address parents or caregivers of children (< 24 months) recently hospitalised due to RSV infection. The first survey ("cross-sectional study") will take place shortly after discharge from hospital with a follow-up ("prospective study") approximately six weeks later. Recruitment will be done primarily through social media outreach, national parent organisations, healthcare professional societies, the ResQ Family expert group as well as related scientific organisations. All statistical analyses will be performed using the statistical software R (R Core Team (2022)). The ResQ Family study received an independent research grant by Sanofi in support of the study.

### Result

Parental/caregiver related quality of life will be operationalised by the acute version (past 7 days) of the PedsQL Family Impact Module. In addition, further relevant information including socio-economic position, parents/caregivers worry and concerns regarding the infants' RSV infection symptoms and disease course, feelings associated with hospitalisation and disease, loss of work productivity, parental health literacy and supporting factors will be considered.

### Conclusion

We will present the background, the objectives as well as the research outline of this ongoing project.



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## Respiratory syncytial virus related complications on pediatric intensive care units (BRICK study)

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Emily Phijffer - ARN0124

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### Background

Respiratory syncytial virus (RSV) is the most common cause of life-threatening lower respiratory tract infections during infancy. In the Netherlands, an increase of RSV-related paediatric intensive care units (PICUs) admissions over recent decades is observed. Data on RSV-related burden on PICU's and -associated complications during PICU stay are necessary before broad introduction of RSV preventive strategies (e.g., maternal and infant immunization) can be implemented. Therefore, we sought to characterise RSV-related complications in infants admitted to the PICUs in the Netherlands.

### Method

A prospective, observational, multicenter study was performed in the Netherlands from September 2021 until April 2022. All seven Pediatric Intensive Care Units (PICU) participated. RSV-related PICU admissions of infants below 12 months of age were included. An RSV-related PICU admission was defined as a PICU admission of  $\geq 1$  day because of respiratory insufficiency with a laboratory confirmed RSV infection. During the study period, RSV testing occurred in all infants with respiratory insufficiency because of the COVID-19 pandemic. Included infants and their parents were followed for one year after PICU admission.

### Result

During the study period, 127 infants with an RSV-related PICU admission were included. Median age at PICU admission was 40 days (range 3-361) and mean length of PICU stay was 6.4 days (SD 4.9). Most patients were term born ( $n=98$ , 77.2%) and did not have comorbidities ( $n=85$ , 69.1%).

In total 37 (29.1%) infants suffered a complication during RSV-related PICU admission, including re-intubation ( $n=11$ , 8.7%), post extubation stridor ( $n=4$ , 3.1%), pneumothorax ( $n=4$ , 3.1%), extravasation injury ( $n=3$ , 2.3%), upper-airway obstruction ( $n=2$ , 1.6%) and delirium ( $n=2$ , 1.6%). 31 (83.8%) of complications occurred in infants below 3 months of age and 6 (16.2%) in infants 3-12 months of age. Additionally, 70 (55.1%) infants were treated for suspected bacterial superinfection, of which 52 (74.3%) below 3 months of age at PICU admission. One year post PICU admission 7 (5.5%) parents were diagnosed with post-traumatic stress syndrome during the psychological consultation.

### Conclusion

This study shows a high rate of RSV-related PICU complications, particularly in infants below 3 months of age. Therefore, the introduction of two RSV preventive interventions, a maternal vaccination and infant immunization, into the Dutch immunization program have the potential to decrease RSV burden in infants admitted to the PICU.



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## Cost-effectiveness of monoclonal antibody and maternal immunization against Respiratory Syncytial Virus (RSV) in Danish children younger than 5 years.

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Xiao Li - ARN10125

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### Background

Promising prophylactic interventions against the respiratory syncytial virus (RSV) are likely to be available soon. Determining their health and economic effects is an important consideration for decision-makers. We aimed to assess the cost-effectiveness of upcoming interventions against RSV in Danish infants.

### Method

We used a static cohort model to project the health and economic burden of RSV disease, and the cost-effectiveness of four intervention strategies: year-round maternal immunization (MI), year-round monoclonal antibody (mAb), seasonal mAb (October to April), and seasonal mAb plus a catch-up program in October for infants <6 months. Input parameters were obtained from Danish national registries and literature. Uncertainty was accounted for with probabilistic sensitivity analysis. Influential input parameters were identified with the expected value of partial perfect information (EVPPPI) and extensive scenario analyses (including the impact of intervention strategies on wheezing and asthma).

### Result

For children < 5 years, the model for Denmark estimated on average 29,585 RSV cases and 3,225 hospitalizations per year, resulting in €8,159,592 treatment costs. From the health care payer perspective, the cost-effective strategy was the seasonal mAb program for willingness-to-pay (WTP) values between €12,000 and €36,000 per QALY gained, and the seasonal mAb plus catch-up program for higher WTP values (Figure 1). This result was most sensitive to the data used for informing the number of hospitalizations. From a full societal perspective (including leisure time lost), the seasonal mAb plus catch-up program was cost-saving and preferred, regardless of the probabilistic uncertainty accounted for.

### Conclusion

We found that the seasonal mAb program with or without catch-up in October dominates the year-round mAb and MI strategies in Denmark. The choice between no program, seasonal mAb or seasonal mAb plus catch-up depends on the most on Denmark's WTP value and the age-specific number of RSV hospitalizations. More evidence on this and other influential factors would reduce decision uncertainty for the health care payer.



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## Characteristics of the unusual inter-seasonal RSV circulation during COVID-19 pandemic, 2021, Germany

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Janine Reiche - ARN10126

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### Background

Due to coronavirus disease 2019 (COVID-19) pandemic and associated non-pharmaceutical interventions (NPI), the respiratory syncytial virus (RSV) season was absent in the 2020/2021 winter season in Germany. Following loosening of NPI measures in early summer of 2021, an unusual inter-seasonal RSV circulation occurred.

### Method

Based on virological data of German national acute respiratory infection (ARI) outpatient sentinel surveillance, we determined start and end of this unusual RSV season (season 2021) according to our recently evaluated method based on lower limit of 95%-confidence interval of weekly RSV positivity rate (PR) in ARI cases aged <5 years. Characteristics of season 2021 were described and further compared to previous RSV seasons from 2011/2012-2019/2020.

### Result

Season 2021 was from calendar week (CW) 35 to 50 in 2021 with a season length of 16 weeks and peak in CW 41, whereas previous RSV seasons were in median from CW 50 to 12 with a median season length of 15 weeks and median peak in CW 8. The total number of identified RSV cases and RSV PR (747, 23%) in season 2021 were significantly higher compared to median number of RSV cases and median RSV PR in previous RSV seasons (207, 11%,  $p < 0.05$ ). In season 2021, 71% of RSV cases were <5 years old. RSV PR in children aged <1 year was 46%, and RSV PR was at an equal high level in children aged 1, 2, 3 and 4 years, respectively (38-40%). In contrast, in previous seasons, median RSV PR decreased substantially with age in 0-4 years old children (39-16%). Typing of RSV-positive specimens of children aged <5 years revealed predominance of RSV group A (73%) as lately seen in season 2019/2020.

### Conclusion

Season 2021 was stronger compared to previous RSV seasons. Besides infants, children aged 1-4 years were at similarly high risk of RSV infection. Year-round population-based RSV surveillance based on different age groups was crucial to detect out-of-season circulation and an age-related shift in primary care consultations. Based on sentinel surveillance data, early warning was possible for action regarding health service management and prevention, especially during COVID-19 pandemic.



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## Genomic characterisation of respiratory syncytial virus (RSV) causing disease in South Africa, 2021 - 2022

Daniel Gyamfi Amoako - ARNI0128

**Daniel Gyamfi Amoako<sup>3</sup>, Josie Everatt<sup>2</sup>, Boitshoko Mahlangu<sup>2</sup>, Naume Tebeila<sup>2</sup>, Dikeledi Kekana<sup>2</sup>, Wilhelmina Strasheim<sup>2</sup>, Anele Mnguni<sup>2</sup>, Noxolo Ntuli<sup>2</sup>, Mvuyo Makhas<sup>2</sup>, Thulisa Mkhencela<sup>2</sup>, Amelia Buys<sup>2</sup>, Fahima Moosa<sup>4</sup>, Mignon Du Plessis<sup>4</sup>, Sibongile Walaza<sup>5</sup>, Jocelyn Moyes<sup>5</sup>, Jinal N. Bhiman<sup>1</sup>, Cheryl Cohen<sup>5</sup>, Anne Von Gottberg<sup>4</sup>, Nicole Wolter<sup>4</sup>**

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### Background

The molecular epidemiology of respiratory syncytial virus (RSV) is complex with high genetic diversity and two antigenically distinct subtypes. This study aimed to characterise RSV in South Africa using whole-genome sequencing (WGS).

### Method

From 1 January 2021 through 31 March 2022, RSV was detected from respiratory specimens collected from individuals of all ages enrolled in sentinel syndromic surveillance for influenza-like illness (ILI) and severe respiratory illness (SRI). Real-time reverse transcription-polymerase chain reaction (rRT-PCR) RSV-positive specimens were subtyped by PCR. Specimens with a cycle threshold (Ct) value  $\leq 30$  were sequenced on the Illumina platform. Genotypes and lineages were assigned using ReSVidex (<https://cacciabue.shinyapps.io/resvidex/>) and glycoprotein (G) gene molecular classification. Comparative phylogenomic analysis was performed using IQ-TREE.

### Result

Among 10,223 individuals, 801 (7%) tested RSV positive with a detection rate of 5.4% (127/2360) in ILI and 8.6% (674/7863) in SRI. RSV cases mostly occurred among children aged  $<10$  years (87%, 700/801). Subtyping showed RSV-A and RSV-B dominance differed by syndrome [ILI (RSV-A: 72%, 91/127; RSV-B: 28%, 36/127) versus SRI (RSV-A: 47%, 320/674; RSV-B: 53%, 354/674),  $p$ -value = 0.001]. Of the 801 cases, 615 (77%) had Ct values  $\leq 30$ , of which 20% (123/615) were randomly selected for sequencing [RSV-A (59%, 72/123); RSV-B (41%, 51/123)]. Comparative phylogenomics of the sequenced subset depicted greater lineage diversity for RSV-A compared to RSV-B: RSV-A formed four distinct clusters while RSV-B formed one large cluster. RSV-A was dominated by the GA.2 genotype (99%, 71/72) and consisted of three lineages: GA.2.3.2b (2%, 1/71), GA.2.3.4 (7%, 5/71), and GA.2.3.5 (91%, 65/71)]. The GB.5 genotype (92%, 47/51) dominated RSV-B and consisted of lineages: GB.5.0.2 (2%, 1/47), GB.5.0.4a (4%, 2/47), and GB.5.0.5a (94%, 44/47). Mutational analysis of the G-protein revealed that mutations P71L, K262E, I265L, and D284G were prevalent in RSV-A (93%, 66/71), whereas A74V, and T226N were prevalent in RSV-B (32%, 15/47). The F-protein consisted of the mutations T122A (41/71), and I384T (30/71) in RSV-A, whereas I17V (17/47) was dominant in RSV-B.



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### **Conclusion**

During the study period, RSV cases mostly occurred in children aged <10 years and RSV subtype dominance differed by syndrome. RSV-A and RSV-B consisted of different lineages, with dominant mutations in the G-protein (RSV-A: K262E, D284G; RSV-B: A74V) and F-protein (RSV-A: T122A; RSV-B: Q I17V). Ongoing WGS of RSV will aid in the characterisation of circulating lineages and help to predict the potential effect of vaccines and monoclonal antibody treatment in South Africa.



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## Seasonal and inter-seasonal RSV activity in the WHO European Region between weeks 40/2020 and 19/2022

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Margaux Mesle - ARN10130

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### Background

Respiratory syncytial virus (RSV) typically co-circulates with influenza viruses in the winter months, causing more severe illness in infants and older adults, and is captured through sentinel primary care influenza surveillance systems in the WHO European Region. The emergence of SARS-CoV-2 in early 2020 and the subsequent public health and social measures (PHSM) implemented for its control have potentially impacted the spread of other respiratory viruses, including RSV. This work aims to describe the RSV epidemiology observed during the 2020/21 and 2021/22 influenza seasons (weeks 40/2020 to 20/2021 and 40/2021 to 20/2022) and one inter-seasonal period (weeks 21 to 39/2021).

### Method

Using data submitted to The European Surveillance System (TESSy) at ECDC by countries or territories in the WHO European Region between week 40/2020 to 19/2022, we calculated aggregated and country specific weekly counts of sentinel surveillance specimens and the percentage positivity for RSV. Results for both seasons and one inter-seasonal period (2021) were compared to 2016/17 to 2019/20 seasons and inter-seasonal periods.

### Result

During the 2020/21 season, a total of 23,150 specimens were tested for RSV, of which 200 (1%) were identified as positive, and a total of 31,888 specimens were tested during the 2021/22 season, of which 3,756 (12%) were positive. This compares to a mean of 1,669 (11% positivity) samples tested positive in prior seasonal periods in 21 countries across the WHO/Euro Region. The percentage positivity of specimens during the 2020/21 season was lower than in previous seasons, even though more samples were tested. However, the 2021/22 season saw a much higher positivity than prior seasons, up to 39% in week 44/2022, although more samples were tested. During the inter-seasonal period, up to 14% of specimens tested positive (peak in week 39/2021) with Germany recording the highest percentage positivity (53% in week 39). This contrasts with inter-seasonal activity not rising above 0.5% average positivity over the four prior seasons.

### Conclusion

Although, countries have tested more specimens compared to prior seasons, seasonal detections of RSV were below levels from prior seasons during the 2020/21 season. However, very high and increasing levels of percentage positivity were detected over the summer months of 2021 which continued during the 2021/22 season. PHSM implemented against the spread of SARS-CoV-2 likely effected the spread of other respiratory viruses, given the absence of RSV detections during the 2020/21 season. The out-of-season RSV activity in summer 2021 and ensuing season was likely linked to easing of PHSM across the region.





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## A Multisite Initiative to Assess Transplacental Transfer of Naturally-Acquired RSV Maternal Antibodies in Preterm and Full Term Infants

Jessica Atwell - ARN0131

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### Background

Protection of infants via maternal immunization (MI) requires efficient transplacental transfer (TPT) of maternal antibodies (Ab) to the fetus. TPT is an active, receptor-mediated process that begins early in the second trimester and continues throughout pregnancy. Full term infants ( $\geq 37$  weeks gestational age (wGA)) are generally born with higher antigen-specific Ab concentrations than their mothers, resulting in cord:maternal titer ratios (CMRs) of  $>1.0$ .

Preterm infants are at high risk of RSV-associated illness; they may also receive a lower relative amount of maternal Ab than full term infants. Observational data on TPT of naturally acquired RSV Ab have either excluded preterm infants or dichotomized wGA as pre/full term, limiting data on how this process varies across wGA. Phase III clinical trials are underway for RSV MI but preterm births are expected to be rare in study populations, which are healthy women screened for preterm risk factors. Detailed data on RSV Ab TPT dynamics throughout pregnancy are needed to better understand the potential for RSV MI to protect preterm infants and inform ideal timing of MI to maximize vaccine effectiveness (VE).

### Method

To address critical data gaps, Pfizer initiated a collaborative project of RSV seroepidemiology studies in preterm infants (PRISERO) across 5 sites: Emory Univ. (Atlanta) and Univ. of Washington/Seattle Children's (Seattle) in the US, Roosevelt Hospital (Guatemala City) via Univ. of Colorado, Univ. of Geneva (Switzerland), and Univ. Medical Center Utrecht (The Netherlands). The primary aim will centrally measure naturally acquired RSV A and B neutralizing Ab titers across a range of wGA at birth in  $\geq 1,000$  mother-infant pairs enrolled approximately 1:1:1:1 across categories of wGA:  $\leq 27$ , 28-31, 32-36,  $\geq 37$ . All sites are independently investigating additional RSV Ab/TPT-related aims.

### Result

Enrollment began in August 2021. Pooled analyses of the primary aim will summarize cord/maternal titers and CMRs, stratified by key variables including wGA at birth. CMR data by wGA at birth will be used along with immunogenicity data from the RSV MI clinical trial to model VE of maternal Pfizer RSVpreF vaccination in preterm infants. The first interim analysis (IA) will occur in the summer of 2022; additional IAs will follow.

### Conclusion

These data will address critical knowledge gaps regarding RSV Ab TPT in preterm infants and inform strategies to achieve maximum population-level RSV MI VE.



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## MOLECULAR EPIDEMIOLOGY OF RSV IN ARGENTINA, 2017-2021

Mara Russo - ARNI0134

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### Background

RSV is the main cause of bronchiolitis and pneumonia worldwide in children under 2 years. Since 2017, Argentina participates in the WHO Global RSV Surveillance in order to standardized it and provide evidence to measure the impact of the implementation of future RSV vaccines. This report presents the results of RSV surveillance in Argentina 2017-2021.

### Method

Between 2017- 2021 the NIC received clinical samples coming from children under 5 years and older adults collected from 5 Health Centres located in different regions of the country: Tucumán, La Rioja, Chaco, CABA and Buenos Aires. The case definitions were: SARI with and without fever, influenza-like illness with and without fever, and ARI. Real time RT-PCR and duplex rt RT-PCR were carried out for RSV detection and subtyping, respectively. A set of A and B RSV viruses were selected for sequencing the G gene. Phylogenetic analysis was performed using Bioedit and Mega 5 software. The sequences were deposited in GISAID database.

### Result

Among 2017-2019, the NIC received 2,164 samples (605 in 2017, 862 in 2018 and 739 in 2019) and the percentage of RSV positivity was 40 % (241/605) in 2017, 34 % (290/862) in 2018 and 48% (352/739) in 2019. During the pandemic 2020, until EW 9 (end of February), 22 samples were received and no RSV was detected. In 2021, the increment of RSV activity started late in comparison with the period 2017-2019; 200 samples were received and 175 (88%) were RSV positive. Between 2017-2019, the predominant clinical presentations in RSV positive patients (most of them under 5 years of age) were SARI followed by extended SARI. In relation with A and B subtyping (982 total viruses), RSV A was detected predominantly in 2017, RSV B was detected predominantly in 2018 and also in 2019 but the difference was not as marked, in 2021 B viruses were prevalent. Fifty-three viruses were sequenced: 20 in 2017, 11 in 2018, 8 in 2019 and 14 in 2021. ON1 genetic clade was identified in 23 A viruses and BA clade in 30 B viruses.

### Conclusion

In the pre Covid-19 pandemic 2017-2019 period, RSV surveillance in Argentina showed that the percentage of positivity was between 34-50 %. In 2021, human circulation increases, children return to classrooms and RSV activity begins to be detected again. The SARI definition predominated over the extended SARI definition but the last one has to be taken into account. The predominant circulating RSV subtype varied according to the season and the only genetic groups detected were ON1 and BA. RSV surveillance allows to obtain information in order to understand its evolution and relationship with the clinical presentation to respond in a properly manner when RSV vaccines become available.



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## A live attenuated codon-pair-deoptimized human respiratory syncytial virus (RSV) vaccine candidate genetically stabilized in vitro is immunogenic and protective in hamsters

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Cyril Le Nouen - ARNI0138

**Cyril LeNouen<sup>1</sup>**

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### Background

Recoding viral genomes by introducing numerous synonymous nucleotide substitutions that generate suboptimal codon pairs provides new live-attenuated vaccine candidates. Because recoding typically involves hundreds to thousands of nucleotide substitutions, the risk of de-attenuation is presumed to be low. However, this had not been thoroughly studied.

We previously generated an RSV vaccine candidate in which the NS1, NS2, N, P, M and SH ORFs were codon-pair deoptimized (CPD) by 695 synonymous nucleotide changes (RSV Min A). Min A exhibited a global reduction in transcription and protein synthesis, was restricted for replication in vitro and in mice and non-human primates, and exhibited moderate temperature sensitivity.

### Method

To evaluate its genetic stability under selective pressure, Min A was subjected to serial passage in vitro at incrementally increasing temperatures. After 18 passages, progeny was sequenced by whole-genome deep sequencing to identify missense mutations. Mutations of interest were introduced into Min A, and their effect on Min A replication in vitro and on immunogenicity and protective efficacy in hamsters was evaluated.

### Result

During the passages, Min A regained replication fitness and lost its temperature sensitivity. Whole-genome deep sequencing identified numerous missense mutations in several genes, in particular, ones accumulating between codons 25 and 34 of the phosphoprotein (P), a polymerase cofactor and chaperone. When re-introduced into Min A, these P mutations restored viral transcription to wt level, resulting in increased protein expression and RNA replication. These P mutations presumably increased the efficiency of the RSV transcription/replication complex, compensating for the reduced protein expression due to CPD. Molecular dynamic simulations suggested that the P mutations increased the flexibility of the N-terminal domain of P, which might facilitate its interaction with the nucleoprotein N, and increase the functional efficiency of the RSV transcription/replication complex.

The mutation P[F28V], which increased Min A replication in vitro paradoxically reduced Min A replication in hamsters but not its immunogenicity. The further addition of one missense mutation each in M and L generated a version of Min A with increased genetic stability. The resulting vaccine candidate exhibited increased attenuation in hamsters and, paradoxically, increased immunogenicity per plaque-forming unit.

### Conclusion

This study provides further insight into the adaptability of deoptimized RNA viruses under selective pressure and identified an improved CPD RSV vaccine candidate suitable for further evaluation.



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## Development of a Mucosal Virus Vectored Vaccine for Bovine Parainfluenza Virus Type 3

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Courtney Hawthorn - ARN10140

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### Background

Bovine PIV3 (bPIV3) is a major aetiological component of the bovine respiratory disease complex (BRDC), which is a leading cause of calf morbidity and mortality worldwide. It also causes major economic losses to the agricultural industry. While there are commercial vaccines available for bPIV3, there is a clear need for improvement.

This project aims to develop recombinant vaccine candidates using a Sendai virus (SeV) vector to express bPIV3 structural proteins. There is no evidence that SeV infects cattle, so the host will have no prior immunity. It also has a tissue tropism for the respiratory tract.

Reverse genetics protocols are in place to enable the generation of these vaccines, including strategies to generate replication competent (rc) and replication incompetent (ri) SeV vectors expressing the bPIV3 genes of interest. The (ri) SeV vaccine strategy eliminates the minimal vaccine biosafety issues that may be associated with rcSeV vaccines.

### Method

SeV infectious clones containing bPIV3 surface glycoprotein genes were generated and screened by colony PCR to confirm correct insert orientation. Infectious clones were further screened by restriction map analysis and Nanopore sequencing.

A reverse genetics rescue system was employed once the infectious clones were produced, to generate recombinant viruses expressing bPIV3 surface glycoproteins. This system uses a T7 RNA polymerase to drive transcription and translation.

### Result

riSeVs expressing bPIV3 surface glycoproteins were successfully rescued. Appropriate expression of the bPIV3 glycoproteins was confirmed by RT-PCR, sequencing, and immunofluorescence. Rescue of the equivalent rcSeV vaccines is underway.

### Conclusion

riSeV vaccines expressing bPIV3 surface glycoproteins have been generated and are suitable for immunogenicity and protective efficacy studies in mice and/or calves. rcSeV vaccines expressing bPIV3 surface antigens remain to be generated and validated in vitro before in vivo studies.



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## Activation of the cGAS-STING pathway following RSV Line 19 infection

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**Ann Miller** - ARNI0141

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### Background

Respiratory syncytial virus (RSV) is considered the single most important cause of serious acute lower respiratory illness in infants and young children worldwide, with virtually all children infected within their first two years of life. Type I interferons play a critical role in control of viral replication and activation of the innate antiviral immune response during RSV infection. Recent studies have demonstrated that RNA viruses can activate the cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS) leading to stimulator of interferon genes (STING) signaling and production of type I interferons. Here we examined the activation of the cGAS-STING pathway following RSV infection.

