Poster Reception III

Alhassan Yakubu - AOXI0005

Molecular characterization of haemagglutinin genes of influenza B viruses circulating in Ghana during 2016 and 2017

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Background

Recent reports of haemagglutinin antigen mismatch between vaccine composition strains and circulating strains, have led to renewed interest in influenza B viruses. Additionally, there are concerns about resistance to neuraminidase inhibitors in new influenza B isolates. To investigate the presence of the new influenza B strains, selected influenza B-positive samples from 2016 and 2017 were genetically characterized.

Method

Viral RNA was isolated from archived influenza B positive samples collected in Ghana. Full-length HA genes were PCR-amplified and amplicons sequenced.

Result

A total of eleven amino acid substitutions were detected in the B/Victoria lineage and six in the B/Yamagata lineage. The strains of influenza B viruses were closely related to influenza B/Brisbane/60/2008 (fig 1) and influenza B/Phuket/3073/2013 (fig 2), vaccine strains for the Victoria and Yamagata lineages, respectively at the time.

Conclusion

Three main amino acid substitutions (P31S, I117V and R151K) were found in B/Victoria lineages circulating between 2016 and 2017, while one strain of B/Victoria possessed a unique glycosylation site at amino acid position 51 in the HA2 subunit. Two main substitutions (L172Q and M251V) were detected in the HA gene of the B/Yamagata lineage. Though these mutations or glycosylation sites were identified, it could not be inferred what characteristics would be conferred on these strains. Perhaps, the glycosylation site could confer immune-escape as shown in other studies, hence warrants further investigation. Close monitoring of the patterns of influenza B evolution is necessary for the efficient selection of representative viruses for the design and formulation of effective influenza vaccines.

Poster Reception III

Cheryl Cohen - AOXI0042

Comparison of characteristics of infection in individuals with wild-type, Beta and Delta SARS-CoV-2 variants in the PHIRST-C community cohort study, South Africa, 2020-2021

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Background

Data on the characteristics of individuals with mild and asymptomatic infection with different SARS-CoV-2 variants of concern are limited, particularly from sub-Saharan Africa. In South Africa the first wave was dominated by SARS-CoV-2 wild-type, the second by Beta, and the third by Delta variants. We aimed to compare the characteristics of individuals with SARS-CoV-2 infection caused by wild-type, Beta, and Delta variants.

Method

We conducted a prospective cohort study of randomly selected households during July 2020-August 2021. Mid-turbinate nasal swabs were collected twice-weekly from household members irrespective of symptoms and tested for SARS-CoV-2 using real-time reverse transcription polymerase chain reaction (rRT-PCR). Sera were collected every two months and tested for anti-SARS-CoV-2 anti-nucleocapsid antibodies. Differences in demographic, symptom and infection episode (duration and viral load inferred through cycle threshold value (Ct) characteristics) by variant were evaluated using multinomial regression.

Result

We included 1200 individuals (643 at the rural site and 557 at the urban site) from 222 households, among whom there were 648 PCR-confirmed infection episodes with onset >14 days after study enrolment with available data on variant. Of these, 66 (10%) were wild-type, 260 (40%) Beta, and 322 (50%) Delta. At the end of follow-up, 57 (4·8%) individuals aged ≥18 years across both sites were fully vaccinated against SARS-CoV-2. Proportions of symptomatic infections were similar for wild-type (7, 11%), Beta (44, 17%), and Delta (46, 14%) infections (p=0.4). On multivariable analysis, compared to wild-type infection, infection with Beta was more common in individuals aged 5-12 years (vs 19-39 years) (adjusted odds ratio (aOR) 2.6, 95% confidence interval (CI) 1.1-6.6) and Ct value <30 (vs >35) (aOR 3.2, 95% CI 1.3-7.9). Infection with Delta was more common in individuals aged <5 years (aOR 6.7, 95% CI 1.4-31.2) and 5-12 years (aOR 6.6 95% CI 2.6-16.7) (vs 19-39 years) and Ct value <30 (aOR 4.5, 95% CI 1.3-15.5) and 30-35 (aOR 6.0, 95% CI 2.3-15.7) (vs >35). Individuals infected with Delta were also more likely to have had a previous SARS-CoV-2 infection (aOR 6.2, 95% CI 2.3-16.4).

Conclusion

Consecutive SARS-CoV-2 waves with Beta and Delta variants were associated with a shift to proportionately more infections in children. Increasing immunity in adults from previous infection or vaccination may have led to a relative immunity gap in children. Changing variant characteristics could also lead to increasing infections in children. Beta and Delta infections were associated with lower Ct values (a proxy of higher viral load) potentially leading to increased infectiousness.

Poster Reception III

Hannah Maier - AOXI0088

SARS-CoV-2 infection-induced immunity and the duration of viral shedding: results from a Nicaraguan household cohort study

Hannah Maier[1], Miguel Plazaola[2], Roger Lopez[3], Nery Sanchez[2], Saira Saborio[3], Sergio Ojeda[2], Carlos Barilla[2], Guillermina Kuan[3], Angel Balmaseda[3], Aubree Gordon[1]

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Background

Much of the world's population has been infected with SARS-CoV-2 and thus, infection-induced immunity will play a critical role in future SARS-CoV-2 transmission. We investigated the impact of immunity from prior infection on duration of viral shedding and viral load.

Method

We used an ongoing household cohort in Managua, Nicaragua with an embedded transmission study that closely monitors participants regardless of symptom status. Real-time reverse-transcription polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assays (ELISAs) were used to measure infections and seropositivity, respectively. Blood samples were collected in Feb/March and Oct/Nov 2020 and 2021, and surrounding household intensive monitoring periods. We used accelerated failure time models to compare shedding times. Participants vaccinated ≥14 days prior to onset of viral shedding were excluded.

Result

There were 600 RT-PCR-confirmed SARS-CoV-2 infections between May 1, 2020 and March 10, 2022 with ELISAs prior to infection and no vaccination ≥14 days prior to infection. Prior infection was associated with 48% shorter shedding times, event time ratio (ETR) 0.52 (95% CI: 0.39-0.69, mean shedding times: 13.7 vs 26.4 days). A 4-fold higher anti-SARS-CoV-2 spike titer was associated with 17% shorter shedding (ETR 0.83, 95% CI: 0.78-0.90). Similarly, maximum viral loads (lowest CT) were lower for previously infected individuals (mean CT 29.8 vs 28.0, p = 4.02x10-3). Shedding was significantly shortened in adults and children ≥10 years old, not in children 0-9 years old, and there was little difference in CT for adults above age 60.

Conclusion

Prior infection-induced immunity was associated with shorter viral shedding and lower viral loads.

Poster Reception III

Hannah Maier - AOXI0089

An immune correlate of SARS-CoV-2 infection and severity of reinfections

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Background

An immune correlate of protection from SARS-CoV-2 infection is urgently needed. Here we investigate the protection associated with seropositivity resulting from prior infection, determine the anti-spike antibody titers associated with protection, and compare the severity of first and second infections.

Method

We conducted a household cohort study of Nicaraguans aged 0 to 94 years. The cohort has an embedded transmission study that closely monitors participants for infection regardless of symptom status. Real-time reverse-transcription polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assays (ELISAs) were used to measure infections, seropositivity, and antibody titer.

Result

Between March 2020 and October 2021, we followed 2,353 people aged 0 to 94 years of age in 437 households. Only 61 (2.6%) had been vaccinated, the earliest on March 20, 2021. In March 2021, 62.3% of the cohort was seropositive. After March 2021, gamma and delta variants predominated in the cohort. Seropositivity in March 2021 was associated with 69.2% protection from any infection (95% CI: 60.7%-75.9%) through-October 2021, with higher protection against moderate or severe infection (79.4%, 95% CI: 64.9%-87.9%). Anti-spike antibody titers of 327 and 2,551 were associated with 50% and 80% protection from any infection, respectively; titers of 284 and 656 were associated with protection against moderate or severe disease, respectively. Second infections were less severe than first infections (Relative Risk (RR) of moderate or severe disease: 0.6, 95% CI: 0.38-0.98) and were twice as likely to be subclinical (RR of subclinical disease: 1.9, 95% CI: 1.33-2.73).

Conclusion

Prior infection-induced immunity is protective against infection and disease. The infection-induced correlates of protection will aid in vaccine development and estimating future SARS-CoV-2 transmission and disease burden. While second infections were somewhat less severe, moderate and severe second infections did occur. Thus communities like this that have already suffered high infection rates will still benefit greatly from vaccination.

Poster Reception III

Amanda Howa - AOXI0094

Neighborhood deprivation and the risk of SARS-CoV-2 infection in households

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Background

The ongoing COVID-19 pandemic has contributed to over six million deaths worldwide. Examining how socioeconomic factors impact SARS-CoV-2 spread in households can help inform and improve access to interventions and public health strategies for prevention and control. Using the Area Deprivation Index (ADI), we examined socioeconomic characteristics of neighborhoods and the risk of SARS-CoV-2 infection in households.

Method

A case-ascertained study was conducted in Nashville, TN from April 2020 to April 2021, recruiting households with an index case infected with SARS-CoV-2. After informed consent, index cases and other household members were enrolled and completed symptom diaries and self-collected nasal swabs and saliva samples daily for 14 days. Specimens were tested for SARS-CoV-2 using RT-PCR. The 2019 United States ADI for each enrolled household was obtained according to the census block level of the neighborhood of residence. The ADI summarizes neighborhood socioeconomic characteristics on a percentile scale, with higher percentiles corresponding to more disadvantaged areas compared to all others nationally. Multivariable logistic regression was used to test the association between ADI rank and the risk of infection among household members exposed to an index case, accounting for age, sex, and the number of people within the household and clustering at the household level.

Result

Among the 162 households, 270 household members were enrolled. The mean age of household members was 33 years (range: 1-77) and 52% were female. The median ADI rank was 27.5% (interquartile range: 12-38%; range 3-81%). Of the 270 household members, 158 tested positive (58.5%; 95% Confidence Interval = 52.6-64.2%). In multivariable logistic regression, the odds of members becoming infected with SARS-CoV-2 was 1.23 (95% CI: 1.03-1.48) higher for every 10 percentile ranks increase in the national ADI, after accounting for relevant covariates. The figure illustrates the model-derived predicted probabilities of infection according to the range of observed ADI ranks (Figure).

Conclusion

These findings indicate that household members living in more disadvantaged neighborhoods are more likely to have household SARS-CoV-2 infections. This increased risk could be driven by individual components of area deprivation or other unmeasured factors, such as possible correlations between ADI measures of unemployment and income and workplace exposures. Further research is needed to clarify the role of socioeconomic and other factors on the risk of infections within households.

Poster Reception III

Stefano Bonazza - AOXI0052

Exposing mechanisms for the addition of RNA modifications on influenza A RNAs, and the interaction between FluPol and host epitranscriptomic writers.

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Background

During infection, IAVs exploit and circumvent the cellular machinery to achieve robust and sustained gene expression, leading to high levels of replication. As such, understanding all facets of virus-host interaction is crucial to develop new antiviral strategies. In the last few years, a whole new layer of gene expression regulation has been uncovered for viruses: post-transcriptional modification of RNAs. These "epitranscriptomic" modifications of viral transcripts constitute a new aspect of host-pathogen interaction, so far almost entirely overlooked and untapped. RNA modifications were found to provide regulation of splicing, translation efficiency, and immune evasion in a variety of viruses.

Recent evidence shows that all IAV transcripts are modified with a host of different modifications and that their ablation results in decreased viral fitness. However, the mechanisms of deposition of epitranscriptomic modifications onto Influenza transcripts are unknown. Host transcripts are physiologically modified by cellular enzymes dubbed epitranscriptomic writers, which often act co-transcriptionally by associating with the host RNA polymerases. IAV encodes for its own RNA polymerase, the FluPol, which is responsible for the synthesis of the three types of viral transcripts. Unbiased interactome screenings have found some writer proteins associated with subunits of the vRNPs, but these interactions were never validated or explored further. This work investigates the relationship between the FluPol and epitranscriptomic writers in the context of RNA modification deposition.

Method

The interactions between several different epitranscriptomic writers and the FluPol were assessed using microscopy approaches. First, we generated two reporter WSN viruses expressing either mNeongreen-tagged or FLAG-tagged PA, to visualise the FluPol. Then, using combinations of smFISH and immunofluorescence we were able to image viral transcripts and host writers at various timepoints post infection. This approach allowed us not only to investigate the interactions, but also their localisation in the infected cell.

Result

Conclusion

This work allowed us to identify host RNA modification machinery hijacked for efficient replication of influenza viral RNAs. This data sets the groundwork for further research into the mechanism of RNA modification deposition during Influenza infection and the role of the FluPol as a post-transcriptional modification platform for viral transcripts. This novel research has a great potential to uncover new fundamental mechanisms for RNA biology as well as Influenza infection, that could guide the development of epitranscriptomic inhibitors to treat IAV infection.

Poster Reception III

Mengying Liu - AOXI0057

Human-type sialic acid receptors contribute to avian influenza A virus binding and entry by hetero-multivalent interactions

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Background

"Avian" IAVs crossing the species barrier require adaptation to human receptors for efficient human-to-human transmission. Pandemic influenza A viruses (IAV) adapt to human receptors by switching from binding "avian-type" α2-3-linked sialic acid (2-3Sia) to human-type 2-6Sia receptors. IAVs bind SIA receptors by their hemagglutinin (HA). The affinity of a single HA-SIA interaction is low and IAVs depend on multivalent interactions to bind with high avidity. Cells to which IAVs bind in vivo expose diverse SIA receptors that bind with different affinities to a specific IAV strain. However, in vitro studies often focus on sialoglycans that bind with highest affinity as "the receptor", without considering simultaneous interactions of a virus particle with multiple receptors, that differ by their individual binding affinities. Here, we studied whether receptors of lower affinity, abundantly present in the diverse receptor landscape exposed on cells, contribute to virus binding and entry.

Method

IAV receptor binding was analyzed by biolayer interferometry (BLI) using receptor surfaces presenting mixtures of synthetic 2-3Sia and 2-6Sia glycan receptors at different ratios. We used a HEK293ï„Sia cell line devoid of SIA expression by knockout of all 2-3 and 2-6 sialytransferases and genetically remodeled its Sia repertoire by transfection with ST6Gal1 and ST3Gal4 for production of a glycoprotein carrying different ratios of 2-3/2-6SIA in order to determine IAV binding to a more natural receptor repertoire. In parallel, IAV entry into equally remodeled HEK293ï„Sia cells was analyzed to correlate binding to entry.

Result

Binding of virus particles to synthetic glycans showed that "human-type" 2-6Sia receptors, by themselves not supporting binding of "avian" IAVs, efficiently enhanced binding of highly 2-3SIA-specific "avian" IAV strains. Efficient binding required only a low density of high-affinity avian-type receptors that on its own hardly supported binding. We refer to this as "heteromultivalent" binding. Next, using the HEK293ï„Sia cells remodeled with different ratios of 2-3/2-6SIA on N-linked glycans, we determined IAV binding to the expressed glycoprotein, in parallel to IAV entry into equally remodeled HEK293 cells. The results show that low-affinity receptors, assumed to be no receptors at all, drastically lower the density threshold for binding to high affinity receptors, thereby enhancing IAV entry.

Conclusion

We conclude that, considering the heterogeneity of sialoglycan receptors encountered in vivo, hetero-multivalent binding is physiologically relevant and will impact evolutionary pathways leading to host adaptation. This finding challenges the canonical view of avian IAVs binding 2-3Sias and human IAVs binding to 2-6Sias.

Poster Reception III

Ibrahim Eldaghayes - AOXI0012

Surveillance and assessment of risk factors for the spread of avian influenza virus type A in live birds markets located in Tripoli, Libya

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Background

This study was conducted to determine the potential role of live birds markets (LBMs) in the transmission of the avian influenza virus (AIV).

Method

A total of 269 cloacal swabs were collected from different birds species in nine LBMs located in Tripoli and its surroundings. The LBMs included in this study were Souq Tajoura, Souq Aljumaa, Souq Althulatha, Souq Alahad, Souq Alkhamees, Souq Alsaeh, Souq Janzour, Souq Suliman Khater, and Souq Alhot. The target species were duck, geese, local chicken, Australian chicken, Brahma chicken, turkey, pigeon, quail, peacock broiler chicks, and pet birds. Following extraction of RNA, the samples were tested against AIV type A using real-time RT-PCR (rRT-PCR).

Result

Out of 269 samples collected from nine LBMs, 28 samples were tested positive for AIV type A, which represents 10.41% of the total samples. The positive LBMs were Souq Aljumaa, Souq Alkhamees, Souq Althulatha, and Souq Tajoura. The highest percentage (35.71%) of AIV was recorded in Souq Aljumaa. Positive samples for AIV were mainly found in three birds' species. Ducks showed the highest percentage (14/65; 21.5%), followed by local chickens (12/98; 12.24%) and goose (2/28; 7.14%). The prevalence of AI type A had three risk factors: The time spent at the market (odds ratio (OR) = 11.181, 95% credibility interval (CI) =3.827-32.669), disposal of dead birds (OR = 2.356; 95% CI = 1.005-5.521) and the last visited LBM (OR = 0.740; 95% CI = 0.580-0.944). The protective factor was the last visited LBM.

Conclusion

The results of this study alarmed a high risk of LBMs for the spread of AI and therefore highlighted the need for future continuing research.

Poster Reception III

Geok Kee Ng - AOXI0036

Evolution of deletions and co-occurring mutations of influenza B viruses in Singapore

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Background

Influenza B viruses were first detected in humans in 1940 and by the 1980s had evolved into two distinct lineages, Victoria and Yamagata. Influenza B viruses cause seasonal epidemics, leading to significant morbidity and mortality worldwide. In Singapore, influenza activity occurs year-round with biannual peaks in May-July and November-February. Influenza B viruses account for at least 20% of all influenza infections in Singapore. Since 2016, multiple clades of both lineages have co-circulated globally. Our previous work has shown that haemagglutinin (HA) variants with 2- or 3- amino acid (aa) deletions have emerged in the Victoria lineage that co-occur with mutations on other genes. Here, we examine the genetic diversity and evolution of the circulating influenza B viruses in Singapore.

Method

Over 320 influenza B positive nasal swabs were collected from Singapore General Hospital. Complete genomes of influenza B viruses were sequenced at the Duke-NUS Genome Biology Facility. The libraries were prepared using Illumina Nextera XT DNA library kits and sequenced on an Illumina MiSeq with 250 bp paired-end reads. We analysed influenza B viruses from Singapore and subsampled global datasets. Individual gene phylogenies were reconstructed using maximum-likelihood methods, and non-synonymous amino acid mutations were mapped on the phylogenies. We used reverse genetics to assess the effect of observed deletions and mutations on the replicative fitness of influenza B viruses.

Result

During 2017-2019, Victoria lineage with 2-aa deletion variants were predominant in Singapore and globally. Recently, 3-aa deletion variants showed clade expansion since early 2021, coinciding with more mutations on the HA surface proteins (i.e., N150K, G184E, R279K, A127T, P144L and K203R), and eventually replaced the 2-amino acid deletion variants. Interestingly, all 3-aa deletions variants from Singapore appeared to form a monophyletic clade, which may have occurred due to limited international travel during the COVID-19 pandemic. We optimized the reverse genetics methods to generate recombinant influenza B viruses based on 12-plasmids for the virus rescue.

Conclusion

We observed changing evolutionary dynamics of influenza B viruses, which could be affected by combinations of viral and human factors, including genetic drift and COVID-19 measures.

Poster Reception III

Michael Zeller - AOXI0037

Diversity of swine influenza A virus in Cambodia

Michael Zeller[3], Sothrya Tum[2], Sorn San[2], Chou Monidarin[5], Arata Hidano[1], Hannah Holt[1], Jordan Ma[3], Foong Ying Wong[3], Geok Kee Ng[3], Gavin Smith[4], Yvonne Su[3]

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Background

Production and consumption of livestock has increased dramatically worldwide in recent decades. Implications of the rapid growth of commercial farms for risk of zoonotic spillover remain unclear, especially in Cambodia. Recent transitions to large-scale swine production operations in Cambodia may have increased the risks for the transmission of influenza A virus (IAV) within the country. Herein, we conducted active IAV surveillance among pigs in Cambodia. Our study aims to understand the current swine influenza diversity in circulation and to identify the risks of zoonotic transmission of IAV between pigs and humans.

Method

During March 2020-July 2021, nasal swabs were collected from 2,220 pigs at 17 slaughterhouses in four provinces. Samples were screened for IAV using real-time reverse transcription polymerase chain reaction (RT-qPCR). Positive samples were sequenced on a MiSeq NGS platform. Consensus sequences were generated and analysed with subsampled global IAV datasets downloaded from the Influenza Research Database. Phylogenetic trees were inferred for each of the eight gene segments using RAxML.

Result

Out of 2,220 swine nasal swab samples, 41 (1.8%) were IAV-positive. Complete and partial swine influenza sequences were retrieved, including 32 H1, 2 H3, 18 N1, and 1 N2. At least 12 full genomes were obtained and co-infections were detected in 4 samples. Phylogenetic reconstruction indicated that 30 HA sequences were closely related to pandemic human H1N1 virus, 2 were H1N1 classical swine virus of the alpha lineage, and 2 were H3N2 human seasonal virus. The NA phylogeny mirrored the diversity of the HA sequences where all pandemic H1 were paired with pandemic N1. Of note, two NA swine sequences were derived from the Eurasian avian-like H1N1 swine lineage, whereas two other NA sequences originated from human H3N2 virus.

Conclusion

Our data suggests that a high diversity of swine IAV is present in Cambodian pigs, with concurrent circulation of both H1 and H3 subtypes, including a relatively high frequency of pandemic H1N1 viruses. Our HA-H1 phylogeny shows that the swine H1N1 viruses are likely derived from a recent human-to-swine transmission event, indicating a complex interplay of human-swine transmission. Cambodia, as well as South East Asia as a whole, remains a surveillance gap in influenza monitoring, despite hosting broad IAV diversity.

Poster Reception III

Krista Kniss - AOXI0085

Influenza and SARS-CoV-2 co-detections in the United States, 2021-2022

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Background

Influenza virus and SARS-CoV-2 have similar routes of transmission and can cause a wide variety of response, ranging from asymptomatic or mild illness to serious disease or death. There is concern that influenza/SARS-CoV-2 co-infection could complicate diagnosis and treatment, and worsen illness severity. Data on the occurrence of influenza/SARS-CoV-2 co-infection are lacking. In this analysis we describe influenza virus and SARS-CoV-2 co-detection identified at Public Health Laboratories (PHLs) using Centers for Disease Control and Prevention (CDC) surveillance data.

Method

SARS-CoV-2 and influenza virus test results are reported to CDC by PHLs, along with basic demographic and clinical information. Specimens that were confirmed by PCR for influenza and SARS-CoV-2 from October 2021 to March 2022 were used to calculate frequencies of co-detections, proportion of co-detections by influenza type (A or B), and by age group and were later compared with influenza trends for this season.

Result

Influenza virus detections increased from early November through mid-December 2021. From late December 2021 through late January 2022, during the rapid rise in SARS-CoV-2 B.1.1.529 (Omicron) variant detections, influenza activity declined; however, since early February 2022, influenza detections increased again. The predominant influenza virus reported so far this season is influenza A(H3N2). The majority of influenza viruses reported by PHLs are from persons aged 5-24 years (52.4%), followed by those 25-64 years (30.2%), ≥65 years (9.2%) and 0-4 years (8.1%). Among 574,059 specimens tested for both pathogens, 8,428 (1.5%) specimens were influenza positive; of these, 473 (5.6%) had a SARS-CoV-2 co-detection. The largest number of co-detections occurred during a period in which influenza and SARS-CoV-2 detections were both at a high level. The majority (461; 97.5%) of the co-detections were SARS-CoV-2/Influenza A; only 2.5% (n=12) were with influenza B. The highest proportion of co-detections were among persons aged 5-24 years (52.7%), followed by those 25-64 years (31.6%), 0-4 years (8.2%), and ≥65 years (7.6%).

Conclusion

Influenza/SARS-CoV-2 co-detections from October 2021 through March 2022 were similar to detections of influenza virus during that time in terms of the predominant influenza virus, age groups most affected, and timing of occurrence. As influenza and SARS-CoV-2 viruses continue to evolve, it will be important to determine whether codetections follow similar patterns as described here.

Poster Reception III

Julie TS Chu - AOXI0020

INVESTIGATION OF VACCINE INDUCED HETEROSUBTYPIC IMMUNE PRESSURE ON GENOME WIDE EVOLUTION OF SEASONAL INFLUENZA A VIRUSES IN A MOUSE MODEL

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Background

Seasonal influenza vaccines have limited efficacy that can be further curtailed by antigenic divergence from the circulating strain. Next generation "universal" vaccines have the potential of conferring an increased breadth of protection against mismatched and pandemic strains, though host immune pressure resulting from widespread vaccine usage has been implied to contribute to antigenic diversification, leading to inherent concern for the emergence of escape phenotypes that can circumvent universal protection.

Method

A murine-based pipeline was developed to investigate the effects of broadly protective vaccine-imposed selection pressure on influenza A virus (IAV) evolution. Mice vaccinated with a live attenuated influenza vaccine (LAIV) generated in a H1 background were subject to lethal challenge with seasonal H3N2. Viral progeny from infected mice lung homogenates were serially passaged in immunized and naïve populations 18 times. Lung viral RNA isolates from distal time points were assessed for genome wide minor variant changes by Illumina Novaseq. High frequency variants identified were characterized for their functionality.

Result

Heterosubtypic immune selection increased the rate of genome wide SNV occurrence, though early variant populations were dominated by stochastic mutants. Drift that occurred within major immunodominant regions of the surface antigen was not dependent on host immune status. Highly virulent phenotypes that ablated vaccine mediated cross-protection manifested after sustained adaptation under heterosubtypic immune selection. Genotypic markers associated with host-specific virulence unique to escape phenotypes were identified within the polymerase encoding segments.

Conclusion

Broadly protective vaccines with the capacity to protect against mismatched IAV strains can increase the incidence of mutations across the viral genome. Sustained exposure to vaccine conferred non-sterilizing immunity drives emergence of virulent escape phenotypes. These findings can serve to inform the potential impacts of population wide broadly protective influenza vaccine usage and as a preliminary assessment of the risk of universal vaccine driven escape.

Poster Reception III

Li Qi - AOXI0022

The 1957 pandemic Influenza HA gene signal peptide plays a role in virus virulence and replication

Li Qi[1], Ashley Freeman[1], Zong-mei Sheng[1], Stephanie William[2], Jeffery Taubenberger[1]

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Background

The 1957 influenza A/H2N2 pandemic was the second major influenza pandemic to occur in the 20th century with an estimated one to two million deaths worldwide. To identify the genetic basis of the virulence of the H2N2 1957 Influenza virus, we constructed a series of chimeric viruses. Intranasal infection of Balb/c mice with these viruses suggests that in addition to the PA gene, the HA gene is an important virulence factor. Specifically, we provide evidence that the 4th amino acid of the H2 HA signal peptide is critical for HA-associated virulence and that virulence requires both presence of the mutation in the HA gene and the PA V100I mutation epistatically. Increased virulence appeared to be associated with increased replication in vitro and in vivo.

Method

To explore the underlying mechanisms, we employed RNA sequencing analysis of 293T cells expressing different "minigenomes" (4 RNP expression plasmids + 1 pol I plasmid H2 avian HA). We used 4 different combinations of the signal peptide and PA mutations for transfection of the 293T cells. The RNA seq was performed and IPA pathway analysis revealed at least seven genes to be differentially expressed by at least four-fold.

Result

The data suggest that in the presence of the PA V100I mutation, the signal peptide mutation promotes upregulation of HA gene expression by downregulation of the expression of the seven genes and the shut off UTF1 and LOC112268313 pseudogene expression. Downregulated genes were ZNF628, TIMP1, PRR36, SRM, SF3A2, HCN2 and VPREB3. To this end we have blocked ZNF628 gene expression by siRNA and we are verifying successful suppression of ZNF628 expression by Western blot analysis. Once we have confirmed suppression of ZNF 628 expression, we will infect siRNA treated A549 cells with mutant and wild-type viruses to measure the effects on virus replication. If this approach proves successful, we will analyze the other six genes in a similar manner.

Conclusion

In summary we show for the first time that the signal peptide of the influenza HA protein plays a role in virus replication and virulence, and we hope that this will be beneficial for future novel anti-influenza drug development.

Poster Reception III

Feline Benavides - AOXI0043

Persistent infection of seasonal and pandemic influenza A viruses in a hiPSC-derived neural model

Feline Benavides[1]

[1]Erasmus MC

Background

Infection with influenza A virus is generally mild, although central nervous system (CNS) complications can occur in the acute phase of infection and occasionally in the post-acute phase. The pathogenesis of influenza viruses associated CNS disease is largely unknown, but evidence suggest that influenza viruses can enter the CNS through cranial nerves. Once inside the CNS, the cell tropism, replication efficiency, and associated immune response of seasonal or pandemic influenza viruses is largely unknown. The aim of our study was to investigate the interaction of seasonal and pandemic influenza viruses with cells of the CNS using a human induced pluripotent stem cell (hiPSC)-derived neural model, consisting of neurons and astrocytes.