### Method

The human monocytic cell line THP-1 was infected at an MOI of 5 with RSV strains A2 and Line 19. Gene expression of IFN- $\alpha$  and IFN- $\beta$  were determined by qRT-PCR at 12 and 24 hours post-infection (p.i.). To examine mitochondrial DNA (mtDNA) leakage from cells, DNA was isolated from the cytosol and mtDNA was quantified by qRT-PCR at 24 p.i.. Activation of the cGAS-STING signaling pathway, as measured by phosphorylation of TBK1, was examined via immunoblotting at 12, 24, and 48 hours p.i.. cGAS protein expression was knocked down in A549 pulmonary epithelial cells using siRNA. cGAS siRNA treated cells and non-target siRNA control treated cells were infected at an MOI of 5 with RSV Line 19. IFN- $\alpha$  and IFN- $\beta$  mRNA levels were determined by qRT-PCR at 24 hours p.i.. STING and TBK1 phosphorylation were examined at 6, 12, 24, and 48 hours p.i..

### Result

THP-1 cells infected with RSV Line 19 exhibited increased gene expression of IFN- $\alpha$  at 12 and 24 hours p.i. compared to cells infected with RSV A2. IFN- $\beta$  gene expression was increased 12 hours post-Line 19 infection compared to A2, however gene expression was decreased at 24 hours p.i.. THP-1 cells infected with RSV Line 19 exhibited increased mtDNA leakage into the cytosol 24 hours post-infection compared to A2 infected cells. Increased phosphorylation of TBK1 was also observed at 12 and 24 hours post-Line 19 infection compared to A2 infected THP-1 cells. A549 cells treated with cGAS siRNA displayed a significant reduction in IFN- $\beta$  gene expression 24 hours post-Line 19 infection compared to non-target control cells. Additionally, A549 cells treated with cGAS siRNA displayed decreased phosphorylation of STING and TBK1 compared to non-target siRNA control cells over a time course.

### Conclusion

RSV Line 19 infection induces a robust type I interferon response in comparison to the infection with the A2 strain. Our data indicate that activation of the cGAS-STING pathway, likely mediated by mtDNA leakage, contributes to the enhanced type I interferon response observed following Line 19 infection.



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## Respiratory syncytial virus provides protection against a subsequent influenza A virus infection

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Stacey Hartwig - ARN0142

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### Background

Respiratory tract infections are a major global health problem and represent a leading cause of morbidity and mortality. Respiratory syncytial virus (RSV) and influenza A virus (IAV) are two of the most common respiratory virus infections observed in hospitalized individuals, particularly in the very young and aged populations. RSV and IAV exhibit overlapping seasonal outbreaks worldwide. Although co-infection with multiple respiratory pathogens is frequently observed in hospitalized individuals, the impact of overlapping respiratory virus infections on the host immune response and disease severity is not well understood. Here we examined how the order in which BALB/c mice were sequentially infected with RSV and IAV impacts the host immune response and disease severity following infection.

### Method

BALB/c mice were infected with either RSV or IAV and days later, as indicated, infected with the heterologous virus. Infected mice were monitored daily for weight loss and lung function using whole-body plethysmography. Viral replication and the mRNA expression of several interferon-stimulated genes were examined at multiple time points by qRT-PCR. Lung pathology was evaluated by histology. Anti-viral CD8 T cell responses were monitored by MHC Class I tetramer staining and intracellular IFN-gamma production following in vitro peptide stimulation. Cytokine production by ELISA was evaluated at multiple time points. Inflammatory cell populations in the lung were examined at multiple time points.

### Result

Sequential overlapping RSV and IAV infections has a substantial impact on both the host immune response and disease severity, with the order of the infections playing a critical role in influencing the outcome of the infections. Mice infected with IAV prior to RSV exhibited similar patterns of disease when compared to the IAV single infection controls. In contrast, mice infected with RSV prior to IAV exhibited significantly reduced weight loss, viral replication, and mortality as compared to the single infection controls. Consistent with the reduced viral replication and disease severity, the magnitude of the anti-viral CD8 T cell response to IAV was reduced when the IAV infection was preceded by an RSV infection. Mice infected with RSV followed by IAV exhibited increased production of IFN-gamma by ELISA as compared to control groups.

### Conclusion

Our data demonstrate that the order of overlapping viral infections has a substantial impact on the host immune response and disease severity.



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## Vaccination with RSV prefusion-stabilized F protein elicits neutralizing site VI antibodies

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Ryan McCool<sup>1</sup> - ARNI0144

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### Background

Respiratory syncytial virus (RSV) causes tens of millions of acute lower respiratory infections and millions of hospital admissions globally; however, effective vaccines have not yet been approved despite continuous efforts since the mid-1950s. Previous antibody repertoire analysis of a human vaccine trial investigating a prefusion RSV F glycoprotein antigen (DS-Cav1) found that only two antibody clonotypes were prevalent in over 80% of evaluated vaccinated individuals, with the most prevalent clonotype targeting the well-defined antigenic site V.

### Method

Here, we used cryo-electron microscopy (cryo-EM) to further our understanding of the second identified clonotype.

### Result

Our 2.7 Å resolution EM map of an RSV F-Fab complex shows that this clonotype recognizes a newly proposed prefusion F-specific antigenic site VI consisting of the membrane-proximal stalk of RSV F. Antibodies recognizing this epitope were able to potently neutralize RSV. Although these antibodies primarily interact with prefusion RSV F via their heavy chains, light chain contacts with the membrane-proximal stalk are important for potent neutralization.

### Conclusion

Taken together, our results show that prefusion-stabilized F protein subunit vaccination boosts neutralizing antibodies against a previously uncharacterized antigenic site.





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## Development and validation of a simplified severity score for RSV bronchiolitis

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**Zakariya Sheikh** - ARNI0146

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### Background

No consensus exists on quantifying disease severity in infants with respiratory syncytial virus (RSV) bronchiolitis; lack of a validated score complicates the provision of clinical care and, the standardised evaluation of trials of therapeutics and vaccines. The Respiratory Syncytial Virus Network (ReSVinet) score is the most promising. We aimed to simplify this score to facilitate its application in routine care.

### Method

We used data from the RESCEU multinational (NL, ES, UK) case-control observational study of infants with RSV (n=311) to develop simplified versions of the ReSVinet score by conducting a grid search to maximise the score's discriminative ability, assessed by the area under the receiver operating curve, across a range of outcomes. Subsequently we externally validated the score using data collected from a prospective cross-sectional study in hospitals in Rwanda of infants (n=100) presenting with respiratory distress (Hakizimana), and from a single-centre prospective observational cohort study of infants (n=188) presenting with an acute respiratory infection in Colombia (Camacho-Cruz).

### Result

Three scores were identified; they were excellent in the development dataset at discriminating for a range of outcomes; their performance was not significantly different to the original ReSVinet score despite fewer parameters. In the external validation datasets, the simplified scores were moderate-excellent across (see Table) all outcomes, they performed at least as well as the original ReSVinet score.

### Conclusion

We recommend that fever may be excluded from the original ReSVinet score (i.e. ReSVinet-6). Two additional promising simplified scores (i.e. ReSVinet-3 & ReSVinet-4) have been identified; further external validation in larger datasets, with larger number of participants with the outcomes of interest, is required before we can recommend their use.



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## Constitutive differences in adult and infant neutrophil transepithelial migration during in vitro modelling of RSV infection of primary differentiated airway epithelial cells

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Elisabeth Robinson - ARNI0148

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### Background

Respiratory Syncytial Virus (RSV) is the leading infectious cause of hospitalisation in infants. Adults are still susceptible to RSV infection but do not suffer bronchiolitis. Neutrophilic infiltration of the airways is observed in infants with severe infection clinically, but whether these neutrophils contribute to recovery or increased pathology is undefined. This study aims to examine differences in adult and neonatal neutrophil migration across RSV infected airway epithelial cells (AECs), using a sophisticated in vitro model, and measure neutrophil apoptosis, cellular and secreted markers of neutrophil activation.

### Method

Cord blood was collected following uncomplicated term caesarean deliveries at University College Hospital, London. Adult venous blood was collected from healthy volunteers at UCL Institute of Child Health. Full research ethics committee approval was obtained. Neutrophils were purified using negative immunoselection (Stemcell). Primary human airway epithelial cells (AECs) were grown at air-liquid interface for 28 days to ciliation, then infected with RSV A2 for 72hrs. Purified neutrophils were then added to the basolateral side of AECs and incubated for 1 hour. Neutrophils basolateral, adhered, and apical to AECs were collected. Neutrophil numbers were determined by CD11b-APC positivity. Viability, apoptosis and activation markers were quantified using flow cytometry.

### Result

Greater numbers of cord blood neutrophils ( $336,684 \pm 48,129$ ) (Mean  $\pm$  SEM) compared with adult neutrophils ( $56,586 \pm 16,139$ ) migrated apically to RSV infected AECs ( $p < 0.0001$ ) (Fig1). Conversely, greater numbers of adult neutrophils ( $171,641 \pm 31,632$ ) remained basolaterally in comparison to cord blood ( $23,870 \pm 3,359$ ) ( $p < 0.05$ ) (Fig1). A greater proportion of apical cord blood neutrophils were apoptotic ( $43.3\% \pm 10.68$ ) in comparison to adult ( $9.49\% \pm 2.7$ ) ( $p = 0.0024$ ); despite this there were still greater numbers of viable cord blood neutrophils ( $191,130 \pm 44,987$ ) compared to adult neutrophils ( $50,389 \pm 16,139$ ), apical to RSV infected AECs ( $p = 0.037$ ) (Fig2). A greater proportion of RSV AEC adhered cord blood neutrophils were apoptotic ( $14.6\% \pm 1.8$ ) in comparison to adult ( $0.803\% \pm 0.31$ ) ( $p = 0.0192$ ) (Fig2).

### Conclusion

Greater numbers of cord blood neutrophils migrate across RSV infected AECs in comparison to adult, of which a greater proportion undergo apoptosis, and which show limited upregulation of activation markers in comparison to adult. These differences may facilitate greater pulmonary neutrophilic infiltration in RSV infection in infants than adults.



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## Severity of RSV infections in hospitalized children under 2 years of age: Portuguese experience from the first year of national surveillance

ANA PAULA RODRIGUES<sup>1</sup> - ARN0149

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### Background

Respiratory Syncytial Virus (RSV) infection is an important cause of hospitalization in children under 5 years. Monoclonal antibodies prophylaxis is recommended for those at higher risk of severe infection and vaccines are likely to become available soon. Thus, RSV surveillance is a priority in order to plan preventive measures and evaluate the impact of vaccine introduction. Following European recommendations, a national hospital-based RSV sentinel network was set up in Portugal. In this work, we describe the results of the first year of surveillance.

### Method

The network was set up in April 2021 with 4 hospitals, being expanded to 17 hospitals (out of 50) in April 2022. All hospitalized acute respiratory infections (ARI) in children under 2 years or sepsis under 6 months had indication to be notified and tested for RSV by sentinel hospitals. Risk factors (prematurity, chronic lung disease, congenital heart disease, immunodeficiency), clinical and laboratorial data were reported. All RSV positive samples were genetically characterized at the National Reference Laboratory for Influenza and Other Respiratory Viruses. Severity of RSV cases was defined by need of ventilation (invasive/non-invasive) or admission to intensive care unit. Crude risk ratios (RR) and 95% confidence intervals (CI) were used to assess association between gestational age and chronic diseases and RSV severe illness. Statistical analyses were performed using OpenEpi v3.01.

### Result

183 RSV cases (52% of ARI cases) were reported. An anomalous RSV outbreak was observed from June 2021 to February 2022. 47% of RSV cases were under 3 months old, 16% were pre-term, 8% had at least one chronic disease, and 16% had severe RSV illness. Both RSV A and B subtypes were detected. Pre-term children [RR: 1.997; 95 CI (0.981-4.065)] and those suffering from a chronic condition [RR: 2.333; 95 CI (1.042-5.223)] had a 2-fold risk of having severe illness comparing to term children and those without chronic conditions. However, not all children with severe RSV illness met the criteria for prophylaxis.

### Conclusion

The network was able to detect an off-season RSV outbreak, despite the inconsistent data reporting during first months of surveillance. The proportion of RSV severe hospitalization was higher than reported by previous national studies, but this result is probably overestimated due to the high number of cases reported by central hospitals. Although, we also hypothesize that absence of RSV circulation during the first COVID-19 pandemic year may be associated with more severe disease course, which need further investigation. Prematurity and chronic diseases predispose to severe RSV infection.



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## Optimizing next-generation RSV prevention in Mali: a cost-effectiveness analysis of pediatric vaccination, maternal vaccination, and extended half-life monoclonal antibody immunoprophylaxis

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### Background

Respiratory syncytial virus (RSV) is the most common cause of early childhood lower respiratory tract infection (LRTI) in low- and middle-income countries (LMICs). Maternal vaccines, birth-dose extended half-life monoclonal antibodies (mAbs), and pediatric vaccines are under development for prevention of respiratory syncytial virus (RSV) lower respiratory tract infection (LRTI) in young children.

### Method

We conducted an analysis of both health and economic impacts of RSV interventions used alone or in combinations in Mali. We modeled age-specific and season-specific risks of RSV LRTI in children through three years of life, using WHO Preferred Product Characteristics and data generated in Mali. Health outcomes included RSV LRTI cases, hospitalizations, deaths, and disability-adjusted life-years (DALYs). We identified the optimal combination of products across a range of scenarios.

### Result

We found that mAb delivered at birth could avert 878 DALYs per birth cohort at an incremental cost-effectiveness ratio (ICER) of \$597 per DALY averted compared to no intervention if the product were available at \$1 per dose. Combining mAb with pediatric vaccine administered at 10/14 weeks, 1947 DALYs would be prevented. The ICER of this combination strategy is \$1514 per DALY averted compared to mAb alone. In an optimization analysis incorporating parameter uncertainty, mAb alone is likely to be optimal from the societal perspective at efficacy against RSV LRTI above 66%. The optimal strategy was sensitive to economic considerations, including product prices and willingness-to-pay for DALYs. For example, the combination of mAb and pediatric vaccine would be optimal from the government perspective at a willingness-to-pay above \$775 per DALY. Maternal vaccine alone or in combination with other interventions was never the optimal strategy, even for high vaccine efficacy. The same was true for pediatric vaccine administered at 6/7 months.

### Conclusion

At prices comparable to existing vaccine products, public health programs using extended half-life RSV mAbs alone or in combination with pediatric RSV vaccines would be impactful and efficient components of prevention strategies in LMICs such as Mali.



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## Molecular and Microbiome/Metagenome Correlates of Recurrent Wheeze in Respiratory syncytial Virus (RSV) Infected Infants

Mary Caserta - ARN0153

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### Background

Respiratory syncytial Virus (RSV) yearly epidemics are a leading cause of hospitalization. Following primary infection, studies have found an increased rate of recurrent wheezing, especially in infants with severe disease. Transcription profiling of upper respiratory tract (URT) epithelial cells has identified associations with RSV disease severity, as have URT microbiome diversity and microbial community functionality as measured by metagenomics. This study was designed to test if clinical factors, airway gene expression and microbiome/metagenome patterns in the nasal epithelium during primary RSV infection can classify infants who develop recurrent wheezing.

### Method

Healthy full-term infants born after May 1, 2019 with PCR confirmed RSV infection were prospectively recruited from inpatient and outpatient locations from Dec 2019 to April 2020. Clinical and demographic data, 2 anterior nasal swabs and a nasal wash were collected at enrollment. Microbiome/metagenome analysis and transcriptome/RNA sequencing were performed. Recurrent wheezing was identified by biweekly phone contact with families through the subsequent winter season.

### Result

48 (40 hospitalized) infants were enrolled. Four were admitted to the Intensive Care Unit. One child was ineligible and one died during follow-up, unrelated to the study, leaving 46 for analysis. The average age was 2.6 (SD 2.0) months. 31 (66%) were male. The hospitalized and non-hospitalized infants were not different in age, sex, race or ethnicity. The Global Respiratory Severity Score (GRSS) and retrained GRSS (iGRSS) were computed. 46 subjects had at least one follow-up call with 1057 phone logs (78%) completed. 20 (43%) of 46 reported one or more episodes of recurrent wheezing. Due to the COVID-19 pandemic, 14 subjects did not have in-person follow-up. Of 6 children seen at a follow-up visit 3 (7%) had confirmed recurrent wheezing. A multivariate linear regression model was used to associate gene expression profiles with visit age, recurrent wheezing status, and iGRSS. After controlling false discovery rate (FDR) at 0.05 level, 422 genes were significantly associated with iGRSS. Using a slightly relaxed criterion (FDR<0.10), 17 genes were significantly associated with recurrent wheezing. Pathway analysis showed that iGRSS was related to infectious diseases (including Staph aureus) and several signaling pathways (TNF $\alpha$ , chemokine, NF kappa-B, NOD-like receptor, and IL-17). Recurrent wheezing was related to Protein processing in endoplasmic reticulum and vesicle-mediated transport.

### Conclusion

We enrolled a cohort of previously healthy term infants with primary RSV infection and identified associations between nasal epithelium host gene expression and recurrent wheezing.



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## RSVpreF subunit vaccine antibody response modeled through two RSV seasons in older adults

Kena Swanson - ARNI0154

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### Background

Immunity following natural RSV infection is considered short-lived. The expected duration of protection following vaccination is unknown. Pfizer's RSV prefusion F-based vaccine, RSVpreF, for older adults is currently in Phase 3 efficacy trial. Although a precise correlate of protection has not been established, modelling of antibody persistence has been used to assess duration of vaccine-elicited protection. To assess antibody persistence, we compared the antibody decay rate after RSVpreF vaccination with the rate after natural RSV infection in older adults and projected antibody persistence levels through the 2nd RSV season after vaccination.

### Method

We included in this analysis: 1) neutralizing titers (NTs) measured at 1, 2, 3, 6, and 12 months after initial vaccination from 81 participants (65-85 years old) who received RSVpreF (120 µg) in a phase 1/2 study and 2) 8 placebo recipients with confirmed RSV infection as measured by non-vaccine antigen seroconversion (4-fold rise) from pre-RSV season to the end of the RSV season. A simple regression model was used to estimate the antibody decay rate in the vaccine group and the placebo group.

To predict antibody persistence at month 18 (mimicking end of the 2nd RSV season following initial vaccination prior to the 1st RSV season), individual NTs measured during the 12-month postvaccination study period were modeled using a conventional power law model and a piece-wise regression model for RSV subgroup A and B, respectively. Fitted models were then used to predict antibody levels at month 18.

### Result

The decay rate of RSV A and RSV B NT induced by RSVpreF vaccination (0.13 log<sub>2</sub>/month [RSV A/B combined]) is slower than the RSV natural infection immunity decay rate (0.20 log<sub>2</sub> /month [RSV A/B combined] and 0.19 log<sub>2</sub>/month [RSV A]). The decay rate following natural infection was similar to that reported in 20 subjects in a published epidemiology study (Falsey et al, J Med Virol. 2006): 0.20 log<sub>2</sub>/month for RSV A strain with microneutralization assay (MNA). Both models fit NTs measured during the 12-month postvaccination study period well and predicted NTs at month 18 were still >2-fold higher than before vaccination (Figure 1).

### Conclusion

Our modeling indicates RSVpreF-elicited neutralizing antibodies remain at least 2-fold above baseline titers through an estimated duration of two RSV seasons. As a correlate of protection is not yet established, it is unknown if the duration of protection following vaccination with a prefusion F subunit vaccine may also persist for up to two RSV seasons and requires further study.





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## Description and assessment of the unusual and unseasonal increase in RSV activity in Japan, 2021

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Miyako Otsuka - ARN10155

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### Background

In Japan, sentinel surveillance for RSV infection in children has been operating nationally since 2003. The observed epidemiology of many infectious diseases has changed since the coronavirus disease 2019 (COVID-19) pandemic set in, and RSV has been no exception. Here, we report on and discuss the trends and distributions of RSV infection notifications since 2018.

### Method

We extracted the weekly number of reported RSV cases from pediatric sentinel sites from the National Epidemiological Surveillance for Infectious Diseases (NESID) system, the national electronic system for infectious disease surveillance, from 2018-2021. We descriptively assessed the RSV case notification counts per sentinel site by epidemiologic week, age, and region.

### Result

In 2018 and 2019, the number of notifications started to increase continuously from around week 25, showing a peak at week 37 with an average of 2.46 cases and 3.45 cases per sentinel site in 2018 and in 2019, respectively. Relative to these years, the epidemiological trend in 2020 and 2021 differed substantially; while there were very few notifications in 2020, an unseasonably early surge with an unprecedented peak (5.99 per sentinel site) occurred in week 28. The unusually early and large increase was observed in multiple regions; geographically, the temporal order in the rise in RSV activity was overall similar through 2018-2021, with the increase starting from the south and spreading nationwide. Demographically, while the slightly higher male gender ratio remained unchanged, the age distribution in 2021 differed considerably from that of the other years. In 2021, the proportion of cases under 1 year of age decreased to nearly half of that of 2018-2019, and both the number and proportion of those aged 2, 3, and >4-years increased.

### Conclusion

The epidemiology of RSV infection changed drastically during the COVID-19 pandemic, with minimal notifications in 2020 followed by a large and unseasonal increase the following year. Notably, the rise in 2021 saw an older pediatric case age distribution-this may be due to reduced exposure to RSV during the early COVID-19 pandemic period, resulting in an accumulation of susceptible children. Other information, such as acute encephalitis surveillance and clinical networks, also indicated high RSV activity in 2021. RSV clearly poses a public health concern, with the potential to burden medical systems in an unpredictable manner. Continued monitoring for RSV will be imperative to maintain situational awareness and facilitate timely, surveillance-informed decision-making (e.g., provision of Palivizumab).





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## RSV Epidemiology and Strain Identification in a Longitudinal Southeast Michigan Cohort of Households with Children

Emily Martin - ARNI0156

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### Background

Understanding the potential connections between RSV infections in adults and children is key to the planning and implementation of future initiatives to reduce infections in infants. Longitudinal cohort studies provide unique insight into RSV infection and reinfection among households with children.

### Method

The Household Influenza Vaccine Evaluation (HIVE) Study follows approximately 250 to 350 households including young children annually, with active surveillance for acute respiratory illness (ARI) meeting a standard symptom-based case definition capturing mild to moderate illness. Respiratory specimens (nasal-throat swabs) collected at illness onset from 2010 through 2020 were tested for RSV by RT-PCR using subtype-specific primers and probes and were subsequently sequenced. Participant and illness characteristics were collected by interview and medical record review.

### Result

Of the 9,822 ARI's evaluated over 10 years, 494 (5%) were positive for RSV overall, ranging from 3.7% in 2014-2015 to 7.6% in 2012-2013. Of these, 36% (n=180) were RSV-A, 34% (n=167) RSV-B, and 30% (n=147) had no subtype determined. Adjusting for gender, children aged 0-4 years and children aged 5-17 years experienced significantly higher odds of RSV detection during ARI compared to adults [ages 0-4 versus 18+ ORadj: 3.32, p-value<0.0001; ages 5-17 versus 18+ ORadj: 1.72, p-value<0.0001]. The mean interval between first-detected and repeat RSV infection was one and a half years (median: one year and one month) and repeated infections were observed most often in younger children (mean age 7 years, 9 months). 65% of repeat infections with complete subtype data were heterologous pairs, meaning they were infected with a subtype opposite of their previously detected infection. 377 RSV specimens were sequenced including 104 RSV-A and 66 RSV-B from HIVE and 97 RSV-A and 110 RSV-B from broader community ARI surveillance. Within the past decade, RSV-A genotype ON-1 and RSV-B genotype BA-11 were the predominant circulating strains in this community.

### Conclusion

In our US-based study, children accounted for the highest burden of symptomatic RSV within the family. While repeated RSV infection was detected during this longitudinal study, this most commonly represented sequential infections with differing RSV subtypes, indicating potentially lowered cross-reactivity of protection. The genomic distribution of circulating strains in this suburban community reflected larger global patterns.