Method

We employed a rapid differentiation protocol to differentiate hiPSCs to neurons by forced overexpression of the transcription factor Neurogenin (Ngn2). Additionally, we directed hiPSCs through neural progenitor cells into astrocytes. Subsequently, a co-culture of neurons with astrocytes was made that support the survival and maturation of neurons. Co-cultures were infected with pandemic (2009 pH1N1) or seasonal influenza viruses (H1N1 and H3N2 isolated in 2019) after which we determined the cell tropism, replication kinetics and associated immune responses.

Result

Inoculation of neural co-cultures with pandemic (2009 pH1N1) and seasonal influenza viruses (H1N1 and H3N2 isolated in 2019) revealed infection of predominantly neurons with all three viruses, although pH1N1 virus and H1N1 2019 virus infected more neurons than H3N2 2019 virus, based on immune fluorescent staining. However, infection of the neural co-cultures did not result in the production of progeny virus, despite an increase in viral RNA over time. Virus-infected neurons could be detected up to 10 days post infection (dpi). The number of infected neurons remained stable in time, without morphological evidence for cell death or induction of cleaved caspase-3 detected by immune fluorescent staining.

Conclusion

Altogether, our data so far suggest that neurons can be infected with seasonal and pandemic influenza viruses. Infection seems persistent as the same number of cells can be detected up to 10 dpi, without evidence for ongoing virus spread in the cultures or the induction of apoptosis. Ongoing experiments aim to elucidate the anti-viral and neuroinflammatory responses and how replication is halted in neurons. In addition, functional studies will be done to assess whether persistent infected neural co-cultures have functional neural networks.

Poster Reception III

Bri Luis - AOXI0053

Most Influenza Researchers Personally Support Open Science, so Why Are So Few Doing It?

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Background

A lack of transparency is not just a crisis in the social and behavioral sciences -- it is also an issue in microbiology (Schloss, 2017). While practices such as pre-registration and data sharing have spread in some communities, they are not widespread in areas such as virology or pre-clinical influenza research. This has become especially evident as a result of the COVID-19 pandemic, which has served as a catalyst for the rapid adoption of practices such as pre-printing (Besançon et al., 2021). Prior to the pandemic, scientists reported that pressure to publish and selective reporting often contributed to issues with reproducibility (Baker, 2016). To this point, replications and null results reporting are often overlooked for publication because cultural incentives emphasize novelty over verification (Nosek et al., 2012), indicating a disconnect between the push for better science and the academic publishing community. This is important for policy makers to know because transparency in influenza research can save lives (Besançon et al., 2021). The Open Scholarship Survey asked influenza researchers about their beliefs, behaviors, and perceptions regarding open science practices for pre-registration and null results reporting.

Method

The Open Scholarship Survey (77 questions total) was distributed from February to March, 2021 and 2022. Data were collected from researchers within the fields of influenza and virology research.

Result

In 2021, influenza researchers (n = 228) reported favorable attitudes toward the open science practices of pre-registration and null results reporting. However, these same respondents perceived their peers to have much less favorable views toward these same activities (see Figure). Respondents also indicated whether they included a pre-registration (19%) in their most recent publication or published null results (51%) the last time they received them.

Conclusion

The presented data demonstrate a disconnect between what influenza researchers believe regarding open science and how influenza researchers think their peers see open science practices. This disconnect may partially explain why influenza researchers report favorable views toward open science practices, such as pre-registration and null results reporting, but do not engage in these practices regularly. Illuminating the overall favorability of influenza researchers toward open science practices may ultimately lead to a more transparent research process. This is important for policy makers to focus on because transparency in influenza research can save lives (Besançon et al., 2021).

Poster Reception III

Carlos Grijalva - AOXI0154

Evolution of contact patterns in households with a confirmed SAR-CoV-2 infection - Nashville, TN - USA. April 2020-April 2021

Carlos Grijalva[3], Pietro Coletti[2], Niel Hens[2], Christel Faes[2], Melissa Rolfes[1], Alexandra Mellis[1], Carrie Reed[1], Ahra Kim[4], Keipp Talbot[4], Yuwei Zhu[4]

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Background

Most studies of SARS-CoV-2 household infections have not collected detailed data on the type of contacts between household members. Not including these exposure differences within households could impact the estimation of epidemiological measurements such as the secondary attack rate. Here, we characterize contact patterns of relevance for the transmission of SARS-CoV-2 infections in households in Nashville, Tennessee.

Method

We enrolled individuals with a laboratory-confirmed SARS-CoV-2 infection (index case) and other household members from April 2020 through April 2021. Households were eligible to enroll within 7 days after symptom onset of the index case and only if the household included at least 1 individual who was not symptomatic at the time of index case onset. Data on social interactions, including physical contacts, among household members were collected through electronic questionnaires at three time points: 1) the day prior to the index case's symptom onset, 2) the day before enrollment (when infection was known to household members) and 3) 14 days later. We examined interactions involving physical contact and we characterized how contact patterns changed throughout the study period using contact density, i.e., the fraction of realized physical contacts relative to the number of possible physical contacts. We used exponential family random graph modeling to identify relevant covariates driving having physical contacts.

Result

We studied 134 households, with 134 index cases and 290 household contacts. The median household size was 3 people (interquartile range: 2 - 4) and 35% households had at least one child. Compared with the contact density measured on the day prior to index case symptom onset, the contact density in households with more than 2 household members decreased on the day before enrollment. However, contact density at 14 days follow-up returned to the levels seen on the day prior to index case symptom onset (Figure). Preliminary modeling results suggest household size ($p<0.005$) and the presence of a child ($p<0.001$) as strong determinants for contact inside the household; and there was a tendency to avoid close contact with the index case right after infection diagnosis ($p<0.001$).

Conclusion

We showed how the presence of a laboratory confirmed SARS-CoV-2 infection leads to a restructuring of social contact patterns in households. These findings indicate that awareness of viral infection introduction into the household reduces close contact with the index case and the overall number of contacts in the household.

Poster Reception III

Ekaterina Stepanova - AOXI0171

In vitro stimulation with live SARS-CoV-2 reveals Th17 dominance in virus-specific CD4 T cell response after COVID-19

Ekaterina Stepanova[1], Igor Kudryavtsev[1], Irina Isakova-Sivak[1], Victoria Matyushenko[1], Polina Prokopenko[1], Anna Chistyakova[1], Arina Goshina[1], Larisa Rudenko[1]

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Background

SARS-CoV-2 and influenza viruses are the main causes of human respiratory tract infections with similar disease manifestation but distinct mechanisms of immunopathology and host response to the infection. These two human respiratory pathogens generate distinct profiles of antigen-specific CD4+ and CD8+ T-cell responses upon infection, which potentially can contribute to the different levels of protection against re-infection with antigenically evolved viruses. Different Th subsets play a main part in synergy between innate and adaptive branches of immunity, resulting in more effective and successful control of an infection. In this study, we determined phenotypes and frequency of main CD4+ T cell subsets specific for SARS-CoV-2 using activation induced cell marker assay and multicolor flow cytometry, and compared the magnitude of SARS-CoV-2- and H1N1 influenza-specific CD4+ T cell responses.

Method

PBMCs were collected from blood samples of nineteen COVID-19 convalescents aged 23 to 74 years, with disease onset ranging between March 2020 and May 2021 and time post symptoms onset between 1 and 15 months. The levels of SARS-CoV-2- and H1N1 influenza-specific CD4+ T responses were determined by stimulating of PBMCs with sucrose gradient-purified live SARS-CoV-2 or H1N1pdm influenza viruses. The following markers were distinguished: CD3, CD4, CD69, CD137, CD45RA, CD62L, CXCR3, CCR4, CXCR5, CCR6.

Result

SARS-CoV-2-specific CD4+ T cells were detected 1-15 months post infection and the frequency of SARS-CoV-2-specific CM CD4+ T cell was increased with the time post-symptom onset. SARS-CoV-2-specific CD4+ T cells predominantly expressed Th17 phenotype, but the level of Th17 cell in this group was lower than in H1N1-specific CD4+ T cells. We distinguished four distinct Th17 subsets within total CCR6-expressing Th17 cells to better define the features of SARS-CoV-2-specific Th17 cells: CCR4+CXCR3- 'classical' Th17, CCR4-CXCR3+ 'non-classical or Th17.1, CCR4-CXCR3- double negative or CCR6+DN Th17 and co-expressing CXCR3 and CCR4 double positive or CCR6+DP Th17. We found that the lower level of total Th17 subset within total SARS-CoV-2-specific CD4+ T cells was linked with the low level of CCR4+CXCR3- 'classical' Th17 cells if compared with H1N1-specific Th17 cells.

Conclusion

Taken together, the above data support the involvement of Th17 cells and their separate subsets in the pathogenesis of pneumonia caused by SARS-CoV-2 and H1N1, and a better understanding of Th17 mediated antiviral immune responses may lead to the development of new therapeutic strategies.

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Poster Reception III

Larisa Labzin - AOXI0181

ACE2 is necessary for SARS-CoV-2 infection and sensing by macrophages but not sufficient for productive viral replication

Larisa Labzin[1], Keng Yih Chew[6], Xiaohui Wang[5], Tyron Esposito[5], Claudia Stocks[5], James Rae[5], Teodor Yordanov[5], Caroline Holley[5], Stefan Emming[5], Svenja Fritzlar[3], Francesca Mordant[3], Daniel Steinfort[2], Kanta Subbaro[4], Anne Lagendijk[5], Robert Parton[5], Kirsty Short[6], Sarah Londrigan[3], Kate Schroder[5]

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Background

Macrophages are a major source of pro-inflammatory cytokines in COVID-19. How macrophages sense the causative virus, SARS-CoV-2, to drive cytokine release is, however, unclear. Macrophages may directly recognise this virus, or alternatively, sense microbial components or alarmins from infected neighbouring cells. While multiple studies have determined that SARS-CoV-2 replication in macrophages is abortive, whether macrophages are susceptible to infection, and whether viral entry and early-stage replication is required for macrophage cytokine responses is unkown.

Method

We assay viral replication and pro-inflammatory cytokine production in primary human macrophages and ACE2-overexpressing THP-1 cells with qPCR, immunoblot, plaque assay, ELISA and electron microscopy.

Result

Human macrophages do not directly sense and respond to infectious SARS-CoV-2 virions because they lack sufficient ACE2 expression to support virus entry and replication. Over-expression of ACE2 in human macrophages permits SARS-CoV-2 entry and early-stage replication and facilitates macrophage pro-inflammatory and anti-viral responses. ACE2 over-expression does not, however, permit the release of newly synthesised virions from SARS-CoV-2-infected macrophages, consistent with abortive replication. Release of new, infectious SARS-CoV-2 virions from ACE2 over-expressing macrophages only occurred if anti-viral mediator induction was also blocked, indicating that macrophages restrict SARS-CoV-2 infection at two stages of the viral life cycle.

Conclusion

These findings resolve the current controversy over macrophage-SARS-CoV-2 interactions and identify a signalling circuit that directly links macrophage recognition of SARS-CoV-2 to restriction of viral replication.

Poster Reception III

Adam Lauring - AOXI0187

Highly transmissible SARS-CoV-2 variants of concern are constrained by a tight transmission bottleneck

Adam Lauring[1], Emily Bendall[1], Amy Getz[1], Amy Callear[1], Drew Edwards[1], Arnold Monto[1], Emily Martin[1]

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Background

Transmission bottlenecks determine how much of the viral diversity generated in one host passes to another. The relationship between the transmissibility of a virus and the size of its bottleneck is unclear. Studies from the "pre-variant" era of SARS-CoV-2 indicate that early strains exhibit a tight bottleneck, typically 1-3 infectious virions. Here, we compare the transmission bottleneck of these non-VOC SARS-CoV-2 to that of the alpha, delta, and omicron variants.

Method

We sequenced samples from individuals with co-incident infection in two household cohorts. The MHome cohort enrolled households based on an index case with a positive clinical test for SARS-CoV-2 and sampled all household members on days 0, 5, 10. The HIVE cohort followed households longitudinally and sampled all household members on days 0, 5, 10 following a symptomatic case. For each individual, the first positive sample with a qPCR cycle threshold ≤30 was sequenced in duplicate on the Illumina platform. Intrahost single nucleotide variants (iSNV) were called at a 2% frequency threshold using iVar. Only iSNV present in both replicates were used in downstream analyses. Bottleneck size was determined using a beta-binomial model for transmission pairs in which both individuals had high quality sequence data and ≥1 iSNV in the donor.

Result

Among 182 samples with high quality sequence data, 120 were from households with ≥2 cases. There were 9 non-VOC transmission pairs and 3 trios; 4 alpha pairs, 1 trio and 2 quartets; 8 delta pairs, 1 trio and 2 quartets; and 8 omicron pairs, 7 trios and 2 quartets. The median time between symptom onset and viral sampling was 5 days (IQR = 0.5-9.5) for index cases and the median time between symptom onset of the index and household contacts was 3 days (IQR = 0.5-5.5). There was a trend toward a shorter sampling interval in delta and omicron variant pairs. In nearly all cases, individuals were sampled prior to or at the peak of viral shedding, as determined by RT-qPCR. Within-host diversity was low, with no iSNV identified in 62 individuals, 1-2 iSNV identified in 48 individuals, and 3-9 iSNV identified in 10 individuals. The transmission bottleneck was calculated on 72 possible transmission pairs. The bottleneck size was 1 (range 1-1) for non-VOC SARS-CoV-2, and for alpha, delta, and omicron.

Conclusion

In this comparative study, we found that despite their increased transmissibility, SARS-CoV-2 variants of concern exhibit a tight bottleneck. Our data suggest that tight bottlenecks are due in large part to the general absence of within-host diversity at the time of transmission. This relationship is more pronounced in variants with a shorter serial interval.

Poster Reception III

Aaron Frutos - AOXI0200

Endemic Coronavirus Infection History and Association with Risk of SARS-CoV-2 Infection in Nicaraguan Children

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Background

Risk factors for SARS-CoV-2 infection in children remain poorly understood. Previous research has found an association between prior endemic human coronavirus (HCoV) and risk of SARS-CoV-2 infection in adults. In a community-based pediatric cohort study in District II of Managua, Nicaragua, we examine if recent HCoV infection and presence of HCoV antibodies are associated with risk of SARS-CoV-2 symptomatic disease or infection from 2020-2021.

Method

Children under 5 years who participated in the Nicaraguan Pediatric Influenza Cohort Study (NPICS) in 2020 or 2021 and had blood sample(s) were selected. Blood samples collected in 2019, 2020 and/or 2021 were tested for IgG spike antibodies to each of the four endemic HCoVs (Alpha: NL63, 229E; Beta: OC43, and HKU1) via an enzyme-linked immunosorbent assay (ELISA). Recent HCoV infections were defined as absence of species-specific endemic HCoV antibodies in one year and presence in the in following (2019-2020, 2020-2021). Samples collected in 2020 and 2021 were also tested for SARS-CoV-2 receptor binding domain (RBD) antibodies. Respiratory samples were collected at the study clinic when participants present with symptoms of respiratory illness. Samples collected from March 2020 to February 2022 were tested for SARS-CoV-2 using reverse transcriptase-polymerase chain reaction (PCR). Risk of symptomatic infection (PCR+) and infection (PCR+ or ELISA+) will be evaluated using a log-binomial model stratified by age (under 2 years, 3+).

Result

895 participants are included in this study from March 2020-February 2022. From 2019 to 2020, there were 121 recent endemic alpha and 128 recent endemic beta coronavirus infections. In 2020, 732 (82%) and 696 (78%) of included participants had spike antibodies to endemic alpha and beta HCoVs respectively. A total of 155 SARS-CoV-2 PCR+ infections were detected with 32 in 2020 and 123 in 2021. By April 2021, 384 (43%) participants had a PCR+ infection or had antibodies against the RBD of SARS-COV-2. ELISA testing for endemic HCoV antibodies is underway and will allow us to compare risk of SARS-CoV-2 infection by recent endemic HCoV infection and presence of HCoV antibodies for the first waves of the pandemic in 2020 with later variant waves (delta and omicron) in 2021 and 2022.

Conclusion

This study will provide critical information on whether recent endemic HCoV infection or presence of endemic HCoV antibodies is associated with SARS-CoV-2 disease and infection risk in young children.

Poster Reception III

Mina Nakauchi - AOXI0169

Analysis of host factors interacting with nonstructural protein 1 of influenza A virus using proximity-dependent biotin identification

Mina Nakauchi[4], Yoko Ino[1], Hitoshi Takahashi[4], Yutaro Yamaoka[2], Eri Nobusawa[4], Kei Miyakawa[2], Yayoi Kimura[1], Akihide Ryo[3], Hideki Hasegawa[4]

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Background

An understanding of the characteristics of virus-host interaction and the development of anti-viral agents are vital to control of infectious diseases. Non-structural protein 1 (NS1) of the influenza A virus has multiple functions, including inhibition of host cell immune responses, promotion of viral replication, regulation of virus particle morphogenesis, and suppression of host apoptotic response. However, host factors involved in each function have not always been identified. In this study, to identify these factors as potential targets for antiviral therapy, we used a novel method, proximity-dependent biotin identification (BioID).

Method

To isolate the host factors being proximal to NS1, we performed BioID assay. In brief, to biotinylate the host factors, a biotin ligase was fused to the N- or C-terminal of NS1 and expressed in 293 cells. The biotinylated host factors were then purified and identified by liquid chromatography with tandem mass spectrometry. Using RNA interference, we confirmed that the expression of the identified host factors was suppressed in A549 cells, then we evaluated the role of these host factors in viral propagation.

Result

Several host factors were identified, including factors involved in RNA modification, transcription, translation, or intracellular vesicle transport in host cells. The gene knockdown analysis demonstrated that some of these host factors positively regulate viral propagation.

Conclusion

The results show that BioID successfully helped screen functional host factors for the propagation of influenza A viruses through NS1 interactions. These host factors are expected to be targets for antiviral therapy

Poster Reception III

Jenny Ching Man Chan - AOXI0195

Tropism, replication competence and innate host response of the novel genotype 4 (G4) swine influenza virus in ex vivo and in vitro cultures of the human respiratory tract

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Background

Influenza A virus (IAV) is a highly infectious respiratory pathogen which causes seasonal epidemics and pandemics globally. Recent studies have reported a novel genotype 4 (G4) reassortant Eurasian avian-like (EA) H1N1 virus in swine demonstrating a potential pandemic threat. Due to the characteristic of swine being susceptible towards human, avian and swine influenza viruses, it is important to assess the risk of the novel swine influenza virus for its likelihood to pose a risk to the public health worldwide.

Method

Tropism, viral replication competence and cytokine induction of the two G4 EA H1N1 strains were compared in parallel with 2009 pandemic H1N1 (H1N1/pdm/09) and A/Quail/HK/G1/1997 H9N2 (G1.H) using ex vivo and in vitro culture of the human respiratory tract. Human lung and bronchus explants were used to study the tropism and viral replication competence, while peripheral blood monocyte derived macrophages were used to investigate cytokines and chemokines induction upon infection.

Result

The two novel G4 strains replicate effectively to a similar competence with H1N1/pdm/09 in bronchus and of similar replication competence with H1N1/09 pandemic and G1.H in the lung. Desialylation-haemagglutination assay suggested that the novel G4 strains had a binding affinity for human-like receptors (α-2,6-linked sialic acid). Human macrophages were susceptible to the G4 virus with a similar replication kinetics compared with H1N1/pdm/09 and G1.H. In general, similar level of cytokine induction were observed in both novel G4 and H1N1/pdm/09 strains. In particular, the G4 and pandemic strains have induced a stronger pro-inflammatory cytokine response than that of G1.H, which has illustrated a significantly high induction of chemokines (MCP-1, RANTES, IP-10 and MIP-1β) in macrophages.

Conclusion

Taken together the replication competence and cytokine induction of the two G4 strains in both in vitro and ex vivo cultures of lung and bronchus, we can therefore extrapolate that there is a potential risk of swine-to-human transmission of the novel G4 EA H1N1 swine influenza with a moderate disease severity in human. The G4 EA H1N1 virus may pose a notable public health threat.

Poster Reception III

Jessica Belser - AOXI0149

Robustness of the ferret model for influenza risk assessment studies: a cross-laboratory exercise

Jessica Belser[1], Eric Lau[7], Wendy Barclay[4], Ian Barr[11], Hualan Chen[3], Ron Fouchier[2], Masato Hatta[10], Sander Herfst[2], Yoshihiro Kawaoka[10], Seema Lakdawala[9], Leo Lee[11], Gabriele Neumann[10], Malik Peiris[7], Daniel Perez[8], Charles Russell[6], Kanta Subbarao[11], Troy Sutton[5], Richard Webby[6], Hui-Ling Yen[7]

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Background

Ferrets represent the preferred animal model for assessing the transmission potential of newly emerged zoonotic influenza viruses, and data from these experiments inform formal risk assessment rubrics established by the WHO and CDC. However, heterogeneity among established experimental protocols and facilities across different laboratories may lead to variable results, complicating interpretation of transmission experiment data generated from multiple laboratories.

Method

Between 2018-2020, a global exercise was conducted by 11 participating laboratories to assess the range of variation in ferret transmission experiments using two common stock H1N1 influenza viruses, A/California/07/2009 (Cal/09) and A/ruddy turnstone/Delaware/300/2009 (ruddy turnstone/09), that possess different transmission potential in ferrets. The inoculation route, dose, and volume were standardized, and all participating laboratories followed the same experimental conditions for respiratory droplet transmission, including the establishment of contact with donor ferrets at 24 hours post-inoculation with a strict 1:1 donor:contact ratio. Additional host (including source of animals, gender, age) and environmental (including humidity, temperature, air changes per hour, cage design) parameters likely to affect influenza transmission kinetics were monitored and recorded throughout all experiments.

Result

Overall transmission outcomes for both viruses across 11 laboratories were concordant, suggesting the robustness of the ferret model for influenza risk assessment despite substantial heterogeneity in numerous non-standardized parameters in experimental setups employed between groups. Among environmental parameters that varied across laboratories, donor-to-contact airflow directionality was associated with increased transmissibility. To attain high confidence in identifying zoonotic influenza viruses with moderate-to-high or low transmissibility, our analyses support that as few as three but as many as five laboratories, respectively, would need to independently perform viral transmission experiments with concordant results.

Conclusion

This exercise facilitates the development of a more homogenous protocol for ferret transmission experiments that are employed for the purposes of risk assessment, and highlights the potential benefit of increased uniformity of employing a 1:1 donor:contact ratio when conducting these assessments.

Poster Reception III

Helen Everett - AOXI0112

Susceptibility of camelids (alpacas) to experimental infection with both influenza C and D viruses

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Background

Influenza D virus (IDV), a new genus within the Orthomyxoviridae family, was initially detected in pigs and cattle. IDV is structurally similar to influenza C virus (ICV). Influenza A, C and D viruses all have non-human maintenance hosts and likely circulate in several mammalian species. Camelids, as a reservoir for zoonotic viruses, were not extensively studied until the emergence of the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012. We previously reported that, in dromedary camels from Kenya, antibody responses to both ICV and IDV could be detected but not differentiated, owing to cross-reactivity (Salem et al, 2017). It was unclear whether these findings reflected a technical issue or suggested a role for camelids in ICV and IDV ecology. The aim of the present project was therefore to investigate the susceptibility of alpaca (Lama pacos), a camelid species, to experimental infection with ICV (C/Victoria/1/2011) and IDV (D/bovine/France/5920/2014).

Method

Groups of seven seronegative alpacas were inoculated intranasally with the respective viruses. Clinical signs were monitored daily and nasal swabs were obtained for 14 days post-infection (dpi) to monitor shedding of viral RNA. Serum samples were obtained pre-inoculation and on 3, 7, 14 and 21dpi. Post-mortem examination (PME) was planned for three alpacas per group on 3dpi and for two alpacas per group on 7 and 21dpi for virus detection in tissues.

Result

Clinical signs remained mild for most animals, and similarly the gross pathology and histology findings were unremarkable. Elevated temperature and slight diarrhoea were observed in some animals in the ICV group. Nasal shedding of viral RNA was detected in nasal swabs between 1-7dpi for both viruses and peaked between 2-4dpi. Assessment of tissues confirmed the presence of RNA for both viruses in the upper respiratory tract (URT) at 3 and 7dpi.

Conclusion

We have demonstrated that alpacas can be experimentally infected with both ICV and IDV with subclinical infection of the URT, suggesting that virus transmission could potentially occur. These findings accord with previous serology results obtained for camelids and indicate a putative role for these species in ICV and IDV ecology. Further studies are warranted to assess whether they may represent asymptomatic reservoirs for IDV.

Poster Reception III

Jenny Ching Man Chan - AOXI0126

Human nasopharyngeal organoid as an experimental platform for the risk assessment of influenza A viruses

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Background

The transmission and pathogenesis of influenza virus is hitherto primarily studied using experimental animal models or in cell cultures in vitro. Organoids, derived from stem cells or organ-specific progenitors, display structures and functions consistent with organs in vivo which can serve as a highly physiological relevant model to study the tropism and pathogenesis of influenza A virus.

Method

By using a non-invasive nasopharyngeal (NP) swab to collect upper airway stem cells, we isolated and differentiated adult NP organoids. The potential application of the NP organoids as a risk assessment platform to study the tropism and pathogenesis of influenza A viruses was determined.

Result

Human adult NP organoids were cultured in chemically defined medium with well-defined supplements and growth factors. NP organoids appeared round with sphere diameters range from 30-80μm. The transmission electron microscope images showed the high expression of basal cells with tight junctions between cells of the NP organoids, which is recapitulating the composition and organization of cell types found in human nasopharynx. To clarify the cell types in NP organoid system, immuno-staining of fixed organoids was performed. The result revealed that the NP organoids contained high abundant of p63a+ basal cells, some SCGB1A1+ secretary club cells, and a few of acetylated-a-Tubulin+ ciliated cells. Lectin staining showed that human NP organoids were highly expressed in SAα2,3Gal and SAα2,6Gal, suggesting that the NP organoid culture contains both human and avian sialic acid receptors. The NP organoid cultures were infected with highly pathogenic avian influenza virus (HPAI) H5N1 and 2009 pandemic H1N1 virus (H1N1pdm). H1N1pdm replicated efficiently with higher replication competence than H5N1, which was comparable to those observed in ex vivo explants model of human conducting airway. Immunohistochemical staining revealed that basal cells, but not club cells were infected by influenza viruses, conferring its role in viral entry and replication.

Conclusion

Overall, we demonstrated that influenza A viruses could infect the NP organoids with similar replication competence and cellular localization to ex vivo human conducting airway explants, and thus provide an alternative physiologically relevant experimental platform for investigating virus tropism and replication competence that could be used to assess the pandemic threat of animal influenza viruses.

Poster Reception III

Jessica Belser - AOXI0139

Detection of influenza A and SARS-CoV-2 viral RNA in exhaled breath of ferrets following ocular inoculation

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Background

While influenza A viruses (IAV) and SARS-CoV-2 are capable of replication within ocular tissue and using the eye as a portal of entry to establish a productive infection, the dynamics of virus spread throughout oculonasal tissues and relative capacity of virus transmission following ocular exposure remain poorly understood. Furthermore, the impact of exposure route on subsequent release of airborne viral particles has not been examined previously.

Method

Ferrets were inoculated by the ocular route with two IAV (H1N1 and H7N9) and two SARS-CoV-2 (early pandemic Washington/01 and Delta variant) viruses (n=3/virus using a high-dose liquid inoculum deposited on the surface of the eye). Replication was assessed in both respiratory and ocular specimens, and transmission was evaluated in direct contact or respiratory droplet settings. Two-stage cyclone aerosol samplers (NIOSH) were employed to quantify viral RNA in aerosols shed by inoculated ferrets. To assess the magnitude of virus released in exhaled breath following a physiologically relevant low-dose exposure, ferrets were inoculated with aerosolized IAV (H1N1 and H7N9) delivered exclusively to the ocular surface, or via respiratory inhalation, at comparable presented doses (n=3/route/virus); viral RNA in aerosols shed by all ferrets inoculated with aerosolized virus was quantified.

Result

All IAV and SARS-CoV-2 viruses mounted a productive and transmissible infection in ferrets following ocular inoculation. Peak viral titers in nasal wash specimens and release of virus-laden aerosols from ferrets inoculated by the ocular route were indistinguishable from ferrets inoculated by previously characterized intranasal inoculation methods. Aerosols shed by infected ferrets with both viruses were primarily in the >4µm size range. Viral RNA was detected in ferret conjunctival washes from all viruses examined, though infectious virus in this specimen was only recovered following IAV virus inoculation. Ferrets were productively infected with IAV following low-dose (<10 PFU) ocular-only aerosol exposure or respiratory inhalation aerosol exposure, highlighting the capacity of IAV to mount productive mammalian infections following multiple exposure routes.

Conclusion

IAV and SARS-CoV-2 viruses can lead to high-titer virus replication in ferrets following ocular exposure, with inoculated animals shedding virus-laden aerosols through day 11 post-inoculation. While the magnitude and kinetics of infectious virus and viral RNA differed somewhat between viruses, these data support that both IAV and SARS-CoV-2 can establish a mammalian infection that is associated with virus in airborne particles in exhaled breath as early as one day post-ocular exposure.

Poster Reception III

Liselotte van Asten - AOXI0152

Severe Acute Respiratory Infections (SARI) in Intensive Care Units: Exploring the reporting delay and surveillance potential in the Netherlands

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Background

Surveillance of Severe Acute Respiratory Infections (SARI) is strongly recommended for pandemic preparedness. In the Netherlands, Intensive care (ICU) admissions are registered in the National Intensive Care Evaluation (NICE) registry, but reported by hospitals with delay. We investigated their potential for near real-time SARI surveillance.