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## Evaluation of Recombinant Live-Attenuated Respiratory Syncytial Virus Vaccines RSV/ $\Delta$ NS2/ $\Delta$ 1313/I1314L and RSV/276 in RSV Seronegative Children

Ursula Buchholz - ARN0157

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### Background

This study evaluated the safety and immunogenicity of two live RSV candidate vaccines, delivered intranasally to RSV-seronegative infants and children 6-24 months of age. The candidates were (i) RSV/ $\Delta$ NS2/ $\Delta$ 1313/I1314L, attenuated by NS2 gene-deletion and stabilized temperature-sensitivity mutation  $\Delta$ 1313/I1314L in the polymerase gene; and (ii) RSV/276, attenuated by M2-2 deletion.

### Method

In a randomized (2:2:1 vaccine:vaccine:placebo), double-blind study conducted at 12 US sites, RSV-seronegative children aged 6-24 months received RSV/ $\Delta$ NS2/ $\Delta$ 1313/I1314L [106 plaque-forming units (PFU)], RSV/276 [105 PFU] or placebo intranasally (Clinicaltrials.gov: NCT03227029, NCT03422237). Participants were monitored for vaccine shedding, reactogenicity, and RSV serum antibodies, and followed over the subsequent RSV season to monitor for RSV-associated medically-attended acute respiratory illness.

### Result

Sixty-two participants received study product and completed safety evaluations through day 56 post-inoculation (p.i.). Through day 28 p.i., upper respiratory illness and/or fever occurred in 16/25 (64%) of RSV/ $\Delta$ NS2/ $\Delta$ 1313/I1314L, 21/25 (84%) of RSV/276, and 7/12 (58%) of placebo recipients. Symptoms were generally mild. Cough was more common in RSV/276 recipients than RSV/ $\Delta$ NS2/ $\Delta$ 1313/I1314L (48% v. 12%) or placebo recipients (17%). There were no lower respiratory illness or serious adverse events through day 56 p.i.. Infectivity analyses included 61 participants, excluding a randomly-selected twin. Twenty-two of 25 (88%) of RSV/ $\Delta$ NS2/ $\Delta$ 1313/I1314L recipients and 23/24 (96%) RSV/276 recipients were infected with vaccine (shed vaccine and/or had  $\geq 4$ -fold rises in RSV antibodies). Sequence analysis of isolates from vaccinees confirmed the stability of the NS2 deletion and the  $\Delta$ 1313/I1314L site, or the M2-2 deletion. Serum RSV-neutralizing titers and



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anti-RSV F IgG titers increased  $\geq 4$ -fold in 60% and 92% of RSV/ $\Delta$ NS2/ $\Delta$ 1313/I1314L and RSV/276 vaccinees, respectively. Among participants exposed to community RSV during the subsequent RSV season, strong anamnestic RSV-antibody responses ( $\log_2$ ) were observed in both RSV/ $\Delta$ NS2/ $\Delta$ 1313/I1314L ( $n=11$ ; median [IQR]: 10.1 [7.7-10.8]) and RSV/276 ( $n=4$ ; 10.6 [8.0-11.3]) vaccine groups; in placebo recipients, the antibody responses during the RSV season ( $n=4$ ; 5.9 [4.5-6.3]) were comparable to responses detected in vaccinees on day 56 post-immunization (RSV/ $\Delta$ NS2/ $\Delta$ 1313/I1314L:  $n=25$ ; 5.1 [4.1, 6.2]; RSV/276:  $n=24$ ; 6.7 [6.0, 7.8]).

### **Conclusion**

Both vaccines had excellent infectivity and were well-tolerated. RSV/276 induced an excess of mild cough. Both vaccines were immunogenic and primed for strong anamnestic responses. Further evaluation of RSV/ $\Delta$ NS2/ $\Delta$ 1313/I1314L in larger safety and efficacy studies is warranted.



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## Incidence and impact of respiratory syncytial virus-associated wheezing in early life: a birth cohort study in a low-income urban community in Dhaka, Bangladesh

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### Background

Respiratory syncytial virus (RSV) is a significant cause of wheezing and respiratory illness in young children and is associated with an increased risk of childhood asthma. We estimated the incidence and impact of RSV-associated childhood wheezing in early life.

### Method

From May 2015 to February 2017, newborn infants in a low-income community in Dhaka were enrolled during the first week of life and were followed until two years of age. Field staff visited households twice weekly and referred children with respiratory symptoms to the study clinic. Physicians collected nasal wash samples from children with acute respiratory infections (ARI), defined as cough and/or runny nose; or pneumonia, defined as cough with tachypnea, and identified wheezing on lung auscultation. Samples were tested for RSV and other respiratory pathogens, including human metapneumovirus (HMPV), influenza viruses, human parainfluenza viruses (HPIV) and adenoviruses by rRT-PCR. We used multivariate Cox's regression and ad-justed for clustering at the child level to calculate hazard ratios to estimate the association between RSV infection during the first year of life and subsequent episodes of wheezing illnesses during 13-24 months of age.

### Result

We enrolled 482 children and observed for 625 child-years. Of 2736 respiratory illness episodes, 2419 (88%) were identified as ARI and 317 (12%) as pneumonia. Wheezing was associated with 10% (267) respiratory illness episodes. Thirty-three per cent (159/482) of children had at least one episode of wheezing, and 10% (49/482) had at least two episodes. Respiratory viruses were detected in 66% (176/267) of wheezing episodes. Of the wheezing episodes, RSV was detected in 18% (47) of episodes, rhinoviruses in 16% (42), HPIV in 12% (33), influenza viruses in 6% (15), HMPV in 5% (14), adenovirus in 4% (12) and multiple viruses were detected in 4% episodes (13). The estimated annual incidence of RSV-associated wheezing was 8.3 per 100 child- years (95% CI: 6-11). RSV infection during the first year of life was not associated with an increased risk of subsequent wheezing illness during 13-24 months of age (HR=1.7, 95% CI 0.6-5.2, P=0.51).

### Conclusion

Young children in Dhaka frequently suffered from respiratory illnesses with wheezing. RSV was the predominant cause of early life wheezing. Further research should explore other contributors to childhood wheezing and the development of asthma.



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## Acute respiratory infections among individuals seeking outpatient care in the states of Washington and Michigan by pregnancy status, 2011-2016

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Collrane Frivold - ARNI0159

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### Background

Respiratory illnesses are considered common during pregnancy, but the disease burden of common acute respiratory infections (ARIs) among pregnant people is not well characterized. Most studies to date on ARIs in pregnant people have focused on influenza, which have demonstrated increased morbidity and mortality among pregnant people. However, the disease burden caused by other respiratory pathogens including respiratory syncytial virus (RSV) is poorly defined. The objective of this study was to compare respiratory virus detection, illness characteristics, and hospitalization rates among pregnant and non-pregnant people with ARIs in outpatient settings.

### Method

This study used data from the U.S. Flu VE network from the Michigan and Washington sites collected between 2011-2016. We included outpatients who presented with symptoms of ARI to healthcare facilities at participating sites and had nasal and oropharyngeal swabs tested for respiratory viruses using RT-PCR. Participants were categorized by self-reported pregnancy status and matched by age, site, and respiratory virus season. We first conducted descriptive analyses on respiratory virus detection. We also calculated the odds ratio and associated 95% confidence interval (CI) of hospitalization by pregnancy status using conditional logistic regression.

### Result

Among the pregnant (n=92) and non-pregnant (n=96) participants, the most common pathogens detected were influenza A (12.0% vs 12.5%), rhinovirus (15.2% vs 12.5%), human coronavirus (17.4% vs 7.3), and RSV (6.5% vs 11.5%) (Table 1). Pregnant participants were more likely than non-pregnant participants to have received a seasonal influenza vaccine (61% vs. 33%), but had similar rates of influenza A/B infection (18.5% vs 16.7%). The odds of hospitalization for a medically attended ARI following enrollment among pregnant participants was 4.0 times that of non-pregnant participants (95% CI: 0.45-35.8).

### Conclusion

Our results suggest that respiratory viruses other than influenza may be playing an important role in ARI morbidity among pregnant people. However, the findings are limited by the small sample size. Additional studies are needed to characterize the burden of respiratory viruses among larger populations of pregnant people across multiple years to account for season-to-season variability. These data are critical to informing the implementation of public health interventions targeted at this high-risk population including maternal RSV vaccines currently in development.



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## Respiratory Syncytial Virus-associated Deaths among Under-five Children before and during COVID-19 Pandemic in Bangladesh

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### Background

Respiratory syncytial virus (RSV) is one of the leading causes of acute lower respiratory tract infections and hospitalization in young children globally. RSV-associated deaths estimate among children <5 years is limited in Bangladesh. We analyzed hospital-based surveillance data to identify the contribution of RSV in SARI mortality among children <5 years before and during the COVID-19 pandemic.

### Method

From August 2009 to March 2022, we analyzed data of SARI patients aged < 5 years from the hospital-based influenza surveillance (HBIS) platform at 14 tertiary level hospitals in Bangladesh. We considered August 2009-February 2020 as the pre-pandemic and March 2020-March 2022 as the pandemic period. Surveillance physicians identified inpatients meeting the WHO-SARI case definition, collected information on demographics, clinical characteristics, and outcomes. We collected oropharyngeal and nasopharyngeal swabs and tested for common respiratory viruses: RSV, influenza, SARS-CoV-2, human metapneumovirus (HMPV), human parainfluenza viruses (HPIV) and adenoviruses by rRT-PCR. We summarized the data using frequency, percentages and chi-squared test.

### Result

We enrolled 8,923 children aged <5 years with SARI during the pre-pandemic period [median age: 6 months (IQR: 2.5 - 12); 67% male] and 2,570 children < 5 years during the pandemic [median age: 6 months (IQR: 3 - 14); 65% male]. A higher proportion of SARI deaths occurred during the pandemic compared to pre-pandemic [(2.6%, 66) vs. (1.8%, 159);  $p < 0.001$ ]. Respiratory viruses were detected among 45% (71) death cases, during the pre-pandemic, and 47% (31) cases during the pandemic. Of the 159 pre-pandemic death cases, detection of RSV (13%, 20) was predominant, followed by HPIV (9%, 14), adenovirus (8%, 12), HMPV (6%, 10), influenza (4%, 6) and viral co-infections were also detected in (6%, 9) death cases including 3 (2%) co-infections with RSV. Of the 66 pandemic deaths, the highest proportion of virus detection was for RSV (12%, 8) and adenovirus (12%, 8), followed by HPIV (6%, 4), SARS-CoV-2 (6%, 4), influenza (3%, 2), HMPV (1%, 2) and co-infection with RSV and adenovirus (3%, 2) and co-infection with HPIV and adenovirus (3%, 2). Of the death cases, solely RSV was detected in 57% (16) children aged <6 months, in 25% (7) children aged 6-12 months, in 11% (3) children aged 1-2 years and in 7% (2) children aged 3-5 years.

### Conclusion

RSV was the major contributor for deaths among young children with SARI both in pre and pandemic periods. Future preventive interventions as maternal RSV vaccination and infant monoclonal prophylaxis should be evaluated to combat these premature deaths.





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## Transcriptional and metabolic dynamics of primary human lung cells acutely infected by RSV

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Thomas Pietschmann - ARNI0162

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### Background

The respiratory syncytial virus (RSV) is the leading cause of severer lower respiratory tract infections in children. The RSV disease severity varies widely, but the principles that control severe courses of infections are not well defined.

### Method

We analyzed RSV infection-induced transcriptional changes of well-differentiated human air-liquid interface cultures from six human donors over a time course of 7 days with single cell resolution. We used a GFP-tagged RSV variant and FACS sorting prior to single cell sequencing allowing us to assess transcriptional changes of both infected and bystander cells. We also quantified cytokines and metabolites secreted at the apical and basolateral cell poles.

### Result

We defined the cell types represented in the culture using data of the human lung atlas (Travaglini et al, Nature 2020). RSV primarily targeted cells annotated as ciliated and basal cells. The number of RSV infected cells and the average virus load remained stable over time, but varied by more than 1,000-fold between cells. The viral mRNA abundance reproduced the 5'-3'-transcriptional gradient with only the G-protein coding message being significantly over-represented. The average virus load was divergent between cell types with goblet cells showing lowest and basal cells the highest abundance of viral RNAs. IFN-I/III mRNAs were only detectable in very few cells, whereas interferon regulated genes (IRGs) were overexpressed in bystander and, to a lower extent, in infected cells. Hypothesizing that virus load increase represents progression through the virus replication cycle, we conducted a pseudo-time trajectory analysis. This analysis revealed six discernable waves of transcriptional signatures along a gradient of increasing virus load within infected cells. These waves featured functional gene ontologies such as IFN-signaling and antigen processing for low virus load (early life cycle) stages and regenerative and/or apoptotic and cell stress responses for high virus load stages. Above mentioned transcriptional responses were accompanied by enhanced uptake of sugars and amino acids.

### Conclusion

Preliminary data implicate a set of transcription factors driving these RSV-dependent perturbations. These findings inform about the RSV pathophysiology and provide orientation for development of host-targeting strategies for control of virus infection and pathogenesis.





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## The impact of COVID-19 restrictions on Respiratory Syncytial Virus circulation in Wales, and the potential impact of the absence of RSV circulation in Wales during 2020

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### Background

Respiratory Syncytial Virus (RSV) related disease burden is highest in those aged under 5 years of age. RSV is the most common cause of bronchiolitis, and childhood cases may require urgent General Practice consultation, or hospitalisation. RSV has a seasonal pattern of circulation, with epidemics occurring each winter in Wales. Following the implementation of COVID-19 social restrictions in Wales in March 2020, no cases of RSV were detected throughout 2020 or the 2020-21 winter period, when an RSV epidemic would usually be seen. We aimed to determine the potential impact of the absence of RSV circulation in Wales in terms of laboratory confirmed RSV cases, GP bronchiolitis consultations and bronchiolitis hospital admissions, and the potential for deferred burden to healthcare.

### Method

Laboratory test data were extracted from the all-Wales laboratory test database; Datastore. Weekly GP Read-coded consultations for bronchiolitis/acute respiratory infection were extracted from GP databases using Audit+ and accessed through the Secure Anonymised Information Linkage (SAIL) portal. Weekly hospital admissions for bronchiolitis/acute respiratory infection were extracted from the Patient Episode Database for Wales (PEDW). A novel approach using a time series regression model, building on published methods, was used to compare the expected and observed numbers for each of these indicators. The number of children remaining susceptible to RSV infection was also estimated.

### Result

Laboratory confirmed RSV cases saw a 100% reduction, with a complete absence of cases, compared to expected, in November and December 2020, before a higher than expected and unseasonal peak in August 2021. GP bronchiolitis consultations were lower than expected across three periods, with estimated reductions of 89% (95% CI: 69.9-93.2); 97% (95% CI: 59.8-98.2) and 91% (95% CI: 85.0-93.2) across each period respectively. Hospital admissions saw an estimated 92% reduction (95% CI: 86.9-94.2) between October 2020 and February 2021.

### Conclusion

A resurgence in RSV-confirmed cases was seen in August 2021 following a prolonged absence, it is unclear whether this resurgent peak reduced the 'immunity debt' in children who were not exposed to RSV during 2020, as high testing rates may have been driving the increased detections. It is unclear how changes in healthcare seeking behaviour impacted on GP consultations and hospital admissions for bronchiolitis during 2021, but current evidence suggests that the RSV immune debt from 2020 has not yet been fully met.



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## The RSV attachment protein affects dendritic cell maturation

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Anke Lakerveld - ARNI0164

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### Background

Respiratory syncytial virus (RSV) infection is a major cause of severe lower respiratory tract infections in infants and elderly. RSV reinfections occur frequently throughout life, suggesting a defective memory response. Dendritic cells (DCs) play an important role in the generation of memory T cell responses. RSV is known to influence DC maturation, but the underlying mechanisms remain unclear.

### Method

Here, we studied the effect of the RSV attachment protein (G) on DC maturation using monocyte-derived DCs from healthy adult donors. To study the effect of the G protein, we stimulated immature DCs with RSV, RSV lacking the G protein (RSVΔG), and RSVΔG in combination with recombinant G soluble protein. Using flow cytometry, we measured the percentage infected DCs and the expression of several maturation markers on DCs. Additionally, a multiplex immunoassay was used to measure the concentration of cytokines secreted by DCs.

### Result

We show that RSVΔG infects an increased percentage of DCs compared to RSV, which is decreased in the presence of recombinant G. In contrast, the expression of several maturation markers, e.g. CD38 and CD86, is reduced upon stimulation with RSVΔG compared to RSV and remains low in the presence of recombinant G. Additionally, secretion of certain cytokines (e.g. IFN- $\beta$ , CXCL10) by DCs is also decreased upon stimulation with RSVΔG compared to RSV.

### Conclusion

These results suggest that the RSV G protein plays a role in DC maturation which might be of importance for the design of novel vaccines. Future research will focus on further elucidating the role of specific regions within the G protein using our in-house RSV reverse genetics platform.



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## Full genome sequencing reveals circulation of 6 HMPV lineages since 2005, with recent predominance of viruses with duplications in the G gene.

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### Background

Human metapneumovirus (HMPV) is one of the leading causes of respiratory tract infections, primarily in infants, the elderly, and immunocompromised individuals. Worldwide, two genetic lineages (A and B) of HMPV are circulating that are antigenically distinct and can each be further divided into genetic sublineages. Surveillance combined with large-scale whole genome sequencing studies of HMPV are scarce but would help to identify viral evolutionary dynamics that could aid future vaccine development efforts. In addition, a robust, unified HMPV classification system for the emerging virus lineages is currently lacking.

### Method

In this study, we analyzed 130 HMPV whole genome sequences obtained from samples collected from hospitalized individuals, and 144 sequences of partial fusion protein (F) genes obtained from samples collected from non-hospitalized patients, between 2005 and 2021.

### Result

Phylogenetic analysis demonstrated that HMPV continued to group in four sublineages (A1, A2, B1, B2), with new lineages appearing in A2 sublineage. However, one sublineage (A1) was no longer detected in the Netherlands after 2006, while the others continued to evolve. No differences were observed in dominant (sub)lineages between samples obtained from hospitalized and non-hospitalized patients. In both populations, viruses of lineage A2 carrying a 180-nucleotide or 111-nucleotide duplication in the attachment protein gene became the dominating genotype within the A2 sublineage. Genetic evolution was further studied based on alignment of full-length F gene sequences obtained in this study combined with those available from GenBank (n=744). Analysis of the genetic map and phylogenetic tree based on this alignment, demonstrated that the viruses clustered in six distinct lineages (A1, A2.1, A2.2.1, A2.2.2, B1 and B2). In both analyses, either based on full genomes or full F sequences, viruses containing a 180- or 111-nucleotide duplication clustered together in lineage A2.2.2.

### Conclusion

This study suggests that using whole genome sequences of HMPV captures similar phylogenetic information as the use of full-length F gene sequences. In addition, the genetic map and phylogenetic analysis were used to propose robust classification criteria for the designation of new genetic lineages, to aid towards a unified HMPV classification system



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## Acute and Long-Term Costs among US Infants with Respiratory Syncytial Virus in the First Year of Life

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### Background

Respiratory syncytial virus (RSV) can cause serious illness among infants aged <1 year, especially pre-term infants; however, the costs of RSV beyond the acute phase of illness are not well characterized.

### Method

A retrospective observational matched-cohort design and data from the IBM MarketScan Commercial Database (2016-2020) were employed. Infants aged <1 year with RSV treated in the hospital (RSV-H), emergency department (RSV-ED), or physician office/hospital outpatient (RSV-PO/HO) setting were matched (1:1) to comparison patients without evidence of RSV before age 1 year. All-cause healthcare expenditures (2020 US\$) were tallied during the acute and long-term phases of illness (defined below) overall and by subgroup (i.e., gestational age at birth, age at onset of RSV); RSV-attributable expenditures were defined as the difference in values between RSV and comparison infants. Inverse probability treatment weights were employed to adjust for differences between groups in baseline characteristics.

### Result

Mean RSV-attributable expenditures spanning both the acute and long-term phases were \$16,586 for infants with RSV-H, \$5,857 for infants with RSV-ED, and \$2,845 for infants with RSV-PO/HO. For RSV-H infants, the highest burden of expenditures occurred during the acute phase of illness; for RSV-ED and RSV-PO/HO infants, expenditures were high during the acute phase and remained elevated throughout the subsequent year. Expenditures were higher for pre-term infants and infants who experienced RSV at a younger age.

### Conclusion

Infants who experience RSV before their first birthday accrue considerable RSV-attributable expenditures during the acute and long-term phases of illness, suggesting the economic burden of RSV extends well beyond the onset of illness.



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## Disruption and resurgence in viral respiratory infections during the COVID-19 pandemic period, in children under 6 in Wales

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### Background

Respiratory viruses present a global disease burden and are a significant cause of paediatric morbidity.

During 2020 and 2021 in Wales, patterns of circulation in a number of respiratory viruses changed. Changes may be associated with temporary public health interventions put in place to reduce community transmission of SARS-CoV2 (including social movement restrictions, closure of educational settings and nurseries, mandatory wearing of masks in some settings). Following easing of restriction measures in 2021, there has been a resurgence in many of these viruses. We summarise trends in common childhood respiratory viruses over 2020-2022.

### Method

Multiplex PCR respiratory screen data for children aged under 6 years from Sep 2017 to May 2022 were extracted from the all-Wales Database (Datastore). Data contained test results of respiratory screens and diagnostic testing carried out in all hospital patients and GPs (non-sentinel). Samples are tested for influenza, adenovirus, rhinovirus, RSV, parainfluenza, mycoplasma, human metapneumovirus (hmpv), enterovirus, bocavirus and seasonal coronaviruses. Testing for SARS-CoV2 was implemented from week 9 2020. Rapid/stand-alone tests limited to SARS-CoV2, flu and RSV are excluded from this analysis.

### Result

Following the start of 'lockdown' in week 13 2020, overall sample positivity (any virus) significantly decreased to < 20%, for 17 weeks, increasing again from week 30 2020. Overall positivity increased again from week 06 2021 to week 38 2021 to pre-pandemic levels.

From week 13 2020, positivity for hmpv and parainfluenza decreased and remained low at < 5% and < 7% respectively (with an absence of 'expected' seasons). Resurgence of parainfluenza was observed from week 21 2021 and peaked at 42.5% (n = 135) in week 26 2021. Positivity for hmpv began to increase significantly from week 38 2021 and peaked at 33.6% (n = 115) in week 48 2021. Not every virus followed the same pattern of resurgence, adenovirus activity increased but peaked at lower than observed in previous years.

### Conclusion

Public health measures in response to COVID-19 are likely to have disrupted the circulation of common respiratory viruses and there has been a subsequent resurgence. Resurgent peaks in samples positive for both hmpv and parainfluenza were the highest seen in at least 5 years. The size of resurgent peaks in test positivity may be related to reduced population immunity, with additional cohorts of the children under 6 who have never been exposed to these viruses. Additionally, increased numbers of detected cases may in part be due to increased testing and respiratory symptom awareness. The changes in seasonality and circulation patterns have not been uniform across all viruses.



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## Microparticle Vaccines Presenting the Respiratory Syncytial Virus (RSV) G Protein CX3C Chemokine Motif in the Context of TLR Signaling Induce Protective Th1 Immune Responses and Prevent Eosinophil Infiltration in Lungs Post-challenge

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### Background

It has been hypothesized that insufficient engagement of the innate immune system by the formalin-inactivated RSV (FI-RSV) vaccine may have primed the host for inflammatory Th2 immune responses and eosinophil infiltration in the lungs upon subsequent infection, thus causing vaccine enhanced disease. The RSV attachment (G) protein is an attractive vaccine target since it contains a chemokine motif that elicits Th2 responses, thus its neutralization may not only block viral infection but also reduce unwanted Th2 inflammation and eosinophilia.

### Method

Layer-by-layer fabrication was used to produce synthetic microparticle vaccines (LbL-MP) presenting a fusion peptide containing the CX3C chemokine motif of the RSV-G protein and a CD8 epitope of the RSV-M2 protein, with or without a TLR2 agonist (Pam3Cys) covalently linked to the fusion peptide. Mice were immunized with either vaccine candidate (GM2 or Pam3.GM2 LbL-MP) in the absence of exogenous adjuvant; control groups were naive, immunized with FI-RSV, or infected with a low dose of RSV and allowed to recover (live RSV). Following challenge with live RSV, local immune responses in the lungs were examined by monitoring RSV-G-specific antibodies, cytokines and chemokines, and cellular phenotypes.

### Result

Mice immunized with either candidate developed G-specific antibody and M2 specific CD8<sup>+</sup> T-cell responses and were protected from infection following challenge with live RSV. Post-challenge, mice immunized with GM2 LbL-MP or FI-RSV developed a Th2 immune response in the lungs, with high levels of IL-4, IL-5, IL-13, and eotaxin, a dominant IgG1 antibody response, and an influx of eosinophils in the bronchoalveolar lavage (BAL) fluid. By comparison, mice immunized with Pam3.GM2 LbL-MP or live RSV yielded much lower to non-detectable levels of Th2 cytokines and chemokines, higher levels of RSV-G-specific IgG2a, and very low numbers of eosinophils in the BAL fluid post-challenge.

### Conclusion

Engagement of the innate immune system upon vaccination yielded a more potent antibody response, greater protection, and a clear shift away from Th2/eosinophil responses. Since the failure of the FI-RSV vaccine tested



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in the 1960s has been hypothesized to be partly due to ablation of host TLR signaling by the vaccine and Th2 inflammatory responses upon subsequent viral infection, these findings stress the importance of appropriate engagement of the innate immune response during initial exposure to the RSV-G epitope to prime the host immune response for a protective, Th1-dominant response.





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## Human Metapneumovirus Fusion Protein Immunogens in Nanoparticle Format

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### Background

Human metapneumovirus (HMPV) is a major cause of respiratory disease worldwide, particularly among children and the elderly. Currently there is no HMPV-specific prevention treatment although it is a heavy medical burden. Surface fusion glycoprotein of HMPV is the major target for vaccine design.

### Method

Here we produced HMPV fusion glycoprotein immunogens in a two-component nanoparticle format (Bale et al., Science 2016). HMPV F was fused genetically to I53-50A component and then was combined with I53-50B component. Assembled HMPV nanoparticle was then purified from the two-component mixture through size exclusion chromatography.

### Result

Negative stain electron microscopy analysis indicated HMPV F trimer displayed evenly on the surface of the nanoparticle core. HMPV neutralizing antibody binding analysis demonstrated antigenicity of the nanoparticle immunogen was enhanced for apex epitopes compared to fusion protein trimer immunogens.

### Conclusion

These nanoparticle immunogens are now under evaluation for their ability to elicit neutralization antibody responses in animal models.



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## Lab adapted vs contemporary RSVs: single amino acid changes modulate F protein mediated cell fusion

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### Background

Respiratory syncytial virus (RSV) is the world leading cause of infant pneumonia and one of the main causes of lower respiratory tract infection in older adults and immunocompromised individuals. The fusion (F) protein is an attractive target for therapeutics and vaccine development due to its role in virus entry and cell-to-cell spread. However, basic, and translational research studies in this area have largely used prototypic RSV-A strains, such as A2 or Long, which have a complicated passage history and exhibit different phenotypes in in vitro and in vivo models in comparison to contemporary clinical isolates. We have therefore quantified the cell-to-cell fusion induced by F proteins, which are derived from RSV-A2 and two recent clinical isolates of RSV-A (0594) and -B (9671). The impact of strain specific amino acid changes in the F protein on cell fusion has also been assessed in the context of transient transfection of expression vectors and recombinant viruses.

### Method

Expression plasmids based on the pCG vector were generated containing the codon optimized open reading frames of RSV-A-0594, RSV-B-9671 and RSV-A2 F protein. Nucleotide changes encoding for desired amino acid substitutions were introduced into these plasmids by Q5 site directed mutagenesis (NEB). Quantitative split beta galactosidase cell fusion assays were performed in Vero cells by transfection of plasmids encoding for each fusion construct. Recombinant (r) RSV-A-0594-EGFP variants were rescued in which specific point mutations in the F protein had been introduced into a full-length cDNA copy of the antigenome via BAC recombineering or NEB HiFi assembly, and assessed for phenotypic differences with respect to induction of cell fusion in infected cell monolayers.

### Result

Introduction of the A2 specific amino acid change E66K into the RSV-A-0594 F protein resulted in enhanced cell-to-cell fusion, but a similar effect was not observed with the RSV B 9671 F protein. Mutagenesis of the RSV-A2 F protein to contain K66E resulted in only a slight decrease in cell fusion. Additional RSV-A2 specific amino acid changes (S25G, P101Q) also resulted in an increase in cell fusion in the context of the RSV A 0594 F protein. The ability of rRSV-A-0594-EGFP variants containing A2 specific amino acid changes in the F protein (E66K, P101Q) to infect and induce cell fusion in different cell lines was assessed in comparison to the unmodified recombinant virus.

### Conclusion

In summary, we show that the ability of the RSV F protein to induce cell-to-cell fusion in immortalized cell lines is strain dependent and single amino acid changes can have a significant impact on modulating fusion activity.



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## Unraveling specific mechanisms of community deaths due to RSV in Buenos Aires, Argentina

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### Background

Respiratory syncytial virus (RSV) is a major cause of respiratory tract illness in infants (LRTI). Its contribution to child mortality has been underestimated. Uncertainty related to RSV death attribution or bacterial coinfection can bias RSV burden estimation. Histological patterns of lung injury associated to RSV have been mainly characterized in hospitalized cases.

### Method

We designed an approach based on active surveillance, minimal invasive tissue sampling technique (MITS), verbal autopsy (VA), molecular methods, and cause of death (COD) attribution based on determination of Cause of Death (DeCoDe) process. This study aimed to define the mechanistic role of RSV in lungs of community deaths of children under 5.

Active surveillance population-based prospective study in a catchment area of 40,027 live births in the Southern region of Buenos Aires were conducted. Tissue samples were subjected to routine processing procedures and immunohistochemistry (IHC) was performed for RSV, CD3, CD20 and MPO. Patterns of lung disease were evaluated and scored.

### Result

In 2019, 63 subjects were enrolled, RSV infection was found in the causal chain of 11/13 cases with positive molecular biology results in respiratory samples. The estimated mortality rate due to RSV among infants was 0.27 deaths/1000 live births. We found in 8/12 (66.7%) deaths a mixed histopathology pattern of mild bronchiolitis and interstitial pneumonia, with peribronchiolar lymphocytic infiltrates and bronchiolitis present in all cases. 16.7% presented with severe acute lung disease, however, none of RSV cases harbored characteristic syncytia in lung parenchyma. When we compared lung injury patterns with non RSV LRTI we found that there weren't significant differences in severity between groups. When we analyzed distribution of specific types of inflammatory cells between RSV and non RSV LRTI, neutrophil infiltration was significantly lower in RSV LRTI deaths compared to non RSV ones.

### Conclusion

The prevailing histological pattern of RSV infection in community deaths was mild bronchiolitis and interstitial pneumonia with no evident syncytia in lung parenchyma. Acute neutrophilic inflammation was significantly lower in RSV cases compared to non RSV LRTI.



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## Respiratory Syncytial Virus Associated Clusters of Severe Acute Respiratory Infections Identified Through Hospital-based Surveillance in Bangladesh, 2009-2022

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### Background

Respiratory syncytial virus (RSV) is one of the leading causes of acute lower respiratory tract infections and hospitalization worldwide, particularly in children. We explored the use of hospital-based sentinel surveillance system in Bangladesh to detect clusters of RSV infection.

### Method

During May 2009-March 2022, surveillance physicians at 14 sentinel surveillance hospitals enrolled SARI cases following the WHO case definition. Among the SARI cases, clusters were identified if  $\geq 2$  SARI cases who developed symptoms within 10 days of each other and lived  $<30$  minutes' walk or 3 km from each other. We collected data on demographics, clinical characteristics and outcome at discharge from clustered cases. Oropharyngeal and nasopharyngeal swabs were collected and tested for RSV by rRT-PCR. We used descriptive statistics to summarize the data.

### Result

We enrolled 37,216 SARI cases; of these cases, we identified 4,88 clusters comprising 1,505 SARI cases (817 were aged  $<5$  years and 688 were aged  $\geq 5$  years). On average, three clusters were identified in each month (range: 0-13). The median age of cases in clusters was 2 years (IQR 0.5 - 25) and 63% were male. Most clusters, 296 (61%) comprised 3 cases. Three hundred thirty-seven (69%) of the identified clusters lived within 5 km of the surveillance hospitals. RSV was detected in 363 (24%) cluster cases: 351 aged  $<5$  years and 12 aged  $\geq 5$  years. Of the RSV clustered cases, 7 (2%) were children  $<1$  month, 198 (54%) were children aged 1-6 months, 79 (22%) were children aged 6-12 months, 43 (12%) were children aged 1-2 years, 24 (7%) were children aged 2-5 years and 12 (3%) were individuals aged  $\geq 5$  years. All cases in each cluster tested positive for RSV in 12% (58) clusters. Although circulation of RSV was observed throughout the year among the clustered cases, the highest proportion of RSV clustered cases (42%, 69/166) were identified during September. Among all RSV clustered cases, the most commonly reported symptoms beside fever and cough was difficulty breathing on admission (92%; 333). Among the 351 RSV positive children aged  $<5$  years, the most common symptom was chest indrawing on admission (91%; 318) followed by inability to drink (21%; 74) and vomiting (16%; 56). Of the RSV clustered cases one died.

### Conclusion

Nearly a quarter of the clustered cases had RSV infection and over half of these cases were children aged  $<6$  months suggesting circulation of RSV in the community and hospitalized children. To reduce RSV transmission, prevention strategies, such as handwashing and respiratory hygiene should be promoted. Immunization strategies should target young infants to reduce the RSV-associated hospitalization.



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## Estimating RSV seasonality from pandemic disruptions: a modelling study

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### Background

The seasonal pattern of RSV is shaped by factors including short-lived immunity, seasonally varying contact rates and pathogen viability. The disruption of the regular seasonality of RSV during the COVID pandemic in 2020-2022 due to control measures, and the ensuing delayed surge in RSV cases provides an opportunity to estimate the strength of seasonal forcing and its implication for vaccination strategies.

### Method

We developed a mathematical model of RSV transmission, which simulates the sequential re-infection and decreasing infectiousness and susceptibility with each reinfection (SEIRRS4). Using MCMC we fit the model to laboratory confirmed RSV incidence from routine surveillance during 2010-2022 in New South Wales (Australia) accounting for the reduced mobility during the pandemic approximated with Google mobility data. We estimated the baseline transmission rate (i.e. the product of per-contact transmission probability and the average contact rates), its amplitude and shape during RSV season as well as the average duration of immunity. We then simulated the expected shifts in peak timing and amplitude under two vaccination strategies: continuous and seasonal vaccination.

### Result

Pre-pandemic NSW peak RSV activity was commonly observed in July. In 2020 only few cases were reported during the typical RSV season followed by an out of season epidemic peak in December. We estimate that RSV dynamics in NSW can be best explained by a high effective transmission rate at baseline (off-season, 2.94/d, 95% CrI 2.73-3.18) but a relatively weak seasonal forcing, with a 13% increase during peak season compared to the baseline (off-season) transmission rate. We also estimate the duration of post infection temporary but sterilizing immunity to be 412 days (95% CrI 391-435). The continuous vaccination strategy led to more condensed seasonal incidence with a delay in the peak timing and a higher amplitude. Seasonal vaccination reduced peak incidence.

### Conclusion

Quantifying the parameters that govern RSV seasonality is key in determining potential indirect effects from immunization strategies as those are being rolled out in the next few years.



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## Immune and inflammatory markers associated with RSV disease in experimentally infected older adults

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### Background

Respiratory syncytial virus (RSV) is widely recognised as a cause of severe, progressive and sometimes fatal lung disease in debilitated elderly persons. This represents a major global disease burden in adults over 65 years. Studies of natural infection in older adults have not clearly identified the factors associated with enhanced severity, and the mechanisms of age-related susceptibility to infection remain poorly understood. We recently established Human Infection Challenge (HIC) with RSV in elderly adults and used this model to investigate the innate and adaptive immune biomarkers that correlate with protection and susceptibility to infection in this clinically relevant population.

### Method

Seventeen healthy volunteers aged 60-75 years old were inoculated with RSV Memphis 37 by intranasal administration, with no pre-selection by antibody titre. Viral load was quantified by qPCR of nasal lavage samples and symptom scores calculated by self-completed diaries. Inflammatory and immune-related cytokines and chemokines were quantified in both the blood and upper respiratory tract throughout infection by Mesoscale Discovery (MSD). Intracellular Cytokine Staining (ICS) was used to analyse the CD4+ and CD8+ T cell response to the viral fusion (F) protein in peripheral blood and airway cells.

### Result

Following inoculation, 76% of the older participants became infected. A third of these infected individuals were asymptomatic, with the remaining two-thirds developing mild, self-limiting upper respiratory tract symptoms. No pausing or stopping rules were met, and all symptoms resolved with no sequelae. Viral load and symptoms peaked on day 6-8 post infection. We observed RSV-mediated up-regulation of pro-inflammatory cytokines and chemokines at early timepoints in the airways and blood following challenge, specifically; IFN $\gamma$ , IL-17A, IL-6, IP-10/CXCL10, MCP-1, MIP-1 $\alpha$  and MIP-1 $\beta$ . These were associated with activated and proliferating F-specific populations of CD4+ T cells producing Th1 cytokines (IFN $\gamma$ /IL-2/TNF $\alpha$ ), which were identified in the lower airways during acute infection. Cytokines associated with generation of memory B cells and induction of Ig producing cells, such as; IL-10, IL-6, IL-15, IL-12 and IL-21 were found to be elevated in the blood later in infection.

### Conclusion

Our previous findings have indicated that both local and peripheral humoral immune responses diverge significantly in older people. This study further shows alterations of the mucosal and systemic response to RSV infection even in healthy aging. Thus, by using this model, further elucidation of biomarkers associated with prevention or enhancement of RSV disease and testing of potential interventions directly in this target age group may be achieved.





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## Role of RSV infection and factors associated with apnea during acute respiratory tract infections

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### Background

Apnea is one of the most severe complications of acute respiratory tract infections (ARI) in infants and it is a life-threatening event. While 99% of fatal ARIs occur in developing countries, the impact of apnea has only been assessed in industrialized nations. Reports often associate apnea with respiratory syncytial virus (RSV) infection, however a specific role for RSV is controversial. The mechanisms and risk factors associated with apnea due to ARI or RSV are poorly understood and the role of immune mediators in apnea inception remains unclear.

### Method

Cross-sectional, multicenter study conducted between 2011-2013 in Buenos Aires, Argentina, that included infants hospitalized due to ARI during RSV season in a catchment population of 52480 infants younger than 2 years. Apnea was defined as an unexplained episode of cessation of breathing for 20 seconds or longer, or a shorter respiratory pause associated with bradycardia, cyanosis, pallor and/or marked hypotonia. Predictors in patients with apnea were compared to those with ARI and no apnea. Nasal aspirates were obtained from all hospitalized children and tested in duplicates for RSV and other viruses by RT-PCR and also tested for biomarkers previously hypothesized to influence the development of apnea (IL1 $\beta$ , TNF $\alpha$ , IFN $\gamma$ , IL4).

### Result

We enrolled 5008 infants younger than 2 years of age hospitalized with ARI. From these, 77 infants presented apnea on admission or during hospitalization, for an estimated population incidence of 1.47 per 1,000 infants (1.54% of infants hospitalized with ARI). Overall, 32 infants (41%) were diagnosed with apnea due to RSV infection. Increased odds for developing apnea during ARI were associated with prematurity, age in months, birth weight, malnutrition, chronic underlying disease (neurological disease or immunodeficiency), and severe crowding (5 or more people per bedroom). Severe crowding (OR 1.02-4.97), age  $\leq 1.5$  months (OR 3.91-15.49), prematurity (OR 2.34-10.16), and weight Z score  $< -2$  (OR 3.52-14.93) were the only variables independently associated with an increasing odds ratio for illness in multivariable hierarchical analyses. Between those patients with apnea and confirmed RSV infection, the variables increasing the odds of illness were the same except for severe crowding. The presence of RSV did not significantly alter the odds of disease. Viral loads of RSV and levels of biomarkers did not differ between groups.

### Conclusion

Apnea during ARI was associated with prematurity, age in months and weight during illness, and it is not confined to infants with RSV infection. We found no association between apnea and the biomarkers evaluated in this study. Further investigation is needed to elucidate immune determinants of apnea.





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## Identification of a good prognostic group (Positive-z-score-weighted Anorectic RSV-associated-SARI Infants, PARI) among infants hospitalized with RSV Severe Acute Respiratory Infection (SARI) during their first year of life in the 2013-2020 birth cohort of a large tertiary care center in Lyon, France

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### Background

RSV is the main cause of Lower Respiratory Tract Infections (LRTI) among infants younger than one year. In temperate climate countries, the acute epidemic burden overwhelms healthcare system capability every winter. Clinical description of hospitalized infants based on thorough review of medical records, severity grading with exhaustive review of brady-apnea, hyporesponsiveness, and description of respiratory and digestive pictures are sparse. We aim to describe the clinical presentation, the outcomes in RSV-SARI-infants, and identify subgroup of interest for potential ambulatory management.

### Method

The 2013-2020 birth cohort of the tertiary care center (Lyon, France) was followed to describe hospitalization of infants younger than 1 year with a positive RSV-RT-PCR. Medical files were reviewed using WHO grading (20% double review). SARI were identified as Very Severe and Severe-LRTI cases. Burden of each season was assessed through area under the curve (AUC) of hospitalization and respiratory support (invasive ventilation [IV] or non-invasive ventilation [NIV]) as infant\*days.

### Result

901 infants were hospitalized for a RSV-Infection, 864 included, among the birth cohort of 62,229 childbirths. Incidence rates for 1,000 infant\*year were 14 (13-15) for hospitalization, 12 (11-12) for SARI-Case, 4 (3-4) for respiratory support (IV, NIV), and 2 (1,7-2,4) for feeding support only; all significantly conversely proportional to



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age. Mean AUC of hospitalization was 838 (671-1005) infant\*days, and mean AUC of IV-NIV was 183 (137-229) infant\*days. Being born during the month of epidemic onset or two months before, was the main risk factor for hospitalization (57%,  $p < 0.001$ ). Among hospitalized infants, 4% presented (brady-apnea or hyporesponsiveness), 61% presented hypoxemia only (80% of them with inability to feed), 18% presented inability to feed only, and 17% met no SARI-Case criteria. SARI-Cases had a mean length of stay in hospital of 6.6 days (6.2-7.0). Linear regression for infants presenting with inability to feed only and positive admission-weight Z-score showed a shorter hospital stay duration trend. Clinical management led to 27% undergoing respiratory and feeding support, 38% oxygen therapy (76% of them with feeding support), 15% exclusive feeding support, and 20% clinical monitoring.

### **Conclusion**

This birth cohort study summarizes the RSV epidemiology at steady state, before SARSCov2 pandemic interaction, with incidences in general population. SARI-Cases description identified 4 severity groups. To lower the epidemic hospital burden, we can consider the possibility of ambulatory feeding support management for infants with inability to feed only using age, underlying conditions, and admission weight Z-score-based-strategy.



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## Airway epithelial cell gene expression following respiratory syncytial virus infection shows differential chronological and gestational age-related responses in early life.

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### Background

Respiratory syncytial virus (RSV) remains the most common cause of acute lower respiratory tract infection (ALRTI) in infants <2 years of age, with the disease burden greatest between 6 weeks and 6 months of life. No licensed vaccine exists, and limited prophylactic options are available for high-risk infants, including those born prematurely. Why infants <6 months are more frequently susceptible to severe disease is unknown. RSV primarily targets ciliated cells of the airway epithelium in humans. The type and robustness of innate immune responses to infection in these tissues likely dictate disease severity. We hypothesised, therefore, that gene expression responses to RSV infection differed by gestational and chronological age. To address this hypothesis, we exploited our novel RSV infection model based on well-differentiated primary paediatric nasal epithelial cell (WD-PNEC) cultures derived from newborn (preterm and term) and 1-year old infants.

### Method

WD-PNEC cultures were derived from the same infants at birth (n=12) and 1-year and categorised as preterm (28-34 weeks gestation) or term (37-42 weeks gestation). WD-PNECs were infected with RSV BT2a (MOI~3) or mock infected. RNA was extracted at 96 hpi and total RNA-sequencing was conducted. QC of FASTQ files was undertaken and differential gene expression (DGE) analysis was performed in R using the edge-R limma-voom package. Several contrasts were undertaken to explore the impact of infection, gestational age, and chronological age on DGE.

### Result

Contrasts were grouped into three categories: DGE with infection (infected vs mock), gestational age (preterm vs term) and chronological age (1-year vs newborn). Numerous genes were differentially expressed between RSV and mock infected WD-PNECs, with gene-set enrichment revealing innate immunity and interferon signaling enriched gene-sets. DGE with gestational age revealed transcriptomic changes present in newborns that persisted at 1-year in both mock and infected cultures. When RSV- vs mock-infected WD-PNECs from newborns and 1-year old's were compared DGE did not reach statistical significance. However, there was a trend toward a more robust expression of innate immune genes following RSV infection at 1-year. In contrast, DGE with chronological age revealed 156 statistically significant differentially expressed genes in infected cultures.

### Conclusion

Innate immune responses to RSV infection of WD-PNECs became more robust with chronological age up to 1-year. In addition, there was a persistent DGE in newborns and 1-year old's that reflected gestational age, irrespective of infection.



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## The cost of care for children hospitalized with respiratory syncytial virus (RSV) associated lower respiratory tract infection (LRTI) in Kenya

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### Background

Respiratory syncytial virus (RSV) is one of the main causes of hospitalised lower respiratory tract infection, in children under five years of age globally. Vaccines and monoclonal antibodies for RSV prevention among infants are in advanced stages of development. However, data are limited on the economic burden of RSV disease from low- and middle-income countries (LMIC) to inform decision making on prioritization and introduction of such interventions against RSV. This study aimed to estimate household and health system costs associated with childhood RSV in Kenya.

### Method

A structured questionnaire was administered to caretakers of children <5 years admitted to referral hospitals in Kilifi, coastal Kenya and Siaya County, western Kenya with symptoms of acute lower respiratory tract infection (LRTI) during the 2020 and 2021 RSV seasons. These children had been enrolled in ongoing in-patient surveillance for respiratory viruses. Household expenditures on direct and indirect medical costs were collected prior to, during and two weeks post hospitalization. Aggregated health systems costs were acquired from the hospital administration and were included to calculate cost per episode of severe RSV illness. Costs paid to the health system by other organizations were not included.

### Result

We enrolled, a total of 241 (79, 32.78% RSV positive) and 184 (21, 11.41% RSV positive) participants from Kilifi and Siaya hospitals, respectively. The total (health system and household) mean costs per episode of severe RSV illness was USD 119 (95% confidence interval (95% CI): 96-142) in Kilifi and USD 521 (95% CI: 404- 638) in Siaya. Household costs were USD 67 (95% CI: 54-80) and USD 172 (95% CI: 131- 214) in Kilifi and Siaya, respectively. Mean direct medical costs to the household during hospitalization were USD 11 (95% CI: 10-12) and USD 67 (95% CI: 51-83) among Kilifi and Siaya participants, respectively. More than 90% of participants from both sites reported family finances were adversely affected.