Method

A descriptive study was performed with retrospective data on ICU admissions (NICE, all Dutch adult ICUs). World Health Organization (WHO) proposed severity parameters explored were: SARI incidence, SARI proportion (of all medical admissions) and in-ICU mortality of SARI patients. The APACHE IV severity of illness score was also included. Parameters were explored at different levels of reporting delay and compared to complete data.

Result

Yearly coverage of ICUs by NICE was stable from 2012/13 to 2018/19, but hospitals report their data at varying time points and intervals. After 2/4/6/8/10/12/26/52 weeks of reporting delay the incidence of ICU SARI admissions was 6/22/39/51/61/69/90/97% complete. However, after 2 weeks, 13% of timepoints in the dataset had no data reported yet (down to 0.3% after 4 weeks). Of weekly medical admissions, 13% (±4%) were a SARI admission in the complete data, with a difference of -2.40% and -0.03% in this average after 2 and after 52 weeks reporting delay.

Conclusion

With an unstable 6% of ICU SARI admissions being reported within 2 weeks, the current NICE registry does not yet allow for near real-time SARI surveillance. Data was almost complete (around 90%) after 26 weeks delay. For strengthening surveillance and pandemic preparedness, investing in daily or weekly reporting of all ICUs or a representative subset is recommended.

Poster Reception III

Ka Ling Lai - AOXI0193

The role of Platelet-derived growth factor receptor (PDGFR) and the therapeutic potential of PDGFR inhibitor in MERS-CoV infection

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Background

Middle East Respiratory Syndrome coronavirus (MERS-CoV) is a zoonotic virus that can induce lethal respiratory illness in human. Cytokine storm caused by MERS-CoV is the pathogenic immune responses that contribute to the acute lung injury and the pathogenesis of MERS-CoV infection. Unfortunately, no clinical approved vaccination or treatment for MERS is available. The lack of treatment for MERS and the pandemic potential of coronaviruses lead to a need to develop MERS-specific treatment. Currently, platelet-derived growth factor (PDGF) is believed to be participated in the replication cycle of MERS-CoV and the PDGFR inhibitor is considered as a potential immunomodulator in the acute lung injury model. In this study, we aim to investigate the role of PDGFR in MERS-CoV replication and evaluate the therapeutic potential of PDGFR inhibitor on MERS-CoV infection.

Method

We examined the replication competences and host immune responses of MERS-CoV infection in human airway epithelial cell line (Calu-3), human alveolar epithelial cell line with over-expressing human dipeptidyl peptidase 4 (A549-DPP4) and human lung microvascular endothelial cells (HMVEC-L) in the presence or absence of PDGFR inhibitor. Prototype MERS-CoV EMC strain and South Korean clinical isolate of MERS-CoV were used in this study. Cytokine and chemokine induction were estimated by quantitative real-time polymerase chain reaction. Time-of-drug addition assays were performed to investigate the mechanism of action of PDGFR inhibitor.

Result

The replication kinetics of MERS-CoV in drug-treated cells were significantly decreased compared to the vehicle-treated cells. Mechanistic studies suggested that PDGFR inhibitor interrupts the post-entry step and pre-entry step of MERS-CoV replication cycle in human lung epithelial cells and HMVEC-L respectively. In addition, the proinflammatory cytokine and chemokine expressions were significantly reduced in PDGFR inhibitor treated Calu-3.

Conclusion

Our data demonstrate that PDGFR plays a role in the post-entry and pre-entry steps of the viral replication in human lung epithelial cells and endothelial cells, respectively. In addition, PDGFR inhibitor shows antiviral and immunomodulatory effects on MERS-CoV infection, which gives us a promising therapeutic option for MERS-CoV infection and enhances our understanding of the pathogenesis of MERS-CoV.

Poster Reception III

Keita Fukao - AOXI0167

Therapeutic treatment with baloxavir acid reduces mortality caused by co-infection with influenza A virus and Streptococcus pneumoniae in mice

Keita Fukao[1], Shinji Kusakabe[1], Motoyasu Onishi[1], Takao Shishido[1]

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Background

Bacterial co-infection following influenza virus infection is a crucial cause of severe pneumonia. Despite the availability of vaccines and antivirals, the aggravation of influenza leading serious complications, hospitalization and death remains a major concern worldwide. Therefore, an effective treatment option should be investigated for severe influenza infection. Baloxavir acid (BXA), the active form of orally available prodrug baloxavir marboxil (BXM), is an inhibitor of cap-dependent endonuclease of influenza virus, which is an enzyme that is essential for viral transcription and replication. BXM is licensed in Japan, US and other countries/areas for the treatment of influenza A and B viral infections. Here, we evaluated the efficacy of BXA on mortality and body weight loss induced by co-infection with influenza A virus and Streptococcus pneumoniae in mice.

Method

BALB/c mice were inoculated with A/Osaka/129/2009, followed by an inoculation with Streptococcus pneumoniae SR1326 on day 2 (2 days after virus infection). From day 2, the mice were treated subcutaneously with BXA suspension at a dose of 10 mg/kg (single dose) or orally with oseltamivir (OSP) solution at a dose of 10 mg/kg (bid for 5 days). Control mice were administered subcutaneously with vehicle (0.5% methylcellulose, single dose). Mice (N=4-5/group) were examined once daily for survival and body weight. When the mice lost more than 30% of their body weight compared to their weight pre-infection of virus, they were euthanized and regarded as dead according to humane endpoints.

Result

Co-infection increased mortality compared to either single infection with A/Osaka/129/2009 or Streptococcus pneumoniae SR1326 in mice. When treatment was delayed for 2 days after virus infection, BXA significantly prolonged survival time compared with vehicle or OSP, resulted in 75% survival in BXA-treated group, whereas none of the mice survived in vehicle- or OSP-treated group. In addition, BXA-treated group showed less reduction of body weight compared with the vehicle- or OSP-treated group.

Conclusion

Oral dosing of BXA significantly reduces mortality and body weight loss induced by co-infection with influenza A virus followed by Streptococcus pneumoniae in mice. These results support potential efficacy of BXM in a patient population with severe infection and complications.

Poster Reception III

Angel Ma - AOXI0192

Mesenchymal stromal cell-derived extracellular vesicles alleviate lung damage induced by influenza A/H5N1 virus infection

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Background

Acute lung injury arising from influenza A/H5N1 virus infection is responsible for a high mortality rate, for which few current therapeutic options are available. Key characteristics of acute lung injury include impaired alveolar fluid clearance (AFC) and increased alveolar protein permeability (APP) observed in the damaged lung alveolar epithelium. Mesenchymal stromal cells have regenerative and immunoregulatory properties; their secretome may harbour similar functions. Within the secretome, extracellular vesicles regulate cell-cell communication by transferring RNA and functional proteins between cells. Therefore, we hypothesized that mesenchymal stromal cell-derived extracellular vesicles (MSC-EVs) can attenuate the lung damage induced by influenza A/H5N1 virus infection.

Method

A physiologically relevant in vitro human lung injury model using primary culture of human alveolar epithelial cells grown on transwell inserts was used in the study. Extracellular vesicles (EVs) were isolated from the MSC culture medium by ultracentrifugation. Cells were infected with influenza A viruses A/HK/483/97 (H5N1), A/HK/415742/2009 (H1N1pdm) and A/Quail/HK/G1/1997 (H9N2), then treated with MSC-EVs post-infection for 24 hours. The rate of AFC and APP across the alveolar epithelium in the transwell were measured. The proinflammatory cytokine and epithelial transporter gene expression profiles were analysed by RT-qPCR.

Result

Influenza H5N1 significantly reduced net alveolar fluid transport and increased APP at 24 hours post-infection, with moderate impaired effects observed in H1N1pdm- and H9N2-infected cells. The administration of MSC-EVs to virus-infected alveolar epithelial cells restored impaired AFC and decreased APP in vitro. MSC-EVs also reduced the mRNA expressions of dysregulated proinflammatory cytokines (including IL-1β, IP-10 and IL-8) and enhanced the virus-suppressed epithelial transporters (CFTR, epithelial sodium channel (ENaC) and sodium potassium pump (NaKATPase)) in influenza H5N1-infected alveolar epithelial cells, therefore minimising epithelial damage.

Conclusion

MSC-EVs can exert similar anti-inflammatory properties as their parent MSC cells in attenuating influenza A-associated lung injury. This study highlights the potential therapeutic role of human MSC-EVs in maintaining alveolar fluid transport and cellular integrity in the human alveolar epithelium damaged by influenza A/H5N1 virus infection, which furthers our understanding on the pathogenesis of influenza infection in humans and provides a potential novel strategy for severe human influenza disease treatment.

Poster Reception III

Takuhiro Sonoyama - AOXI0194

Post-Hoc Analyses of Ensitrelvir Phase 2b part: Exploratory Clinical Endpoints for Phase 3 part in Phase 2/3 Study (SCORPIO-SR) in Patients with Mild to Moderate COVID-19 Symptoms

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Background

Ensitrelvir is a novel oral selective inhibitor of 3C-like protease of SARS-CoV-2 currently under clinical development. Multicenter, randomized, placebo-controlled, double-blinded phase 2b part was conducted during the omicron phase in 428 patients with mild to moderate COVID-19 symptoms. Ensitrelvir 125 mg group (375 mg once daily on Day 1 and 125 mg once daily on Day 2 to Day 5) and 250 mg group (750 mg once daily on Day 1 and 250 mg once daily on Day 2 to Day 5) showed significant reduction of infective viral tier and viral RNA load vs placebo. However, ensitrelvir did not result in significant improvement on Day 6 in the total score of 12 COVID-19 symptoms vs placebo. Here, we conducted post-hoc analyses to explore suitable clinical endpoints for this population.

Method

Participants recorded 12 COVID-19 symptoms using a 4-point scale (0, none; 1, mild; 2, moderate; 3, severe), and anosmia and dysgeusia themselves using a 3-point scale (0, same as usual; 1, less than usual; 2, no sense) twice daily until Day 9. Pre-specified clinical outcome was based on time to first improvement of 12 symptoms. Definition of improvement was all symptoms rated as mild or none except for pre-existing symptoms before COVID-19 onset. Post-hoc analysis was based on time to first complete resolution of 12 symptoms. Definition of complete resolution was all symptoms rated as none except for pre-existing symptoms. Post-hoc analyses also assessed time to onset of anosmia or dysgeusia.

Result

No difference was observed between ensitrelvir and placebo in time to improvement of 12 symptoms, whereas median time to first complete resolution was 169.4 hours, 154.7 hours, 243.4 hours in ensitrelvir 125 mg, 250 mg, and placebo group, respectively (Figure). The differences in the median time [95%CI] to symptom resolution between ensitrelvir and placebo were -74.0 [-175.8, 14.1] hours (p=0.0939, Stratified log-rank test) for 125 mg and -88.7 [-188.4, -1.8] hours (p=0.0406) for 250 mg. Ensitrelvir reduced anosmia onset (p<0.001 and 0.082 for 125 mg and 250 mg, respectively, log-rank test) and dysgeusia onset (p<0.001 and 0.074 for 125 mg and 250 mg, respectively) vs placebo.

Conclusion

Time to first improvement of symptoms did not discern clinical benefit for Omicron infection which is less severe vs infection with previous variants of concern. In post-hoc analyses based on time to first complete resolution of symptoms, ensitrelvir showed a favorable trend compared to placebo. Endpoints based on time to first complete resolution of symptoms will be used to assess clinical benefit of ensitrelvir in the ongoing phase 3 part. Ensitrelvir reduced onset of anosmia or dysgeusia vs placebo.

Poster Reception III

Amanda Seekings - AOXI0262

In vivo modelling of SARS-CoV-2 skin-to-skin and bioaerosol transmission

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Background

SARS-CoV-2 remains a major global health concern with substantial research focusing on charactering genetic variants, pathogenesis and virus transmission. Identifying the mechanisms of virus transmission from fomites and infectious bioaerosols from the environment are critical to further our understanding on SARS-CoV-2 infection. Porcine skin was identified as a surrogate for human skin to investigate the risk of skin-to-skin virus transmission to establish infection in ferrets as a model for mild/asymptomatic SARS-CoV-2 infection in humans.

Method

The oral-nasal skin surfaces of naïve ferrets were exposed to porcine skin spiked with SARS-CoV-2 B.1.617.2 Delta variant to determine if skin can act as a fomite for establishing infection. A separate group of ferrets were directly infected intranasally with the same virus and co-housed with uninfected naïve ferrets to assess virus transmission and bioaerosol generation. Virus shed from mucosal orifices and into the environment were analysed.

Result

Ferrets exposed to porcine skin spiked with SARS-CoV-2 became infected with shedding primarily seen from the oral-nasal route up to 14 days post-infection (dpi). Directly infected ferrets shed virus from 2 dpi with evidence of efficient virus transmission to contact ferrets. Comparable levels of viral RNA were detected in all groups at the early stages of infection. Extended oral-nasal shedding up to 22 dpi was detected in the directly infected and contact ferrets compared to ferrets exposed to contaminated skin. All ferrets cleared infection and seroconverted after 28 dpi. Viral RNA was detected from the air sampled from within cages providing evidence of bioaerosol environmental contamination. Genome sequencing will compare aerosolized samples with samples obtained from the ferret upper respiratory tract to assess viral adaptation.

Conclusion

Ferrets exposed to SARS-CoV-2 contaminated skin can act as a route for establishing infection, highlighting a risk pathway for public health and veterinary health with regards to zoonotic and reverse-zoonotic transmission.

Poster Reception III

John Kubale - AOXI0292

SARS-CoV-2 Seropositivity, Symptomatic Reinfection, and Influenza Co-infection in Children in Nicaragua

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Background

Better understanding of the burden of COVID-19 among children and their risk of re-infection is crucial as children have high infection rates, low vaccination rates and will likely play an increasingly important role in transmission as immunity is established in the population.

Method

We conducted a prospective, community-based pediatric cohort study of 2021 non-immunocompromised children aged 0-14 years in Managua, Nicaragua from March 1, 2020, through March 25, 2022. Respiratory samples were collected from children presenting to the study clinic and blood samples were collected yearly in February/March. SARS-CoV-2 infection was confirmed by positive anti SARS-CoV-2 antibodies (receptor binding domain [RBD] and spike protein) or real time RT-PCR. PCR positives occurring in children with prior antibodies or with confirmed SARS-CoV-2 infection ≥60 days prior to current positive were considered re-infections. Influenza infection was confirmed by RT-PCR.

Result

In this cohort study the mean age of participants was 6.9 years, and 50.2% were male. Serology was available for 1817 (89.9%) participants of whom 56.4% (95% Confidence Interval [CI]: 54.1, 58.6) were seropositive by November 15, 2021. There were 299 PCR-confirmed COVID-19 cases, 12 (4.0%) of which were severe enough to require hospitalization. Incidence of COVID-19 was highest among children aged <2 years-18.8 per 100 person-years (95% CI: 15.3, 22.9)-approximately three times that of children in any other age group assessed. Additionally, 101 (33.8%, 95% CI: 28.4, 39.1) symptomatic SARS-CoV-2 episodes were documented re-infections with 36 (35.6%) occurring in the wave driven by the Omicron variant in January/February 2022. This wave coincided with the circulation of influenza A/H3N2, representing the first substantial transmission of influenza in Nicaragua since the beginning of the COVID-19 pandemic in 2020. A total of 15 COVID-19/influenza A co-detections were observed, however, this number did not differ from what would be expected by random chance (p=0.9).

Conclusion

In this prospective community-based pediatric cohort rates of symptomatic and severe COVID-19 were highest among the youngest participants, with rates stabilizing around age 5. Additionally, reinfections represent a large proportion of symptomatic COVID-19 cases in the cohort.

Poster Reception III

Haowei Wang - AOXI0300

Community prevalence and risk factors for SARS-CoV-2 infection prior to widespread vaccination in England: results from the REal-time Assessment of Community Transmission swab-positivity (REACT-1) study

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Background

The rapid spread of SARS-CoV-2 caused high levels of hospitalisation and death in England in late 2020 and early 2021, prior to the widespread uptake of vaccination. A detailed understanding of the risk factors for infection, as a precursor for disease, will help prioritise interventions during possible future waves of severe SARS-CoV-2 variants or during the initial phases of the emergence of similar respiratory viruses.

Method

We analysed trends in prevalence and risk factors associated with SARS-CoV-2 infection, using swab-positivity data from the REal-time Assessment of Community Transmission-1 (REACT-1) study between September 2020 and March 2021. Samples were randomly collected from populations aged above 5 years across England. Risk factors were assessed by fitting multivariable logistic regression models.

Result

Between 18th September 2020 and 31st March 2020, we observed that the prevalence of SARS-CoV-2 infection changed over time, with a continuous increase after round 5, reaching its highest point in round 8 at 1.57% (1.49%, 1.66%), followed by significant fall during round 9 and 10, giving a prevalence of 0.20% (0.17%, 0.23%) at the end of March 2021. School-age children (5-17 years) and young adults (18-24 years) were over-represented among those testing positive. Regional prevalence showed substantial heterogeneity, with an initial higher prevalence in five northern regions. But with the emergence of the Alpha variant, the prevalence was highest in London at 2.83% (2.53%, 3.16%) between 6th and 22nd January 2021, with surrounding areas of South East and East of England also high at 1.61% (1.46%, 1.77%) and 1.78% (1.57%, 2.02%) respectively. After the start of the third national lockdown in January, substantial falls in prevalence were seen in all regions, with northern regions in England again higher than the south. Compared to the White ethnic group, Asian and Black ethnic groups showed a higher risk of infection with odds of 1.46 (1.27, 1.69) and 1.35 (1.11, 1.64) respectively. Among ethnic subgroups, the highest and the second-highest odds were found in Bangladeshi and Pakistani at 3.29 (2.23, 4.86) and 2.15 (1.73, 2.68) respectively compared to British white participants. Health care workers with direct patient contact and care

home workers with or without direct contact with clients showed higher odds of infection compared to other essential/key workers.

Conclusion

Our findings show substantial differences in community prevalence and risk of SARS-CoV-2 infections among subgroups of the population in England during a period where the risk of disease and death from infection was substantial. Planning for future severe waves of respiratory pathogens should include policies to reduce inequality driven by ethnicity, household size, and occupational activity.

Poster Reception III

Julie McAuley - AOXI0310

A naturally occurring human SARS-CoV-2 variant has an increased affinity for murine ACE2 and productively infects mice.

Julie McAuley[1], James Cooney[2], Stefanie Bader[2], Leigh Harty[1], Samantha Grimley[1], Rebecca Plavcak[1], Vincent Corbin[1], Lachlan Coin[1], Raelene Pickering[3], Merlin Thomas[3], Pellegrini Marc[2], Damian Purcell[1]

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Background

Murine infection models enable delineation of disease aetiology and are an essential component of pre-clinical vaccine, antiviral and therapeutic testing. However, early SARS-CoV-2 isolates were unable to infect mice unless they were genetically modified to express human ACE2, and infection caused extreme pathology. Genetically modified SARS-CoV-2 to alter binding affinity for mouse ACE2 enabled murine infection, but potentiated problems for evaluating vaccine and antiviral efficacy. Therefore, we sought a naturally occurring human SARS-CoV-2 variant to establish a murine infection model and enable exploration of COVID-19 progression and pre-clinical testing.

Method

Using www.GISAID.org, we probed the genomic sequences of SARS-CoV-2 isolates from Australian infections for the mouse adaptive Spike N501Y mutation and selected isolate hCoV-19/Australia/VIC2089/2020 for testing. Computational prediction of docking of the VIC2089(N501Y) variant indicated the binding interface of murine ACE2 supported interaction. We used soluble human- and mouse-ACE2 in a plaque inhibition assay to confirm murine-ACE2 binding. We then established the murine infection potential of the VIC2089(N501Y) isolate via both aerosolization and intranasal inoculation of adult and aged (6 month) wild-type mice and assaying respiratory organs for viral load, histopathology and immune responses.

Result

During the Victorian June-September 2020 outbreak, the Spike N501Y mutation dominated SARS-CoV-2 isolate consensus sequences and we confirmed these viruses could productively infect wild-type mice. Adult mice infected with the VIC2089(N501Y) isolate exhibited minimal weight loss and illness, while aged mice had an enhanced lung cytokine storm response. Findings were congruent with the majority of otherwise healthy adults experiencing mild COVID and enhanced vulnerability of the elderly during this outbreak. Passaging of VIC2089(N501Y) through mice resulted in host-adaptive mutations and increased virulence in adult mice.

Conclusion

Our findings reveal a naturally occurring human SARS-CoV-2 can infect mice and provides a robust model to pre-clinically test vaccines, antivirals and therapeutic treatments and explore long-COVID development.

Poster Reception III

Luis Antonio Haddock - AOXI0299

Narrow bottlenecks constrain influenza A virus genetic diversity during direct contact transmission

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Background

Influenza A viruses (IAV) exist within a host as dynamic and diverse populations where related variants arise by low-fidelity replication. Transmission of IAV between hosts is subject to physical and biological barriers, imposing genetic bottlenecks that can sharply reduce IAV genetic diversity. Population bottlenecks therefore play an important role in determining the evolutionary pathways taken by IAV on larger scales. Here we sought to characterize the mechanisms that govern IAV population bottlenecks within and between hosts.

Method

We created diverse IAV libraries bearing molecular barcodes on the Hemagglutinin (HA) and Polymerase Acidic (PA) genes, enabling high-resolution tracking and quantification of over 105 unique viral lineages within hosts. We performed site-specific inoculation of barcoded IAV in the upper respiratory tract of ferrets and tracked viral diversity as infection spread throughout the respiratory tract and by direct contact transmission to naïve hosts. We quantified molecular barcodes via targeted deep sequencing approaches and custom bioinformatic analysis.

Result

We show that within-host infections of IAV are readily influenced by bottlenecks that result in anatomical compartmentalization, leading to genetically distinct populations throughout the respiratory tract. Barcode quantification and bottleneck size estimations revealed that most viral diversity is lost following compartmentalization, where viruses are stochastically sampled, resulting in dramatic expansions of dominant genotypes in different lung lobes. Direct contact transmission markedly reduced viral diversity between donor and recipient animals. However, 1000s of unique viral lineages were present in recipient animals 1 day post-exposure, followed by a further substantial reduction in diversity 3 days post-exposure. These data suggest that infection in a new host is sustained by a small subset of viruses from the larger pool that is initially transferred from the donor.

Conclusion

Anatomical compartmentalization and narrow transmission bottlenecks may contribute significantly to the low rates of intrahost adaptation observed during acute infections. Our findings suggest that IAV diversity may be shaped by not just bottlenecks and genetic drift occurring before and during transmission, but also in the recipient host. These within- and between- host populations appear to be stochastically shaped by genetic drift and founder effects, with no apparent evidence for positive selection, further limiting the evolution of IAV. Understanding the population dynamics shaping IAV diversity may shed light on the spread and evolution of these viruses.

Poster Reception III

Taronna Maines - AOXI0313

Risk assessment of emerging influenza viruses using the ferret model

Taronna Maines[1], Jessica Belser[1], Xiangjie Sun[1], Joanna Pulit-Penaloza[1], Nicole Brock[1], Terrence Tumpey[1]

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Background

Influenza A viruses from avian, swine, and other zoonotic sources continue to jump species barriers resulting in human infections. While the majority of these cases are self-limiting, each introduction into humans provides an opportunity for the zoonotic influenza virus to adapt to the human host and potentially lead to a pandemic. Avian influenza viruses (H5, H7, H9), as well as H1 and H3 variant, swine-origin influenza viruses, have caused hundreds of human infections in recent years. There is a critical need for timely and continuous evaluation of these novel and emerging influenza viruses to determine the threat they pose to public health. Risk assessment rubrics (such as IRAT) consider virus, population, ecological and epidemiological properties to estimate the relative risk to humans. Transmission in laboratory animals is one of ten risk elements used to calculate an IRAT score.

Method

s: Serologically naïve ferrets were inoculated with contemporary influenza A viruses that were associated with human infections, including both North American and Eurasian lineages. Inoculated ferrets were each housed with a naïve ferret either in the same cage (Direct Contact Transmission Model) or in an adjacent cage that prevents contact between animal pairs (Respiratory Droplet Transmission Model) to assess transmissibility. All animals were monitored for clinical signs of infection and virus shedding and systemic dissemination were measured.

Result

s: A variant H3 influenza virus that was isolated from an individual in North America in 2017 transmitted well in both models, although not to all animal pairs, while low pathogenic avian influenza H7N1, H7N2, and H7N3 viruses also isolated in North America (2016-2018) did not transmit as efficiently, despite replicating productively throughout the ferret respiratory tract. H9N2 Eurasian influenza viruses that caused human infections (1999-2018) exhibited lineage-specific transmissibility in the absence of severe disease. Highly pathogenic avian influenza H5 viruses (clade 2.3.4.4b, 2017-2021) did not display consistent transmission among cohoused ferrets.

Conclusion

s: There is substantial diversity in the transmission phenotypes of influenza viruses that are capable of jumping the species barrier. Currently, assessments of viral pathogenicity and transmissibility cannot be ascertained solely by antigenic, genetic, or molecular analyses. As zoonotic influenza viruses continue to expand their host range and cause human infections, data generated from the ferret model is necessary to inform risk assessment rubrics and contribute towards the evaluation of vaccine and antiviral strategies to prevent and control the spread of influenza worldwide.

Poster Reception III

Tian Bai - AOXI0258

Influenza A(H7N9) virus infection in men is associated with testosterone depletion: a retrospective study

Tian Bai[3], Yongkun Chen[4], Sebastian Beck[3], Stephanie Stanelle-Bertram[3], Nancy Kouassi Mounogou[3], Tao Chen[1], Jie Dong[1], Bettina Schneider[2], Tingting Jia[4], Jing Yang[1], Lijie Wang[1], Lothar Kreienbrock[2], Dayan Wang[1], Yuelong Shu[4], Gülsah Gabriel[3]

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Background

Human infections with avian influenza A (H7N9) virus that emerged in East China in 2013 causing high mortality rates were more frequently detected in men compared to women over the last five epidemic waves. However, molecular markers associated with poor disease outcome in men are yet unknown.

Method

In this study, we systematically analyzed sex hormone and cytokine levels in men and women with laboratory-confirmed H7N9 influenza in comparison to control cohorts, including H7N9 negative close contacts, H7N9 negative poultry workers as well as men and women with laboratory-confirmed seasonal H1N1/H3N2 influenza (n=369). The underlying association of H7N9 infection and dysregulation of sex hormones was further investigated in vivo using a mouse model for influenza infection.

Result

Multivariable analysis revealed that H7N9 infected men present significantly reduced testosterone levels associated with poor outcome compared to non-infected control cohorts. Regression analysis revealed that testosterone levels in H7N9 men are negatively associated with several pro-inflammatory cytokine levels such as IL-6 and IL-15. In male mice, H7N9 influenza virus infection depleted testosterone levels up to 3 days post infection. Moreover, viral RNA as well as a proinflammatory cytokine profile were observed in the testes of H7N9 infected mice.

Conclusion

Collectively, these findings suggest that low testosterone levels pose a poor prognostic marker in H7N9 infected men. Monitoring sex hormone levels may therefore support individualized patient management in infections with avian influenza viruses.

Poster Reception III

Lisa Bauer - AOXI0260

The systemic inflammatory response to influenza A virus infection is fueled by endothelial cells

Lisa Bauer[2], Laurine Rijsbergen[1], Lonneke Leitjen[1], Feline Benavides[1], Rory De Vries[1], Rik De Swart[1], Debby Van Riel[1]

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Background

Influenza A virus infections can lead to severe disease which is often associated with a dysregulated systemic inflammatory response in the lungs and circulation. Even though the primary target cells of influenza A viruses in humans are respiratory epithelial cells, it is becoming increasingly clear that endothelial cells play a key role in the pathogenesis, especially in modulating systemic inflammatory responses. Here, we investigate the contribution of endothelial cells to the systemic inflammatory responses in an in vitro co-culture model.

Method

To study the contribution of endothelial cells to the systemic inflammatory response in a model that mimics the pulmonary environment, we made use of a transwell system with well-differentiated organoid derived lung epithelial cells on the apical side and primary lung microvascular endothelial cells (LMEC) on the basolateral side. In LMEC cultures and epithelial-LMEC co-cultures, we studied susceptibility to infection with pandemic 2009 H1N1 virus (pH1N1) and seasonal H1N1 and H3N2 viruses from 2019, and studied the associated inflammatory responses.

Result

Direct inoculation of LMECs resulted in infection, based on nucleoprotein expression, without evidence for productive infection and spread. In contrast, in epithelial-LMEC co-cultures, inoculation of epithelial cells with influenza A virus did not result in infection of LMECs, despite abundant infection of epithelial cells and breakdown of the epithelial barrier. In LMEC single cultures virus inoculation triggered a modest cytokine response (IL-6, IL-8, IP-10). However, when we infected epithelial cells in co-cultures, a stronger immune response was observed in the LMECs compared to single LMEC cultures. This included production of type-I-IFN, IP-10 and several interleukins. In lung tissues from experimentally inoculated ferrets, we confirmed the induction of inflammatory cytokines in endothelial cells by in situ RNA hybridization. In general, responses were stronger after inoculation with pH1N1 virus compared to seasonal H1N1 and H3N2 viruses

Conclusion

We show that LMECs do not become infected when co-cultured with influenza A-infected epithelial cells. However, the interaction with infected epithelial cells amplifies the inflammatory response in LMECs. In vivo, cytokines and chemokines produced by endothelial cells will directly enter the circulation, thereby contributing to the systemic cytokine response. Ongoing studies aim to identify factors that trigger inflammatory responses in endothelial cells.

Poster Reception III

Amanda Seekings - AOXI0261

The role of gamebirds as a bridging host for the introduction of H5N8 and H5N1 high pathogenicity avian influenza viruses into galliform poultry

Amanda H Seekings[2], Yuan Liang[1], Caroline J Warren[2], Charlotte K Hjulsager[3], Saumya S Thomas[2], Fabian Z X Lean[4], Alejandro Nunez[4], Paul Skinner[2], David Selden[4], Marco Falchieri[4], Hugh Simmons[4], Ian H Brown[2], Lars E Larsen[1], Ashley C Banyard[2], Marek J Slomka[2]

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Background

Incursions of clade 2.3.4.4 H5Nx high pathogenicity avian influenza viruses (HPAIVs) were identified in farmed pheasants in the UK during the 2020/2021 and 2021/2022 epizootic seasons, resulting in high pheasant mortality. Interestingly, the affected pheasant farms in the UK included adjacently-housed partridges, yet H5Nx infection was not identified in this species. H5Nx HPAIV infection in partridges has been rarely observed and it is postulated that these birds may be more resistant to HPAIV infection in the field than other gamebird species.

Method

Pathogenesis followed by intra- and inter-species transmission of H5N8-2021 and H5N1-2021 HPAIVs isolated from pheasants in the UK was investigated experimentally in pheasants and partridges. The ability for onward virus transmission to chickens was assessed to understand the role of gamebirds as a bridging host between wild birds and galliform poultry.

Result

Directly inoculated pheasants required a lower H5N8-2021 virus dose compared to H5N1-2021 to become infected and shed detectable virus. However, when directly inoculated with the highest virus dose (106 EID50), more rapid systemic infection was detected with H5N1-2021 compared to H5N8-2021. Efficient intra-species transmission to contact pheasants was successful for both viruses, with inter-species transmission to a first chicken-contact group also shown to be efficient. However, further onward transmission to additional chickens was only evident with H5N1-2021. Gross pathological changes were identified in pheasants and chickens with a stronger systemic distribution evident for H5N1-2021. Transmission of both viruses from pheasants to partridges was very limited, with an inability for further onward transmission among partridges. Partridges directly inoculated with the highest dose of H5N1-2021 all became infected and transmitted to all contact partridges. Viral genetic evolution and adaptation was analysed to evaluate genetic adaptations as a result of sequential passage both within game birds and following cross-species transmission.

Conclusion

Our data demonstrates that gamebirds are susceptible to H5Nx HPAIV infection although partridges appear refractive to infection when compared directly with pheasants. H5N8-2021 and H5N1-2021 pheasant infection, mortality and onward transmission to chickens demonstrated that pheasants can contribute to the ongoing risk of virus introduction into galliform poultry settings. Futher, these data support a low level possible zoonotic risk to operatives working in the gamebird industry.

Poster Reception III

Joseph Bresee - AOXI0285

Partnership for Influenza Vaccine Introduction (PIVI): sustainable expansion of influenza vaccine programs in low and middle-income countries, 2013-2021

Joseph Bresee[1]

[1]Task Force for Global Health

Background

PIVI is a public/private partnership with Ministries of Health, technical agencies and industry that supports the development of seasonal influenza vaccination programs and pandemic response capacity in low and middle-income countries by providing low-cost or donated vaccine and technical assistance in program planning, implementation, and evaluation. We evaluated the success of the PIVI model in creating sustained influenza vaccination programs in partner countries.

Method

We analyzed 2013-2021 data about vaccine doses acquired by partner countries collected before and after each PIVI-supported vaccination campaign. Annual vaccine wastage was calculated, and information about vaccine program policies, uptake, and challenges were collected via intermittent surveys. PIVI surveyed at least one vaccine target group in 10 partner countries about vaccine acceptability between 2018 and 2020. Influenza vaccine program information about non-PIVI countries was collected from WHO's Immunization Portal.

Result

During 2013-2021, PIVI expanded support from one to 18 low and middle-income partner countries in 4 WHO regions and delivered 4,745,478 influenza vaccines (annual median 475,411; range: 18,500-799,179). Countries vaccinated persons with underlying conditions (33% of doses), older adults (21%), health workers (12%), children 2-5 years (8%), children >5 years (6%) pregnant women (4%), and children <2 years (1%); 16% were used in other groups. All countries conducted surveillance for vaccine adverse events, developed five-year sustainability plans and promoted influenza vaccination through various sources. Three countries transitioned to 100% nationally funded programs; five are expected to transition to national funds in 2023. Of 27 countries that reported new influenza vaccination introductions to WHO since 2013, 14 (52%) were PIVI partners. During 2015-2021, national influenza vaccine purchases among partner countries increased 15-fold (from 166,565 doses to 2,488,128 doses); reported vaccine wastage was ≤2%. Seventy-seven percent of health workers and 84% of pregnant women self-reported willingness to accept vaccines. Challenges included finding low-cost vaccines and matching available formulations and timing delivery to countries' needs.

Conclusion

PIVI successfully supported partner countries to introduce, expand and sustain influenza vaccination programs. Modest vaccine donations were replaced with substantial increases in annual partner country purchases. Although challenges remain in vaccine access and affordability, PIVI demonstrates remarkable progress in the introduction, expansion, sustainable use of influenza vaccines and promoting their benefits.

Poster Reception III

Akira Endo - AOXI0289

Simulating respiratory disease transmission within and between classrooms to assess pandemic management strategies at schools

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Background

The global spread of COVID-19 has emphasised the need for evidence-based strategies for safe operation of schools during pandemics that balance infection risk with the society's responsibility of allowing children to attend school. Due to limited empirical data, existing analyses assessing school-based interventions in pandemic situations often impose strong assumptions, for example, on the relationship between class size and transmission risk, which could bias the estimated effect of interventions such as split classes and staggered attendance.

Method

To fill this gap in school outbreak studies, we calibrated parameterised an individual-based model that accounts for heterogeneous contact rates within and between classes and grades to a multi-school outbreak data of influenza. We then simulated school outbreaks of respiratory infectious diseases of ongoing threat (i.e. COVID-19) and potential threat (i.e. pandemic influenza), under a variety of interventions (changing class structures, symptom screening, regular testing, intermittent schooling to reduce school days, cohorting and responsive class closures).

Result

Our results suggest that interventions changing class structures (e.g. reduced class sizes) may not be effective in reducing the risk of major school outbreaks upon introduction of a case and that other precautionary measures (e.g. screening and isolation) need to be employed. Class-level closures in response to detection of a case were also suggested to be effective in reducing the size of an outbreak.

Conclusion

We propose two approaches for pandemic management in school settings: a routine 'preemptive' approach that attempts to keep the within-school reproduction number low by e.g. regular screening, reducing school days and cohorting; and a 'responsive' approach where fixed-period class closures are employed upon detection of a symptomatic case.

Poster Reception III

Kristine Moore - AOXI0290

Development, monitoring, and evaluation of the global Influenza Vaccines R&D Roadmap

Kristine Moore[5], Julie Ostrowsky[5], Anje Mehr[5], Angela Ulrich[5], Michael Osterholm[5], Joseph Bresee[4], Bruce Gellin[3], Christopher Chadwick[7], Martin Friede[7], Stacey Knobler[2], Ann Moen[1], Peter Hart[6], Josephine Golding[6], Charlotte Weller[6]

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Background

Despite extensive funding and R&D investment over recent years, current influenza vaccines fall short. Improved influenza vaccines are urgently needed to reduce the burden of seasonal influenza and to enable a rapid and effective public health response to future influenza pandemics. The Influenza Vaccines Research and Development (R&D) Roadmap (IVR) was created to: (1) improve the production and effectiveness of strain-specific seasonal influenza vaccines and (2) advance the development, licensing, and production of universal influenza vaccines.

Method

The IVR is the product of a 2-year collaborative, consensus-building process among a diverse group of stakeholders representing public and private sector perspectives from nearly 100 organizations and 29 countries. We conducted extensive discussions with subject-matter experts to identify key scientific gaps and barriers to improved influenza vaccines and to build consensus on a set of strategic goals, milestones, and additional R&D priorities related to influenza vaccine R&D.

Result

The roadmap covers a 10-year timeframe and is organized into six topic areas: virology; immunology; vaccinology for seasonal influenza vaccines; vaccinology for universal influenza vaccines; animal and human influenza virus infection models; and policy, finance, and regulation. We identified 113 specific technical milestones for measuring progress over time, with 37 designated as high priority by the IVR expert taskforce.

Conclusion

Recent experience with the COVID-19 pandemic has shown what is possible with coordination of efforts and political will. The IVR provides a critical framework to coordinate the work of existing influenza researchers while encouraging research aimed at new solutions. The roadmap also demonstrates to funders the efficiency of investing in research connected to an accountable, inclusive, and urgently needed global endeavor. To ensure the roadmap is a success and to monitor impact, in 2022 we began a three-year program of Monitoring, Evaluation, and Adjustment (ME&A) for tracking research progress, updating the goals and milestones to reflect achievements and ongoing unmet needs, promoting activation and acceleration of research efforts aimed at meeting IVR goals and milestones, and facilitating academic, public sector, and industry engagement. The IVR website (https://ivr.cidrap.umn.edu/) provides a public forum for information sharing and stakeholder engagement.

Poster Reception III

Melissa Rolfes - AOXI0301

Testing strategies to detect SARS-CoV-2 in household contacts: implications for surveillance, research, and public health decision-making during pandemics

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Background

Studies of respiratory virus transmission during pandemics may be biased if contacts are tested infrequently or only when symptomatic. Using a case-ascertained household transmission study with daily testing for 14 days, we compared SARS-CoV-2 infection detection in alternative testing scenarios.

Method

In Wisconsin and Tennessee during April 2020-April 2021, we enrolled household contacts of individuals testing positive for SARS-CoV-2 within 6 days of index symptom onset or test date if asymptomatic. Daily nasal swabs (NS) were self-collected for 14 days following enrollment. We considered contacts infected if they were positive for SARS-CoV-2 RNA (real-time RT-PCR) from any collected swab. We examined 20 testing scenarios that we gathered from available study protocols and published public health guidance. Scenarios included combinations of 1 to 10 days of NS collected at various times after symptom onset (of contact or index case), the index initially tested positive, or study enrollment. We calculated proportions of infected contacts that would have been detected by each scenario and used Wilcoxon ranked sum tests to examine contact characteristics associated with detection frequency.

Result

Of 577 household contacts enrolled a median of 4 days after index's symptom onset (IQR: 3-4 days), 295 contacts had SARS-CoV-2 infection detected during 2 weeks of daily testing. No alternative testing scenario detected all 295 infections (Figure). In a scenario with daily tests for 10 days after study enrollment (most sensitive scenario), 284 (96%) contact infections were detected. One NS collected 3 days after index case's symptom onset detected 23% of infections and 1 NS collected at the contact's symptom onset detected 53%. Asymptomatic contacts had their infection detected less frequently than symptomatic contacts ($p<0.001$) but age was not associated with detection ($p=0.9$).

Conclusion

Compared with daily testing for 2 weeks, infrequent testing or tests only when symptomatic failed to capture most infections, which would lead to underestimated attack rates, and may bias conclusions on transmission or susceptibility. Guidance and research protocols for when to test close contacts after exposure to SARS-CoV-2 during a pandemic will need to balance resources with the level of detection needed for transmission control and study objectives.

Poster Reception III

Mark WC Sze To - AOXI0256

Therapeutic effects of synthetic ion channels on acute lung injury caused by influenza virus infection

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Background

Acute lung injury (ALI) is a respiratory disorder causing pulmonary distress, it can be found in severe respiratory infections such as influenza with high mortality rates. Significant downregulation of major ion channels in alveolar epithelial cells during infection impairs alveolar fluid clearance (AFC) and results in ALI. The downregulation of ion channels hinders treatments using existing ion channel modulators. Hence, we investigated the potential of synthetic ion channels as novel therapy in restoring AFC and thus treating ALI caused by influenza virus infection.

Method

Ion transporting activity of synthetic ion channels was detected by 8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) assay. Compounds of synthetic ion channels were tested with cell viability assay for their cytotoxicity and non-toxic concentrations of the compounds are determined by the dosage treated resulting in over 80% cell viability. Compounds were treated to human alveolar epithelial cells infected with avian influenza virus A/Quail/Hong Kong/G1/97(H9N2) or A/Hong Kong/483/97 (H5N1), and effects of the compounds on pro-inflammatory cytokine expression and influenza virus replication were determined by quantitative PCR. AFC and alveolar protein permeability (APP) of the cells was determined by in vitro lung injury model.

Result

According to HPTS assay results, compounds for forming synthetic ion channel possess ion transporting activity as channels or transporters for major electrolytes (Cl-, Na+ and K+), which possess physiological roles in driving AFC. Cytotoxicity of the compounds to human alveolar epithelial cells is moderate as shown by cell viability assay, the non-toxic concentrations of the compounds range from 2.5μM to 20μM. Treatment of synthetic ion channel compounds to influenza infected alveolar epithelial cells did not alter expression of pro-inflammatory cytokines or replication of virus based on the results of quantitative PCR, indicating that the compounds do not show any antiviral and immunomodulatory activities. The in vitro lung injury model demonstrated that compounds (e.g. C11, LPY5.2) restored the impaired AFC and reduced APP of alveolar epithelial cells infected with avian influenza virus A/Quail/Hong Kong/G1/97(H9N2) or A/Hong Kong/483/97 (H5N1).

Conclusion

Synthetic ion channels are able to restore impaired AFC of avian influenza virus A/Quail/Hong Kong/G1/97(H9N2) or A/Hong Kong/483/97 (H5N1) infected alveolar epithelial cells without interfering the viral infection, providing a novel therapeutic option to treat acute lung injury caused by influenza virus infection.

Poster Reception III

Benjamin Meyer - AOXI0347

Infectious viral load in unvaccinated and vaccinated individuals infected with ancestral, Delta or Omicron SARS-CoV-2

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Background

Infectious viral load (VL) is one determinant of secondary transmission of SARS-CoV-2. Emergence of variants of concerns (VOC) Alpha and Delta was ascribed, at least partly, to higher VL. RNA VL measured by qRT-PCR is only a weak proxy for infectiousness. Studies on the kinetics of infectious VL are important to understand the mechanisms behind the different transmissibility of SARS-CoV-2 variants and the effect of vaccination on transmission. This will help to guide public health measures such as the duration of the isolation period and the benefit of vaccination certificates.

Method

In this study we quantified infectious and RNA VL in SARS-CoV-2 infected individuals during the first 5 symptomatic days by focus-forming assay and qRT-PCR in unvaccinated or vaccinated individuals infected with pre-variant of concern (pre-VOC) SARS-CoV-2 (unvaccinated n=118), Delta (unvaccinated n=127, vaccinated 2 doses n=166), or Omicron (unvaccinated n=33, vaccinated 2 doses n=91, vaccinated 3 doses n=30).

Result

Unvaccinated individuals infected with pre-VOC SARS-CoV-2 had lower infectious VL but higher RNA VL compared to Delta-infected unvaccinated individuals. Full vaccination (defined as >2weeks after reception of 2nd dose during primary vaccination series) significantly reduced infectious and RNA VL for Delta breakthrough cases compared to unvaccinated individuals. For Omicron breakthrough cases, reduced infectious VL was only observed in boosted but not in fully vaccinated individuals compared to unvaccinated subjects while RNA VL was similar regardless of vaccination status. In addition, infectious VL but not RNA VL was lower in fully vaccinated Omicron- compared to fully vaccinated Delta-infected individuals.

Conclusion

In conclusion, this study provides strong evidence for higher infectiousness of SARS-CoV-2 Delta as well as a significant impact of full vaccination on infectious VL and its speed of clearance. In addition, we show that Omicron has lower infectious VLs compared to Delta in fully vaccinated subjects, suggesting that other mechanisms than increased infectious VL contribute to the high infectiousness of SARS-CoV-2 Omicron. Last, after Omicron infection, lower infectious VL is only observed in boosted individuals. Our findings highlight the beneficial effect of vaccinations beyond the individual protection from severe disease and underscore the importance of booster vaccination. Thereby we provide guidance for public health measures such as shortening of the isolation period and vaccination certificates.

Poster Reception III

Tonia Tong - AOXI0373

Risk Assessment of SARS-CoV-2 Omicron BA.2 in human respiratory tract ex vivo

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Background

The Omicron BA.2 sublineage has become the most dominant variant circulating globally in the context of the BA.1 surge and has similar neutralization to BA. These observations suggest that enhanced antibody evasion is not related to increased transmissibility of BA.2.

Method

We compared the replication competence and tissue tropism of the Omicron variants-BA.1 and BA.2, with two previous strains, wild-type and Delta variants, in human nasal, bronchus and lung explants ex vivo. Replication competence was measured by TCID50 assay. Immunohistochemical staining of the SARS-CoV-2 nucleocapsid protein was performed to assess the tissue tropism.

Result

BA.2 replicated efficiently in nasal and bronchial tissues at 33°C than wild-type, Delta and BA.1. Similar replication competence of BA.2 and BA.1 with a higher replication capacity than wild-type and Delta was found in bronchi at 37°C. Besides, replication competence of BA.1 and BA.2 was lower in lung compared to previous strains. More extensive staining of SARS-CoV-2 nucleocapsid protein in BA.2 infected bronchi than BA.1 at 33°C was found while similar intensities of viral protein staining was observed between BA.1 and BA.2 infected bronchi.

Conclusion

Higher replication capacity of Omicron BA.2 in the human nasal tissues and bronchi infected at 33°C than BA.1 may help to explain the current expansion of BA.2 over BA.1. A lower replication of the tested Omicron variants in human lungs is in line with the clinical manifestations of decreased disease severity of Omicron patients.

Poster Reception III

Helen Wagstaffe - AOXI0375

Viral and immune kinetics during SARS-CoV-2 human challenge in young, seronegative adults

Helen Wagstaffe[1], Richard McKendry[1], Nana-Marie Lemm[1], Stephanie Ascough[1], Loukas Papargyris[1], Ashley Collins[1], Ben Killingley[2], Mariya Kalinova[3], Alex Mann[3], Andrew Catchpole[3], Christopher Chiu[1]

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Background

Human challenge has the unique capacity for detailed characterisation of the response to infection, to accelerate understanding of pathogenesis, transmission, immunity and mechanisms of resistance to disease in a controlled setting. Human challenge with SARS-CoV-2 was therefore developed to investigate viral and immune dynamics, with the longer-term aim of enabling rapid and early-stage testing of novel diagnostics, treatments and vaccine candidates.

Method

Thirty-six healthy individuals (seronegative to SARS-CoV-2, aged 18-30 years) were inoculated with a D614G-containing pre-alpha strain manufactured to GMP standards. The primary aim was to identify a well-tolerated inoculum dose that resulted in infection in more than 50% of participants. Viral load, symptom, antibody and peripheral blood T cell responses (ELISpot and flow cytometry) were also measured before and after intra-nasal inoculation. SARS-CoV-2 spike and N antigen specific T cells were tracked using MHC class I pentamers and MHC class II tetramers.

Result

Eighteen (53%) participants became infected after an inoculation dose of 10 TCID50. Infection was detectable by qPCR after a minimum of 40 hours, viral loads rose steeply and peaked at day 5 after inoculation. Mild-to-moderate symptoms were reported by 16 (89%) of infected participants and began 2-4 days after inoculation. All participants developed spike-specific IgG and neutralising antibody responses by day 28 post-inoculation. T cell activation and proliferation was observed, peaking at day 10 in CD4+ T cells and day 14 in CD8+ T cells and returning to baseline at day 28. Antigen-specific T cells were largely CD38+Ki67+ and displayed central and effector memory phenotypes. Upregulation of chemokine receptors, including CXCR3 that promote migration to the inflamed airway, was identified at days 10 and 14 post-infection.

Conclusion

A novel SARS-CoV-2 human challenge model revealed viral and immune kinetics over the course of primary infection with SARS-CoV-2 in young, healthy adults. Mild disease following human challenge with SARS-CoV-2 is associated with robust T cell activation and proliferation in the majority of infected participants, with phenotypic features suggestive of migratory potential to the infected airway. Future studies will identify factors associated with protection and investigate the effect of prior immunity on outcome.

Poster Reception III

Lieve Naesens - AOXI0384

Impact of SARS-CoV-2 spike mutations on its activation by TMPRSS2 and the alternative TMPRSS13 protease

Annelies Stevaert[1], Ria Van Berwaer[1], Cato Mestdagh[1], Julie Vandeput[1], Els Vanstreels[1], Manon Laporte[1], Lieve Naesens[1]

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Background

The continuous emergence of new SARS-CoV-2 variants with mutations in the spike (S) protein is not only linked to immune evasion but also to fine-tuning of the spike's biological activity. To mediate membrane fusion, S requires cleavage at the S2´ motif to release the fusion peptide. Remarkably, though located in a strong immunogenic region, the S2´ cleavage motif, has, thus far, remained highly conserved. Another striking feature is the dibasic (= KR) scissile sequence that is present in SARS-CoV-2 but missing in all four endemic human coronaviruses. Here we investigated which residues in the S2´ motif are critical for pseudovirus activation by TMPRSS2 and the alternative TMPRSS13 protease.

Method

We conducted a variation analysis of the SARS-CoV-2 S2´ motif in ~10.5 million S sequences in the GISAID database. Mutations of residues P2 to P5 were introduced into pseudoviruses bearing the spike of Wuhan-Hu-1, its G614 mutant and the Delta and Omicron variants. Analogous S2´-mutant pseudoviruses were created for HCoV-229E and MERS-CoV. The efficiency of TMPRSS2 and TMPRSS13 to activate the S2´-mutant pseudoviruses was assessed in transfected HEK293T cells and in Calu-3 cells, a cell line with endogenous expression of TMPRSS2 and TMPRSS13.

Result

Though belonging to an immunogenic region, the SARS-CoV-2 S2´ motif (811-KPSKR-815) has shown hardly any variation, with its three basic (K/R) residues being >99.99% conserved thus far. We show that residue K814 (preceding the scissile R815) is dispensable for TMPRSS2 yet favored by the alternative TMPRSS13 protease. The Wuhan-Hu-1, G614, Delta and Omicron spikes showed no difference in this regard. Activation by TMPRSS13 was drastically reduced when the SARS-CoV-2 S2´ motif was swapped with that of the low pathogenic 229E coronavirus (685-RVAGR-689), and also the reverse effect was seen. This swap had no impact on recognition by TMPRSS2. Pseudovirus entry experiments in Calu-3 cells confirmed that the S2´ mutations that we studied have minor impact on TMPRSS2.

Conclusion

Our findings are the first to demonstrate which S2´ residues are important for SARS-CoV-2 spike activation by these two airway proteases. We conclude that TMPRSS2 readily accepts variations at the S2´-motif, whereas TMPRSS13 is more fastidious with a preference for K/R rich motifs such as present in SARS-CoV-2 but missing in less pathogenic coronaviruses. This may suggest that S2´-recognition by TMPRSS13 contributes to coronavirus virulence. Being the first in its kind, our study will help to assess the impact of S2´ variations as soon as they are detected during variant surveillance.

Poster Reception III

Jet Van den Dijssel - AOXI0411

Parallel detection of SARS-CoV-2 epitopes reveals dynamic immunodominance profiles of CD8 T memory cells in convalescent COVID-19 patients

Jet Van den Dijssel[1], Ruth Hagen[1], Rivka De Jongh[4], Maurice Steenhuis[4], Dionne Geerdes[5], Juk Yee Mok[5], Angela Kragten[5], Mariël Duurland[4], Niels Verstegen[4], Wim Van Esch[5], Klaas Van Gisbergen[3], Pleun Hombrink[3], Anja Ten Brinke[4], Carolien Van de Sandt[2]

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Background

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic leads to global morbidity and mortality. The virus is expected to remain endemic, with new variants emerging regularly, stressing the importance of cross-reactive immunity. Infection induced SARS-CoV-2-specific CD8+ T cells recognize conserved viral sequences present in multiple variants of concern, including Omicron. Furthermore, CD8+ T cell responses are associated with mild COVID-19 disease outcome. However, the antigenic breadth and the immunodominance hierarchies of epitope-specific CD8+ T cells remain largely unexplored and are essential for the development of next-generation broad-protective vaccines. Our study aims to identify a broad-spectrum of conserved SARS-CoV-2 CD8+ T cell epitopes and define their respective immunodominance and phenotypic profiles following SARS-CoV-2 infection.

Method

Based on prediction programs (NetMHC4.0 and NetMHCpan4.1), literature and homology with seasonal coronaviruses 133 potential SARS-CoV-2 derived peptides restricted by 11 common HLA class I were selected. After confirming HLA binding, combinatorial encoded HLA class I tetramers were used to screen 93 of those peptides on PBMCs from 50 HLA-typed convalescent COVID-19 patients directly ex vivo. Combining heterotetramer combinatorial coding (HTCC) with phenotypic markers allowed in-depth profiling of CD8+ T cell responses at quantitative and phenotypic levels directly ex vivo.

Result

A comprehensive panel of 49 mostly conserved SARS-CoV-2-specific CD8+ T cell epitopes, including 5 newly identified, was established. For 9 out of 11 HLA class I allotypes we identified at least one epitope which was recognized by 90-100% of the donors expressing that specific HLA class I allotype. The novelty of this study lies in the immunodominance evaluation of up to 30 epitopes in the same donor, which revealed 3 immunodominant epitopes HLA-A*01:01/ORF1ab(1637-1646), B*07:02/N(105-113) and B*35:01/N(325-333). Furthermore, the magnitude of subdominant epitope responses, including A*02:01/S(269-277) and A*03:01/N(361-369) largely depended on the donors' HLA class I context. Prevalent memory phenotypes were observed for all epitopes, with the highest memory frequencies in severe COVID-19 donors.

Conclusion

Overall, SARS-CoV-2 infection induces a strong CD8+ T memory response directed against a broad spectrum of conserved SARS-CoV-2 epitopes, which likely contributes to long-term protection against severe disease. The observed immunodominance hierarchy emphasizes the importance of CD8+ T cell epitopes derived from non-spike proteins to the overall protective and cross-reactive immune response, which could aid future vaccine strategies.

Poster Reception III

Cynthia Tang - AOXI0415

SARS-CoV-2 transmission dynamics among urban and rural populations prior to the introduction of the COVID-19 vaccine

Cynthia Tang[2], Tao Li[3], Jane McElroy[2], Detlef Ritter[2], Christopher Schulze[1], Richard Hammer[2], Christopher Sampson[2], Robin Trotman[2], Kate Burke[3], Grace Lidl[3], Jun Hang[3], Xiu-Feng Wan[2]

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Background

Approximately 60 million individuals in the United States live in rural areas, with rural populations having known disparities in health-related behaviors, risk factors, and health conditions. During the second half of 2020, rural areas experienced higher incidences of COVID-19 disease and mortality compared to urban regions. This suggested a shift in pandemic burden from urban to rural populations, particularly in the Midwest. Despite this, disease mitigation strategies are largely informed by urban-centric data. Thus, as SARS-CoV-2 continues to evolve, it is urgently necessary to understand urban-rural differences in virus evolution and transmission dynamics.

Method

Here, we study the urban-rural transmission patterns of SARS-CoV-2 variants in Missouri. We analyzed 1,157 geocoded SARS-CoV-2-positive samples collected between March 2020 and January 2021, the period prior to the rollout of the COVID-19 vaccine to the general public. Evolutionary dynamics and transmission events and patterns were studied using Bayesian inference phylogenetic and phylogeographic analyses.

Result

Our results revealed high genomic diversity among SARS-CoV-2 viruses throughout the early phases of the pandemic, with consistently greater lineage diversity seen among urban populations than in rural populations. Of interest, we also found that transmission events occurred more often within and into urban areas during the early phases of the pandemic in Missouri, whereas during the latter phase of the pandemic, the majority of transmission events occurred within rural areas and from rural to urban areas.

Conclusion

These results suggest that rural communities play a critical role in SARS-CoV-2 evolution and transmission and that understanding the genomic variation and directionality of SARS-CoV-2 spread can facilitate COVID-19 prevention and control, particularly in rural areas.

Poster Reception III

Victoria Lawson - AOXI0443

Identifying the neurological impact of COVID-19 in a mouse of model of SARS-CoV-2 infection

Julie McAuley[2], Connor Daymond[4], Natalia Fortunato de Miranda[4], Tim Smith[4], Jesslyn Ong[4], Peter Crack[1], Damian Purcell[4], Victoria Lawson[3]

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Background

The long-term health outcomes of brain injury arising from infection with SARS-CoV-2 are yet to be realised. Understanding the pathogenesis of acute and chronic (long) neurological symptoms of COVID-19 is essential to developing effective interventions. Mouse models of infection can be used to assess neural invasion and pathology of acute viral infection, study the long-term health outcomes and identify and test therapeutic targets. We have used a naturally mouse tropic variant of SARS-CoV-2 to infect wildtype mice to assess the acute and long-term neurological impact of COVID-19.

Method

The mouse tropic VIC2089(N501Y) isolate of SARS-COV-2 was identified though computational prediction of docking to murine ACE2 and shown to induce a productive respiratory infection and disease in wildtype mice. The neurological phenotype of this isolate was compared to the ancestral VIC01 isolate in cultures of human neurons (SH-SY5Y), astrocytes (U87) and microglial (HCM3) cells and neural invasion and neuropathology of VIC2089(N501Y) was assessed in wild-type mice following intranasal infection.