### Conclusion

RSV-associated disease among children leads to a substantial economic burden to both families and the health system in Kenya. This burden may differ between counties in Kenya and similar multi-site studies are advised to support cost-effectiveness analyses.



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## Potential impact of maternal vaccination on respiratory syncytial virus-related childhood mortality in low-income and middle-income countries: a modelling study

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### Background

Respiratory syncytial virus (RSV) represents a substantial burden of disease in young children, with over 99% of mortality occurring in low-income and middle-income countries (LMICs). Currently, several maternal RSV vaccine candidates are in late clinical development. It is important to understand the potential vaccine impact on RSV mortality to assess value for money for local decision makers and organisations. Scheltema et al. (2018) developed a mathematical model that can be used to predict the potential impact of maternal vaccination. We updated the model according to current literature and made some extensions to predict country-specific impact of maternal vaccination.

### Method

The model estimates individual antibody levels at time of birth based on time of vaccination and gestational age (GA) at birth, and predicts the corresponding number of days protected after birth. For each case the number of days protected after birth are compared to the age at time of death distribution, to estimate the percentage of deaths potentially averted.

We used a subset of the RSV GOLD database, which includes the age and GA distribution of RSV-related mortality cases in LMICs in children < 6 months of age. To account for biases in our dataset, we simulated 1000 datasets to improve the reliability of our estimates. Parameter values were fixed according to the Phase 3 RSV Novavax vaccine characteristics (Madhi et al. 2020).

To validate our model, we estimated the vaccine efficacy of the Novavax trial based on the reported GA and GA at time of vaccination of their participants. Additionally, we used the simulated datasets based on the RSV GOLD data to estimate country-specific percentages of mortality cases averted given vaccination, taking the reported country-specific timing of antenatal care visits during pregnancy as a proxy for GA at time of vaccination.

### Result

Our model predicted that the Novavax vaccine would protect their LMIC vaccine trial participants for 82 days on average (IQR=73; 94), and a predicted vaccine efficacy of 51.2% (95%CI= 46.9; 55.5) in RSV-related mortality in children <180 days of age. The Novavax trial reported a vaccine efficacy of 50.4% (95%CI=26.2; 66.7) with hospitalisation <180 days of age. Hence our model showed predictive ability to predict vaccine impact.

Our country-specific estimates for the percentage of RSV-related mortality cases of children <6 months that could be averted range from 30% to 36% assuming full vaccine coverage and 14% to 35% taking into account coverage of antenatal care visits, service availability and vaccine acceptance estimates per country.

### Conclusion

We show that this mathematical model can be a useful tool to make subgroup-specific predictions for future vaccine impact.



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## Respiratory Syncytial Virus Sequelae among Adults in High Income Countries: A Systematic Literature Review

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<sup>1</sup>P95 - Turning Data Into Evidence, <sup>2</sup>Pfizer Vaccines

### Background

Respiratory syncytial virus (RSV) is a common pathogen that infects children and adults and can cause severe respiratory illnesses. In contrast to children, information on sequelae risk following RSV infection resolution in adults is limited. A systematic literature review was conducted to identify and assess sequelae that occur in adults after resolution of acute RSV infection or post hospitalisation.

### Method

Studies published from 2000-2021 were searched in Embase, PubMed, LILAC, SciELO, and grey literature sources. Studies were included if they reported the incidence or risk of RSV sequelae post hospitalisation or after resolution of acute illness. Data were descriptively analysed and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Risk of bias of included studies were assessed using National Institutes of Health tools.

### Result

Following the interim analysis, a total of 52 studies were included out of 6502 title and abstracts reviewed (Figure). Among the 21 articles extracted to date, the most commonly reported RSV sequela at different periods (1, 3, 6, 12 months) following infection was bronchiolitis obliterans syndrome (new onset or progressive), with the risk higher in immunocompromised population, especially lung transplant recipients. Other reported respiratory sequelae of RSV were dyspnoea, chronic lung allograft dysfunction, and pneumonia. Non-respiratory RSV sequelae were not commonly reported. However, in some patients, viral gastroenteritis, paroxysmal atrial fibrillation, ischemic cardiomyopathy, and arrhythmias were diagnosed at discharge or 6 months after RSV diagnosis.

### Conclusion

Reported RSV sequelae included bronchiolitis obliterans syndrome and other respiratory conditions as well as cardiac events. Sequelae following RSV infection should be included in RSV disease burden of disease estimates. Additional efforts should be undertaken to systematically document and quantify RSV sequelae frequency, type, and duration to determine the true burden of RSV in adult population.





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## Saliva as an alternative to nasopharyngeal swabs for detection of respiratory syncytial virus (RSV) in children

Annefleurlangedijk - ARN10195

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### Background

Nasopharyngeal swabs (NPS) are considered the gold standard for diagnosis of respiratory syncytial virus (RSV). Although highly sensitive, NPS come with sampling burden, a time interval between sampling and PCR test results, and high personnel and resource costs; it is an invasive sample type that requires trained healthcare workers to collect. Moreover, collection can cause discomfort in the patient making healthcare workers hesitant to collect additional samples for research purposes. A saliva-based method for RSV detection would make large-scale and frequent clinical and community sampling more feasible. Saliva is easy to obtain and has been validated for detection of SARS-CoV-2 and can yield greater SARS-CoV-2 detection sensitivity and consistency throughout the course of infection. In this study, we evaluated detection of RSV in paired NPS and saliva samples collected from hospitalised infants.

### Method

Infants <12 months of age admitted to the Wilhelmina Children's Hospital, Utrecht, the Netherlands with a respiratory infection were invited to enrol in the study. Matched NPS and saliva samples were obtained by trained study personnel. Two saliva collection methods were compared: collection with a bulb pipette directly from the mouth and collection with an ORACOL sponge swab. Burden assessment was done for all collection methods and included cry duration and pain scores. NPS and saliva samples were tested with SalivaDirect+, a saliva-based PCR test authorized by the US FDA for detection of SARS-CoV-2, expanded to also target RSV.

### Result

To date, 29 paired samples have been collected from infants with medically-attended respiratory infections. As a proof of concept, we analysed the first 10 samples including 5 samples from RSV positive infants and 5 from infants with other respiratory viruses. RSV could be detected in all 5 samples in both NPS and saliva. Viral load of NPS and saliva did not differ significantly ( $p=0.2$ ). Moreover, other respiratory viruses including enterovirus and rhinovirus could be detected in the other 5 matched samples. Sampling discomfort was considered to be significantly higher for NPS compared to saliva samples ( $p=0.001$ ). More data will be available at the time of the conference.

### Conclusion

We detected RSV in all saliva samples. Our preliminary findings indicate that saliva is a viable and preferable alternative to NPS for RSV detection. Our limited data show that the sensitivity of RSV detection in saliva samples is comparable to NPS and that the sampling burden is significantly reduced. Saliva could allow for frequent repeated testing. With further validation, widespread implementation of saliva sampling could transform RSV diagnosis and surveillance in children.





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## One-step multiplex RT-PCR amplicon-based next generation sequencing of human respiratory syncytial virus subgroups A and B genomes

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Yi-Mo Deng - ARN0197

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### Background

Human Respiratory Syncytial Virus (RSV) infections pose a significant risk to human health worldwide, especially for young children. Whole genome sequencing (WGS) of RSV is a useful tool for global surveillance by helping us to better understand the evolution and epidemiology of RSV as well as providing essential information that may impact on antibody treatments, antiviral drug sensitivity and vaccine effectiveness.

### Method

Here we report the development of a rapid and simplified one-step multiplex reverse-transcription polymerase chain reaction (mRT-PCR) for RSV WGS that is suitable for two popular next generation sequencing (NGS) platforms. Our multiplex RT-PCR was designed to amplify both RSV-A and RSV-B genomes, requiring only two reactions for each virus, which significantly cuts the cost and time in the RSV WGS set up compared to the highly utilised four reaction method.

### Result

In silico analysis revealed that the selected primers for the new method cover all currently known circulating RSV-A and RSV-B. Amplicons generated were used successfully for NGS on both Illumina and Oxford Nanopore Technologies (ONT) platforms. The method was further tested on 177 clinical samples collected in Australia in 2021, and it achieved a high success rate (77.4 %) with high depth and a full coverage for the whole genome of 73 RSV-A and 64 RSV-B. In addition to its simpler set up compared to the original method, this assay resulted in a reduced cost of RSV WGS by at least 20%.

### Conclusion

With its robust performance, faster and more scalable preparation this assay is a valuable addition to existing NGS RSV WGS methods.



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## Removal of the N-glycans in p27 of the respiratory syncytial virus fusion protein

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Lotte Jacobs - ARN0199

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### Background

Respiratory syncytial virus (RSV) is worldwide the leading cause of acute lower respiratory tract infections (ALRI), among young children. In the search for a vaccine, the fusion (F) protein is the major focus. Depending on the strain, 5 or 6 N-glycosylation sites are present on the RSV F protein of which 2 to 3 of these glycans are located in p27, a peptide that is assumed to be removed during maturation of the protein. Previously, we have shown that removal of the N-glycan sequon for N116, located in p27, resulted in higher neutralizing antibody responses and better protection upon RSV challenge. As we previously only evaluated the role of conserved N-glycosylation sites, we now aim to elaborate on these findings by investigating all glycomutants within the p27 peptide, both single, double and triple mutants.

### Method

F p27-specific glycomutants were constructed by substituting the N codon at the 3 N-glycosylation sites in the p27-peptide by a Q codon, resulting in single (N116Q, N120Q and N126Q), double (N116-120Q, N116-126Q and N120-126Q) and triple (N116-120-126Q) mutants. We evaluated surface expression and fusion capacity of these F p27-glycomutants by immunofluorescent analysis. Total and neutralizing antibody responses in mice were observed after immunization with the glycomutants and correlated with viral loads after RSV challenge. And lastly, we performed a cross neutralization assay using post RSV-challenge serum from these mice and different RSV isolates.

### Result

Immunofluorescent analysis showed that all F p27-glycomutants were expressed on the cell surface and induced syncytia formation without major differences. Immunization of BALB/c mice with F p27 glycomutants induced total and neutralizing antibody responses that were similar to wild type F. After challenge of these mice, similar trends in antibody titers were seen but only two (N116Q and N120-126Q) showed significantly higher neutralizing antibody responses compared to F WT. Interestingly, all three single mutants (F N116Q, F N120Q, F N126Q) and the double mutant (F N116-120Q) resulted in significantly lower lung viral loads after RSV challenge, compared to F WT.

### Conclusion

Removal of glycans from the p27 peptide has no major effect on both surface expression and fusogenicity of the F protein. Upon immunization of mice, subtle differences are observed in the neutralizing antibody response, which are better defined upon challenge, which may suggest differences in priming of the antibody response upon immunization. Our findings thus indicate that N-glycans located in the p27 peptide can influence the F-specific immune response. The mechanism involved remains however not clear and more study is needed.



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## Risk Factors Associated with Severe RSV Infection in Infants: what is the Role of Viral Co-infections?

Kim Stobbelaar - ARNI0200

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### Background

Respiratory Syncytial Virus (RSV) is a major cause of viral respiratory tract infections in young children. Despite the high medical, societal and economical burden, it remains largely unclear why some children develop serious illness, while others do not even have symptoms at all. The role of viral co-infections in clinical severity in children suffering from an RSV infection is not fully understood. We aim to investigate the impact of viral coinfections on several disease severity indices, such as need for intensive care, duration of hospital admission, supplementary oxygen need and bronchiolitis severity scores, using a series of clinical RSV isolates.

### Method

During two consecutive winter seasons from October 2018 until February 2020, we included previously healthy children up to 2 years of age presenting with an acute lower respiratory tract infection, both ambulatory and hospitalized, at a tertiary hospital. We collected clinical data and tested nasopharyngeal secretions for a panel of 16 different respiratory viruses with multiplex RT-qPCR. Disease severity was assessed with both traditional clinical parameters and bronchiolitis severity scoring systems.

### Result

120 patients, with a mean age of 7 months, were included, of which 91.7% were RSV positive. Within the RSV positive group, 43% of patients (47/110) had a co-infection with at least one other respiratory virus, with human Rhinovirus (24.1%) and human Adenovirus (23.3%) the most commonly detected. We found that patients suffering from a single RSV infection had higher pediatric intensive care unit (PICU) admission rates (OR = 5.4, 95% CI 1.5-19.8), longer length of hospital stay (median= 4 vs. 3 days, p=.024), and higher Bronchiolitis Risk of Admission Scoring System (BRASS) scores (median= 3 vs. 2, p=.010) compared to patients with RSV co-infections, even though the presence of certain well known risk factors for severe disease, such as male gender, daycare attendance and parental atopy was significantly higher in the RSV co-infections group.

### Conclusion



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In our study, patients with a single RSV infection had increased disease severity compared to patients with RSV co-infections, as indicated by higher rates of PICU admission, longer length of stay and higher BRASS scores. Additional, larger-scale studies are warranted to confirm these findings.



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## Protective efficacy of an inactivated whole virus RSV vaccine in mice

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Valentina Eberlein - ARN10205

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### Background

The respiratory syncytial virus (RSV) is one of the leading causes of acute lower respiratory infections in children associated with numerous paediatric hospitalizations, with no approved vaccine available.

Previously our group showed a development of a safe and efficacious vaccine produced by a versatile and non-chemically electron beam inactivation. This low-electron energy irradiation inactivation showed high conservation of the antigenic structure of RSV particles. After homologous intramuscular immunization using this vaccine a 10,000-fold reduction of viral load after RSV challenge was induced. Since the whole-virus chemical inactivated RSV vaccine induced an unwanted enhanced immune mediated disease in children after natural RSV infection, we questioned if an atraumatic mucosal application is able to induce a strong and immune balanced mucosal immune response associated with protective efficacy. Hence, the ELLI inactivated RSV was formulated with different procedures to optimized mucosal uptake and protection upon RSV challenge.

### Method

Based on Phosphatidylcholine (PC), leading to neutral liposomes, and Dioleoyl-3-trimethylammoniumpropane (DOTAP), inducing cationic liposomes, various formulations were generated with different virus to liposome ratios. Formulations were tested in vitro with the Zeta-Sizer, ELISAs and immunoblot analysis and in ex vivo murine precision cut lung slices (PCLS). For the immunogenicity and protective efficacy trials mice were immunized twice via intranasal route. Blood samples were taken before and 3 weeks after prime and boost immunization. One month after boost mice were challenged with 1\*10<sup>6</sup> focus forming units of RSV and scored for 5 days. We analysed protective efficacy by the viral load in lungs of challenged animals and immunogenicity by RSV-binding and -neutralizing antibodies in serum samples.

### Result

The in vitro analysis confirmed strong antigenicity in all formulation variants with the different liposomes. Consequently, after application on PCLS no cytotoxicity was detected and we observed secretion of IP-10, whereas IL-6, KC/GRO, MCP-1, MIP-1 $\alpha$  and RANTES levels were reduced in comparison to untreated PCLS.

PC-formulated vaccines showed RSV-binding and -neutralizing antibodies. Furthermore, after two immunizations protective efficacy with a 171-fold reduction of the virus load in the lungs after RSV challenge was shown with the PC-formulation.

### Conclusion

In conclusion, we found a promising inactivated vaccine candidate for a mucosal application against RSV. After optimization we will translate the mucosal inactivated vaccine delivery into clinical trials to evaluate safety and efficacy against RSV in human challenge experiments.



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## Clinical course of outpatient RSV disease in healthy term-born European infants - a prospective birth cohort study (RESCEU)

Sarah Hak - ARNI0207

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### Background

While the vast majority of respiratory syncytial virus (RSV) infections does not require hospitalization, knowledge on the clinical course and societal impact of RSV outside the hospital is limited. This lack of knowledge leads to an incomplete appreciation of the true burden of RSV on the population level. This information is essential for policymakers to decide on the implementation of soon-to-be available RSV immunoprophylaxis or maternal vaccination in national immunization program. Therefore, we aimed to describe the clinical course of outpatient RSV disease in healthy term-born infants in comparison with acute respiratory tract infections (ARTI) of other etiology.

### Method

The RESCEU birth cohort study is a multicenter prospective, observational birth cohort study that enrolled healthy term-born infants (n=9164) between July 2017 and April 2020 in 5 European countries. We performed active RSV surveillance in a nested cohort (n=993) until the age of one year. In case of an ARTI episode, parents kept a 14-day diary on symptoms, medicine use and medical attendance. Infants with at least one ARTI episode were included in analyses. Episodes which required hospitalization were excluded, as well as episodes for which no RSV test result was available or no diary was kept. This is a preliminary analysis; a more comprehensive analysis will follow later.

### Result



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A total of 1210 outpatient ARTI episodes in 600 infants were included in the analysis, of which 211 were RSV-positive and 999 were RSV-negative ARTI. Median age at onset was similar for RSV-positive and RSV-negative ARTI (6.0 vs. 6.0 months,  $p=0.25$ ). Symptoms were generally more severe in RSV-positive than in RSV-negative ARTI; shortness of breath (61.6% vs. 33.1%), cough (97.2% vs. 82.5%), fever (64% vs. 47.9%), wheeze (67.8% vs. 40%), and feeding difficulties (76.3% vs. 52.8%) (all  $p<0.001$ ). If present, these symptoms also lasted longer in RSV-positive ARTI than RSV-negative ARTI (all  $p<0.05$ ). Infants with RSV ARTI were more likely to seek medical attendance (46.7% vs. 24.5%,  $p<0.001$ ) and used more medication (65.2% vs. 52.4%,  $p=0.001$ ), compared to RSV-negative ARTI. Caretakers more often took days off work in case of RSV-positive ARTI than RSV-negative ARTI (28.4% vs. 13.8%,  $p<0.001$ ).

### **Conclusion**

This preliminary study report shows that the majority of young infants with outpatient RSV ARTI exhibit significant respiratory symptoms and nearly half of them seeks medical attendance. Symptoms were more frequent and lasted longer in RSV ARTI compared to other ARTI. These findings indicate that outpatient RSV infections contribute substantially to the total burden caused by RSV disease in Europe.





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## Efficacy of PreF subunit BRSV vaccine in calves: towards a translational model for infant vaccination.

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**Sabine Riffault** - ARNI0208

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### Background

Bovine and human respiratory syncytial viruses (bRSV and hRSV) are two genetically and antigenically closely related pneumoviruses that are responsible for lower respiratory tract infections and severe pulmonary diseases in bovine and human neonates. Achieving safe and protective vaccination against RSV in infants and in calves has proven a challenging task. In fact, no vaccine is available yet for infants and knowledge from bRSV vaccine trials may prove valuable in the development of safe hRSV vaccines able to elicit long-lasting, protective immune responses in the presence of maternally derived antibodies (MDA).

The design of recombinant antigens with a conformation close to their native form in virus particles has been a major breakthrough these last ten years.

### Method

We compared two subunit vaccines combining the prefusion form of the bRSV F protein (BRSVF DS2-v1, stabilized by two artificial disulfides) and hRSV N nanorings formulated in a water-in-oil adjuvant (Montanide<sup>TM</sup> ISA 61 VG) administered to calves with MDA in a single intramuscular vaccination.

### Result

Both subunit vaccines (preF alone or preF combined with N nanorings) provided safe and highly protective immunity (at the clinical and virological levels) against an experimental challenge with bRSV. Analysis of immune parameters pointed to neutralizing antibodies and antibodies to preF as being significant correlates of protection. The duration of protective immunity afforded by PreF vaccination was further compared with that obtained after mucosal immunization with either a commercial live attenuated bRSV vaccine, or a recombinant bRSV attenuated via deletion of the SH gene.

### Conclusion

Such bRSV vaccination studies considered under the "one health" point of view will pave the way to safe and effective hRSV vaccines, even in the context of maternal immunity.



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## Incidence of respiratory syncytial virus in older adults: Limitations of current data

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**Samantha Kurosky** - ARNI0209

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### Background

Respiratory syncytial virus (RSV) is an important cause of respiratory illness in older adults but its incidence and prevalence may be underestimated. A literature review was undertaken to characterise factors resulting in under/over-estimation of RSV epidemiology in adults in high-income countries.

### Method

Primary research studies reporting RSV incidence or prevalence in adults in English since 2000 were identified from Ovid Medline and snowballing. Data on study characteristics, populations, outcomes and author-reported limitations were extracted. Additional known factors that could impact RSV incidence estimates were also captured.

### Result

71 studies met the inclusion criteria, covering a range of countries, settings and populations; the great majority were in symptomatic patients presenting for healthcare. Common limitations that may lead to under- or over-reporting of RSV (Table 1) were use of influenza-based case definitions (particularly requirement for fever); sampling not tailored to the RSV season; sampling a single season (could also result in over-reporting); lack of reporting by age group; and reliance on only PCR testing of upper respiratory swabs. Reporting on the percentage of eligible patients sampled was inconsistent.

### Conclusion

Accurate estimation of RSV incidence will be critical for decision-making on the value and cost-effectiveness assessment of RSV vaccines when approved. A substantial proportion of current studies are likely to underestimate the case burden of RSV. Lack of routine RSV testing promotes underestimation in studies relying on diagnostic codes. Reliance on influenza surveillance consistently underestimates RSV incidence. Additional specimen types can increase detection of missing cases. Results should report specifically on older adults and those with underlying cardiopulmonary conditions, and potential for sampling bias should be documented and addressed.



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## RSV vaccine acceptability among pregnant and lactating individuals in the wake of the COVID-19 vaccine experience

Alisa Kachikis - ARN0210

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### Background

The roll-out of COVID19 vaccines among pregnant and lactating individuals was unconventional due to lack of inclusion of these populations in COVID-19 vaccine clinical trials. Given this experience, our study aimed to investigate knowledge of respiratory syncytial virus (RSV) and acceptability of a novel RSV vaccine among pregnant and lactating persons.

### Method

A follow-up survey including questions regarding potential receipt of RSV vaccine as well as timing and administration questions was sent to participants in an online prospective cohort study of primarily adults residing in the United States (US) who received a COVID-19 vaccine while pregnant, lactating or planning pregnancy. This study was Institutional Review Board-exempt by the University of Washington Human Subjects Division. Participants self-reported opinions about potential RSV vaccines by completing REDCap surveys online.

### Result

As of May 30, 2022, 12615 individuals had responded to the follow-up survey. The majority lived in the US (n=12143, 96.8%), identified as female (n=12493, 99.7%), had a masters or professional degree (n=8385, 67.0%) and worked in healthcare (n=6793, 55.8%). Of these, 1568 (12.4%) were pregnant at the time of the survey, 5667 (44.9%) were lactating and 5380 (42.6%) were recently pregnant or lactating. Overall, 61.5% (n=7754) reported a good understanding of RSV and implications with infection and 70.9% (n=8947) knew of someone who had RSV infection in the past. While currently pregnant individuals were less likely to report that they would be "likely" or "extremely likely" to consider being part of a Phase 1 clinical trial for a novel RSV vaccine in pregnancy compared to currently lactating individuals and those recently pregnant or lactating (n=562, 35.9%; n=2497, 44.2%; n=2316, 43.2%, respectively; p<0.001), over one third of pregnant participants gave a favorable response. Most respondents preferred protein-based (n=9470, 76.6%) and mRNA-based (n=9622, 77.8%) vaccine platforms for an RSV vaccine and stated they would prefer as many vaccines as are recommended in pregnancy (n=7130, 57.0%) to be administered over multiple separate visits (n=6267, 50.1%).

### Conclusion

Participants in our cohort, who were generally highly educated and often worked in healthcare, demonstrated familiarity with RSV and acceptability of a novel RSV vaccine during pregnancy and lactation. With new vaccines in development, ascertaining perspectives of pregnant and lactating individuals is imperative and these populations must be included early on in clinical trials.



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## Heterologous Prime-Boost Immunization Increases Cross-Reactive and Neutralizing Antibodies against Respiratory Syncytial Virus

Christopher Anderson - ARNI0211

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### Background

The Centralized Conserved Domain (CCD) of the Respiratory Syncytial Virus (RSV) G protein contains a human-CX3CR1 binding motif (CX3C) which supports binding to host lung epithelium. Antibodies specific to CCD have been shown to be neutralizing in both in vitro and in vivo systems. We hypothesized that a heterologous prime-boost immunization strategy would boost the number of cross-reactive, neutralizing antibodies compared to monovalent or bivalent vaccine formulations.

### Method

Using an agent-based computational algorithm (ssMod.v2) of humoral immune system and RSV G protein antigen interactions, immune responses were simulated to three RSV G protein vaccine regimens: (1) Monovalent vaccine comprised of RSV G- protein antigen derived from the RSV A2 strain given in two doses (MA2A2), (2) Bivalent formulation comprised of equal amounts of A2-derived G protein and B1-derived G protein given in two doses (BA2B1), (3) A heterologous prime-boost monovalent vaccine formulation comprised of A2 G protein antigen prime-immunization followed by a boost with B1 G protein antigen (HetA2B1). Antibody levels and specificity in the simulation was determined 14 days post-boost for each group. Additionally, BA2B1 and HetA2B1 strategies were additionally tested in mice. Mice were hind-leg i.m. injected with recombinant G protein (15ug) per dose. Serum antibody was collected 14 days post-boost and G specific antibodies determined by ELISA. CCD specific antibody was determined by CX3CR1-Gprotein-binding blocking ELISA.

### Result

In the simulations, the HetA2B1 group showed a significant (1.4 fold) increase in total antibody levels compared to the MA2A2 group. The BA2B1 group also showed a significant increase in antibodies, although to a lesser extent (1.1-fold). The HetA2B1 group showed the highest level of CCD specific antibodies compared to the MA2A2group (1.6-fold) in the simulations. Moreover, the HetA2B1 group showed increased cross-reactivity to ten genetically distinct G protein antigens in the simulations. Mouse serum antibodies levels to recombinant A2 G and B1 G recombinant proteins were significantly greater in HetA2B1 mouse group compared to the BA2B1 group of mice (7.9-fold, 2.4-fold, respectively). We also found a greater ability (1.8-fold) of HetA2B1 mouse group serum antibodies to block RSV A2 G protein binding to recombinant hCX3CR1 protein.

### Conclusion

The heterologous prime-boost vaccination strategy, HetA2B1, shows promise as an effective vaccination strategy for RSV.



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## Transcriptomic changes in blood and bronchus associated with RSV disease in experimentally infected adults

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**Pete Dayananda** - ARNI0212

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### Background

Respiratory syncytial virus (RSV) is a common cause of respiratory viral illness throughout life and severe lower respiratory tract infection in infants and older adults. Previous transcriptomic analysis of nasal tissue from experimentally infected adults showed an association between pre-exposure neutrophilic inflammatory signals and symptomatic infection, characterised by upregulation of interferon signalling pathways. Here, we extended transcriptomic analysis to the lower airway and peripheral blood to identify changes that might progress to trigger severe disease in high-risk groups.

### Method

Twenty-four young adults (aged 18-55) were inoculated with RSV A Memphis 37. Whole blood and bronchial samples were collected, and analysis of the transcriptome was performed using microarray to investigate differential gene expression. To validate these findings, broncho-epithelial cells were grown at air-liquid interface and infected with RSV M37. Changes in ciliary activity were observed via an inverted microscope system equipped with motorised stage.

### Result

Following inoculation, 12 (50%) young adults became infected and reported self-limiting, mild-to-moderate upper respiratory tract symptoms. The viral load peaked at Day 7 post inoculation. The highest number of differentially expressed genes (DEGs) were seen at Day 7 post inoculation in the infected participant cohort and of the top 30 DEGs, interferon and cytokine signalling genes were among the most highly upregulated genes across all sample types. Pathway analyses confirmed the enrichment of cytokine and interferon signalling across all sample types and showed a downregulation of genes involved in cilium assembly pathways in the bronchial brushings. Ciliary beat frequency analysis showed significant differences between the mock and RSV infected cultures.

### Conclusion

Maximal differentially expressed gene signatures occur at Day 7 post inoculation in the infected participant cohort, correlating with the peak in viral load. At this peak in viral load, gene expression in blood and bronchial tissues is dominated by interferon and cytokine signalling genes. RSV infection downregulates genes involved in cilium assembly in vivo which may translate to changes in ciliary function ex vivo associated with reduced mucociliary clearance. This may contribute to the plugging of bronchioles seen in severely ill patients.



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## RSV Health Care Resource Utilization (HCRU) in Children's Hospitals Across The United States Before and After the COVID-19 Pandemic, 2015-2022

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Robert Suss - ARN0213

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### Background

An RSV surge has been noted following the COVID-19 pandemic. Recent literature suggests the 2021-2022 RSV season to be more severe than typical seasons. This analysis examines the relative change in RSV hospitalizations in children under 5 years of age, from 2015-2019 to 2021-2022.

### Method

Data from 42 US children's hospitals in the Pediatric Health Information System (PHIS) database were included in the analysis, with data through March 2022. Children had a diagnosis of RSV, bronchiolitis, or both. Hospitalizations by care unit and age for 2015-2019 (annual mean with margins of error) was compared to those in 2021-2022, and relative changes in hospitalization were calculated.

### Result

There were relative increases in RSV associated inpatient (IP) emergency room (ER) and observational unit (Obs) use during 2020-21 in all age groups. In those <12 months of age these were offset by decreases in bronchiolitis HCRU in the 3 units. There were significant relative increases in RSV and all HCRU in older children (12-59 months) after the pandemic. (Table 1).

### Conclusion

For infants <12 months of age, there was no change in either the overall HCRU use or severity (ICU use was similar). The increases in RSV HCRU in those <12 months of age appear to be due to an increase in testing in younger ages after the start of the pandemic. Children older than 12 months did not have exposure to RSV in 2021-2022, and therefore probably had more severe RSV as suggested by relative increases across all care units for those older than 12 months with RSV and bronchiolitis diagnoses.



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## IGF1R and PKCzeta activation mediate plasma membrane remodelling during RSV Entry

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Quinten Kieser - ARNI0214

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### Background

Recently, our group reported that insulin-like growth factor 1 receptor (IGF1R) is a receptor for respiratory syncytial virus (RSV) and that activation of IGF1R recruits nucleolin (NCL), the viral co-receptor, to the cell surface via protein kinase C zeta (PKCzeta) signalling. Here, we aim to elucidate the role that IGF1R signalling plays during the RSV entry process.

### Method

Immunofluorescence microscopy was used to visualize the viral entry process. We studied a number of targets that mediate the RSV entry process including actin, caveolin-1, clathrin, PKCzeta, and IGF1R, to investigate the effects on virus internalization.

### Result

NCL could not be substantially detected on the cell surface immediately following inoculation with RSV. However, NCL surface expression peaked, and subsequently decreased, 30- and 90-minutes following infection with RSV, respectively, suggesting that the bulk of viral particles are internalized within 90 minutes. Actin staining showed that actin projections, a common phenotype of micropinocytosis, formed around viral particles 30 minutes post-infection and virus particles had been completely internalized within 90 minutes. Inhibiting actin polymerization significantly reduced viral titre by approximately 10-fold. Additionally, inhibiting PKCzeta had similar effects on attenuating viral entry and reduced the actin remodelling observed during infection with RSV.

### Conclusion

RSV has been shown to interact with IGF1R to stimulate recruitment of the coreceptor NCL to the cell surface via PKCzeta signalling. Here, we show that interruption of both actin polymerization and PKCzeta signalling results in attenuated viral entry and suggests that the actin remodelling events associated with viral entry are mediated by PKCzeta.





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## CIRCULATION PATTERN OF HUMAN RESPIRATORY SYNCYTIAL VIRUS IN THE NORTH AND NORTHEAST REGIONS OF BRAZIL: EPIDEMIOLOGICAL AND EVOLUTIONARY ANALYSIS.

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Jessylene Ferreira - ARN0219

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### Background

The Human respiratory syncytial virus (HRSV) acts as the main triggering agent of bronchiolitis in children. This virus belongs to the Pneumoviridae family and has two distinct subgroups, A and B, which imply the variability and dispersal and circulation patterns of this agent worldwide. This study aimed to describe the epidemiology, evolution and circulation dynamics of HRSV strains detected in the North and Northeast regions of Brazil, between 2011 and 2017.

### Method

During this period, 19,567 clinical specimens were obtained from patients with ARI symptoms from both Brazilian regions. The HRSV genome was detected and typed by real-time polymerase chain reaction, preceded by reverse transcription, then sequenced via Illumina platform. For sequence analysis, the following steps were performed: genome assembly and curation, multiple sequence alignment, phylogenetic inferences, evaluation of the temporal structure of nucleotide sequences, evolutionary inferences and phylogeographic dispersion.

### Result

Thus, of the 19,567 cases analyzed, 1,644 (8.40%) were positive for HRSV, in which 935 (56.87%) subgroups were identified, being 209 (22.35%) belonging to subgroup A, and 710 (75.93%) to subgroup B, with 16 (1.71%) HRSV A and B codetections occurring. The seasonal HRSV period in the North and Northeast regions occurred from March to June. The positivity in the group of children under one and two years old was significant, being also the group that presented the highest proportion of severe acute respiratory infections. NA1 and ON1 genotypes for HRSV A and genotypes BA4, BA9 and BA10 for HRSV B were identified. The estimated evolution rate for HRSV A was  $5.05 \times 10^{-4}$  s / s / a (4, 78 -  $5.32 \times 10^{-4}$  s / y, 95% HPD) with TMRCA around 1936 (1933 - 1940, 95% HPD), and for HRSV B the evolution rate was estimated at  $6.82 \times 10^{-4}$  s / s (6.47 -  $7.21 \times 10^{-4}$  s / s, 95% HPD with TMRCA around 1967 (1965 - 1968, 95% HPD).

### Conclusion

HRSV phylogeographic dispersion demonstrated that South America received a clear influx of HRSV imports mainly from North America, Northwest Europe and Oceania. Given the results presented, the frequency of detection of HRSV was similar to the detection described in other countries, occurring mainly in the first half of the year in the two study regions. As expected, children under two years of age were the group with the highest occurrence of HRSV infections, including the most severe infection, thus being considered the priority age group for future immunization actions. The data found about migration patterns may support the discussion of relevant issues in the conduct of prevention and control of infections by this virus, especially those aimed at future immunization for the Brazilian tropical region.



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## Circulation of Respiratory Syncytial Virus in Nashville, Tennessee Before and During the COVID-19 Pandemic

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### Background

Nonpharmaceutical interventions (NPIs) introduced during the coronavirus disease 2019 (COVID-19) pandemic altered the circulation of common respiratory viruses, including respiratory syncytial virus (RSV). We sought to describe the circulation of RSV in Nashville, Tennessee before and during the pandemic.

### Method

We retrieved records of all respiratory samples tested using the BioFire® FilmArray® Respiratory 2.0 Panel at Vanderbilt University Medical Center between April 2018 and April 2022. This platform is a provider-ordered multiplex PCR assay that tests for 21 common acute respiratory pathogens, including RSV. Using the results of BioFire® testing, we evaluated the proportion of RSV-positive tests before and during the COVID-19 pandemic, including by age groups. We used April 1, 2020, as the referent date because it was the first day Tennessee's stay-at-home order was in effect. We defined the beginning and end of each RSV season by a minimum monthly threshold of 20 RSV detections.

### Result

Providers ordered 49,687 tests during the 49-month study period. RSV was the most common pathogen detected (n=2,994 [6.0%]). The median age of those who tested RSV-positive was 1.0 years (interquartile range, 0.0-3.0 years) and 1,586 (53.0%) were male. Before the pandemic (2018-19 and 2019-20), RSV primarily circulated over a 6-month period between October and March and peaked in December (Figure 1). During the pandemic (2020-21 and 2021-22), RSV did not circulate during the usual period, but resurged in April 2021 and continued to circulate over the following 10 months until January 2022. The distribution of detections across age groups was comparable before and during the pandemic. However, during the pandemic, RSV exclusively circulated in children 0-4 years old at the beginning of the season (April 2021).

### Conclusion

The circulation of RSV in Nashville, Tennessee was disrupted during the COVID-19 pandemic, resurged out-of-season (relative to pre-pandemic circulation), and circulated for an extended period. Our findings suggest NPIs may be effective in controlling RSV circulation and that children 0-4 years old may serve as a reservoir of RSV between seasonal epidemics.



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## Structure-based Design of Human Metapneumovirus Fusion Protein Yields Improved Neutralizing Antibody Responses

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Tongqing Zhou - ARN0222

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### Background

Human metapneumovirus (HMPV) is in the paramyxovirus family along with respiratory syncytial virus (RSV), its infection causes respiratory disease in people of all ages, especially among children, elderly, and people with weakened immune systems. Currently, there is no licensed vaccine for this worldwide-distributed virus. Previous structure-based designs of the fusion glycoprotein (F) trimer in the prefusion conformation have identified promising candidates of RSV and Human parainfluenza viruses; However, immunizations with HMPV F trimers stabilized in either prefusion or postfusion conformations have been reported to elicit equivalent neutralization responses.

### Method

We hypothesize that further stabilization of prefusion conformation will elicit higher neutralizing responses. Here we engineered soluble prefusion-stabilized versions of the HMPV F glycoprotein in which various combinations of inter-protomer disulfide bonds, cavity-filling mutations, and proline substitutions were introduced. We screened the designs for their expression and antigenicity using a panel of HMPV antibodies with site- and conformation-specificities.

### Result

Antibody screening identified promising designs that had improved protein expression yields and could be specifically recognized by prefusion conformation-specific antibodies. Cryo-EM of one of the designed HMPV F with 3 engineered disulfide bonds was obtained in complex with antibody MPE8. The structure confirmed the prefusion conformation and formation of inter- and intra-protomer disulfide bonds and revealed the MPE8 epitope at the junction of neighboring HMPV F protomers. Immunization with HMPV F variants elicited superior neutralizing antibodies in mice compared to previous postfusion and prefusion versions.

### Conclusion

Iterative structure-based design combined with antibody-based screening can therefore identify potential immunogens with improved immunogenicity.



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## RSV Hospitalization Rates in Colorado: Changing Epidemiology 2006-2019

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**Robert Suss** - ARNI0223

**Robert Suss<sup>1</sup>, Eric Simoes<sup>2</sup>**

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### Background

RSV hospitalization rates have been used to inform recommendations and policy for prophylaxis, especially in infants and children under 5 years of age. We compared changes in RSV and bronchiolitis hospitalizations between 2015-2019 and 2006-2010 in the state of Colorado.

### Method

Hospital admissions in the state of Colorado were obtained from the Colorado Health and Hospital Association (CHA) database between 1 Jan 2006 and 30 June 2021. Children from 0 to 59 months of age were included from 75 hospitals. ICD codes were used to identify RSV bronchiolitis cases and comorbidities. Census data was used to calculate child-years (CY) at risk for each of four age groups: 0-5, 6-11, 12-23, and 24-59 months of age. IRR of RSV and bronchiolitis hospitalizations from 2006-2010 to 2015-2019 were stratified by age group, and comorbidities.

### Result

There was no significant change in overall rates of RSV hospitalizations in the two periods (Table 1). However, there was a significant decrease in IRR in children without comorbidities (n=4,677), with a concomitant significant increase in those with comorbidities (n=14,701). The increase was also significant by age group, except for those 0-5 months of age. The same patterns were observed for combined RSV and bronchiolitis hospitalizations (except for the 6-11 month age group).

### Conclusion

Colorado children have differing RSV hospitalization risk. The rate of RSV hospitalization in children with comorbidities has significantly increased for all children between 6 months and 5 years of age, and decreased in those with no comorbidities for all age groups. This is not due to decreased RSV testing except perhaps in the 6-11 month age group.



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## Inhibition of the TLR4 signalling pathway with TAK-242 reduces RSV infection and cytokine release in primary airway epithelial cells

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Lindsay Broadbent<sup>2</sup> - ARNI0226

**Lindsay Broadbent<sup>2</sup>, Grace Roberts<sup>3</sup>, Jonathon Coey<sup>3</sup>, Judit Barabas<sup>3</sup>, Michael Shields<sup>4</sup>, Ultan Power<sup>3</sup>, Breathing Together Consortium<sup>1</sup>**

<sup>1</sup>Wellcome Trust-funded strategic project on the origins of paediatric wheeze involving 8 UK and 1 Australian institutions,

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### Background

Respiratory syncytial virus (RSV) infection is a leading cause of hospitalisation in children worldwide. Toll-like receptor 4 (TLR4) expression and several single nucleotide polymorphisms are associated with severe RSV disease. While the role of TLR4 in innate immune responses to RSV has been well-studied, its role in RSV infection remains poorly defined. We therefore elucidated the role of TLR4 in RSV infection in a physiologically relevant primary airway epithelial cell model using a RSV prototypic strain and clinical isolate.

### Method

Expression of TLR4 in mock- and RSV-infected well-differentiated primary airway epithelial cell (WD-PAEC) cultures and continuous cell lines (Calu-3, A549 and BEAS-2B) was determined by immunofluorescence. WD-PAECs and cell lines were exploited to study the role of TLR4 in RSV infection. To determine the role of the TLR4 signalling pathway on viral growth kinetics, WD-PAECs or cell lines were treated with inhibitors of TLR4 (TAK-242) or its co-receptor MD-2 (L48H37) before infection with the low passage clinical isolate RSV BT2a or prototypic strain A2 (MOI=0.1). Heparan sulphate (HS) is a receptor for RSV in immortalised monolayer cells. To study the role of TLR4 in RSV infection of immortalised cells, they were treated with heparinase to remove HS, followed by TAK-242 treatment and infection with either RSV strains A2 or BT2a. RSV growth kinetics and inflammatory responses were quantified. The impact of inhibition of several TLR4 pathway intermediates in WD-PAECs, including PI3K, MEK1/2, p38 MAPK and NF- $\kappa$ B, on virus growth kinetics and IFNL1 was also assessed.

### Result

Inhibition of TLR4 dramatically reduced RSV titres and proinflammatory chemokines in our WD-PAEC model ( $> 2\log_{10}$  TCID<sub>50</sub>/mL) but not in immortalised cell lines. In contrast, inhibition of MD2 had no significant effect on RSV growth kinetics. Removal of HS from A549 cells reduced RSV A2 infection but not BT2a, suggesting that the clinical isolate is less reliant on HS for infection. However, TLR4 inhibition in A549 cells did not alter infection with either virus, irrespective of heparinase treatment. Interestingly, inhibition of the TLR4 signalling intermediate p38 MAPK significantly reduced both RSV titres and IFNL1, while NF- $\kappa$ B inhibition reduced IFNL1 without affecting viral titres.

### Conclusion

There is a clear dichotomy in RSV infection mechanisms in WD-PAECs compared to immortalised cell lines. TLR4 is important for efficient RSV infection/replication in our WD-PAEC model, but not in monolayer cells. This work highlights the need for physiologically relevant experimental models to study RSV infection. Our data also suggest that TLR4 represents an attractive pharmacological target for RSV.



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## Systematic Literature Review of Risk Factors for Poor Outcomes among Adults with RSV Infection

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<sup>1</sup>Pfizer Vaccines, <sup>2</sup>RTI Health Solutions

### Background

There is limited data on risk factors that increase the likelihood of progressing from an upper respiratory tract infection with respiratory syncytial virus (RSV) to lower respiratory tract infection (LRTI) and associated poor outcomes such as hospitalization requiring mechanical ventilation. We reviewed the literature on comorbid conditions and other risk factors linked to severe RSV infection in adults aged 18+ years in high-income countries.

### Method

A systematic literature search was performed in PubMed and Embase according to a prespecified protocol and following recognized review standards for publications in last 10 years. Screening of identified studies was conducted by two researchers; data extraction was performed by one researcher and checked by a second researcher. Data extracted included proportions of patients with serious RSV infection and comorbidities or other risk factors, and risk factors associated with poor outcomes (e.g., pneumonia, hospitalization, mechanical ventilation, and mortality).

### Result

In total, 1,494 articles were identified and screened for eligibility. Of these, 90 met eligibility criteria and were selected for data extraction. The table presents the most frequently identified risk factors for poor RSV-associated outcomes (pneumonia, hospitalization, mechanical ventilation, mortality) and the range of relative risk estimates for those risk factors.

### Conclusion

Older age, male gender, chronic obstructive pulmonary disease, cardiovascular disease, chronic kidney disease, and immunodeficiency were associated with more severe RSV outcomes. Identification of risk factors for severe RSV disease may facilitate vaccine recommendations for at-risk adults once efficacious RSV vaccines are developed and licensed.



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## A characterisation of epithelium-leukocyte crosstalk induced by RSV infected airway epithelial cells from healthy and wheezing children

Lena Glaser - ARNI0228

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### Background

Respiratory syncytial virus (RSV) is an important cause of respiratory symptoms in some, but not all, young children. The nasal airway epithelium plays an important role in the pulmonary immune responses to RSV and other inhaled pathogens. Characteristics of airway epithelium/leukocyte crosstalk during RSV infection and its relation to symptomatic disease in childhood remain poorly understood. We hypothesized that intrinsic differences in the airway epithelial cells from non-wheezing and wheezing children induce differential responses in leukocytes when exposed to RSV.

### Method

To address this hypothesis, we developed a co-culture system based on mock- or RSV-infected well-differentiated primary nasal airway epithelial cells (WD-PNECs) from wheezing (n=4) or non-wheezing (n=5) paediatric donors (mean age: 4 y [range 2.6 - 5.4 y]) and peripheral blood mononuclear cells (PBMCs) from healthy adult donors. The consequences of co-culture on epithelial integrity (transepithelial electric resistance [TEERs]), viral growth kinetics, PBMC activation (CD69 expression on T-cells, monocytes, and natural killer cells), and cytokine/chemokine gene expression and secretions were evaluated.

### Result

Co-culture of WD-PNECs and PBMCs resulted in reduced epithelium integrity, independent of, but aggravated by, RSV infection, compared to WD-PNECs cultures alone. Interestingly, TEERs were lower in co-cultures involving WD-PNECs from wheezing compared to non-wheezing children. RSV growth kinetics were reduced in co-culture conditions, irrespective of wheeze phenotype. PBMC activation was increased following RSV infection of WD-PNECs, with a trend towards lower activation in co-cultures with wheezer-derived WD-PNECs. There were increased secretion levels of a panel of chemokines/cytokines in the basolateral medium following RSV infection of WD-PNECs in co-cultures compared to epithelial cultures alone, except for GM-CSF, TSLP and CXCL16. Indeed, CXCL16 levels returned almost to baseline levels under co-culture experimental conditions. Cytokine/chemokine secretion levels were generally lower following infection of WD-PNECs from wheezing compared to non-wheezing children.





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## **Conclusion**

In conclusion, we present a novel WD-PNEC/PBMC co-culture model that facilitated the study of airway epithelium phenotype (wheezing v non-wheezing) on responses to RSV infection. Airway epithelium derived from wheezing children appeared to diminish activation of PBMCs and cytokine/chemokine responses following RSV infection compared to non-wheezing peers. This co-culture model could provide an excellent platform to further investigate lung pathologies, such as wheeze and asthma, under physiologically relevant conditions.



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## B cell-dependent protection during acute pneumovirus infection in immunodeficient mice

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### Background

The pathogenesis of RSV infection in immunocompromised patients is poorly characterized due to lack of suitable permissive small animal models. Here, we used the RSV-related murine pneumovirus (MPV) as surrogate model for pneumovirus infections in mouse strains of incremental increasing immunodeficiency.

### Method

T cell-deficient TCR $\beta$ <sup>-/-</sup> mice, T and B cell-deficient Rag1<sup>-/-</sup> and T, B and NK cell-deficient Rag1.C $\gamma$ 0<sup>-/-</sup> mice were infected with 300 Pfu of MPV and analyzed for virus load, disease outcome, immune cell recruitment, and, when possible, antibody response.