Result

In vitro VIC01 and VIC2089(N501Y) had a similar neurological phenotype with viral RNA detected in neurons, astrocytes and microglia 48 hours after infection and evidence of viral replication and IL-6 production in neurons. Following intranasal infection of wildtype mice with VIC2089(N501Y) pathology consistent with a vacuolating encephalitis was detected in the brain of adult (10 week) and aged (6 month) mice during the acute respiratory infection which persisted for at least 14 days post inoculation and after clearance detectable respiratory infection.

Conclusion

This natural model of COVID-19 associated brain injury will be used to assess routes of SARS-CoV-2 neural invasion, identify the mechanism of infection associated brain injury, assess the long-term health and behavioural outcomes of infection and identify and test the efficacy of therapeutic interventions.

Poster Reception III

Hannah Coutts - AOXI0342

Revealing the epitranscriptomic landscape of Influenza A virus RNAs in infected eukaryotic cells

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Background

Within the past decade, the importance of the viral epitranscriptome has become increasingly apparent. However, the impact of RNA modifications on viral fitness and gene expression remains unclear. The epitranscriptome concerns post-transcriptional modifications to RNA that do not alter the nucleotide sequence. Such modifications have been implicated in affecting several post-transcriptional regulatory functions such as RNA secondary structure, translation efficiency, and immune recognition. Recent technologies and methodologies have opened new avenues that allow virologists to pinpoint the precise location of RNA modifications, determine their evolutionary conservation, and explore their phenotypic effects.

Influenza A Virus (IAV) manipulates host-cell machinery to post-transcriptionally modify bases across its viral transcripts and genome. Data from our lab and others have shown both IAV vRNAs and mRNAs to be extensively modified, but the precise location, number and conservation of modifications between species is not clear. Of particular interest is what purpose RNA modifications may serve for the vRNAs.

Method

A number of RNA modifications, but primarily m6A, were mapped on WSN vRNAs and mRNAs using a combination of meRIP-seq, DART-seq, and Nanopore sequencing. These techniques allowed for the identification of high confidence sites of modification at single-nucleotide resolution, with further validation performed by knocking down primary modification writer proteins, with the main writer protein for m6A being METTL3.

Result

Conclusion

These data shed some light on the epitranscriptomic landscape across the IAV genome and transcripts of WSN, with potential implications for more clinically relevant influenza A strains. Knowledge of the location of RNA modifications sets the foundation for future studies investigating the specific roles these modifications may play during the IAV lifecycle and comparing modifications between different strains of IAV.

Poster Reception III

Justine Oliva - AOXI0380

Bacterial superinfections induce a major transcriptional regulatory switch of interferon-stimulated genes

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Background

Respiratory tract infections constitute a significant public health problem and one of the main death causes worldwide. Another critical point is the limited therapeutic arsenal, which is threatened by the emergence of antiviral and/or antibiotic resistance. Viral-bacterial co-infections are very often associated with the severity of these respiratory infections and have been explored mainly in the context of bacterial superinfections following primary influenza infection. However, our current knowledge comes primarily from observational or in vivo studies, and the underlying mechanisms are still relatively poorly understood, requiring the development of new models and approaches to the study of virus/bacteria co-infections.

Method

In this study, we aimed to decipher the molecular mechanisms involved in bacterial superinfection by S. aureus or P. aeruginosa after a primary influenza viral infection, in a model of human lung epithelial cells.

Result

Comparative analysis of the transcriptional profiles of the different experimental conditions (single infections versus superinfections) revealed a specific signature for the superinfection, with a higher number of deregulated genes compared to single infection and more than 40% overlap between the two scenarios of infection (S. aureus or P. aeruginosa). Functional analyses revealed an enrichissement of genes involved in specific biological processes, such as calcium signaling for example, rather than immunological mechanisms.

To better understand the mechanisms involving the IFN response in the context of bacterial superinfections, we have further investigated the transcriptional regulation of a large and representative subset of interferon-stimulated genes (ISGs). Our results reveal that bacterial superinfections, in comparison with single influenza infection, induce a switch in transcriptional regulation of ISGs, reflecting a major change in the signaling pathways associated with the IFN response.

Conclusion

This study shows that bacterial superinfections, in the context of primary infection with influenza viruses, induce a specific host response distinct from those observed in simple viral or bacterial infections. This study also showed that bacterial superinfections induce a specific downregulation of ISGs. Overall, these results are a first step in understanding the mechanisms underlying the severity of bacterial superinfections in patients.

Poster Reception III

Nigel Temperton - AOXI0406

The Influenza Virus Toolkit: a reagent sharing resource for influenza virus research

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Background

Influenza viruses are one of the leading causes of respiratory illness, and decades of intensive research on influenza have created a highly active but dispersed research environment. A wealth of non-commercial influenza reagents exists, but it is challenging for researchers to identify these reagents, as well as to archive and redistribute them. In addition, the breadth of influenza reagents is limited, with most targeting a small number of well-studied viral components (mainly the HA, M1 and NP proteins of influenza A and B viruses).

Method

To improve access to influenza virology reagents we have created the 'Influenza Virus Toolkit,' a reagent sharing initiative with two aims. (i) To create a reagent repository offering a sustainable, long-term framework for the archiving and redistribution of reagents for influenza virus research, for academics and industry, in the UK and overseas, using a well-established cost-recovery model. (ii) To generate and validate additional reagents for influenza virology. We are currently generating and characterising panels of polyclonal sheep antisera and associated expression plasmids for the core and accessory proteins of all four genera of influenza virus (A, including H1N1 and H3N2; B, including Victoria and Yamagata, C and D), and validating a set of plasmids to generate pseudotype viruses carrying the HA proteins of all influenza A-D virus subtypes.

Result

We present here data validating our newly generated reagents, including those for use with widely studied viral proteins (e.g. polyclonal antisera against influenza A virus NP) and previously unstudied targets (e.g. the 'UFO' accessory proteins). Here we also launch our reagent-sharing website, and encourage the influenza research community to deposit reagents for archiving and redistribution at https://www.influenza.bio/

Conclusion

The Influenza Virus Toolkit will facilitate resource sharing and archiving in the highly active field of influenza virology, fostering open-science and a culture of collaboration among influenza researchers. Importantly, it will also create a key resource for the rapid provision of research reagents during the next influenza pandemic.

Poster Reception III

Michael Lutz - AOXI0430

Host adaptive mutations in influenza A virus polymerase gene enhance translation efficiency of viral mRNA

***Michael Lutz*[1], *Jordana Schmierer*[1], *Toru Takimoto*[1]**

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Background

The emergence of pandemic influenza A viruses (IAVs) is a significant public health concern but the mechanism(s) of how pandemic viruses emerge is still poorly understood. The most recent IAV pandemic, the 2009 H1N1 virus (pH1N1), was a reassortant virus containing genes from avian, human and swine IAVs. The PA and PB2 genes were of avian IAV origin but lacked key mammalian host-adaptive mutations previously identified in PB2. We and others found that PA was the most significant factor in adaptation of the viral RNA-dependent RNA-polymerase (vRdRp). We identified several new host-adaptive mutations in PA, but the mechanism(s) of how these mutations contribute to mammalian host adaptation are unknown. In this study, we determined if these PA mutations directly increase vRdRp activity and produce more transcripts/genomes or enhance translation of viral transcripts.

Method

We measured both the transcriptional and translational outcomes of vRdRps from the prototypical avian strain A/chicken/Nanchang/3-120/01 (Nan), the 2009 pH1N1 virus A/California/04/2009, and a recently circulating 2017 pH1N1 virus A/Michigan/272/2017. PA genes of these viruses were also mutated. Translation efficiency was measured by reporter gene assay and qRT-PCR. Nuclear/cytosolic fractionation was performed to determine the nuclear export of viral mRNA. In addition, recombinant pH1N1 viruses containing vRdRp mutations of interest were rescued and analyzed for the trafficking and efficiency of translation of viral mRNAs in infected cells.

Result

Avian vRdRp was found to produce viral mRNAs that were poorly exported to the cytosol and translated. However, substitution of pH1N1 PA, or PA with individual mutations rescued these defects. By characterizing the PA mutations, we found that residues within the endonuclease domain of PA enhanced nuclear export and translation efficiency. Analysis of recombinant pH1N1 viruses confirmed the role of these mutations during viral infection. Increased translation efficiency and nuclear export of viral mRNA lead to greater protein synthesis, viral replication, and growth in infected human airway cells.

Conclusion

We have uncovered a novel mechanism of host adaptation driven by mutations in polymerase gene of IAV, regulating translation efficiency of viral mRNAs. This study also identified the nuclear export of viral mRNA as a process which can be manipulated by the IAV polymerase and contributes to host adaptation. These findings highlight the importance of efficient trafficking and translation of viral mRNAs during the process of mammalian host adaptation of IAVs.

Poster Reception III

Charlotte Hjulsager - AOXI0370

Transmission of clade 2.3.4.4b H5 HPAI to commercial turkey farms in Denmark in seasons 2020/2021 and 2021/2022

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Background

In recent years, European countries have experienced severe epizootics of highly pathogenic avian influenza viruses (HPAIVs) belonging to clade 2.3.4.4b. During the seasons 2020/2021 and 2021/2022, clade 2.3.4.4b H5 HPAIVs were detected in more than 400 wild birds and in 27 poultry herds in Denmark. Especially turkey broiler farms suffered from HPAI. H5N8 HPAIV was confirmed in four turkey broiler farms in March 2021. The farms had the same owner and was located with 2-10 km apart. H5N1 HPAIV was confirmed in three turkey broiler farms on November 1st 2021, January 1st and 3rd 2022, respectively. The farms were located 28-74 km apart, and had the same owner as the turkey farms affected in the previous season. One farm was infected in both seasons.

Method

Whole genome sequencing of HPAIVs from the Danish outbreaks in poultry and approx. 150 wild birds from the 2020/2021 and 2021/2022 seasons was performed. Sequences were analysed by phylogenetic analyses including related sequences obtained from public databases. The genetic data combined with epidemiological information was used to explore the most likely route of introduction into the infected turkey farms.

Result

Phylogenetic analyses showed that the turkey H5N8 HPAIVs from March 2021 were more closely related to each other than to other contemporary poultry and wild bird HPAIVs, albeit Danish wild bird HPAIVs also were closely related.

Phylogenetic analyses of H5N1 HPAIVs from the November 2021 and January 2022 outbreaks showed they were closely related to contemporary wild bird HPAIVs. Viruses from the two January outbreaks were genetically more closely related to each other than to viruses from the November outbreak.

The four turkey farms infected in March 2021 shared staff, straw storage and equipment prior to the outbreaks. Except for geographical location and ownership, no additional concerning links between turkey farms were identified.

Conclusion

The data suggest that the Danish poultry outbreaks in 2020/2021 and 2021/2022 were the result of direct or indirect contact with HPAIV-infected wild birds. However, inter-farm transmission of HPAIVs is likely to have occurred in the March 2021 turkey outbreaks, although a common HPAIV source cannot be excluded. The timing of clinical symptoms indicate that HPAI started in one herd and spread horizontally to the other three farms. Potential modes of inter-farm transmission pinpointed, was mechanical transmission of virus by staff, straw and/or equipment.

Clade 2.3.4.4b H5 HPAIVs have been sporadically detected in mammals in Europe, including a habour seal in Denmark. Clade 2.3.4.4b H5N6 have been implicated in human fatal cases in Asia. Altogether, this raises a zoonotic concern for HPAIVs belonging to clade 2.3.4.4b.

Poster Reception III

YiJu Han - AOXI0348

Splice site signature determines influenza A virus pathogenicity in a subtype-specific manner

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Background

Recently, many research were reported that the splicing of M1 mRNA is related to the host determinant. Including the expression of M2 ion channel and M1 matrix protein. Between them, M2 proteins had been found that it is correlated to pathogenicity. However, association between the M segment splicing and pathogenicity remains ambiguous in human influenza A viruses.

Method

We compared the sequences of different strains and also performed the evolutional analysis between H1N1 and H3N2. Then we use RNP reconstitution and reverse genetic (RG) to investigate the impacts of single nucleotide variants (SNVs) on M1 mRNA splicing and viral growth. Finally, we challenge mice with RG virus to study the pathogenicity.

Result

We discovered a cis-acting 55C/T SNV that is unique to human IAV and modulates the M segment splicing. The human H3N2 virus presents a lower splicing efficiency of M2 under both infection and RNP reconstitution conditions. By generating different subtypes of RG viruses harboring distinct SNVs, we demonstrated that a mutation at the 55 nucleotide position impairs the replication of H1N1 but not of the H3N2 virus. Importantly, we discovered that the low level of H3N2 M2 fulfills the functional requirement of H1N1 M2 in the chimeric RG viruses. However, elevated H3N2 M2 protein levels induced by the T55C mutation has deleterious effects on H1N1 replication. Moreover, mice challenged with the C55T mutant WSN virus showed an improved survival rate and reduced weight loss as compared with those infected with wild-type H1N1 viruses. Noteworthily, the pathogenicity of chimeric H1N1 viruses remained unchanged if the wild-type H3N2 M segment was present, whereas milder symptom developed when mice were infected with chimeric viruses carrying the mutant H3N2 M segment. The discrepancy in the M2- dependence is unique to human IAVs, which derived from an adaptive evolution route undertaken by H3N2 M.

Conclusion

We found a preferential expression of M2 in H1N1 than in H3N2 viruses. Analysis of the M sequence splice sites revealed an evolutionarily conserved single nucleotide variant, 55C change to T, in H3N2, which impairs M2 expression accompanied by collinear M1 and mRNA3 production. Therefore, aberrant M2 splicing results from splice-site selection rather than a general defect in the splicing process. The C55T substitution significantly reduces both M2 mRNA and protein levels regardless of the virus subtype; however, a lower M2 expression only attenuated H1N1 virus replication and in vivo pathogenicity. Such attenuated phenotype is restored by M replacement of H3N2 M in a chimeric H1N1, despite low M2 levels. The discrepancy in M2-dependence further elucidates an important role of M2 in the pathogenicity of human IAV in a subtype-specific manner.

Poster Reception III

Ka Ling Lai - AOXI0374

Interactions between influenza A and SARS-CoV-2 viruses in respiratory epithelial cells

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Background

Seasonal influenza is a common respiratory tract infection in Hong Kong and worldwide. Most patients manifested with mild symptoms and recover within a week, and the majority of patients stay in the community during the course of infection instead of being quarantined. We found that infection with influenza virus in human alveolar epithelial cells up-regulated the expression of angiotensin-converting enzyme 2 (ACE2)-the receptor of SARS-CoV-2, which is the virus causing COVID-19 pandemic. This may enhance the replication of SARS-CoV-2. Therefore, we investigated the interactions between influenza A virus and SARS-CoV-2 on the host responses.

Method

Seasonal H3N2, pandemic H1N1 and highly pathogenic avian influenza H5N1 viruses were co-infected with a SARS-CoV-2. Human airway and alveolar epithelial cells were used to assess the infectivity and host immune responses of SARS-CoV-2. Viral load was measured by TCID50 assay or real-time PCR of viral genes and the induction of cytokine and chemokine was monitored using real-time PCR.

Result

In airway epithelial cells, influenza virus infection did not affect the replication of SARS-CoV-2 while SARS-CoV-2 reduced the viral load of H3N2 virus. We found that SARS-CoV-2 induced lower levels of cytokine and chemokine than H5N1 virus but they were higher than that induced by H3N2 or H1N1 viruses. Co-infection with SARS-CoV-2 increased H1N1-induced cytokine levels while reduced H5N1-induced cytokines and chemokines. Co-infection with H3N2 only elevated IFN-beta when compared to SARS-CoV-2 infection.

In alveolar epithelial cells, all three subtypes of influenza viruses reduced the replication of SARS-CoV-2. Interestingly, SARS-CoV-2 induced similar levels of cytokine and chemokine as H1N1, while the cytokine and chemokine induction of H5N1 and H3N2 was higher than that of SARS-CoV-2. Co-infection with H1N1 had no effects on SARS-CoV-2-induced cytokine levels. Both H5N1 and H3N2 co-infection elevated the cytokine induction of SARS-CoV-2 infection.

Conclusion

Though, the influenza viruses have different tropism and exert different levels of innate immune responses, our findings suggest that co-infection of influenza viruses and SARS-CoV-2 probably cause more inflammation and hence tissue damage to respiratory epithelial cells.

Poster Reception III

Nancy Candide Mounogou Kouassi - AOXI0428

IgE depletion increases vulnerability to influenza in an experimental allogeneic mouse asthma model.

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Background

Asthmatics were more likely to be hospitalized than non-asthmatics during the H1N1 influenza pandemic in 2009. The underlying mechanisms of increased vulnerability of asthmatics to influenza remained largely unknown due to the lack of clinically relevant animal models.

Method

Here, we developed an allogenic asthma mouse model (1SFB6F1), which is more susceptible to pandemic influenza compared to syngeneic C57BL/6 mice. Using comparative and longitudinal transcriptome analysis in susceptible 1SB6F1 and resistant C57BL/6 mice, we identified immunoglobulin-related genes as a correlate of vulnerability to pandemic influenza.

Result

We found that infection with the 2009 pandemic H1N1 influenza virus results in a specific and prolonged reduction in serum IgE levels in asthmatic 1SB6F1 mice. Depletion of serum IgE levels converted resistant C57BL/6 mice highly vulnerable against influenza.

Conclusion

Our data show that serum IgE plays a key role in influenza susceptibility in asthmatic mice. Moreover, our findings suggest that serum IgE has a protective role in respiratory viral infection.

Poster Reception III

Eric Bortz - AOXI0323

Better practices using deployable nanopore sequencing for influenza and respiratory disease pathotyping in resource-limited laboratories

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Background

Respiratory diseases often present with an overlapping spectrum of symptoms, making diagnosis of pathogen strains a challenge. While high throughput next-generation sequencing (NGS) technologies have transformed the real-time identification of respiratory viruses, including genotyping of influenza A/B and SARS-CoV-2, widespread application of these advanced sequence-based molecular diagnostics are often limited by resources in low- and middle- income countries.

Method

To address this capacity gap, we have been working to develop protocols for sequence-based virus pathotyping and lineage identification using deployable, long-read Oxford Nanopore (MinION) sequencing technology. We applied tiling primer (ARTIC V3) and pan-influenza virus genome amplification and end-ligation library protocols, and common cloud-accessible, open access bioinformatics workflows for virus genome assembly, annotation, and phylogenetics, to analyze human and animal influenza, paramyxoviruses, and coronaviruses. We tested this platform with biobanked nasal swabs samples from human respiratory disease hospital surveillance in Laos (2017/18), the COVID-19 pandemic in Alaska (2020-2021), and other specimens identified by RTPCR as human and animal respiratory viruses with pandemic potential.

Result

By MinION sequencing and phylogenetics analyses, we genotyped twenty (H1N1)pdm09 influenza A viruses circulating in Laos (2017/18) as 6B.1A.1 subclade (genetic group) that clustered with the 2019-2020 Southern Hemisphere vaccine strain A/Brisbane/02/2018 (H1N1). The Laos isolates' HA1 encoded S183P, a substitution adjacent to the Sb antigenic site that is associated with escape from neutralizing antibody response generated by 6B.1 vaccination. Applying this rapid nanopore sequencing method to SARS-CoV-2, we analyzed the lineages of SARS-CoV-2 variants in Alaska in two waves of the COVID-19 pandemic in 2020-2021, and identified multiple independent introductions of clades 20A, B, and C in 2020, and Delta variant of concern (VOC) in 2021. We tested the deployability and robustness of this approach by nanopore sequence-based pathotyping of SARS-CoV-2 (Delta and Omicron variants), measles (group d8), and avian H5N8 and H5N5 highly pathogenic avian influenza in Ukraine, marine mammal influenza (H5N3) in Alaska, and bat and rodent coronaviruses.

Conclusion

This Pathogenomics toolkit is cost-effective, deployable, and teachable in any diagnostic or sentinel laboratory receiving primary clinical samples. With democratization of pathogen genome sequencing capacity and bioinformatics skills, novel virus genotypes might be identified close to introduction or spillover events, enhancing pandemic preparedness.

Poster Reception III

Brian Yau - AOXI0363

WHO's activities in building infodemic management capacity and competency: strengthening preparedness for respiratory disease pandemics and accompanying infodemics

Atsuyoshi Ishizumi[1], Tina Purnat[1], Catherine Bertrand-Ferrandis[1], Brian Yau[1], Becky White[1], Sylvie Briand[1], Tim Nguyen[1]

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Background

As evidenced by the COVID-19 pandemic, respiratory disease pandemics can be accompanied by infodemics, which make it difficult for people to discern what they see and hear to make good health decisions amid an excess of information. Due to their multifaceted impacts on health and society, building competency and capacity for managing infodemics to support uptake of vaccines, public health and social measures, treatments, and health behaviours has become a priority for health authorities. Since mid-2020, the World Health Organization (WHO) has developed a competency framework and capacity-building tools for infodemic management (IM), including a comprehensive training programme to support health professionals.

Method

The WHO infodemic management multiformat and transdisciplinary training program builds the skills and knowledge needed to prepare for and respond to infodemics. The trainings are built on WHO competency framework for building an infodemic response workforce. The methodology used relies on human-centred and emotional design evidence and practice and includes evaluations for continuous learning design improvement. Furthermore, several practical tools and job aids for training participants are currently being developed through technical consultation with experts.

Result

Since November 2020, three WHO global trainings organized online in partnership with US Centers for Disease Control and Prevention, UNICEF and other partners, including a four-week-long simulation exercise, have enabled the creation of a network composed of 754 infodemic managers from 133 countries. A "train-the-trainers" companion package was prepared and by April 2022 delivered in Iran. Deep dive training modules on specialist infodemic management practice topics have been prepared for use at the country level. In addition, a comprehensive set of self-paced free online courses enhances infodemic literacy and resilience to misinformation. Launched in December 2021, the OpenWHO Infodemic Management 101 course has achieved over 17,000 enrolments by April 2022. The training programme will be updated based on evaluations, the feedback from practitioners in the field and the evolution of the WHO competency framework for infodemic management workforce. Following the comprehensive training in June 2022, various toolkits will be deployed to help guide practitioners responding to infodemics in the field.

Conclusion

The WHO multiformat blended training program allows an efficient and rapid dissemination of infodemic management skills and knowledge. IM capacity and competency development needs to be mainstreamed into epidemiological preparedness plans to enable effective emergency response to pandemics in the future.

Poster Reception III

María Elena Dattero - AOXI0403

RESPONSE OF THE NATIONAL INFLUENZA CENTER OF ARGENTINA TO THE THREAT OF THE SARS-COV-2 PANDEMIC

María Elena Dattero[1], Estefanía Benedetti[2], Martín Avaro[1], Mara Russo[1], Ana María Campos[1], Fabián Pardón[1], Erika Machicado[1], Andrea Pontoriero[1], Elsa Baumeister[1]

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Background

On March 11, 2020, WHO declared SARS-CoV-2 outbreak as pandemic. The response was a priority for WHO and countries around the world. In Argentina, the experience and lessons learned by the National Reference Laboratory (NRL) in the 2009 Influenza pandemic, allowed the rapid decentralization of diagnosis from the NRL to the National Network of Laboratories of Influenza and other Respiratory Viruses (NNLIRV). The objective was to expand the diagnostic capacity and make it accessible to the majority of the population and allow the authorities to take sanitary measures with evidence.

Method

At the beginning of the pandemic, the NRL had an end point RT-PCR pan coronavirus technique. The last week of January 2020, the Pan American Health Organization (PAHO) provided training for the SARS-CoV-2 RT-qPCR method of Corman et al., at the Osvaldo Cruz Institute of Brazil. At the beginning of February, Argentina ordered specific reagents in bulk quantity to be distributed to the NNLIRV. On Friday 20 and Monday 30 March 2020, the NRL conducted two virtual trainings to the NNLIRV. On Friday, March 27, the probes and primers were received at the NRL and were distributed to the country on Saturday, March 28.

Result

On February 11, the NRL had already implemented the SARS-CoV-2 diagnosis by the RT-qPCR and was the only laboratory in the country that performed the diagnostics. On March 3, the NRL obtained the first positive diagnostic in an international traveller. The 35 laboratories of the NNLIRV were called for the two trainings and 310 health professionals and technicians were trained in the reference diagnostic test. Detection protocol was distributed and commented on in detail, results interpretation of different clinical cases, reporting and communication of the cases to the National Health Surveillance System were discussed during the trainings. An update on the epidemiological situation and health measures taken at the global and local levels was given. 57,000 determinations were distributed in the first shipment. The amount of tests for each jurisdiction was defined based on the number of inhabitants of each one. On April 1st 2020, the NRL received first result reports obtained by the NNLIRV.

Conclusion

The NRL alerted health authorities about SARS-CoV-2 outbreak at the end of December 2019. This action made it possible to request the necessary reagents prior to the declaration of the pandemic and it allowed the rapid decentralization of the diagnosis. Besides, to have developed a National Laboratory Network with more than 20 years of experience in respiratory virus surveillance allowed to respond to the diagnostic demand throughout the country.

Poster Reception III

Silke Rimaux - AOXI0387

Evaluation of influenza virus fusion inhibitors against contemporary H1 and H3 hemagglutinins

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Background

During influenza virus entry, the viral hemagglutinin (HA) undergoes low pH-triggered refolding to induce membrane fusion. Efforts are ongoing to develop small molecule antivirals and anti-HA antibodies that prevent this fusion process. Most fusion inhibitors reported in the literature were discovered with HA proteins of outdated laboratory strains. In this study, we evaluated three different compound series against HAs representing currently circulating strains, i.e. pandemic 2009-H1 HA and Victoria 2011-H3 HA. We interpret their activity in light of the presumed binding pocket, also used by arbidol, at an inter-monomer interface in the HA trimer.

Method

Compound series [1] targets H3 HA, whereas series [2] and [3] target H1 HA. Expression vectors for the HA proteins of A/Virginia/ATCC3/2009 and A/Victoria/361/2011 were constructed and used to determine the inhibitory effect of these compounds on: (i) HA-mediated cell-cell fusion in HeLa cells; and (ii) HA-driven pseudovirus entry in MDCK cells. Both assays were luciferase-based and optimized for 96-well format, enabling easy evaluation of larger compound series. The cell-cell fusion assay was derived from Pöhlmann, J. Virol., 2011 and the pseudovirus assay was adapted from Laporte, J. Virol., 2019.

Result

The spirothiazolidinone compounds of series [1] are presumed to bind to the same HA pocket as arbidol but engage in more interactions, resulting in superior biological activity. The most active compound had an EC50 value of 1.39 µM in the cell-cell fusion assay with Victoria 2011-H3 HA, which is comparable to the activity of this series against A/Aichi/2/68 HA. This agrees with our analysis that the binding pocket of [1] is highly conserved among H3 HAs.

Series [2] (aniline derivatives) and series [3] proved nicely active against pandemic 2009-H1 HA, having higher activity in the pseudovirus entry than the cell-cell fusion assay. The best compound of series [3] had an EC50 value as low as 0.032 µM. This corroborates that some influenza virus fusion inhibitors are concentrated in endosomes, a property that enhances their antiviral effect. Also the H1 HA binding pocket was analyzed to identify which points of interaction are conserved among all H1 HAs, and understand the SAR of our tested inhibitors.

Conclusion

Our findings indicate that fusion inhibitors targeting H1 HA or H3 HA can be developed to cover all strains of the respective subtype. The low micromolar inhibitors of series [1], [2] and [3] are relevant for antiviral drug development, and also useful to study the key role of this pocket in the HA refolding process.

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Poster Reception III

JIE ZHOU - AOXI0478

Omicron breakthrough infections in vaccinated or previously infected hamsters

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Background

The 2nd and 3rd years of the SARS-CoV-2 pandemic have been marked by the repeated emergence and replacement of 'variants' with genetic and phenotypic distance from the ancestral strains. The lack of cross-neutralization between Omicron and earlier variants likely accounts for the observed high transmission of Omicron in populations that are heavily vaccinated and/or have a high rate of previous infection.

Method

We applied a hamster contact exposure challenge model to assess protection conferred by vaccination or prior infection against re-infection. Donors were inoculated with Delta or Omicron variants, 1 day later, naïve, vaccinated, or previously infected sentinels were introduced to co-house with the donor. Nasal washes and weight were collected daily. We performed pseudovirus neutralization assays with the hamster sera. We also related these in vivo results to the antibody responses in humans by measuring neutralization activity of antisera from naïve, unvaccinated individuals whose only known exposure was to the named variant.

Result

We found that self-amplifying RNA vaccine based on the ancestral spike ameliorated weight loss following Delta infection and decreased viral loads, but had minimal effect on BA.1 infection. Prior infection with ancestral or Alpha was partially protective against BA.1 infection, whereas all animals previously infected with Delta and exposed to Omicron became infected, although shed less virus. However, measurements of infectious virus in exhaled air suggested the potential for onwards transmission from the re-infected animals. We further tested if prior infection with BA.1 protected from re-infection with Delta or BA.2. BA.1 was protective against BA.2, but not Delta infection, reinforcing that Delta and Omicron have a very large antigenic distance. In addition, cross-neutralisation assays with human antisera confirmed a large antigenic distance between Delta and Omicron. Prior vaccination followed by Omicron or Delta breakthrough infection led to a higher degree of cross-reactivity to all tested variants.