### Result

Infection of TCR $\beta$ <sup>-/-</sup> mice with MPV was asymptomatic with partially controlled but persistent virus replication. B lymphocytes were efficiently recruited to the lung tissue of these infected mice. In addition, the serum of these mice also contained MPV-neutralizing activity that correlated with an increase of MPV-specific IgM, but not IgG antibodies synthesized in a T cell-independent manner. Interestingly, B lymphocytes in the lung tissue displayed an incomplete or delayed maturation phenotype illustrated by simultaneous expression of more than one Ig subclass, e.g. IgM and IgG, on day 14 post infection.

In contrast, infection of Rag1<sup>-/-</sup> and Rag1.C $\gamma$ 0<sup>-/-</sup> mice with the same MPV dose was characterized by significant heterogeneous disease progressions and unpredictable outcome. Also, a three-to-fivefold increase of the infective dose caused similarly heterogeneous disease causes in TCR $\beta$ <sup>-/-</sup> mice. Lethal disease progression in all these mice was associated with loss of control over virus replication. Adoptive B cell transfer into Rag1<sup>-/-</sup> mice influenced the disease progression and outcome after a MPV infection to some extent: some recipient mice survived the infection without any signs of disease, meanwhile other mice showed rather homogenous weight loss progressing to the humane endpoint similarly to lethally infected wildtype mice.

### Conclusion

A functional B cell compartment represents the major difference between TCR $\beta$ <sup>-/-</sup> mice and the Rag1<sup>-/-</sup> mouse strains, this indicates that functional B cells can provide protection against morbidity and mortality by MPV-infection in T cell-deficient mice by via a neutralizing IgM response. There are indications that these results are comparable to observations of natural RSV infections in immunocompromised patients.



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## Integrated surveillance: monitoring coinfections of influenza, SARS-CoV-2, RSV and other respiratory viruses

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### Background

The emergence and rapid spread of SARS-CoV-2 saw an unprecedented drop in influenza and RSV circulation early in the pandemic. The resurgence of influenza and RSV since, raises concern of coinfections and potential health impact. This paper aims to review the prevalence of coinfections of influenza, SARS-CoV-2, RSV, and other respiratory viruses from data sourced from integrated sentinel surveillance systems.

### Method

Countries were invited to share data sourced from their SARI or ILI sentinel surveillance or from suspected COVID-19 patients that were tested by RT PCR for at least two respiratory viruses including SARS-CoV-2. Twelve countries responded of which three country were excluded as it provided only the number of coinfections without the total number of specimens that were tested. Data from nine countries that had tested SARI or ILI specimens during the period 2021 - 2022 for at least two pathogens, were analyzed to estimate the country-specific prevalence of coinfections. A pooled prevalence estimate was not calculated as the data was heterogenous across countries in terms of age groups, clinical presentation, the type of surveillance, whether hospital or primary care based.

### Result

The number of specimens tested for at least two respiratory pathogens ranged from 200 to 12,000 depending on the age groups reported by the country. The prevalence of SARS-CoV-2 and influenza coinfection was less than 1% in all countries. Coinfections of SARS-CoV-2 and RSV was high (8.2% in children less than 2 years age; 6.3% in children 2 to <5 years age) in Argentina where RSV resurged in children with high intensity. Of the 208 ICU admissions in children 12 years age and less from Tunisia, 4% coinfections were due to RSV and other respiratory viruses, 2% due to SARS-CoV-2 and other respiratory viruses.

### Conclusion

The prevalence of coinfections in children was high in countries that reported a surge in activity of RSV or other respiratory viruses along with SARS-CoV-2. There is a need to monitor co-circulation and potential impact on clinical severity of coinfections in the context of the ongoing COVID-19 pandemic.



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## Coinfection of RSV with SARS-CoV-2 results in altered RSV infection kinetics and diminished innate immune responses

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### Background

RSV remains an unmet medical need, with over 33 million cases and >100,000 deaths in children worldwide each year. There are currently no licensed vaccines or therapeutics available.

The emergence of SARS-CoV-2 and subsequent lockdown and hygiene measures to help control transmission resulted in a major disruption of RSV circulation. However, when pandemic mitigations were lifted out of season surges in RSV cases occurred, coincident with widespread SARS-CoV-2 transmission. This presented the possibility for co-infections with both RSV and SARS-CoV-2, with unknown consequence in terms of infection outcomes. The primary site of replication of both viruses is the respiratory airway epithelium. To explore the consequence of RSV and SARS-CoV-2 coinfections we used well-differentiated primary bronchial airway epithelial cell (WD-PBECs) cultures, which closely mimic airway epithelium in vivo both physiologically and morphologically.

### Method

WD-PBECs were infected with RSV (BT2a), SARS-CoV-2 (Omicron BA.1) or were coinfecting (staggered or concurrently). Apical and basolateral washes were collected every 24 h post infection. Viral growth kinetics were determined by TCID<sub>50</sub> for RSV in HEp-2 cells (refractory for SARS-CoV-2; in the presence of sotrovimab) and plaque assay for SARS-CoV-2 on VeroE6 cells expressing human ACE2 and TMPRSS2 (in the presence of palivizumab), and viral loads for both viruses by RT-qPCR. IFN $\lambda$ 1 release was measured by ELISA, while expression of a panel of inflammatory genes, including RSAD2, ISG15, Mx1, NLRP3, IFNL1, and IL-18, are under investigation by RT-qPCR.

### Result

Single infection of WD-PBECs with SARS-CoV-2 or RSV resulted in titres peaking at 48 and 72 hpi for SARS-CoV-2 and RSV, respectively. SARS-CoV-2 infection 48 h before RSV resulted in significantly reduced RSV titres. However, RSV infection prior to SARS-CoV-2 did not significantly alter SARS-CoV-2 growth kinetics.

SARS-CoV-2 infection prior to, or concurrent with, RSV infection of WD-PBECs resulted in reduced expression and secretion of IFN $\lambda$ 1. This correlated with reduced expression of interferon stimulated genes when WD-PBECs were infected with SARS-CoV-2 prior to, or concurrently with RSV infection. Alterations in expression of inflammatory genes following single or coinfection are under investigation.

### Conclusion

Prior SARS-CoV-2 infection of WD-PBEC interfered with the replication kinetics of RSV and results in diminished IFN $\lambda$ 1 secretion. While the mechanism behind this remains to be elucidated, this study highlights the importance of studying respiratory viral co-infections, especially as novel SARS-CoV-2 variants emerge and circulate, while infants experience another out of season surge in RSV.



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## Novel paramyxovirus vectored vaccines induce high neutralising antibody titres and specific T cell responses against bovine Respiratory Syncytial virus

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### Background

Bovine RSV (bRSV) is an important cause of bovine respiratory disease complex (BRDC), a leading cause of calf morbidity and mortality worldwide, but current vaccines are poorly efficacious. To address this, we developed novel recombinant vaccine candidates based on 2 paramyxovirus vector platforms expressing structurally defined bRSV antigens. Both replication-competent (rc) and incompetent (ri) viral vector strategies were pursued for mucosal delivery.

### Method

Two bRSV genes encoding immunogenic viral proteins were individually inserted into (rc) and (ri) paramyxovirus infectious clone plasmids. The (rc) and (ri) bRSV recombinant virus vectors were recovered by reverse genetics and thoroughly characterised in vitro to ensure expression of the appropriate bRSV antigens. To assess immunogenicity of the bRSV vaccine candidates, BALB/c mice were immunised once or twice intranasally with one or both vectors. Total anti-bRSV IgG serum responses were quantified by ELISA. Presence of systemic and mucosal neutralising antibodies (nAb) was tested with a live virus neutralisation assay. RSV-specific T cell recall responses were investigated by stimulating splenocytes from immunised mice with bRSV vaccine antigen-derived peptides.

### Result

Presence of paramyxovirus vector genomic RNAs and viral proteins was confirmed by RT PCR and SDS PAGE, respectively, and was consistent with successful recovery of both (rc) and (ri) bRSV vaccines. Expression of the vaccine antigens was confirmed by immunofluorescence and western blot upon infection of LLC-MK2 cells. As anticipated, spread of (ri) viral vectors was evident in an engineered packaging cell line, but not in LLC-MK2. No significant weight loss was recorded in mice, irrespective of vector used. Importantly, immunisations with either (rc) and (ri) vectors elicited significant anti-bRSV antibody responses. Total IgG ELISA titres were highest after 2 immunisations. The nAb activity was elevated in serum, limited in bronchoalveolar lavages (BALs) and very low/undetectable in nasopharyngeal lavages (NALs) of immunised mice. There was evidence of dose-effects, immunogenicity and virus neutralising activity differences between the bRSV antigens and the vectors used. Initial data indicated that a CD8+ cytotoxic T cell memory response was induced in mice immunised with (rc) bRSV vaccines. Additional characterisation of the anti-bRSV IgG and IgA antibody responses in NALs and BALs is ongoing as are the CD4+ and CD8+ T cell responses.

### Conclusion

We have generated promising bRSV vaccine candidates based on paramyxovirus vectors that warrant further assessment of their protective efficacy in the calf model of bRSV.



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## Estimation of the timing and intensity of the RSV season preceding and during the COVID-19 pandemic in South Africa 2010-2021

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### Background

Describing seasonal patterns like timing, length and peak of the RSV season are important for awareness, prevention and healthcare service planning. In South Africa, prior to COVID-19, the RSV season timing and impact was defined using proportion of positive tests from surveillance data among hospitalised individuals of all ages. During the COVID-19 pandemic, high numbers of respiratory illness hospitalisations in adults may have biased proportion positive downwards. We aimed to compare estimates of RSV timing and impact in South Africa preceding and during the COVID-19 pandemic using data for all age groups, and for children aged <5 years.

### Method

Data from syndromic surveillance for severe respiratory illness (SRI) was used to define the start, peak and end of the RSV season. The epidemic threshold for RSV for each of the two age groups was calculated using the Moving Epidemic Method (MEM). MEM uses the 40th, 90th and 97.5th percentiles established from available years of historical data to calculate thresholds of activity. To set thresholds, surveillance data collected from 2010 to 2016 were used and RSV seasons for 2017 to 2021 were described.

### Result

From 2017-2019, using data from all age groups and children <5 years respectively, the average start of the season was week 8 (range 8-10) and week 7 (range 6-9) and the length of the RSV season was 19 (range 16-21) and 21 (range 20-23) weeks. The peak was in week 18 (17-22) for both groups. From 2017-2019, the impact reached high level in one season when using all ages, compared to three seasons reaching very high level in children only. In 2020, using all age groups, impact reached low level in week 36, which persisted for 7 weeks. However, in children, the 2020 season started in week 13, peaked in week 16 and ended in week 18, followed by a second increase in RSV activity, which started in week 29, peaked at high level in week 42 and continued until



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week 20 in 2021. In 2021, a second increase started in week 21, peaked at low level in week 25 and ended in week 29.

### **Conclusion**

From 2017-2019, restricting data to children aged <5 years for defining the RSV season resulted in a slightly earlier start, higher level of impact and longer epidemic periods as compared to using all ages. During COVID-19 using all age groups, a low-level increase in activity was found later in the year during 2020 and no season was reported in 2021. Whereas restricting to children revealed a prolonged season with a substantial shift in RSV seasonality through COVID-19. This highlights how emerging pathogens can bias surveillance indicators based on proportion positive. Reporting pathogen seasonality during an emerging pathogen may need adjustments.





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## The effect of case fatality estimates on RSV prevention cost-effectiveness

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### Background

A long-acting monoclonal antibody (mAb) has been developed for the prevention of RSV lower respiratory tract infection (RSV LRTI). Cost-effectiveness analyses of mAb programs in low-income settings have identified the inpatient case fatality ratio (CFR) as an influential parameter. This parameter is difficult to measure with precision, and different studies have produced varied CFR values.

### Method

We constructed a birth cohort model of RSV LRTI among Malian infants in their first year of life. We assessed the health and economic impact of mAb programs, using age-specific CFR values for inpatient RSV (Figure): 1) PERCH conventional, the Mali-specific RSV-associated CFR from the Pneumonia Etiology Research for Child Health (PERCH) study, calculated by dividing the total number of fatal cases with RSV detected by the total number of cases with RSV detected; 2) PERCH integrated analysis (PIA), the Mali-specific RSV-attributable CFR from PERCH, estimated by summarizing the CFR for fatal cases with RSV assigned as the cause of their pneumonia; 3) Li 2020 age gradient, an age-specific CFR for low-income countries derived from a cost-effectiveness analysis published in 2020; 4) Li 2020 without age gradient, a CFR calculated as the mean across age groups of the previous CFR, applied uniformly across ages, and; 5) Li 2022, the age-specific CFR for low-income countries from a meta-analysis published in 2022. The status quo was defined as no mAb program.

### Result

We identified considerable variation in the deaths that could be prevented by mAb due to the choice of CFR input. Preventable deaths among the birth cohort of 79,000 infants ranged from 16 when using PERCH PIA (method 2) to 133 when using PERCH conventional (method 1). The incremental cost-effectiveness of mAb compared to the status quo accordingly decreased from \$1152 per DALY to \$141 per DALY for these scenarios, respectively.

### Conclusion

The inpatient RSV CFR is a critically important and influential parameter. Replacing the RSV-associated CFR with the RSV-attributable CFR from the same study resulted in an 8-fold change in the cost-effectiveness ratio for mAb programs in Mali. From a policy perspective, mAb is far less economically favorable when the RSV-attributable CFR is used. Decision-makers should carefully evaluate the choice of CFR when considering any RSV intervention.



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## RSV surveillance in the context of the COVID-19 pandemic: lessons learnt and opportunities offered

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### Background

Respiratory Syncytial Virus (RSV) is a significant cause of acute lower respiratory infections in infants and young children leading to hospitalizations. RSV surveillance can provide evidence-based guidance for developing preventive and therapeutic strategies and inform immunization policy and practice.

After demonstrating the feasibility of leveraging the Global Influenza Surveillance and Response System (GISRS) for RSV surveillance, the second phase was expanded to 25, to include low- and middle-income countries across all six WHO regions with a focus on children aged less than 2 years. The emergence of SARS-CoV-2 virus in 2020 resulted in significant disruptions in influenza and RSV surveillance. This paper aims to document the challenges, lessons learnt and the opportunities offered, in the context of the COVID-19 pandemic.

### Method

Information was collected on RSV surveillance practices, challenges and adaptations during the COVID-19 pandemic, from the national focal points for surveillance and laboratory through a survey and a progress review meeting in June 2021.

### Result

RSV surveillance was disrupted in most of 25 countries. Two countries could not start the RSV surveillance and two provided inadequate information to the survey; therefore, they were excluded from the analysis. Two countries did not have significant disruptions. The major reasons for disruptions were changes in healthcare seeking pathways (12 of 19 countries), diversion of patients to COVID-19 diagnostic and treatment centers (4/19



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countries) and repurposing of sentinel surveillance hospitals as COVID-19 treatment centers (7/19 countries) and clinical staff for COVID-19 (12/19 countries).

## **Conclusion**

The COVID-19 pandemic resulted in significant disruptions to influenza and RSV surveillance as GISRS capacities were repurposed for national COVID-19 response. It highlights the need for a resilient GISRS with an ability to surge, and quickly adapt to threats from other respiratory viruses. It also provides an opportunity to leverage GISRS to integrate influenza, SARS-CoV-2, RSV and other novel respiratory pathogens as a sustainable strategy to address future threats from novel respiratory viruses of pandemic and epidemic potential.



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## Survival and long-lived functional enhancement of murine alveolar macrophages targeted early following RSV infection

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### Background

Alveolar macrophages (AM) are the most abundant immune cell within the lower airways and are one of the first cells to encounter respiratory pathogens. AMs are known to play a critical role in the induction of the host immune response following respiratory syncytial virus (RSV) infection, however, the additional functions AMs play early following infection remains poorly defined. Our research demonstrates that murine alveolar macrophages are the initial cellular target of RSV early during the infection. RSV-infected AMs survive infection and exhibit enhanced functional capacity upon heterologous re-challenge.

### Method

To track virally infected cells, mice were infected with a panel of fluorescent reporter-expressing RSV and influenza viruses and cellular dynamics were assessed by spectral flow cytometry. In order to track cells that survive infection, we infected floxed-tdTomato-reporter with a novel recombinant RSV engineered to express the cre recombinase (RSV-Cre). Using this system, all infected cells are indelibly marked due to continuous expression of the tdTomato reporter. Reporter-positive and -negative AMs were isolated at 30 days p.i., and their functional capacity was assessed ex vivo.

### Result

AM numbers significantly declined 6-12 hrs following RSV infection. The remaining AMs accounted for the majority of virus-infected cells at early time points, far surpassing the epithelium, in both RSV and influenza virus infected mice. Strikingly, AMs represented over 80% of all RSV-infected cells which was confirmed by confocal and intravital microscopy and ImageStream flow cytometry. Blockade of TNF prior to infection reduced AM loss resulting in increased total numbers of RSV-infected AMs in vivo as well as a decrease in viral titers, suggesting that AMs may act as a barrier to the respiratory epithelium by allowing direct infection. Cre-mediated reporter activation by RSV-Cre required direct infection by replication competent virus. Reporter-positive AM numbers remained constant up to 30 days p.i., long after viral clearance. Both reporter-positive and -negative AMs exhibited increased basal levels of pro-inflammatory cytokines as compared to naïve AMs which was further increased upon heterologous infection. Reporter-positive AMs displayed an enhanced inflammatory response as compared to reporter-negative AMs taken from the same lung, indicating AM cytokine production is enhanced following direct RSV infection.

### Conclusion

Our results confirm that AMs play an essential role in limiting early viral access to the respiratory epithelium through direct infection which results in long-lived functional enhancement.



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## Systematic Literature Review: Under-ascertainment of Respiratory Syncytial Virus infection in adults due to diagnostic testing limitations

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Elizabeth Begier - ARN0247

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### Background

Accurate assessment of RSV incidence is critical for evaluating RSV preventive interventions. While it is known that the choice of diagnostic testing approach can contribute to RSV disease underestimation, this has not been formally quantified across testing methodologies and specimen types. We sought to quantify RSV under-ascertainment in adults related to RSV diagnostic testing approach via systematic literature review.

### Method

EMBASE, PubMed, and Web of Science were systematically reviewed (Jan 2000-Dec 2021) for studies in adults ( $\geq 18$  years) using or comparing  $>1$  RSV testing approach. Studies reporting RSV diagnostic test performance or quantitative comparison of testing methods, or that compared test sensitivities using different specimen types or processing conditions were eligible. We quantified test performance characteristics (sensitivity, specificity, and detection rate) and calculated weighted averages and the increase in RSV-case ascertainment. Data were analyzed descriptively; quality assessment for included studies was done using the Quality Assessment of Diagnostic Accuracy Studies checklist (QUADAS-2).

### Result

We identified 8066 unique references and 154 met our inclusion criteria. Overall, 64% of studies included had a high risk of bias in  $\geq 1$  domain using QUADAS-2 tool (patient selection and flow: 33% in each domain). RT-PCR (68.5% of studies) and nasopharyngeal (NP) swab (66%) were the most common diagnostic test and specimen type used. Using RT-PCR as the gold standard, other tests were less sensitive: rapid antigen detection ( $n=24$  comparisons; sensitivity weighted average 78.8%), direct fluorescent antibody testing (4; 87.3%), and viral culture (2; 86.7%), but specificity was comparable (all  $>90\%$ ). Compared to simplex PCR, multiplex PCR had lower sensitivity (7; weighted average: 92.7%). In comparison to RT-PCR of NP/nasal swab alone, the pooled percent increases from the addition of another sample type (with range) were as follows: sputum RT-PCR - 3 studies, 65% (29-100%); 4-fold rise by paired serology - 6 studies, 46% (29-64%); and oropharyngeal swab RT-PCR - 3 studies, 25% (0-30%) (Figure/Table). Based on one study each, adding RT-PCR of NP wash or saliva boosted RSV detection by 8%.

### Conclusion

While RT-PCR - particularly simplex testing - is the most sensitive RSV diagnostic test in adults (usually performed on NP swab), adding other specimen types substantially boosts RSV detection. Research on synergistic effects of adding multiple specimen types should be undertaken and results should be incorporated as a multiplier into disease burden and economic models.



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## Incidence of respiratory syncytial virus (RSV)-associated hospitalization among American Indian and Alaska Native children

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Laura Hammitt - ARNI0249

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### Background

Historically, American Indian/Alaska Native (AI/AN) children have experienced high rates of acute respiratory infection (ARI) hospitalization, up to half of which are due to respiratory syncytial virus (RSV).

### Method

We conducted active surveillance for ARI in hospitalized AI/AN children age <5 years in the US southwest (Navajo Nation and White Mountain Apache Tribal lands) and Alaska (Yukon Kuskokwim [YK] Delta and Anchorage) over three RSV seasons (November-May). Mid-turbinate nasal swabs were tested by PCR for RSV and other respiratory viruses. We calculated incidence of RSV hospitalization by season and age. Interseason RSV cases observed in summer of 2021 were not included in seasonal rates.

### Result

We enrolled 469 children: 324 in 2019-20; 22 in 2020-21; and 123 in 2021-22. RSV was the pathogen most commonly detected (53%) in 2019-20. RSV hospitalization seasonal incidence per 1,000 children was greatest in the first year of life and ranged from 19.2 (Anchorage) to 112.2 (YK Delta; Table 1). RSV incidence declined during the SARS-CoV-2 pandemic. No RSV was detected among participants in the 2020-21 season and incidence was lower in 2021-22 compared to 2019-20.

### Conclusion

These data document high incidence of RSV hospitalization among AI/AN children and disruption of RSV transmission during the SARS-CoV-2 pandemic.



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## Whole genome sequencing of RSV on ONT platform

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**Kseniya Komissarova** - ARNI0250

**Kseniya Komissarova<sup>1</sup>, Artem Fadeev<sup>1</sup>**

<sup>1</sup>Smorodintsev Research Institute of Influenza

### Background

Respiratory syncytial virus (RSV) causes a respiratory tract infection in humans. It is especially dangerous for children under 2 years old and adults over 65. More than 100,000 people die each year from RSV, most of them are children. Moreover, every fifth death is not associated with risk factors in the patient's history. At the moment, there is no licensed vaccine against RSV in the world, and the only monoclonal therapy is used. RSV belongs to the Paramyxoviridae family, subfamily Pneumovirinae, genus Pneumovirus and has a negative-sense RNA genome consisting of 15000 nucleotides. There are two subtypes of hRSV: A and B, based on antigenic reactivity to monoclonal antibodies. The whole genome sequencing can be used in RSV surveillance projects and help us answer questions about the evolution of these viruses.

### Method

Reverse transcription was made with Lunascript RT SuperMix kit (NEB), PCR - Q5® High-Fidelity 2X Master Mix (NEB) with 4 pairs of primers (Annefleurf C. Langedijk and co-authors, doi.org/10.1186/s12879-020-05175-4). Libraries for nanopore sequencing were prepared with Ligation Sequencing kit LSK-109 and Native Barcoding expansion EXP-NBD196 (ONT). Sequencing was performed on GridION sequencer with R9.4.1 flowcell. Guppy v.6.1 in super-accurate mode was used for basecalling and debarcoding of data. Reads were aligned to the reference sequence with Minimap2. Samtools and ivar were used for consensus sequence generation.

### Result

Whole genome sequences of two original specimens and two tissue culture isolates were obtained to demonstrate the method's performance. Sequencing run produced 55000 - 160000 reads per sample 2000 - 3000 bp long with mean Phred quality value 21 (Fig.1). This amount of data was sufficient for covering more than 98% of genome length of each sample with median coverage 250 - 2400 (Fig.2).

### Conclusion

Nanopore sequencing is a very flexible approach for whole genome surveillance of important viral pathogens such as human respiratory syncytial virus.