Conclusion

Cohorts whose only immune experience of COVID is BA.1 infection may be particularly vulnerable to future circulation of Delta or Delta-like derivatives. In contrast, repeated exposure to antigenically distinct spikes, via infection and or vaccination drives a more cross-reactive immune response.

Poster Reception III

Mark Anthony Casel - AOXI0480

Critical role of neutralizing antibody for SARS-CoV-2 reinfection and transmission

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Background

Cases of laboratory-confirmed SARS-CoV-2 reinfection among recovered COVID-19 patients continue to increase and are reported in several countries. Further, the role of induced immunity after SARS-CoV-2 infection, especially the serum neutralizing antibody (NAb), has not been well characterized. In this study, we adapted a ferret reinfection model with a dose-dependent SARS-CoV-2 NAb to determine the correlation between NAb titer and reinfection rate, virus replication, shedding period, and changes in antibody titers during reinfection with heterologous SARS-CoV-2.

Method

For primary infection, groups of 12 to 24-months-old ferrets (n=6) were inoculated with three different doses of NMC-2019-nCoV02 virus (S Clade) through the intranasal route and serum NAb assay was conducted at 3 weeks post-infection. Ferrets were then grouped according to their NAb titers (NAb < 20 (G2), 20-40 (G3), 80 (G4), 160 (G5)) including a naive control group (G1). Each group of ferrets was inoculated with CBNU-nCoV02 virus (GH Clade), a heterologous virus with 99.9% homology with the NMC-2019-nCoV02 in the spike protein, at a dosage of 105.0 TCID50/mL via the intranasal route. To confirm virus infection, nasal washes and rectal swabs were collected every other day for virus titration in Vero cells and qRT-PCR. At two weeks post-reinfection, changes in serum antibody titers were evaluated between the primary and the reinfected SARS-CoV-2.

Result

Although, infectious viruses were only isolated in the nasal washes of control and low NAb titer groups (G1 and G2) from 2dpi until 6dpi, however, qRT-PCR results revealed that CBNU-nCoV02 was detectable in all directly infected groups in both nasal wash and rectal swabs. Wherein only G1 and G2 showed transmission to naïve contact ferrets. Moreover, lung histopathology demonstrated that high NAb titer groups (G3, G4, and G5) showed limited inflammatory regions than the low Nab and control groups. These results suggest that high NAb titers are associated with low infectious virus shedding and rapid viral clearance in the ferret reinfection model. To evaluate changes of the serum antibody titers, sera were collected at the primary infection (0 dpi) and after reinfection (14 dpi). Results revealed that most of the reinfection groups exhibited a significant increase in both IgG and NAb antibody titers.

Conclusion

Although, we primarily focused on the NAb-dependent cross-protection against heterologous SARS-CoV-2, results of the ferret reinfection with dose-dependent induction of SARS-CoV-2 NAbs provides detailed insight into the possibility of reinfection in humans and emphasizes a close correlation between NAb titres and SARS-CoV-2 reinfection.

Poster Reception III

Maarten van Wijhe - AOXI0489

Persistent symptoms and sequelae after SARS-CoV-2 infection not requiring hospitalization: Results from Testing Denmark, a Danish cross-sectional survey

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Background

SARS-CoV-2 infection has been associated with persistent symptoms (long-COVID). We assessed the burden of long-COVID among non-hospitalized PCR-confirmed

adults.

Method

In the fall of 2020, a cross-sectional survey was performed in the adult Danish general population. This included a self-administered point-of-care test for SARS-CoV-2 antibodies, the Short Form Health Survey (SF-12), and COVID-19 associated symptom

questions. Non-hospitalized respondents with a positive SARS-CoV-2 PCR-test three or more months before the survey (cases) were matched (1:10) to seronegative controls on age, sex, and BMI. Propensity score weighted odds ratios (OR) and ORs

for risk factors were estimated for each health outcome.

Result

In total, 728 cases and 7280 controls were included. The attributable risk of at least one long-COVID symptom was 25.1 per 100 cases (95% confidence interval (CI): 22.3, 27.6). Compared to controls, cases reported worse general health (OR: 6.0, CI: 5.0, 7.2) and had higher odds for a broad range of symptoms, particularly loss of taste (OR: 12.2, CI: 9.8, 15.2) and smell (OR: 11.7, CI: 9.4, 14.5). Physical and Mental Component Summary scores were also significantly reduced with differences of -2.5 (CI: -3.1, -1.8) and -2.3 (CI: -3.1, -1.6) respectively. Female sex and severity of initial infection were major risk factors for long-COVID symptoms.

Conclusion

Non-hospitalized SARS-CoV-2 PCR-positive individuals had significantly reduced physical and mental health, and one in four reported persistence of at least one long-COVID symptom.

Poster Reception III

Se-Mi Kim - AOXI0491

Coinfection with SARS-CoV-2 and Influenza A virus enhanced viral pathology and reduced neutralizing antibody responses

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Background

The coronavirus disease 2019 (COVID-19) pandemic caused by SARS-CoV-2 has resulted in millions of confirmed cases and deaths worldwide. Moreover, seasonal influenza A virus infections stand to compound the challenges posed to the public health by the COVID-19 pandemic, especially in the winter season. In addition to the rapid evolution and emergence of SARS-CoV-2 variants. Secondary respiratory infections are believed to have been one of the main causes of death during the last major influenza pandemic of the twentieth century. While, recent clinical studies suggest that the coinfection of SAR-CoV-2 with influenza virus results in more severe disease manifestations in humans, the immunopathogenic mechanisms are still largely unknown.

Method

To investigate the pathogenic (body weights, survival rates, and viral titers) and immunological (IgG titer, SN test, cytokine/chemokine levels, and T cell response) consequences of SARS-CoV-2 and IAV H1N1 coinfection in the K18-hACE2 transgenic mouse model, compared to a single infection with SARS-CoV-2 or IAV.

Result

Our results demonstrate that coinfection of SARS-CoV-2 and IAV leads to an increased mortality rate and prolonged presence of the virus in the lungs in K18-hACE2 mice compared to the single infection groups. Results also show that SARS-CoV-2 and IAV coinfection induce higher immune cell recruitment to the lungs than either single infection by mediating cytokines/chemokines production. Moreover, SARS-CoV-2 and IAV coinfection is accompanied by severe lymphopenia that leads to impaired total IgG, neutralizing antibody titer, and CD4+T cell responses against each virus.

Conclusion

Taken together, the data presented here shed light on the virological and immunological interactions between SARS-CoV-2 and IAV and suggest their coinfection could lead to increased disease severity. Furthermore, these studies may be of value for the rational development of therapeutic strategies for the effective treatment of coinfected patients.

Poster Reception III

Young-Il Kim - AOXI0493

Age-dependent pathogenic characteristics of SARS-CoV-2 infection in ferrets

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Background

While the seroprevalence of SARS-CoV-2 in healthy people does not differ significantly among age groups, those aged 65 years or older exhibit strikingly higher COVID-19 mortality compared to younger individuals. Therefore, to investigate the differential and diverse clinical manifestations in COVID-19 patients of different ages, age-related disease severity of COVID-19 was assessed in ferret animal models of three different age groups.

Method

We demonstrate the age-related disease severity observed in COVID-19 patients by performing SARS-CoV-2 infection in ferrets of three different age groups: ferrets under 6 months to simulate juveniles/children (G1), one to two-year-old to simulate young adults (G2), and 3 years or older to simulate patients over 50 years old (G3). We compare disease severity among the three groups by examining clinical symptoms, viral load in the respiratory tract, and lung histopathology. Furthermore, RNA sequencing analysis with lung tissues from SARS-CoV-2-infected young adult or aged ferrets reveals differences in global and dynamic gene expression.

Result

As a result, SARS-CoV-2 is isolated from all ferrets regardless of age, aged ferrets (≥3 years old) show higher viral loads, longer nasal virus shedding, and more severe lung inflammatory cell infiltration, and clinical symptoms compared to juvenile (≤6 months) and young adult (1-2 years) groups. Furthermore, contact ferrets co-housed with infected aged ferrets sheds significantly high viral titers through their respiratory tracts and exhibits high clinical disease scores. In addition, although an IgG antibody response to SARS-CoV-2 is not induced in the juvenile group, the young adult and aged contact groups show detectable IgG in an age-dependent manner. Transcriptome analysis of aged ferret lungs reveals strong enrichment of gene sets related to type I interferon, activated T cells, and M1 macrophage responses, mimicking the gene expression profile of severe COVID-19 patients.

Conclusion

Taken together, aged ferrets showed significantly higher virus loads and more severe lung pathology compared to juvenile and young adult ferrets. Moreover, these differences were closely associated with enhanced type I IFN responses and activated M1 macrophages as well as hyper-inflammatory responses in infected aged ferrets. This aged immune-competent ferret model demonstrates for the first-time age-dependent pathogenesis of SARS-CoV-2 infection, making it an invaluable animal model to understand the age-dependency of COVID-19 pathogenesis and the detailed underlying mechanism of asymptomatic infection in juveniles and young adults.

Poster Reception III

Tonia T. Kam - AOXI0495

SARS-CoV-2 and SARS-CoV-2 variants infection of human cardiomyocytes

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Background

The current coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been a major threat to the public health-care system over the world. Cardiac complications, particularly myocarditis, has been commonly found in COVID-19 patients, especially in critically ill patients. Infection and replication of cardiomyocytes by wild-type SARS-CoV-2 has been demonstrated previously. However, little is known about the detailed mechanism of cardiac injury and inflammation of cardiomyocytes upon SARS-CoV-2 infection. This study aims to investigate the tropism and pathogenesis of SARS-CoV-2 and SARS-CoV-2 variants infection in human cardiomyocytes. Since cytokine dysregulation is known be associated with COVID-19 severity, the induction of pro-inflammatory cytokine by cardiomyocytes upon SARS-CoV-2 and related signaling pathways were investigated.

Method

Using cardiomyocytes derived from human embryonic stem cells (hESC) as a model, we compared the viral tropism, replication competence and innate host response upon infection of the wild-type SARS-CoV-2 and SARS-CoV-2 variants: the Delta and the Omicron. hESC-derived cardiomyocytes were infected with the viruses to study their replication efficiencies. Alterations in the mRNA expression of cardiac markers, proinflammatory cytokine profile, and signaling pathways were investigated.

Result

Productive viral replication of SARS-CoV-2 and SARS-CoV-2 variants, delta, omicron was observed in human cardiomyocytes with wild-type SARS-CoV-2 showing a significantly higher replication than Omicron. All three variants of SARS-CoV-2 induced pro-inflammatory cytokines (e.g. IL8, IL6) in hESC-derived cardiomyocytes. VEGF signaling pathway was found to be altered upon infection.

Conclusion

Our study suggest that both the wild-type and the variants of SARS-CoV-2 are capable of infecting and productively replicating in human cardiomyocytes, thereby inducing proinflammatory cytokine release and alterations in VEGF signaling pathways. These results have implications for understanding the pathophysiological mechanism of cardiovascular injury in COVID-19 patients.

Poster Reception III

Yukiko Muramoto - AOXI0524

Studies on SARS-CoV-2 replication in human nasal organoids

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Background

The nasal cavity is a replication site of novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Olfactory dysfunction has been reported as a typical symptom in coronavirus disease 2019 (COVID-19) patients infected with SARS-CoV-2, however, the pathogenesis of olfactory dysfunction remains unclear due to the lack of appropriate models recapitulating human nasal neuroepithelium.

Method

Here, we generated human nasal epithelium (NE) organoids from human embryonic stem cells, which comprise nasal respiratory epithelium (NRE) and olfactory epithelium (OE). The NE organoids were infected with SARS-CoV-2 to evaluate virus replication property and identify susceptible cell types of SARS-CoV-2 infection.

Result

Immunostaining showed that angiotensin-converting enzyme 2 (ACE2), which is a receptor of SARS-CoV-2, was expressed in NRE in the NE organoids. SARS-CoV-2 replicated well in the NE organoids. In contrast, it did not replicate in ACE2-knockout NE organoids, suggesting that SARS-CoV-2 infection in human NE is ACE2-dependent. Immunostaining of SARS-CoV-2-infected NE organoids showed that SARS-CoV-2 efficiently infected and replicated in cells in NRE, and rarely infected in cells in OE. Interestingly, ACE2 expression was induced in OE following SARS-CoV-2 infection in NRE. Single-cell RNA sequencing revealed that type I and type III interferons were expressed in SARS-CoV-2-infected cells in the NE.

Conclusion

These findings provide novel insights into SARS-CoV-2 replication and the host responses in human NE and suggest underlying mechanisms of olfactory dysfunction in COVID-19.

Poster Reception III

Jack Hassard - AOXI0531

Investigation into the Interactions between SARS-CoV-2 and the Gylcosaminoglycan Heparan Sulfate

Jack Hassard[1], Yuan Chen[1], Thomas Peacock[1], Wengang Chai[1], Yan Liu[1], Wendy Barclay[1]

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Background

SARS-CoV-2 uses its Spike glycoprotein to bind to angiotensin converting enzyme 2 (ACE2) and facilitate entry into cells. However, multiple studies have highlighted the potential role of the glycosaminoglycan (GAG) heparan sulfate (HS) in SARS-CoV-2 cell entry. The mechanism by which SARS-CoV-2 Spike binds to HS is poorly understood despite HS having been demonstrated to enhance SARS-CoV-2 infection in vitro.

Method

Lentivirus pseudotypes (PVs) expressing both SARS-CoV-1 and SARS-CoV-2 Spike proteins were generated and screened on neoglycolipid-based GAG oligosaccharide microarrays for HS binding. PVs expressing chimeric SARS-CoV-2 Spike with the SARS-CoV-1 Spike receptor binding domains (RBDs) or N-terminal domains (NTDs) were then generated and tested for HS binding to attempt to map the domains responsible.

Result

PVs expressing SARS-CoV-2 Spike protein bind to heparin/HS but not other types of GAG oligosaccharide probes on glycan arrays. This is also the case for recombinantly expressed SARS-CoV-2 Spike protein. Conversely, SARS-CoV-1 PVs do not bind to the GAG oligosaccharide arrays. SARS-CoV-2 PVs containing either a SARS-CoV-1 Spike RBD or NTD no longer have detectable binding to any GAG probes on the arrays.

Conclusion

SARS-CoV-2 may utilise HS as a potential secondary receptor. Both the receptor binding domain and N-terminal domain of SARS-CoV-2 Spike protein are likely to contribute to HS binding.

Poster Reception III

Rare Rollon - AOXI0532

Pathogenesis and Transmission of SARS-CoV-2 in Ferrets

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Background

The outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in China and has been found to have high human-to-human transmission through close contact with infected patients, leading to a rapid global spread of the virus. To prevent SARS-CoV-2 dissemination, understanding the in vivo characteristics of SARS-CoV-2 is a high priority. Where additional animal models that mimic high human-to-human transmission of SARS-CoV-2 infections are warranted.

Method

To demonstrate ferret-to-ferret transmission in an experimental setting, ferrets (n = 2) were inoculated via the intranasal route with 105.5 TCID50 of NMC-nCoV02 (S clade), a strain that was isolated from a COVID-19-confirmed patient in South Korea. Moreover, to evaluate the transmission mode of the virus, naive ferrets (n = 2/group) were placed in direct contact (DC) (co-housed) or indirect contact (IC) (housed in cages with a permeable partition separating them from infected ferrets) with infected ferrets two days after the primary infection. Clinical features of SARS-CoV-2 infections were recorded. Replication and shedding (virus isolation and titration) of SARS-CoV-2 were evaluated in blood, nasal washes, saliva, urine, and fecal specimens. Nasal turbinate, trachea, lung, kidney, and intestine tissues were collected to assess the replication of the virus in various ferret organs. In addition, to further confirm viral replication in infected ferrets, immunohistochemistry (IHC) and histopathological examinations were conducted.

Result

SARS-CoV-2- infected ferrets exhibit clinical manifestation of SARS-Cov-2 infection, such as elevated body temperatures, lethargy, and coughing. Although fatalities were not observed, SARS-CoV-2-infected ferrets shed the virus in nasal washes, saliva, urine, and feces up to 8 dpi. The highest amount of viral RNA was detected in nasal washes and peaked at 4dpi. Interestingly, SARS-CoV-2 was detected in all naive direct contact ferrets as early as 2 days post-contact with few naive indirect contact ferrets showing positive viral RNA in the nasal washes and fecal specimens which persisted for 4 days, suggesting airborne transmission. Moreover, immunohistochemistry revealed detection of viral antigens in intestine, nasal turbinate, trachea, and lungs with acute bronchiolitis in infected lungs. Hence, results revealed multiple routes of virus shedding, serving potential sources for viral transmission.

Conclusion

In summary, we demonstrated a ferret model of SARS-CoV-2 infection and transmission that recapitulates aspects of human disease, hence, would be a useful tool to facilitate development of SARS-CoV-2 therapeutics and vaccines.

Poster Reception III

Alex Wing Hong Chin - AOXI0534

Increased stability of SARS-CoV-2 Omicron variant over ancestral strain

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Background

The Omicron SARS-CoV-2 variant of concern (VOC) is highly transmissible in humans. As of April 2022, the Omicron VOC has been spreading quickly around the world and outcompeting other circulating strains.

Method

We tested the stability of the Omicron VOC and its ancestral strain on different smooth and porous surfaces. Viral droplets of five microliters were applied on the surfaces and were incubated at room temperature for different time points. The treated surfaces were then immersed in viral transport medium to recover the virus on the surface. The viable virus titre recovered were determined by TCID50 assay.

Result

Compared with the ancestral virus, the Omicron VOC was found to be more stable on the tested smooth and porous surfaces. On smooth surfaces such as stainless steel, glass and polypropylene plastic sheet, viable ancestral virus was unable to be detected on day 4 or day 7 post-inoculation. However, viable Omicron VOC was still detectable on these applied surfaces. For porous surfaces, no viable virus was recoverable from the inoculated printing paper and tissue paper in 15 and 30 minutes, respectively. But viable Omicron VOC was still detectable on these surfaces at these time points.

Conclusion

Our results show that the Omicron VOC is more stable than its ancestral strain on both smooth and porous surface. This finding implies that this variant may have an increased likelihood for transmission by the fomite route and this should be taken into consideration when recommending control measures against COVID-19 infection.

Poster Reception III

Jianyu Lai - AOXI0543

Evolution of SARS-CoV-2 Shedding in Exhaled Breath Aerosols

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Background

The transmissibility of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to increase as new variants emerge. It is unclear if all highly transmissible variants of concern (VOCs) are associated with high viral aerosol shedding.

Method

We collected 30-minute fine (≤5 μm in diameter) and coarse (>5 μm) exhaled breath aerosol (EBA) samples with a Gesundheit-II using a loud speaking and singing protocol. Samples were assayed using the TaqPath COVID-19 Real Time RT-PCR Assay (Thermo Scientific) and aliquots were cultured using TMPRSS2-expressing VeroE6 cells and A549-ACE2 cells.

Result

From June 2020 to March 2022, we collected EBA from 93 people with mostly mild SARS-CoV-2 infections (Table 1).

We detected SARS-CoV-2 RNA in EBA from 44 (47%) people. Infectious virus was recovered from EBA of one Alpha and one B.1.2 (Adenaiye et al, 2021), two Delta, and four Omicron (two BA.1.1 and two BA.2) cases (three singly boosted), including seven fine and one coarse EBA sample. Overall, fine EBA viral RNA loads were similarly high for Alpha, Delta, and Omicron cases (Figure 1), and among Omicron subvariants; the maximum was 1.8x107 RNA copies from an Omicron BA.1.1 case.

Conclusion

Evolutionary selection for SARS-CoV-2 variants associated with high viral aerosol shedding appears to be occurring. Immune evasion and enhanced transmissibility are likely responsible for Omicron's ascent even as infection- and vaccine-acquired immunity increases. As current vaccines and boosters cannot prevent shedding of infectious virus via aerosols, non-pharmaceutical interventions including masking and indoor air cleaning (ventilation, filtration, germicidal UV) are still needed to mitigate COVID-19 transmission and protect vulnerable populations.

Poster Reception III

Zijian Guo - AOXI0454

Influenza A Virus Neuraminidase Activity Modulates Cellular Co-infection

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Background

During influenza A virus (IAV) infection, the surface proteins hemagglutinin (HA) and neuraminidase (NA) mediate virus attachment and detachment from the viral receptor, sialic acid. Functional balance between these competing proteins is critical for IAV spread. While genetic determinants of HA binding affinity and NA activity are well-established, other factors that may contribute to HA-NA balance remain less clear. By investigating the activity and function of NA in situ during productive infection, we find that its ability to cleave sialic acid both in cis and in trans modulates virus attachment to neighboring cells and the extent of viral co-infection that occurs during multicycle growth.

Method

Using selective oxidation and fluorescent hydrazide coupling, we quantify the abundance of sialic acid on NA-expressing and neighboring cells. Sialic acid depletion provides a measurement of in situ NA activity in cis (on the surface of infected cells) and in trans (on the surface of uninfected neighboring cells). Using complementary fluorescence techniques, we monitor the binding and spread of virions during infection, connecting viral spread to cell-surface sialic acid abundance and NA activity.

Result

We show that cell surface NA cleaves sialic acids on both infected and neighboring uninfected cells and that the extent of cleavage does not necessarily follow the intrinsic activity of NA measured by traditional assays (e.g. MUNANA). Trans cleavage of sialic acid leads to a reduction in virus binding capacity and thus virus uptake by neighboring cells (Fig. 1). Modulating A/California/04/09 NA activity through inhibition with oseltamivir, supplementation with exogenous sialidase, or genetic replacement with weaker NA from A/WSN/33, reveals that the amount of virions shed to neighboring cells is inversely correlated with in situ NA activity, leading to differences in co-infection (Fig. 2) and multi-round spread (Fig. 3).

Conclusion

Cell surface NA changes virus binding capacity through depleting sialic acids in cis and in trans. This gives rise to different spatial distributions of progeny virions and modulates co-infection. As most virions are not capable of independently initiating productive infection, this may have important implications in the progression of infection.

Poster Reception III

MUHEMEDI SALEH KAYUMBA - AOXI0460

Determining influenza seasonal characteristics in the Democratic Republic of Congo

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Background

Since 2007, the DRC has set up a sentinel influenza surveillance system across the country with 11 sentinel surveillance sites and a national influenza reference laboratory. Due to logistical difficulties, the number of sites was gradually reduced to four.

This study was conducted to determine the characteristics of each influenza season, particularly the beginning, the end, the intensity, and the different alert thresholds to fully understand the dynamics of influenza transmission and prepare the country to deal with epidemics and the next influenza pandemic.

Method

In this cross-sectional study, we used the weekly percentage of positive ILI and SARI to produce the average curve of 21(from 2010 to 2022) past seasons compared to the epidemic curve of the current influenza season (2021/2022). We have also determined average and epidemic curve thresholds, including epidemic, moderate, high, and extraordinary thresholds. The process was performed by using a specific WHO web tool located at https://worldhealthorg.shinyapps.io/averagecurves/.

Result

Regarding ILI data, the average curve showed two waves: The first runs from the 40th to the 15th week with a peak in the 52nd week, and the second wave extends from the 10th to the 40th with a peak in the 19th week. For SARI data, two waves were also observed in the same period that ILI data, with peaks in the 52nd and during the 19th week. Overall, the average intensity for those two waves was moderate.

Conclusion

The use of PISA indicators to assess the severity of seasonal epidemics has been a useful way to better understand influenza seasons in the DRC and to better prepare for the upcoming influenza pandemic.

Poster Reception III

Nancy Leung - AOXI0506

Detection of airborne respiratory viruses in pediatric patient rooms

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Background

Respiratory virus infections have been thought to transmit via droplets of various sizes including fine particle aerosols, but there are few direct comparisons between different respiratory viruses. We aim to determine whether common respiratory viruses of public health importance in the pediatric population could be detected in the air in healthcare settings.

Method

We sampled air with two-stage cyclone (NIOSH) sampler in 5-bed pediatric patient rooms in a tertiary hospital in China where at least one patient was admitted with influenza-like illness and was later confirmed with one of the targeted respiratory virus infections. The NIOSH samplers collected air particles in size fractions of <1 µm, 1-4 µm and >4 µm. Collected air samples were tested for common pediatric respiratory viruses and quantified by reverse transcription polymerase chain reaction (PCR).

Result

From December 2017 to January 2020, on 44 occasions, we recovered influenza A virus, influenza B virus, respiratory syncytial virus (RSV), adenovirus (ADV) and parainfluenza virus (PIV), in 70%, 27% 27%, 23% and 5%, respectively. Moreover, on occasions with patient(s) positive for infection, influenza A virus was the most frequently recovered ($p<0.01$), of influenza A detected in 67% (five to 8) compared to influenza B in 50% (two to 4), and lesser of other respiratory viruses in ADV in 39% (seven to 18), RSV in 25% (six to 24), and even much lesser in PIV (one to 16). Influenza virus was most frequently recovered in all-size fraction mainly in flu season. RSV and ADV were detected both in droplets and fine particles, while PIV was mainly in droplets.

Conclusion

Influenza virus, respiratory syncytial virus (RSV) and adenovirus (ADV) were frequently recovered in the air, in frequencies that were consistent with the prevalence of respiratory virus infections in the children in those rooms. The detection of various respiratory viruses in the air in pediatric patient rooms may suggest the need of strengthening indoor ventilation in healthcare settings.

Poster Reception III

Adamou Lagare - AOXI0519

SARS-CoV-2 and RSV detection through the framework of influenza surveillance in Niger, 2020-2021

Adamou Lagare[1]

[1]CERMES

Background

Acute respiratory infections (ARI) cause more than four million deaths particularly among children annually, with the overwhelming majority occurring in developing countries. Influenza and respiratory syncytial virus accounted as major viral pathogens of ARI. However, with the advent of Covid-19 pandemic, SARS-CoV-2 has become a serious threat. In Niger, part of influenza sentinel surveillance, SARS-CoV-2 and RSV have been monitored to determine predominating strains and coinfections.

Method

From January 2020 to December 2021, nasopharyngeal swab samples were collected from the eight sentinel sites located in three regions according to the WHO case definition for Severe Acute Respiratory Infections (SARI) and Influenza Like Illness (ILI) patients. Influenza and SARS-CoV-2 virus were detected by qRT-PCR using the CDC Influenza SARS-CoV-2 Multiplex Assay (RUO) kit. RSV was detected using specific primers and probe.

Result

A total of 2,689 suspected influenza cases were sampled during the study period from which 1,383 (51.4%) were SARI cases. The average age was 3.6 years with predominance of male 1,476 (54.8%). Influenza types A and B viruses were detected in 252 (9.4%) cases. H1N1pdm and H3N2 were the predominating influenza A subtypes with respectively 100/206 (48.5%) and 47/223 (22.8%). Influenza B Victoria was the only lineage detected with 33/39 (84.6%). Covid-19 and RSV infections were confirmed among 59 (2.2%) and 171 (6.3%) cases. 28 cases of coinfections were detected with Covid-19 and RSV representing 14 (50.0%), influenza and Covid-19 8 (28.6%) and influenza and RSV 6 (21.4%).

Conclusion

Although the impact of the pandemic on the health system, influenza sentinel surveillance in Niger contributed to monitor both Covid-19 and RSV infections. These data sustain the new WHO strategy for integrated surveillance of influenza and other respiratory pathogens including Covid-19.

Poster Reception III

Nicole Rockey - AOXI0530

Transmission of human seasonal influenza viruses in ferrets under realistic environmental conditions for comparison to childcare centers

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Background

Ferrets are widely used as a model to study influenza virus infection and transmission. Current ferret transmission systems allow researchers to gain insights into the pandemic risk posed by emerging influenza viruses, but they do not mimic environmental parameters (air exchange rates, flow patterns) or exposure times in real-world settings. Here, we describe a novel transmission setup that better represents environmental characteristics in childcare settings and includes multiple aerosol collection strategies and video recording to characterize host behaviors. Ultimately, this setup will allow us to identify behaviors that affect transmission and assess the effectiveness of various interventions that reduce onward transmission in a controlled experimental setting.

Method

Our transmission setting consists of an exposure space housed within a biobubble that restricts air exchange to ~1 per hour. During each exposure, four naïve recipient ferrets were exposed to a donor ferret infected with 2009 H1N1 pandemic virus (A/CA/07/09) for either one or three hours of total exposure. Air samples were collected inside the exposure area and around the perimeter using NIOSH bioaerosol cyclones and a condensation sampler (Spot) for virus quantification. Surface samples inside the exposure space were also collected for virus quantification. Multiple air sensors monitored CO2, relative humidity, and temperature in the biobubble. Video monitoring allowed for individual host tracking to characterize interactions. Influenza virus transmission was assessed through viral titration of ferret nasal washes and seroconversion post-exposure.

Result

Following a one-hour exposure of naïve recipients to an infected donor, infectious influenza virus from surface samples was recovered, including from toys and on the wall of the exposure area. However, no infectious virus was detected in air collected from the Spot sampler. Influenza virus transmitted to one of four recipients after a one-hour exposure. Extending the exposure timeframe to three hours increased transmission to three of four recipients. In this extended exposure setting, infectious virus was still undetectable in collected air, while it was detected in two surface samples. Similar temporal trends in CO2 concentrations measured in two locations in the space suggest a well-mixed atmosphere.

Conclusion

Our findings demonstrate that influenza virus is readily culturable from surfaces after exposure to an influenza virus-infected ferret. Additionally, in this reduced air exchange setting, increased exposure time results in higher transmission efficiency. Ongoing analysis will reveal the behaviors correlated with successful transmission events and contamination of environmental surfaces.