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## Epidemiologic Modeling to Inform RSV Trial Operations during the COVID-19 Pandemic

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Eileen Farnon - ARNI0252

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### Background

Usual RSV seasonality has been skewed during the COVID-19 pandemic. Non-pharmaceutical interventions (NPIs) like mask requirements and school closures at the start of the pandemic aborted or delayed the onset of RSV epidemics, resulting in off-season RSV epidemics, first in the Southern Hemisphere. In order to plan enrollment and respiratory illness surveillance for RSV vaccine studies, we created a model for South Africa based on characteristics of the off-season RSV epidemic in Australia, to estimate the timing of future RSV epidemics in study regions to inform the timing of enrollment and respiratory illness surveillance.

### Method

We evaluated the off-season epidemic in Australia and identified the presence of NPIs and RSV susceptibility as the major variables. We used mobility as a proxy for NPIs, using regional mobility data from Google trends and subtracting mobility among households. Susceptibility was determined by calculating the area between the historical and current RSV epidemic curves. We created a predictive model with the predictors of mobility and susceptibility using surveillance data from New South Wales (NSW), Australia. The model was optimized and applied to data from two surveillance systems in Johannesburg and Cape Town, South Africa to estimate the timing of the current and next RSV epidemics during 2020-2022. Models were rerun every 1-2 months using updated data.

### Result

The model had a 4-stage pattern: 1) mobility <-30%, RSV suppressed, susceptibility increases; 2) mobility >-30%, susceptibility peaks and declines; 3) RSV epidemic, mobility >-30%, susceptibility declines; 4) RSV epidemic ends, mobility high, susceptibility increases. An early model using data from week 23, 2021 estimated the ensuing epidemic correctly for the next 3 months. As the model was optimized and run using updated data, predictions shifted 1-4 weeks and the overall prediction was more accurate. The model was applied to other regions.

### Conclusion

Uncertain timing of RSV epidemics during the COVID-19 pandemic has been a challenge for RSV trials. This model has been used to estimate the timing of RSV epidemics in South Africa and other regions in our trials, recognizing that it is most accurate in the near term. The model is subject to some limitations: lack of environmental factors, possible future NPIs, and other possible variables; less reliability in regions with small numbers of reported cases, reporting lags, and incomplete surveillance where RSV is not nationally notifiable. Despite these limitations we have found the model to be useful for clinical trial operations. Improved RSV surveillance would allow for greater utility of modeling during COVID-19 and future pandemics.



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## Genomic surveillance strategy for Respiratory Syncytial Virus (RSV) in Brazil

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### Background

Respiratory Syncytial Virus (RSV) is the main cause of paediatric morbidity and mortality. Several RSV vaccine candidates are in advanced clinical trials and possibly we will have a safe and effective vaccine against RSV shortly. As such, RSV genomic features and monitoring viral evolution may be an important tool to epidemiological surveillance update and strategies of viral control.

### Method

In this study, we aimed to perform a critical analysis and curation of RSV Brazilian genomes available at EpiRSV database on GISAID. Additionally, we implemented an adapted RSV whole-genome sequencing protocol using an RSV-A and RSV-B primer set in the Illumina COVIDSeq Test commercial kit to recover new RSV genomic sequences from Brazil. Genomes were assembled by using the ViralFlow tool and classified by genotype according to Goya (2020) by maximum likelihood phylogenetic analysis.

### Result

Up to 20 RSV (14 RSV-A and 6 RSV-B) from 2020 to 2022 whole-genome sequences were successfully recovered using the protocol adapted in this study (others are in progress). This protocol allows an outstanding coverage of both RSV-A and RSV-B genomes. Until June 1st, 2022 a total of 24,401 genomic and subgenomic RSV sequences were available at the EpiRSV. Of those, 1,352 were sampled in Brazil between 1995 and 2017, divided between RSV-A (n = 1,103) and RSV B (n = 246), with only 3 genomic ones. Regarding the attached metadata, only a small fraction (17%, n = 232) of those Brazilian sequences are accompanied by their complete collection date. 72%, (n = 978) of those were associated with their source location in the country. Among the identified locations were states from Northern (5%, n = 50), Northeastern (35%, n = 341), Southeastern (47%, n = 464), and Southern, (13%, n = 123). The genotypic assignment of Brazilian samples based on their composition of the G gene 2HR (~300 nts) revealed the dominance of GA.2.3 (65%, 1995 - 2017) among RSV A type genotypes (n = 368), and the majoritarian participation of the GB5 (> 99%), among the RSV B type ones (n = 258).

### Conclusion

Given the promising control strategies for RSV and the clinical impact of RSV on the population, improving the laboratory and epidemiological surveillance in a country is needed and important to guide control strategies. This study shows an easy friendly protocol to recover the RSV whole-genome and brings insights into how to improve the genomic surveillance in Brazil with more robust, representative, and homogeneous data from the whole country per year.



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## EPIDEMIOLOGICAL AND DYNAMIC ANALYSIS OF CIRCULATION OF HUMAN RESPIRATORY SYNCYTIAL VIRUS STRAINS ISOLATED IN THE STATES OF ACRE AND PARAÍBA, BRAZIL, FROM JANUARY 2018 TO JUNE 2020

Jessylene Ferreira - ARN0257

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### Background

Human Syncytial Respiratory Virus (HRSV) is one of the main pathogens related to lower respiratory tract infections, which mainly affects children under 5 years of age. HRSV is classified in the order Mononegavirales, family Pneumoviridae, genus Orthopneumovirus. The viral particle has 11 viral proteins and, according to the antigenic and genetic differences of glycoproteins G and F, the virus is divided into two distinct subgroups, HRSV A and HRSV B. This paper presents the epidemiology and circulation pattern of HRSV strains, isolated from samples of the states of Acre and Paraíba from January 2018 to June 2020.

### Method

Viral RNA samples extracted from clinical specimens from patients with acute respiratory infection (ARI), seen at health units in the states of Acre and Paraíba, were analyzed. Data analysis was carried out following three main steps: (a) detection of the subgroups by RT-qPCR; (b) collection of clinical and epidemiological data; (c) statistical analysis with the application of the  $\chi^2$  test with a 5% significance level ( $p < 0.05$ ).

### Result

During the period studied, 2753 samples were collected, of which 268 (9.73%) were positive for HRSV, with no positive samples detected for the year of 2020 until the month of June. During the 2018-2019 season, there was a predominance of HRSV A in 2018 of 86.95% (100/115) and a more expressive circulation of HRSV B in 2019 of 77.78% (119/153). Regarding the seasonal profile, it was observed that, in 2018, there was greater viral circulation in the period from April to August and, in 2019, in the period from January to April, with peaks of HRSV infections in June and March of each year, respectively. A strong significant correlation of cases with age has been demonstrated ( $p < 0.0001$ ), with a notable difference in children under 5 years old, especially those under the age of 1 year. There was no difference between men and women. As for signs and symptoms, clinical conditions were not associated with cases of symptoms due to HRSV.

### Conclusion

Thus, the results found demonstrated the importance of continuous epidemiological surveillance of HRSV, due to its great impact on public health, especially in children under 5 years of age. In addition, these data may promote research in the states of Acre and Paraíba in future seasons.



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## Genetic Diversity of Respiratory Syncytial Virus and its Influence on Clinical Outcome in Adult Populations

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<sup>2</sup>Northwestern University

### Background

Respiratory Syncytial Virus (RSV) is a single-stranded, negative-sense RNA virus that is a major cause of global acute respiratory tract infections (ARTIs). Among infants, RSV results in nearly 34 million severe ARTIs annually and it is similarly responsible for large disease burdens in vulnerable adult populations, including seniors and the immunocompromised. While some studies have associated RSV serotype with disease severity in children, it is unclear if the same holds true in adult populations and what the mechanism underlying this association might be. Here, we hypothesize that differences in RSV genotype are associated with differences in disease severity and presentation in adults.

### Method

To test this hypothesis, we will assess the viral load, and genetic diversity across a cohort of adult patients diagnosed with RSV and test for association with clinical and demographic data extracted from electronic health records. Since May 2022 and over the course of 5 consecutive RSV seasons, we have collected over 400 residual diagnostic nasopharyngeal swabs from hospitalized adults at Northwestern Memorial Hospital (NMH) in Chicago, IL who tested positive for RSV by PCR-based testing.

### Result

Of the 402 residual clinical specimens collected, 168 RSV genomes (61 subtype A and 107 subtype B) have been sequenced using an optimized amplicon-based strategy for Illumina next-generation sequencing. Consensus sequences from NMH samples in addition to publicly available RSV sequences from the United States were aligned and analyzed using Maximum Likelihood (ML) phylogeny. These data will be integrated with our extracted clinical and demographic data to assess genotype-specific correlations.

### Conclusion

Future studies will focus on mechanistic interrogation of RSV genetic differences associated with higher disease severity or worse patient outcome, including viral entry and immune evasion assays. Ultimately, this work will lead to an expanded mechanistic and epidemiologic understanding of RSV pathogenesis with clinical applications to hospitalized adult populations.



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## RSV vaccines/prophylactics: key learnings from the RSV challenge model and Efficacy of Vaccines

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**Alex Mann** - ARNI0259

**Alex J Mann<sup>1</sup>, Andrew Catchpole<sup>1</sup>, Francois Aubin<sup>2</sup>, Alison Tyler<sup>1</sup>, Alan Bell<sup>1</sup>, Karen Keun<sup>1</sup>, Nicolas Noulon<sup>1</sup>**

<sup>1</sup>hVIVO Services Ltd., <sup>2</sup>VennLife, Open Orphan

### Background

Challenge studies can be a powerful tool in the development of both therapeutic agents and vaccines. However, key decisions made in the study design process can have a profound impact on the outcome of the efficacy assessment. These include factors such as the selection criteria and screening of subjects as well as timing and frequency of the virological sampling and illness symptom data. A critical factor is selecting which efficacy endpoints to include and how precisely that is defined and calculated. While the principles of the endpoints are the same in challenge studies and field trials, subtle but vital differences in the endpoint definitions need to be made between the two clinical trial formats to account for the different context.

### Method

We have compared the study design, disease definitions and endpoints across the different studies to assess and identify key success criteria in the study design and analysis of RSV human challenge study data. Our analysis incorporates both data that is in the public domain for vaccines tested in the model, as well as a significant body of unpublished RSV non-IMP challenge model data. Utilising an extensive database of individual subject data we have further explored a range of different disease parameters in greater depth. Using a meta database from a range of studies facilitated the analysis of data and the explore different analysis endpoints.

### Result

Our analysis highlight some important study design aspects that should be carefully considered when designing RSV vaccine challenge studies. The data demonstrate the importance of a thorough understanding of the expected variation in response to the infection and how this affects the powering considerations for challenge studies as well as expected impact on these endpoints from an efficacious vaccine/prophylactic. We discuss the relative pros and cons of different endpoints and study design approaches used. In addition, we provide an analysis of the impact of subtle changes in endpoint definitions, such as symptomatic incidence, and how changes can facilitate an improved product efficacy assessment. This is exemplified by the RSV vaccines that have recently obtained fast track status that showed efficacy in the challenge model

### Conclusion

This insightful assessment of publicly available vaccine challenge data, as well as hVIVO's metadata, highlight key learnings and success criteria as well as illuminating as yet poorly understood or explored aspects that could further enhance the utility of RSV challenge models.



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## Strategic Advocacy to support equitable access to vaccine-like RSV mAbs

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Alexandra Bhatti - ARNI0262

**Alexandra Bhatti<sup>1</sup>, Susan He<sup>2</sup>**

<sup>1</sup>Merck & Co. Inc., <sup>2</sup>National Coalition for Infant Health

### Background

In the United States, RSV affects most children by the time they are two years old and is the leading cause of hospitalization for all infants under age one. RSV poses a serious risk to all infants and young children, but preventive monoclonal antibodies can make a life-saving difference. With these passive immunizations on the horizon, it is critical to ensure that once available, that they can be equitably accessible for all infants. We have sought to understand the legal landscape to identify how products that provide primary prevention protection, population wide, can be afforded vaccine-like access in national public programs and further have built an awareness campaign to drive education and awareness of this urgent need across the community.

### Method

Built a multi-media and stakeholder digital advocacy and awareness campaign to drive disease awareness and urgent need for equitable access to future RSV mAbs. NCfIH took a top down and bottom up approach in driving tailored communication and engagements with policy stakeholders and government agencies while simultaneously, generating surround sound and community awareness and engagement.

### Result

RSV Campaign Overall Impressions since 03/01/22: 559,311

- NCfIH developed a position paper and associated press release, articulating the public health imperative of equitable access to vaccine-like products for RSV mAbs. While this was shared broadly, the team leveraged digital media to amplify

Impressions: 332,459

Since then, NCfIH has developed a paper on the burden of RSV highlighting disparate impact on sub-populations, developed patient/parent stories and blogs, infographics and more. These have helped drive community awareness. See attachment for more with links embedded.

To further support parent advocate voices, NCfIH stood up a Parent Advisory Council. This enables a unified and coordinated voice that can be supported. For example, in a recent ACIP meeting, where RSV mAbs were discussed, parents successfully submitted public comment.

### Conclusion

Policy must advance with innovation. RSV impacts nearly every infant born and unfortunately no wide prevention modality exists. Early strategic advocacy and awareness efforts are essential to ensuring that once these products are available, they may be equitably accessible for all Americans. This provides a helpful model for other countries exploring similar efforts.



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## RSV G protein monoclonal antibodies targeting the CX3C motif improve the antiviral response and protect against RSV mucogenic disease

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Harrison Bergeron - ARNI0263

**Harrison Bergeron<sup>3</sup>, Jackelyn Crabtree<sup>3</sup>, Tamas Nagy<sup>3</sup>, Ana Castrejon<sup>2</sup>, Rebecca Dubois<sup>2</sup>, Lawrence Kauvar<sup>1</sup>, Ralph Tripp<sup>3</sup>**

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### Background

Respiratory syncytial virus (RSV) is a major cause of serious lower respiratory disease with no vaccine available and limited therapeutic choices. Most candidate vaccines target the fusion (F) protein to induce neutralizing antibodies (Abs) and do not control attachment (G) protein-mediated disease. The RSV G protein contains a highly conserved CX3C chemokine motif implicated in modifying RSV immunity and enhancing disease. Previous findings have shown anti-G protein Abs can neutralize RSV and protect against disease.

### Method

Anti-G protein monoclonal Abs (mAbs) targeting opposing G protein epitopes (i.e. clones 3D3 and 2D10) were compared to the current standard of care i.e. the anti-F protein mAb, palivizumab. In vitro studies were examined using RSV Line19 infected mouse lung epithelial cells (MLE15) treated with 3D3, 2D10 or palivizumab. BALB/c mice were prophylactically treated 24h before Line 19 infection, or therapeutically treated 72h pi and evaluated at subsequent timepoints pi for interferon (IFN) responses, virus neutralization, BAL cell influx, cytokine and chemokine responses, and lung histopathology.

### Result

Mice treated with anti-G protein mAbs had substantially improved type I and type III IFN responses while palivizumab treatment reduced IFN responses. As expected, anti-G protein mAbs neutralized the virus, and significantly reduced the BAL cell influx while palivizumab treatment led to exaggerated BAL cell responses. Treatment with anti-protein G mAbs also improved Th1/Th2 cytokine and chemokine responses and markedly improved lung histopathology.

### Conclusion

These data support the view that anti-G protein mAbs improve RSV disease by blocking CX3C mediated immune dysregulation and improving the protective early antiviral responses.





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## Quantification of Clesrovimab (MK-1654), an Investigational, Half-life Extended, Anti-Respiratory Syncytial Virus Protein F Human Monoclonal Antibody in the Nasal Compartment of Healthy Adults

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Kalpita Vora - ARN10264

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### Background

Respiratory syncytial virus (RSV) is a respiratory pathogen that poses a mortality risk for infants and elderly. Antibodies binding to Pre-fusion F protein have demonstrated potent neutralizing RSV activity both in vitro and in vivo and hence have been used prophylactically in the clinic to prevent RSV infection. To that end, Clesrovimab (MK-1654), is an investigational fully human, half-life extended monoclonal antibody (mAb) against RSV F glycoprotein in clinical trials as a prophylactic agent preventing RSV infection in infants.

### Method

MK-1654 concentrations in humans and non-human primates can be measured easily from sera to establish a pharmacokinetic profile. However, establishing MK-1654 levels at the site of RSV infection, such as the nasal compartment, is essential to correlating the serum PK levels and exposure in the target tissue for RSV neutralization. This study measures MK-1654 concentrations in the serum as well as nasal compartment to establish the partitioning of the mAb, enabling assessment of their relationship.

### Result

For mAbs with YTE mutations, ~1-2% of serum antibodies were detected in nasal mucosa. We have used urea concentrations to normalize the MK-1654 concentrations from nasal samples from two separate adult human clinical trials and find ratios of 1:28-1:48 (nasal:serum) which translates to 2-4% of serum concentrations. The PK in the nasal compartment mirrors that of the serum and corroborates estimates of peripheral volume of distribution that suggest extravascular distribution of MK-1654. In a small phase Ib/IIa dose escalating study (P002), a lower proportion of RSV upper respiratory tract infections was observed in the infants who received MK-1654 than in infants who received placebo.

### Conclusion

These ratios for a FcRn mediated t<sub>1/2</sub> extended mAb would signal higher tissue penetration and could be an added advantage along with extended half-life in protecting infants against RSV using passive prophylaxis approaches.



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## Pediatric burden and seasonality of Human Metapneumovirus over five years in Managua, Nicaragua.

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**Kathryn Hacker** - ARNI0269

**Kathryn Hacker**<sup>1</sup>

<sup>1</sup>University of Michigan

### Background

Human Metapneumovirus (hMPV) is an important cause of pediatric respiratory infection. We leveraged the Nicaraguan Pediatric Influenza Cohort Study (NPICS) to assess the burden and seasonality of symptomatic hMPV infection in children.

### Method

NPICS is an ongoing prospective study of children in Managua, Nicaragua. We assessed children for hMPV infection via RT-PCR. We used classical additive decomposition analysis to assess the temporal trends and Generalized Growth Models (GGMs) were used to estimate effective reproduction numbers.

### Result

From 2011-2016 there were 564 hMPV symptomatic infections yielding an incidence rate of 5.74 cases per 100 person-years (95% CI 5.3, 6.2). Children experienced 3,509 Acute Lower Respiratory Infections (ALRIs), of which 160 (4.6%) were associated with hMPV infection. Children under the age of one had 55% of all symptomatic hMPV infections (62/112) develop into hMPV-associated ALRIs and were five times as likely as children over one to have an hMPV-associated ALRI (Rate Ratio 5.5 95% CI 4.1, 7.4  $p < 0.001$ ). Additionally, symptomatic reinfection with hMPV was common. In total, 87 (15%) of all observed symptomatic infections were reinfections. The seasonality of symptomatic hMPV outbreaks varied considerably. From 2011-2016, four epidemic periods were observed, following a biennial seasonal pattern. The mean ascending phase of the epidemic periods were 7.7 weeks, with an overall mean estimated reproductive number of 1.2 (95% CI 1.1, 1.4).

### Conclusion

Symptomatic hMPV infection was associated with substantial burden among children in the first year of life. Timing and frequency of symptomatic hMPV incidence followed biennial patterns.



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## Respiratory Syncytial Virus Knowledge, Attitudes, and Perceptions Among Older Adults in the United States

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Elizabeth La - ARNI0274

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### Background

Respiratory syncytial virus (RSV) is associated with considerable morbidity and mortality in the United States (US), including an estimated 177,000 hospitalizations and 14,000 deaths among older adults each year. Despite this burden of disease, no previous studies have assessed the knowledge, attitudes, and perceptions (KAP) of RSV among older adults in the US, with previous studies focusing on RSV-related KAP among other patient populations or health care providers. This study evaluates RSV-related KAP among US adults who are at risk of RSV infection, with particular focus on older adults.

### Method

A cross-sectional, web-based survey was administered between May to June 2022 to better understand respiratory infection- and RSV-related KAP among US adults who are at risk of RSV infection. The survey included ≥200 adults in each of 4 subgroups: older adults aged 60-89 years and adults aged 18-59 years with ≥1 chronic cardiac condition, chronic pulmonary condition, or diabetes.

### Result

The survey was completed by 827 adults, including 224 older adults. Awareness of RSV was generally lower than for other respiratory infections, with fewer than 1 in 3 older adults (n=72/224; 32.1%) reporting that they had ever heard of RSV (Figure 1). Although 68.3% (n=153/224) of older adults considered themselves to be knowledgeable about respiratory infections, only 18.1% (n=13/72) of those aware of RSV reported being knowledgeable about it. Examples of identified knowledge gaps included the bacterial vs. viral nature of infections, RSV seasonality, common symptoms of RSV, and the extent to which RSV is a cause of respiratory infections for specific patient populations. Among older adults aware of RSV, more than half were unsure about their risk of RSV-related hospitalizations and deaths compared to influenza. Most older adults aware of RSV (n=53/72; 73.6%) agreed that they rarely consider RSV as a potential cause of illness when they have cold- or flu-like symptoms.

### Conclusion

Despite repeat RSV infections throughout life and 3-7% of healthy older adults being infected with RSV each year in the US, results from this study highlight important knowledge gaps related to RSV, as well as gaps in perceived risk and severity of RSV. These findings emphasize the need for education efforts ahead of the availability of vaccines to prevent RSV in older adults.



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## THE EFFICIENCY OF P27 PEPTIDE CLEAVAGE DURING IN VITRO RSV INFECTION IS CELL LINE AND RSV SUBTYPE DEPENDENT

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### Background

The Fusion protein (F) is highly conserved between RSV/A and RSV/B subtypes. Enzymatic cleavage of the Respiratory Syncytial Virus (RSV) F protein precursor yields two subunits (F1 and F2) and releases a 27 amino acid peptide (p27), whereas partial cleavage retains p27 in the mature F protein. For virus-cell fusion to occur, the F protein precursor undergoes a dramatic conformational change from a metastable pre-F to a stable post-F state, and p27 must be cleaved entirely. Our objective is to determine the amounts of p27 on RSV/A and RSV/B subtypes and if its detection depends on F protein conformation.

### Method

Western Blot, ELISA, and Imaging Flow Cytometry were used to detect p27 epitope and antigenic sites II and Ø in three prototypical RSVs (GA1 and GB1 genotypes) and two contemporary strains (ON and BA genotype) in sucrose purified RSV (spRSV) and on infected HEp-2 and A549 cells.

### Result

Sucrose purified RSV/A (spRSV/A) had higher levels of p27 (22% vs. 14%) and Site Ø (35% vs. 12%) than spRSV/B; the spRSV/A F protein on pre-F conformation was able to withstand higher temperatures than spRSV/B. Similar trends were observed in RSV-infected cells. Between 60%-80% of HEp-2 cells infected with any RSV/A retained p27, but only 10%-20% of HEp-2 cells infected with RSV/B of any genotype retained p27; levels of Site Ø were comparable between subtypes on infected HEp-2 cells. On A549 cells, less than 20% of cells infected with any RSV showed F protein retaining p27; levels of Site Ø were subtype dependent on infected A549 cells. On either cell line, cells infected with RSV/A showed a higher proportion of F proteins on pre-F conformation able to withstand higher temperatures than RSV/B.

### Conclusion

The efficiency of p27 cleavage by furin-like enzymes after F protein synthesis is lower on RSV/As, as they retained more p27 than RSV/Bs. The efficiency of cleavage of p27 was also cell-line dependent, as HEp-2 cells showed a higher proportion of F proteins retaining p27 than A549 cells. RSVs which F proteins harbored higher levels of p27 could sustain the pre-F conformation better during temperature-stress studies. Thus, the incomplete cleavage of p27 may confer higher stability to the pre-F conformation, and the cleavage efficiency is subtype and cell line dependent.