Poster Reception III

Juryeon Gil - AOXI0479

Characterization estimation of avian influenza viruses in migratory birds

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Background

Several subtypes of avian influenza (AI) viruses have caused critical human infections in recent years; however, there is a severe knowledge gap regarding the capacity of wild bird viruses to infect mammals. Therefore, the characterization of AI viruses isolated from their natural hosts is critical to understand their pathogenic and pandemic potentials. Herein, we characterize the genetic and biological properties of various wild bird-isolated low pathogenic AI (LPAI) viruses.

Method

To understand the pathogenic potentials of these viruses, 34 LPAI of varying subtypes were genetically and phylogenetically characterized to evaluate molecular markers which may indicate enhanced pathogenic-phenotype among avian or mammalian hosts. Further, we evaluated replicative and pathogenic properties of these viruses using various animal models and solid-phase direct binding assay was performed to determine receptor-binding preference of the LPAI viruses.

Result

All selected AI virus subtypes were found to predominantly possess Eurasian lineage, although reassortment with North American lineage AI viruses was also noted in some isolates. When used to infect chickens, 20 AI isolates could be recovered from oropharyngeal swabs at 5 days post-infection (dpi) without causing significant morbidity. Similarly, mild to no observable disease was observed in mice infected with these viruses although the majority replicated efficiently in murine lungs. As expected, wild bird AI isolates were found to recognize avian-like receptors, while a few strains also exhibited detectable human-like receptor binding. Selected strains were further tested in ferrets, and 15 out of 20 were found to shed the virus in the upper respiratory tract until 5 dpi. Overall, we demonstrate that a diversity of low-pathogenic AI viruses carried by wild migratory birds have the capacity to infect land-based poultry and mammalian hosts while causing minimal signs of clinical disease.

Conclusion

This study reiterates that there is a significant capacity for interspecies transmission of AI viruses harboed by wild aquatic birds. Thus, these viruses pose a significant threat to human health underscoring the importance of continued virus surveillance in wild aquatic birds.

Poster Reception III

Fuhan Yang - AOXI0450

Annual and non-annual cycles in the respiratory disease dynamics in tropical regions

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Background

Respiratory diseases have been a research focus because they are one of the leading causes of global morbidity and mortality. Sufficient evidence has showed regular annual seasonality of influenza and other respiratory diseases in temperate regions. However, the seasonality of respiratory diseases in the tropics is less well-defined because of the lack of regular winter-forced environmental and behavioral change.

Method

We built a community-based surveillance system including 89 outpatient clinics in a tropical city, Ho Chi Minh City, Vietnam, from 2010 to 2019. We monitored the patients that have the symptoms of respiratory diseases, defined as influenza-like-illness (ILI), using the daily messages sent from the clinicians. We detected the periodic signals of ILI time series using periodogram and wavelet spectra. We estimated the cycles using a simple cyclic step function. And we confirmed the existence of the cycles using regression models.

Result

We found that there are both annual and non-annual (around 200 days) periodic signals present in the ILI time series. ILI activity showed 8.9% [95% CI: 8.8% - 9%] difference in the annual cycle, and 6.9% [95% CI: 6.6% - 7%] difference in the non-annual cycle, leading to all-year transmission pattern with 8.9% lower ILI activity from the beginning of March to the middle of May every year. The regression model predicts the ILI dynamics better when adding either of the cycle in the model, confirming the existence of both cycles. Compared to temperate regions, the non-annual signals are unique in the ILI activity in Ho Chi Minh City.

Conclusion

We found that the respiratory disease dynamics showed both annual and non-annual cyclic and weak fluctuations in a winter-absent tropical setting. The climate factors and the school term contribute less to the prediction of ILI dynamics, suggesting the lack of climate-forcing ILI dynamics in tropical regions.

Poster Reception III

Bjarke Frost Nielsen - AOXI0462

Superspreading: Effects on lockdowns and pathogen evolution

Bjarke Frost Nielsen[1], Lone Simonsen[1], Kim Sneppen[2], Andreas Eilersen[2]

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Background

Although COVID-19 has caused severe suffering globally, the efficacy of lockdown-type non-pharmaceutical interventions has been greater than typical models would predict. Meanwhile, increasing evidence indicates that superspreading plays a dominant role in COVID-19 transmission. Recent estimates suggest that the dispersion parameter k for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is on the order of 0.1, which corresponds to about 10% of cases being the source of 80% of infections. This is in contrast to pandemic influenza, which is seen to spread more homogeneously.

Method

Using dynamical as well as statistical modelling, we probe the effects of superspreading on the effectiveness of non-pharmaceutical interventions which rely on reductions in personal contact number.

Result

We find that superspreading drastically affects the effectiveness of lockdowns and general reductions in overall personal contact number. When transmission is overdispersed, these types of interventions have the potential to be highly effective.

Lastly, we discuss how overdispersion may affect the evolutionary trajectory of a pathogen.

Conclusion

Pathogens exhibiting overdispersed transmission ('superspreading') are found to be more vulnerable to non-pharmaceutical interventions which rely on reductions in personal contact number, such as lockdowns. This highlights the importance of considering transmission statistics when designing control programmes for emerging pathogens. Overdispersion is also likely to play a role in the evolutionary trajectory of a pathogen, in a fashion which is modulated by contact structure and non-pharmaceutical interventions.

Poster Reception III

Zhor Zeghari - AOXI0668

Knowledge, attitude and practice associated with Covid-19 among physicians in Morocco: a cross sectional study

Background

Since the start of the Covid-19 pandemic, the Moroccan Directorate of Epidemiology and Disease Control elaborated guidelines, which it continuously reassesses, that provides a framework to help navigate Covid-19 related issues. Evaluating access, understanding and adherence to these guidelines by healthcare professionals is essential to improve the country's national healthcare response to the crisis. The aim of this study is to subsequently assess the knowledge, attitude and practice associated with Covid-19 among physicians in Morocco.

Method

This cross sectional study was conducted from January 28, 2022 to February 8, 2022. The including criteria was to be a physician currently working in Morocco and the exclusion criteria was to be still in training. This study was approved by the national ethics committee. Healthcare professionals were reached through regional and local professional networks using WhatsApp or emails. Data was collected using an online Google Forms self-administrated questionnaire. The knowledge section contained questions about Covid-19 prevention, clinical characteristics and management. The attitude and practice parts were about pandemic perception, the guidelines acceptance and daily practice. Three scores were calculated based on the answers provided in each section. Categorical variables were expressed in numbers and percentages, while quantitative ones were expressed with mean and standard deviation. Using Jamovi 2.3, analysis was conducted with linear regression and linear correlation.

Result

Among the 780 participants that completed the survey, 775 physicians were included in the analysis. Of these, 65.7% (477) were females. The median age was 47.4 [39.7, 55.4]. All Moroccan regions were represented. The majority of the physicians (82.5%, 639) worked in the public sector. The last guideline was read by 76.3% of the participants (591).

The mean knowledge score was 29.9±4.1 of 38. It was significantly higher ($p<0.001$) among older physicians, females, participants from public sector, those who received a training about Covid-19, who knew about the last guidelines and finally who were previously infected by Covid-19.

The attitude score was positively correlated with knowledge one ($r=0.185$, $p<0.001$).

The practice score was also correlated to the knowledge one ($r=0.145$, $p<0.001$) but it was more narrowly correlated to the attitude one ($r=0.246$, $p<0.001$).

Conclusion

The physicians working in Morocco had a strong knowledge of Covid-19 guidelines with a rather positive attitude and good practice besides some disparities among some groups who may need a targeted communication like young doctors or private practitioners.

Poster Reception III

Alison Han - AOXI0475

Long-term Changes in Antibody Titers to Hemagglutinin and Neuraminidase After Influenza Challenge-an update

Alison Han[1], Lindsay Czajkowski[1], Adriana Cervantes-Medina[1], Rani Athota[1], Luca Giurgea[1], Holly Baus[1], Susan Reed[1], Monica Gouzoulis[1], Jenna Sherry[1], Jeffery Taubenberger[1], Matthew Memoli[1]

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Background

Circulating anti-influenza antibodies play an important role in protection, both after natural infection and vaccination, but generally do not persist at high levels and often wane over time. Human influenza challenge models provide a unique ability to follow individuals from a well-characterized exposure to measure long-term changes in serum antibody titers from pre-exposure baselines.

Method

Healthy volunteers who completed influenza challenge studies at the National Institutes of Health Clinical Center were invited to enroll in a long-term study for 2 years after completing an influenza challenge study. Participants were seen in the clinic once every 12 weeks which included blood collection for serum antibody titers, specifically H1 and H3 hemagglutination inhibition (HAI) and N1 and N2 neuraminidase inhibition (NAI) titers. They also completed monthly online questionnaires to monitor for symptoms of influenza-like illness in between clinic visits.

Result

Since July 2015, 39 participants enrolled and attended at least one study visit. Twenty-six (67%) participants completed an Influenza A H1N1 challenge study, 7 (18%) completed an Influenza A H3N2 challenge study, and 6 (15%) completed both H1N1 and H3N2 challenge studies. At enrollment, participants ranged in age from 19-53 years with 21 (54%) female, 16 (41%) White, 15 (38%) Black, and 7 (18%) Hispanic or Latino participants. Participants completed between 1 and 18 study visits. Among H3N2 influenza challenge participants, the H3 HAI titers and N2 NAI titers did not rise significantly after challenge, and all titers remained similar through follow-up. Among H1N1 influenza challenge participants from 2 different challenge studies, H1 HAI and N1 NAI titers continued to rise after challenge, peaked at Visit 1 (approximately 5 months after challenge), and returned to pre-challenge levels after 2 years. H3 HAI titers and N2 NAI titers in H1N1 influenza challenge participants remained similar to pre-challenge levels.

Conclusion

The H3N2 challenge virus caused more limited disease compared to H1N1 challenge and may have resulted in a reduced antibody response in those challenged with H3N2 who maintained a baseline titer from previous exposure. The clear increase and then decrease over time of H1 HAI and N1 NAI titers after 2009 H1N1 challenge was similar after 2 different H1N1 challenge studies despite an interruption in follow-up due to the COVID-19 pandemic suggesting that these responses were robust after exposure but waned over time.

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Poster Reception III

Charlotte George - AOXI0473

Towards a scalable DNA vaccine platform: lower doses of plasmid and synthetic DNA encoding a SARS-CoV-2 vaccine improves neutralising antibody responses

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Background

A prompt vaccine response to future pandemics requires rapidly scalable and globally accessible vaccine platforms. DNA vaccines benefit from thermostability at room temperature without the need of a cold-chain, making them ideal candidates for global distribution. However, manufacturing of traditional plasmid DNA (pDNA) vaccines require large bio-fermenters and stringent purification requirements in micro-organism dependent stages of multi-gram scale cGMP production. This can create a bottleneck in the scale and timeline of DNA vaccine production when a rapid response is required.

As an alternative to bacterial plasmids, Touchlight Genetics Ltd. has developed a synthetic, enzymatic manufacturing process resulting in linear DNA constructs (Figure 1), termed Doggybone DNA™ (dbDNA™). This yields cGMP, multi-gram scale linear DNA on a clean room bench within a shorter timeframe than pDNA.

This study set out to evaluate and compare the immunogenicity of pDNA and dbDNA™ encoding a modified SARS-CoV-2 receptor-binding domain antigen, M7, in BALB/c mice. The effect of DNA dose on vaccine elicited immunogenicity was assessed.

Method

BALB/c mice were immunised subcutaneously with either pEVAC or dbDNA™ encoding M7 and bled via the saphenous vein according to the regime in Figure 2. Serum neutralising (nAb) and binding antibody (bAb) responses were assessed using SARS-CoV-2 pseudotyped based micro-neutralisation assays and by direct ELISA, respectively.

Result

dbDNA™ and pDNA encoding M7 elicited potent bAb and nAb titres in BALB/c mice, which increased over time (Figure 3a and b). Low doses of dbDNA™ and pDNA elicited greater neutralising antibody responses compared to high doses (Figure 3b and c).

Conclusion

This study demonstrated that low doses of the M7 candidate vaccine expressed either as dbDNA™ or pDNA elicited greater nAb responses compared to higher dose of either DNA vaccines. These findings have important implications in DNA vaccine dose selection and vaccine efficacy. Improved DNA manufacturing, immunisation, and dose sparing delivery would make DNA vaccines a desirable and globally accessible vaccine alternative to current cold-chain dependent methodologies.

Poster Reception III

Sarah BELKALEM - AOXI0561

Spread of multiple lineages of SARS-CoV-2 in Algeria, may 2020 to november 2021

Sarah BELKALEM[5], Happi Christian Tientcha[1], Hadjal Baya[5], Gradi Alia[5], Izri Kahina[5], Mehdi Zahida[5], Mohamed Amine Beloufa[3], Aicha Bensalem[4], Fawzi Derrar[2]

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Background

The COVID-19 pandemic has revealed the importance of whole genome sequencing (WGS) to guide public health interventions to control virus transmission and understand SARS-CoV-2 evolution.

Analysis of early Algerian sequences showed multiple disease introductions of SARS-CoV-2 and consisted of several lineages.

In Algeria, few data concerning lineages spread are available.

Herein, we describe the genetic diversity of circulating SARS-CoV-2 by WGS in Algeria mostly in central region throughout the first three pandemic waves.

Method

Samples from nasopharyngeal swabs collected from patients positive for SARS-CoV-2 that were obtained mostly(90.62%) from the central region of the country during may2020-november2021 were used for WGS.

Samples were selected based on low cycle threshold value(< 28) of the RT-PCR assay and were sequenced using Illumina MiSeq(n = 30) and Oxford Nanopore MinION(n = 45) next-generation sequencing platforms following respectively for each technology: Midnight and COVIDseq protocols.

All sequences have undergone both quality control for adequate coverage, indels, frameshifts and mutation calling using Nextclade. Sequences were classified according to PANGO-lineages system and have been submitted to GISAID database. To analyse trends of lineages over time, we retrieved those sequences from Algeria in GISAID.

Result

Among 75 samples tested in this study, we obtained 62 sequences that met the standard quality assessment parameters employed in NextClade.

During the first and second waves of the epidemic in Algeria, multiple SARS-CoV-2 phylogenetic lineages were detected consisting of:B.1(7/62,11.30%), B.1.1(6/62,9.66%), B.1.160(4/62,6.46%), B.1.597(3/62,4.83%), B.1.356(1/62,1.61%) and B.1.1.317(1/62,1.61%).

Before the first wave, which occurred in Algeria between june and september 2020, we reported B.1, B.1.525 and B.1.597 in circulation(1/62,1.61% for each lineage).

Of note, the Alpha variant (B.1.1.7)(5/62,8.07%) and the Eta variant (B.1.525) (7/62,11.30%) were both co-circulating during the period between the second and third waves in addition to B.1 (1/62,1,61%) and R.1 (1/62,1.61%).

Results showed that the Delta variant (19/62,30.65%) (B.1.617.2 and sublineages AY.20, AY.46.6, AY.54, AY.109 and AY.131) were predominant during the third wave. While B.1.1.7 remains in circulation (4/62,6.46%).

Conclusion

Overall, our analysis shows co-circulation of different SARS-CoV-2 lineages during the first and second waves rapidly replaced by the Delta variant that became dominant until the Omicron variant emerged.

A major limitation of this study was suboptimal sampling. However, a national genomic surveillance system is being implemented to ensure geographically and timely representative sampling.

Poster Reception III

Ben Cowling - AOXI0576

Temporal characteristics of case profile and case isolation shaped the distributions of serial interval of COVID-19 in Hong Kong

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Background

The serial interval distribution is used to approximate the generation time distribution, an essential parameter to predict the effective reproductive number "Rt", a measure of transmissibility. However, serial interval distributions may change as an epidemic progresses rather than remaining constant.

Method

We examined detailed contact tracing data on laboratory-confirmed cases of COVID-19 in Hong Kong during all four waves from January 2020 to May 2021. We constructed the transmission pair data from the epidemiological link information between infector and infectee. We estimated effective serial intervals and assess the association with age and severity profile of cases and case isolation at a temporal scale.

Result

We show that serial intervals in Hong Kong varied over time with variations in patterns across the waves. The serial intervals were found to be shortened and lengthened and closely associated with the impact of temporal variation in COVID-19 case profiles and public health and social measures that were implemented in response to surges in community transmission.

Conclusion

The age and severity profile of infector and infectee and case isolation could primarily shape the mean serial intervals in Hong Kong. Methodological developments require to incorporate in estimating effective serial interval distributions when inferring the time-varying transmissibility.

Poster Reception III

Teresa A. Aydillo Gomez - AOXI0587

ANTIBODY IMMUNOLOGICAL IMPRINTING IN CHILDREN WITH COVID-19

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Background

There has been a great effort to monitor specific serologic responses after SARS-CoV-2 infection and vaccination, however the level and clinical consequences of pre-existing immunity, immunodominance and antigenic hierarchy of human coronaviruses (HCoVs) remains unclear. In addition to SARS-CoV-2, humans are also susceptible to six other coronaviruses, for which consecutive exposures to antigenically related seasonal coronaviruses are frequent. Here, we investigated the role of pre-existing immunity and immunological imprinting on COVID-19 patients' antibody response.

Method

A comprehensive longitudinal antibody profiling against SARS-CoV-2 and seasonal HCoVs (alpha- 229E; and beta-OC43) was performed, including characterization of antibodies against full-length spike (S), the receptor binding domain (RBD)/S1 and S2 domain.

Result

We previously showed that immune memory recall to conserved regions of the S of HCoVs can interfere with the newly induced immune response to SARS-CoV-2 upon infection in adults. This phenomenon -termed immune imprinting- led to delayed antibody responses against variable but immunodominant domains of SARS-CoV-2 S protein. Next, we explored the role of immune imprinting according to age in a pediatric cohort of different ages upon COVID-19 infection. Eighty-eight children were enrolled. Of these, 13 (15%) were infants (0-1 years) and 75 (85%) were children and adolescents (>1 years-19.9 years). All patients developed detectable levels of antibodies against SARS-CoV-2 antigens that remained stable at post-convalescent phase. However, infants showed higher levels of induction compared to pediatric patients. Antibody profiling against seasonal HCoVs showed preexisting and detectable levels in all pediatric patients in an age-dependent manner. However, a significant back-boosting effect was noted only for the beta- HCoVs OC43 S2 domain upon SARS-CoV-2 infection. Interestingly, no increase was detected overtime for patients <1 year, suggesting that these are maternal antibodies. Importantly, a negative correlation was found between IgG responses against SARS-CoV-2 antigens and pre-existing immunity to HCoVs-OC43.

Conclusion

Our results demonstrate that primary infection with HCoVs can occur early in childhood. Infection with beta-HCoV results in imprinting of B cells specific for the S protein and this correlates with reduced induction of specific antibodies against SARS-CoV-2 S protein upon SARS-CoV-2 infection.

Poster Reception III

Kathleen Subramoney - AOXI0590

Impact of intra-host immune adaptations on the evolution of SARS-CoV-2 S protein among individuals with SARS-CoV-2 infections, South Africa, 2020 to 2022

Kathleen Subramoney[8], Beauty Kalenga[3], Nkhensani Mtileni[3], Avani Bharuthram[3], Ashlyn Davis[3], Francinah Maoko[3], Andiswa Simane[3], Jennifer Giandhari[6], Yeshnee Naidoo[6], Sureshnee Pillay[6], Upasana Ramphal[6], Yajna Rampha[6], Houriiyah Tegally[6], Eduan Wilkinson[1], Nadia Harris[4], Nombuso Nxele[5], Bhaveshan Reddy[5], Bonolo Mashishi[5], Nonhlanhla Mbenenge[5], Tulio De Oliveira[7], Zinhle Makatini[5], Burtram Fielding[2], Florette K. Treurnicht[5]

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Background

Intra-host diversity studies can be used to characterise the frequency of mutations and possible heterogeneity of SARS-CoV-2 infections to assist with understanding the impact of virus-host adaptation on the emergence of new lineages. This study investigated the frequency and diversity of spike (S) protein mutations within individuals with SARS-CoV-2 infection in Gauteng South Africa.

Method

Study population included SARS-CoV-2 positive respiratory samples obtained from July 2020 to May 2022. Genotyping was performed with single nucleotide polymorphism (SNP) assays for key S protein mutations. Next-generation sequencing was performed with the Illumina MiSeq or Oxford MinION platforms. SNP assays were analysed using the QuantStudio 5 analysis software and fastq sequencing data were analysed using galaxy.eu, with specific COVID-19 workflows to determine the allele frequency across each sample.

Result

SARS-CoV-2 lineages or variants of concern identified in 2020 were B.1, B.1.1, B.1.1.1, B.1.1.448, B.1.1.52, C.1 and Beta; in 2021 B.1, C.1, C.1.2, Alpha, Beta, Delta, Omicron BA.1 and Omicron BA.2 whereas in 2022 B.1.1.529 and Omicron lineages BA.1, BA.2, BA.4 and BA.5 dominated. Heterogeneous infections occurred in 7% (249/2872) of the study population. The SNP assays identified 5% (50/946) of cases with heterogeneity at delY144 (4%; 2/50), E484Q (6%; 3/50), N501Y (2%; 1/50) and P681H (88%; 44/50). The latter were confirmed as infected with Delta variants. Sequencing confirmed the allele frequency (AF) for delY144 as 0.22, and at position E484 as 0.38 for 484Q and 0.5 for 484A in the aforementioned cases. In addition, variant calling identified 11% (201/1808) cases infected with C.1.2, Beta, Delta, Omicron BA.1, BA.2 and BA.4 that had heterogeneity in the S protein. Amino acid heterogeneity was mainly identified at positions 371 (90%; 181/201) with S371FP (AF 0.3-1.0), and 484 (2%; 4/201) with E484KQ (AF 0.1-0.4), E484AK (0.2-0.7) and E484QA (AF 0.4-0.5).

Conclusion

Here we show that SARS-CoV-2 heterogeneity or quasispecies are present in more than 10% of infected persons. Our study identified E484KQ heterogeneity only among Delta variants. The E484K and E484Q mutations were shown to reduce sensitivity to vaccine-induced antibodies with increased transmissibility due to higher affinity for the host receptor. S371FP heterogeneity were primarily identified in Omicron BA.1. Mutations at position 371 reportedly result in reduced recognition of the RBD antibodies. We hypothesise that intra-host SARS-CoV-2

quasispecies with heterogeneity in the S gene facilitate outgrowth of variants that are able to fully or partially evade host and vaccine-induced immune responses, and thus continue to spread.

Poster Reception III

Jasmin Sidhu - AOXI0592

Distinct inflammatory responses in the nasal mucosa and blood of individuals hospitalised with COVID-19

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Background

Defensive and pathogenic immunological pathways in COVID-19 can be broadly designated as antiviral, pro-inflammatory, anti-inflammatory or coagulation/thrombotic. Changes in circulating mediator levels denoting these pathways often scale with severity. However, mediator levels at the mucosal site of infection are relatively understudied.

Method

Samples collected during the ISARIC-4C study of patients hospitalised with COVID-19 allowed us to compare mediator levels in blood and nasal mucosa in patients with defined disease severity and outcome. Mucosal lining fluid was sampled by nasosorption, a standardised clinically certified non-invasive method that enables nasal fluid sampling. Nasal fluid was obtained from 251 patients with COVID-19 (Mild n=144; Moderate n=75; Fatal n=32) and 25 healthy controls (HCs).

Result

Several mediators were elevated in nasal mucosal fluid regardless of peak disease severity. Whilst many cytokine alterations were similar in the nasal mucosa and plasma, there were notable differences. In the nasal fluid, IFN-γ, TNF-α, IL-2, IL-6, IL-12p70, IL-18, G-CSF and IL-1β generally increased with severity, whereas in plasma, IL-6, IFN-γ, Angiopoietin-2, G-CSF, GM-CSF and IL-10 reflected disease severity. Notably, plasma IL-1β, IL-2, IL-4, IL-12p70 and IL-18 showed little change and nasal fluids showed minor or absent changes in IL-8, D-dimer or Angiopoietin-2.

These trends may reflect COVID-19 activation of the NLRP3 inflammasome in the nasal mucosa leading to IL-1β and IL-18 secretion. IL-12 (produced by myeloid cells) together with IL-18 may stimulate Th1 immunity and IFN-γ secretion by T cells and NK cells, help deliver an effective local antiviral response. Plasma IL-6 and TNF-α provide evidence of a systemic inflammatory response that is especially present in severe COVID-19, but was also seen in nasal samples from those with severe disease. Coagulation abnormalities were evident in plasma but not in the nasal samples.

Conclusion

We conclude that sampling of nasal mucosal fluid offers a simple and practical method by which to assess local responses to respiratory infection applicable to large-scale clinical studies. Using this method, we show evidence of antiviral and inflammasome activation at the site of infection which contrast with findings in the systemic circulation.

Poster Reception III

Xiu-Feng (Henry) Wan - AOXI0602

Surveillance of coronaviruses in the wild rat population in the municipal wastewater system of New York, New York, United States

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Background

Multiple prior studies showed that partial genomes of coronaviruses (CoVs) were detected in municipal sewer systems, and the quantities of the RNA copies in the sewer systems followed the patterns of SARS-CoV-2 outbreaks in the geographically associated human communities. A few unique viral sequences, particularly those at receptor binding domains (RBD) reported to facilitate murine adaptation, were detected frequently in a few (but not all) municipal sewer systems across the United States, including a signal in New York City (NYC) in the Fall of 2021.

Method

To investigate, we captured 82 wild Norway rats that inhabit three sites within the NYC municipal sewer systems in the fall of 2021, when the Delta variants predominated the human outbreaks in the region. We collected the sera, respiratory and gastrointestinal samples from these animals and performed both virological and serological analyses.

Result

s showed that >50% of rats were tested seropositive against both spike- and RBD- specific IgM and IgG detection but were negative in microneutralization assays with live viruses in both lineages B.1 and B.1.617.2 (Delta). The qRT-PCR showed that five respiratory samples were positive against both N1 and N2 primers by using the CDC SARS-CoV-2 diagnosis panel, but viruses failed to be recovered from multiple cells, including Vero E6 cells, 293T/hACE2+TMPRSS, rat lung epithelial cells L2, and rat primary tracheal epithelial cells. After subjecting these samples to pan-virus discovery genome sequencing through capture enrichment, we identified rat coronaviruses (rat-CoV) among these qRT-PCR positive samples.

Conclusion

Our results indicate rat-CoV is likely enzootic in the wild rat population in the NYC municipal sewer systems, and further study needs to validate genomic similarity and serological cross-reactivities between SARS-CoV-2 and rat-CoV. These findings highlight that genetically diverse CoVs are enzootic in wildlife, and it is important to continue pan-CoV surveillance in wildlife to understand the natural history of CoVs and assess the risks these CoVs pose to both agriculture and humans.

Poster Reception III

Paola Resende - AOXI0613

SARS-CoV-2 recombination events detected in Brazil in 2022

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Background

SARS-CoV-2 (SC2) recombination events have been reported from around the world. Viral recombination is a rare event, but it may happen when different lineages co-circulate in a population at the same time and eventually coinfect a given patient. Monitoring for these events is relevant to detect possible emergence of new recombinant lineages with altered phenotypes. The aim of this work was to investigate the genomic features and epidemiological links of SC2 recombinant events in Brazil to help assess their significance as part of ongoing surveillance in Brazil.

Method

Whole genome sequencing was performed using the COVIDSeq Illumina test protocol adapted by the Fiocruz Genomic Network, genomes were assembled by the ViralFlow and classified by Pangolin and NextClade. Additional recombinant genomes reported in Brazil were recovered from GISAID. Epidemiological investigation where these events occurred was performed to establish possible transmission chains.

Result

Thirty-eight samples containing SC2 recombinant strains were found since February 2022 (Fig1). Among them, 1 case of XS in Rio Grande do Sul (RS), 1 case of XF in Bahia. In Pará, an unassigned recombinant minor variant was detected. These were all associated with the recombination between the VOCs Omicron (BA.1) and Delta. In Brazil four cases of recombinant XE in São Paulo (SP), one case of recombinant XQ in Minas Gerais, 2 in SP, 1 in Santa Catarina and 25 in RS were also detected. In addition, one case of XG was detected in SP, associated with the recombination of Omicron strains (BA.1 and BA.2). To better understand the impact of these recombinants, SC2 genomic surveillance was intensified in the populations where they were detected. As a result, 824 genomes from RS collected from February to May 2022 revealed the replacement of BA.1/BA.1* (97.3% in February to 39,13% in May) by BA.2/BA.2* (2.4 % in February to 72.0% in May and the detection of a cluster of the recombinant XQ (0.3% in March to 7,3% in May). This cluster has mutations in Orf1a:C2857T, T5386G, L1774I, S2023del, L2084I and A12334G and Orf1b:C17502T, which were found only in samples from the Brazilian XQ cluster. In contrast, the Orf1b: K1383R mutation was not present in this cluster. These characteristics support the hypothesis that these viruses share the same ancestral clade.

Conclusion

Recombinant viruses need to be part of future surveillance due to their epidemiological importance, since these recombination events have the potential to create new genotypes with different virulence and transmissibility characteristics, which can lead to a major public health burden. Continuous laboratory-based RT-PCR testing and representative sampling are important to maintain timely and sensitive SC2 genomic surveillance.

Poster Reception III

Marilda Siqueira - AOXI0614

SARS-CoV-2 reinfection: A series of reinfection cases from Brazil documented by the genomic features of first and second infecting strains

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Background

Reinfection cases of Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2) have been reported globally. These events have become more frequent since the emergence of the variants of concern (VOCs) in the last quarter of 2020. The first case in Brazil was reported in December 2020. In this study, we evaluated a series of well documented reinfection cases in Brazil from 2020 to 2022.

Method

SARS-COV-2 was detected in two samples of COVID-19 cases collected at least 90 days apart. Up to 386 reinfection suspected cases from different Brazilian regions (North, Northeast, South, and Southeast) were evaluated and the case confirmation was performed by real time RT-PCR followed by whole genome sequencing. SARS-CoV-2 lineages were characterized, and the presence of minor variants characterized using the Viralflow.

Result

A total of 65 reinfection cases were confirmed and all cases had satisfactory clinical progression. Lineages responsible for the first infection of cases were: B.1.1.33, B.1.1.28, and P.2 in 2020 (30 cases) and P.1, P.2, B.1.1.7, N.9, AY.99.2, and AY.101 in 2021 (35 cases). The second SARS-CoV-2 infection of these cases were related mainly by the variant of interest (VOI) P.2 (2 cases), or Variant of Concern (VOC) Gamma/P.1/P.1* (9 cases), Delta/AY*/B.1.617.2 (5 cases) and Omicron/BA* (47 cases). The VOC Omicron has been predominant in reinfection cases (72,3%, 47/65) caused by different lineages BA.1. * and BA.2. From these confirmed reinfection cases 7,7% reported prior complete vaccination course. The analysis of minor variants in the SARS-CoV-2 genomes from the first infection and second infection showed 90% (39/43 cases analyzed) presented minor

variants, 53% (n = 23/43) presented minor variant in the first and second episode and 34% (n = 15/43) presented with a minor variant only in the second infection. Compared to the VOC Delta, the VOC Omicron contains almost twice as many mutations in the Spike protein (S) and important mutations in the ORF1A region, mutations already present in other VOCs and others known to evade antibody neutralization and increase the chances of reinfection cases.

Conclusion

These findings add to data indicating that natural SARS-CoV-2 infection does not necessarily prevent subsequent infections and further demonstrate the important contribution of VOCs in these cases.

Poster Reception III

Marilda Siqueira - AOXI0616

SARS-CoV-2 infection pos vaccine: clinical outcome and genomic features and intra host variants post vaccination

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Background

Coronavirus disease (COVID-19) has affected more than six hundred million people causing more than six million deaths. In January 2021, the Brazilian immunization program started vaccination against SARS-CoV-2 with the aim to control COVID-19 severe cases. It began at a moment when the country was facing the emergence and spread of the VOC Gamma (P.1/P.1. *). As expected, SARS-CoV-2 cases were reported following vaccination in Brazil. The objective of this study was to evaluate a series of cases who reported a previous complete vaccine course and to observe the clinical outcome, to describe viral genomic features of these cases, including intra-host minor variants.

Method

Samples from SARS-CoV-2 infection cases post-vaccine were collected throughout the country. We considered a vaccinated patient, someone who had taken two doses of vaccine with at least 15 days interval between the last dose and the date of the onset of symptoms or collection date. All viruses had the whole genome sequenced and using the ViralFlow we assembled the genomes and observed the presence of intra-host minor variants.

Result

539 post vaccination SARS-CoV-2 cases were recovered from 8 different Brazilian states (Alagoas, Amapá, Espírito Santo, Paraná, Pernambuco, Rio de Janeiro, Rio Grande do Sul e Santa Catarina). The median age of the post-vaccination infected population was 51 (ranging 15 to 98) years-old (y.o). The vaccines administered were AstraZeneca (n = 239), CoronaVac (n = 223), Janssen (n = 9), Pfizer (n = 68), with 83 individuals having received a booster dose of Pfizer. The interval between the last dose and the onset of symptoms was 141 days (ranging 16 to 331 days). In most cases, 77,6% (n = 418) patients recovered well, 15,6% (n = 86) were hospitalized and 6,4% (n = 35) died. It is important to consider that hospitalized cases and death may be associated with other factors such as comorbidities and complications. The lineages that affected these individuals were Delta (n = 167), Gamma (n = 9) and Omicron (n = 363). Up to 127 (79%, 127/163 analyzed) genomes presented minor variants. The selective pressure of immune system may contribute to the emergence of the intra-host diversity found in this study.

Conclusion

In summary, vaccination is a great strategy to reduce severe cases. Monitoring infections post vaccination is interesting to observe strains that can escape from host immune system. Additionally, phenotypic assays and viral neutralization using sera from convalescent and vaccinated are important to monitor new variant.

Poster Reception III

Ana Gonzalez-Reiche - AOXI0622

First direct evidence of onward transmission of a novel SARS-CoV-2 variant emerging from an immunocompromised host

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Background

Prolonged SARS-CoV-2 infections have been reported in immunocompromised individuals and people undergoing immunomodulatory treatments. It has been speculated that the emergence of antigenically diverse SARS-CoV-2 variants such as the SARS-CoV-2 Omicron variant may be the result of intra-host viral evolution driven by suboptimal immune responses, which are then followed by onward transmission. However, although intra-host evolution has been documented, to our knowledge no direct evidence of subsequent onward transmission is available to date.

Method

Whole-genome sequencing was performed on residual nasopharyngeal and anterior nares swab specimens, collected after completion of the diagnostic process, as part of passive SARS-CoV-2 surveillance conducted by the Mount Sinai Pathogen Surveillance Program (IRB approved HS#13-00981).

Result

We identified a persistent SARS-CoV-2 Omicron BA.1 infection in an immunocompromised individual during a 12-week period, with progressive accumulation of eight additional amino acid substitutions in the already antigenically distinct Omicron BA.1 spike protein (E96D, L167T, R346T, L455W, K458M, A484V, H681R, A688V). In addition, we identified five subsequent cases harboring a related genotype in the same geographical region; three of which were identified in the same health system. The presence of a unique combination of mutations in the primary case and 5 subsequent cases is indicative of onward transmission of this novel Omicron BA.1 sub-lineage. Although most amino acid changes in this sublineage occurred at positions known to confer either immune escape or

improved viral fusogenicity, some of the mutations have been seen rarely in other lineages, and their overall constellation is unique.

Conclusion

Our findings show that the Omicron BA.1 lineage can further diverge from its exceptionally mutated genome during prolonged SARS-CoV-2 infection. Considering that prolonged SARS-CoV-2 infections lasting more than 21 days are not uncommon (at least 3% of cases in our health system), this underscores an urgent need to employ therapeutic strategies that limit the duration of infection and spread in vulnerable patients.

Poster Reception III

Andra Banete - AOXI0646

SARS-CoV-2 variants of concern show differential replicative fitness in primary human respiratory cells and transmission in Syrian golden hamsters

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Background

As a result of the widespread circulation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in humans since its emergence in 2019, several novel variants of concern (VOC) have been identified that spread more easily, cause greater disease severity, and are capable of partial escape of host immune responses against prior SARS-CoV-2 variants, monoclonal antibodies, or vaccines. As additional VOCs will presumably continue to be identified for the foreseeable future, it is crucial that we understand the molecular basis for the differences in disease severity and spread that have been observed. Evidence is accumulating that VOCs induce differential immune responses (both innate and adaptive) that ultimately result in varied disease severity as well as quality and durability of immunity.

Method

Here, we investigate replication kinetics of SARS-CoV-2 VOCs in primary human nasal epithelial (HNE) cells, and lung bud organoids (LBO), as well as the pathogenesis and transmission in the Syrian golden hamster model of SARS-CoV-2 infection. HNEs and LBOs were infected with SARS-CoV-2 VOC (Alpha, Gamma, Delta, Omicron BA.1 and BA.2) and infectious virus production was quantified. Male and female 5-7 week old hamsters were infected with VOCs and co-housed in a transmission cage with naïve animals in groups of two. Oropharyngeal swabs were taken every other day until day 10 to quantify infectious virus shedding. To assess viral pathogenicity weight loss, tissue viral burden, and serum cytokine production were measured.

Result

The dynamics of SARS-CoV-2 VOC (Alpha, Gamma, Delta, Omicron BA.1 and BA.2) replication were evaluated in HNEs and LBOs and differences in viral replication kinetics were observed for different VOCs. Omicron isolates BA.1 and BA.2 rapidly replicate in HNE compared to all other VOCs, yielding ~100-1000-fold higher viral tires. While infection of LBOs with BA.1 and BA.2 was productive, viral tires at 3dpi were significantly lower that other VOCs. Viral pathogenesis was examined in vivo in Syrian golden hamsters. Infection with SARS-CoV-2 VOCs resulted in ~10-15% reduction in body weight by 7dpi, with P.1 and Delta causing the greatest weight loss. We observed sex differences in pathogenesis, with males being more affected. A comparison in infectious viral shedding in oropharyngeal swabs at 3dpi shows no difference between VOCs. Airborne transmission of D614G, P.1, and Delta VOCs was more efficient than Omicron.

Conclusion

This work aims to unravel some of the differences observed in spread, disease severity, and immunity for SARS-CoV-2 VOCs. Overall, infection with Omicron isolates demonstrate attenuated replication in lungs in rodent models and primary human cell models of SARS-CoV-2 infection.

Poster Reception III

Connor Bamford - AOXI0582

A novel mode of super-infection enhancement of influenza A virus infection by a gram-negative bacteria

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Background

Co-infection with more than one pathogen (e.g. viruses and bacteria) is common under natural conditions, particularly at mucosal surfaces like the respiratory tract. Despite often being associated with a worse prognosis and more complex clinical management, the full complexity of diverse dual infections is only beginning to be uncovered. Greater knowledge of the molecular interactions at this "tri-kingdom" interface of host cells, invading viruses, and co-infecting bacteria, is essential to understand "real-world" pathogenesis and develop safer and more efficacious clinical interventions.

Method

Human airway epithelial A549 cells and Madin-Darby canine kidney cells were used throughout. Relevant respiratory co-pathogens including viruses: influenza A virus (IAV) (rPR8-mCherry and WSN), SARS-CoV-2, and respiratory syncytial virus (RSV); and bacteria: the gram-negative Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa. Cells were infected with viruses at low MOI for 30 minutes before removal and washing. Bacterial infection (MOI of 100) was carried out in antibiotic-free media for 3 hours after which the inoculum was removed, washed, and incubated overnight in the presence of antibiotics without exogenous trypsin. Virus infectivity was assessed by quantifying the number of infected cells, RT-qPCR and infectivity assays.

Result

No effect on IAV infection was observed with bacterial pre-infection but surprisingly, super infection of IAV-infected A549 or MDCK cells with a hyper-virulent strain of K. pneumoniae enhanced the number of productive viral infections under single-cycle conditions. While the enhancement phenotype was independent of IAV strain used, K. pneumoniae failed to enhance SARS-CoV-2 or RSV infectivity. All members of a panel of clinical isolates of K. pneumoniae - including multidrug resistant strains - enhanced IAV infection. However, no enhancement was observed with A. baumannii and P. aeruginosa. This co-infection enhancement was not observed when challenged with K. pneumoniae conditioned media or UV-inactivated bacterial cells. Mechanistically, enhancement was associated with greater IAV gene expression and replication at early time points and was linked to an inhibition of RIG-I-mediated signalling by K. pneumoniae, occurring independently of interferon signalling.

Conclusion

Although the precise mechanism(s) through which K. pneumoniae enhances IAV infectivity remain unknown, our work uncovers a clinically-relevant and unexpected co pathogen-specific relationship, highlighting the complexity of bacterial/viral co-infection and knowledge of which may open unpredictable avenues for therapeutic development.

Poster Reception III

Xiu-Feng (Henry) Wan - AOXI0603

Loss of the Neu5Gc specific receptor binding ability for subtype H7N9 influenza A viruses is associated with its host preference in chickens over domestic waterbirds

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Background

A novel H7N9 avian influenza A viruses (IAVs) emerged and became enzootic in Chinese domestic poultry, having caused devastating economic losses to domestic poultry as well as significant disease burdens to public health. Of interest, this virus has been detected primarily in chickens and rarely among domestic waterbird species, and the molecular mechanism for the host tropisms of this novel H7N9 is unclear.

Method

In this study, through glycan receptor binding analyses, the novel H7N9 virus bound exclusively to Neu5Ac whereas all the other 21 H7 isolates tested, including 4 wild bird origin isolates and 14 domestic poultry isolates, showed a strong binding specificity to both Neu5Gc and Neu5Ac.

Result

Mutations E174S and I179V in the H7 receptor binding sites resulted in the loss of Neu5Gc specific receptor binding ability during virus adaption to chickens. The cell expressed Neu5Gc facilitated the virus growth for those wild bird origin isolates but not those H7 viruses adapted to domestic poultry. Immunochemistry test demonstrated that domestic poultry such as chickens expressed only Neu5Ac whereas domestic duck expressed both Neu5Ac and Neu5Gc throughout respiratory and gastrointestinal tracts; similar results were observed in a set of wild bird species which are commonly detected with H7 infections.

Conclusion

In summary, this study suggested Neu5Gc affects spillover and adaption of subtype H7 avian IAVs from wild birds to domestic poultry, and the loss of Neu5Gc specific receptor binding ability contribute to the host adaptation and host tropisms of the novel H7N9 virus.

Poster Reception III

Joshua Klonoski - AOXI0647

Gene Expression Profiles Associated with Survival and Death During Influenza A Virus Bacterial Super Infections

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Background

Bacterial super-infections (BSIs) increase morbidity and mortality during influenza epidemics and pandemics, but not all strains of influenza virus uniformly increase host susceptibility to BSIs. Infection of mice with a swine virus isolate, A/swine/Texas/4199-2/98- H3N2 (TX98), does not significantly predispose the host to BSI mortality regardless of whether the BSI was caused by S. aureus, S. pneumoniae, or S. pyogenes. Comparison of this survival phenotype with the laboratory influenza virus strain A/Puerto Rico/3/1934- H1N1, which demonstrates lethal synergism, showed that cellular recruitment profiles and cytokine responses differ between the two viruses in a manner that implicates host responses against the invading virus as a contributor to secondary infection severity.

Method

We utilized metagenomic analysis of 559 genes, the NanoString nCounter Immunology Panel-Plus kit and FFPE mouse lungs from a published BSI time course.

Result

s show less downregulation of host genes during BSIs associated with survival at all time points. Significantly increased gene expression in complement, phagocytosis, TLR, TGF-β, TNF signaling, apoptosis, Th2, T-cell receptor signaling, regulatory T-cell, CAM, lymphocyte trafficking, MHCI, and MHCII pathways are observed during both late TX98 viral infections (day 7) and during TX98:GAS BSIs. Importantly, late (day 7) PR8 viral infections are distinctly associated with increased Th17 (IL-17b) and IFN-α as well as decreased complement (C5a and C7). Subsequent lethal PR8:GAS BSIs show decreased MR1 and Tfrc as well as increased MIP-1α, Fcgr4, S100A8, and S100A9.

Conclusion

To our knowledge this is the first side by side comparative gene expression study of influenza BSIs associated with death and survival. The results offer mechanistic insight into clinical outcomes.

Poster Reception III

Rafael A. Medina - AOXI0653

N-Glycosylation near the receptor binding domain induces major antigenic changes in H1N1 Influenza virus

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Background

N-glycosylations have been increasingly studied due to their impact in virus biology and vaccine development. As a consequence of antigenic drift during its evolution in humans, Influenza virus (IV) have acquired several N-glycosylation motifs. While these modifications can result in variations in the antigenicity, virulence and immune responses, exhaustive analysis of the antigenic changes associated with these N-glycosylations remain limited.

Method

We investigated the impact on antigenicity after the introduction of N-glycosylations in the globular head of hemagglutinin (HA) near the receptor binding domain (RBD). Recombinant soluble H1 HAs (sHA) derived from IAV containing N-glycosylations in the globular head at amino acid positions 142, 144, 172 and 144-172 or the WT unglycosylated form were produced in 293F cells. These sHAs were then probed against a panel of monoclonal antibodies (mAb) directed to the H1 antigenic sites (Sa, Sb, Ca1, Ca2, Cb).

Result

Coincident with antigenic shielding of immunodominant antigenic site Sa, the reactivity of mAbs directed to this site was abolished when N-glycosylations were introduced near the RBD, while partial reactivity or no effects were observed when using mAbs directed against secondary antigenic sites (Sb, Ca1, and Cb). Surprisingly, sHAs containing an N-glycosylations at position 144 (N144 and N144/172), also abolished the reactivity of mAb directed the distant antigenic site Ca2. To confirm the role of N-glycosylations in the observed antigenic changes, we evaluated the effect of the complexity of the HA N-glycans in the reactivity or lack reactivity of the studied mAbs. Thus, we used either mannose rich (simple type) glycans containing sHAs or glycosidase (PNGaseF)-treated sHAs (trimmed glycans) to probed them against the mAb panel. While no difference was observed using mannose rich glycans, remarkably the reactivity against Sa and Ca2 was restored after treatment with PNGAseF.

Conclusion

Our results indicate that the introduction of N-glycosylations near the RBD can induce major antigenic changes not only affecting the antigenic sites proximal to the N-glycosylation motif, but also at distant antigenic sites.

Poster Reception III

Gregory QUEROMES - AOXI0608

Whole-genome intrahost viral diversity from influenza-confirmed hospitalized patients with severe illness

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Background

The genomic and antigenic evolution of human influenza viruses have been described on a global and long term scale spanning decades. However, the rapid evolution of influenza within a human host over the course of infection warrants closer investigation. Furthermore, the genetic profile of influenza viruses from patients hospitalized for severe influenza-confirmed respiratory distress remains unclear.

Method

To elucidate this, nasopharyngeal swabs and bronchoalveolar lavage samples were collected at days 0, 3, and 5 of hospital admission from a cohort of hospitalized adults admitted for severe influenza-confirmed illness dating from 2013-2019 flu seasons. The samples were fluidified for RNA extraction, quantified, and analyzed for intrahost diversity through next-generation sequencing via Illumina. Minority variants were determined through a custom variant-calling pipeline with a minimal threshold of 5% retrieved in duplicate runs. Non-synonymous amino acid substitutions were compared between days of sampling and between all viral genome influenza segments with ANOVA.

Result

Of 163 included patients, 38 patients had respiratory samples meeting sequencing criteria with at least two time points during hospitalization. Overall, the intra-host diversity and the normalized viral load per sample seem to be positively correlated.

For A(H1N1) viruses from patient samples on day 1 of hospital admission, the intra-host diversity was similar between all 8 influenza genome segments (3.5±0.9). Subsequent intra-host diversity at days 3 and 5 was slightly lower yet comparable (2.9±0.9 and 1.6±0.8, respectively; $p>0.05$).

For A(H3N2) viruses from patient samples on day 1 of hospital admission, the number of intra-host substitutions was significantly higher for both HA and NA segments (6.6±1.5 and 9.7±1.9, respectively; $p<0.05$) compared to the other 6 genome segments (2.5±0.6; $p>0.05$). At days 3 and 5, relatively lower diversity was observed for all genome segments (1.1±0.8; $p>0.05$).

Interestingly, for both A(H1N1) and A(H3N2), the HA region with the greatest diversity is the HA2 stalk region and not the primarily immunogenic HA1 region, while the NA diversity is distributed across its globular active site region.

Conclusion

The greater number of intra-host amino acid substitutions for HA and NA segments form A(H3N2) at admission attest to high intra-host viral minority populations in the earliest stage of infection, with a gradual decline in diversity as soon as day 3. These influenza minority variants, also known as quasispecies, give valuable information on the intra-host selection of specific sites in segment evolution and could inform on the pathogenic impact of such selections within the course of an infection.

Poster Reception III

SZE SZE NING - AOXI0557

Acceptance and preference of deep throat saliva and combined nasal and throat swab for the diagnosis of COVID19 infection

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Background

Although self-collected deep throat saliva (DTS) may confer benefit of a reduced risk exposure among healthcare workers (HCW) and represented an alternate sampling approach for COVID19 diagnosis, public perception for self-sampling method remains unclear. The objective of this observational study was to study the comparative acceptability, capability and preference of DTS and combined nasal and throat swab (NTS) among patients presenting to the Accident & Emergency Departments (AED) during the third wave of COVID19 pandemic in Hong Kong.

Method

This study was conducted among adults ≥18 years of age presenting with suspected COVID19 symptoms to an AED and being classified as Tier 4 (Clinically stable outpatients with fever or respiratory symptoms or new loss of taste/smell or gastrointestinal symptoms, with no travel or close contact history). All participants self-collected an early morning DTS as instructed by a simple written instruction manual, and have a NTS collected by a HCW on the same day. Their understanding, perception and preference of the two sampling approaches were assessed by a self-completed questionnaire.

Result

A total of 127 patients participated and submitted both specimens. Although acceptance of these 2 sampling approaches were grossly comparable (52% vs 48%), the majority (78%) of respondents perceived NTS as a more accurate sampling approach for diagnosing COVID19. For assessment on the confidence on performing self-sampling, majority of patients (80%) were confident on self-collection on DTS. Although 54% also expressed that they were confident in performing a self-collected NTS accurately, most individuals (84%) preferred to have the swab collected by a HCW. Common concerns affecting the acceptability of sampling approaches included accuracy of sampling procedure (46%), followed by ease of specimen collection (34%), procedural convenience (14%), comfortability (9%), and timeliness (3%), but not significantly affected by patient's age, gender or exposure history. On stratified analysis 55% of older patients aged ≥65 preferred DTS for its ease of collection, an attribute considered more important by them over accuracy, in contrast to the higher preference for self-collected NTS in younger patients aged <65 (53%) for its accuracy on COVID19 diagnosis.

Conclusion

Although self-collected DTS and NTS had comparable acceptance for using as an alternate sampling approach for diagnosing COVID19, not all people perceived themselves as capable of performing the self-collection procedure accurately under the guidance, with a sizable proportion preferring NTS collected by HCW. Further work is needed to improve patients' understanding and proper self-collection skills to ensure the accuracy of diagnosing COVID19.

Poster Reception III

Herve KADJO - AOXI0579

Circulation of RSV through the influenza surveillance network, case of children under five

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Background

Viral etiology of acute respiratory infections is not often investigated. In Côte d'Ivoire, a network of health centers has been set up to monitor the circulation of influenza viruses. this network has made it possible to integrate the surveillance of other respiratory viruses such as hMPV, SARS-CoV-2 and RSV. Most studies on cute Respiratory Infections (ARI) point to RSV as the primary cause of these acute respiratory infections. In order to improve knowledge on RSV epidemiology, seasonality clinical signs and genotypes circulating associated with RSV acute respiratory infections a study was conducted over four consecutive years.

Method

During these years, 5648 nasopharyngeal samples collected in children aged 0-5 years, through influenza sentinel surveillance were analysis by real-time PCR targeted RSV A and B genes. The amplification of the second hypervariable region of the G(RHV2) gene and the F gene of the positive samples were performed to identify circulating genotypes.

Result

Our results revealed 564 (9.98%) RSV positive children. A number of 181 (32.09%) were positive in inpatients, and 383 (67.91%) in outpatients. The 0-12-month age group was the most affected with 51.95% of positive cases. Cumulative monthly RSV activity for the 4 years of the study was relatively lower during the months of January to March and higher during the months of May to September. This distribution of RSV was superimposed on rainfall. Our work has also linked RSV positivity to the presence of clinical signs, including fever, cough, diarrhea and vomiting. In addition to the results obtained, the current study also provided for the first time data on the genotypes of RSV present in Côte d'Ivoire. The genetic characterization of glycoprotein G, indicated two genotypes ON1 and BA9 which co-circulated during the study period.

Conclusion

It would be important to encourage health personnel to prescribe a diagnostic test for the early detection of RSV in children aged 0 to 5. the search for circulating genotypes would make a considerable contribution to vaccine research and strategy in a context where little data on the molecular carcterization of RSV is available.

Keywords: Respiratory Syncytial Virus, Surveillance, Children, Seasonality, Genotype

Poster Reception III

George Okoli - AOXI0599

Correlation between country-level numbers of coronavirus disease 2019 (COVID-19) cases and mortalities, and country-level characteristics: A global study using the World Health Organization data

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Background

Despite increased understanding of individual and population-level risk factors for COVID-19, not much is known regarding relationships between country-level characteristics and COVID-19 infection and mortality. Understanding the relationships could aid comparison of data from different countries and the development of more effective national public health mitigation measures against the disease.

Method

We conducted an ecological study utilizing publicly available country-level COVID-19 data and other data from the World Health Organization, and other publicly available country-level data from the United Nations, the United States Central Intelligence Agency (CIA) and the World Bank. The data are all at population level and are publicly-available. The study period was from January 2020 to August 2021. We summarized country-level COVID-19 case and mortality counts per 100,000 population and case fatality rate. We conducted adjusted linear regression analysis to assess relationships between these counts/rate and certain country-level characteristics, and we reported adjusted regression coefficients, β and associated 95% confidence intervals (CI).

Result

We included 130 countries in the analyses. The mean number of COVID-19 cases and mortalities per 100,000 population were 5,148 (CI 4,405 - 5,891) and 94 (CI 78 - 110). There was a positive correlation between the number of cases and country-level male/female ratio; and positive correlations between the numbers of cases and mortalities, and country-level proportion of 60+ year-olds, Universal Health Coverage index of service coverage (UHC) and tourism. Country economic status correlated negatively with the numbers of cases and mortalities. COVID-19 case fatality rate was highest in Peru, South American region (9.2%), and lowest in Singapore, Western Pacific region (0.1%). A negative correlation was observed between case fatality rate and country-level male/female ratio, population density, and economic status. These observations remained mostly among mid/low-income countries; particularly, a positive correlation between the number of cases and male/female ratio and proportion of 60+ year-olds.

Conclusion

Country-level characteristics such as male/female ratio, proportion of older adults, country economic status, UHC, and tourism appear to be correlated with country-level number of COVID-19 cases and/or mortalities. It may be necessary to consider these country-level characteristics when designing country-level COVID-19 epidemiological studies and comparing COVID-19 data between countries.

Poster Reception III

Gina Samaan - AOXI0601

Preparedness and Resilience for Emerging Threats (PRET): Building a global initiative for respiratory pathogen pandemic planning

Gina Samaan[1], Shoshanna Goldin[1], Britney McMurren[1], Isabel Bergeri[1]

[1]World Health Organization

Background

As the world learns lessons from the COVID-19 pandemic, emerging and re-emerging respiratory pathogens pose a constant threat to society. Accordingly, the World Health Organization (WHO) recently commenced a global initiative 'Preparedness and Resilience for Emerging Threats' (PRET) to prepare and build resilience for future respiratory pathogen pandemics.

Method

PRET takes an innovative mode-of-transmission approach to pandemic planning recognizing that similar systems, capacities, knowledge and tools can be leveraged for pathogens based on their mode of transmission. Lessons are being synthesized from past events including the COVID-19 and the influenza A(H1N1) pandemics to develop respiratory pathogen-agnostic guidance that links to core pathogen-specific components, and to provide capacity strengthening support to countries from this integrated planning lens. To increase harmonization of global preparedness efforts, WHO has established a partners' forum and a global monitoring framework. Moving forward, WHO and partners are building the PRET online resource pack and setting up the peer learning network to support countries in updating their preparedness plans and to facilitate global sharing of resources and good practices.

Result

Key lessons synthesized to date include the criticality of having functional systems for detection and response, multi-level and multi-sectoral planning, risk communication and community engagement, and having triggers to calibrate medical, public health and social measures. Based on these learnings, WHO published a policy brief in April 2022 to provide high-level advice to countries on developing or updating respiratory pathogen pandemic preparedness plans. A process has been established to update guidance, and to work with countries and partners to make technical and advocacy-oriented resources available to facilitate investments and preparedness actions.

Conclusion

PRET will facilitate efficiency and coherence in respiratory pathogen pandemic preparedness recognizing that collaboration, innovation, and partnerships are needed for success. Having a respiratory focused lens complements the broader National Action Plans for Health Security (NAPHS) and the all-hazard National Health Emergency Response Operations Plans (NHEROP), under the rubric of the International Health Regulations 2005. WHO now calls on countries and stakeholders to leverage the current momentum and learnings from the COVID-19 pandemic to assess gaps and priorities, strengthen functional capacities for preparedness and response, and share knowledge and technical resources through partner engagement and peer learning mechanisms.

Poster Reception III

Supriya Bezbaruah - AOXI0654

Translating science for evidence-informed actions and policies during a pandemic through an innovative multi-disciplinary global network

Supriya Bezbaruah[1], Ramona Ludolph[1], Tim Nguyen[1]

[1]World Health Organization

Background

The COVID-19 pandemic has highlighted the importance of evidence-informed actions and policies by the public and decision makers in managing a pandemic, including an influenza pandemic. To achieve this, the public, health workers and decision makers need timely access to accurate scientific information, that is both understandable to them, and is meaningful and perceived to add value.

However, challenges include public uncertainty, high levels of misinformation, and sometimes greater emphasis on politico-socio-economic considerations, than on the science.

A multidisciplinary global Science Translation Network is intended to strengthen understanding of science in public health emergencies. The goal of the network is to foster a symbiotic engagement between scientists, health professionals, policy makers, community decision makers and media persons. And to make a difference by using the individual strengths and collective weight of such a collaboration to provide accurate, meaningful, easily accessible information.

Method

For decision makers, 'EPI-WIN updates', simple but nuanced explanations of key technical issues, were developed, translated, and disseminated to WHO Representatives in countries and others.

Regular EPI-WIN webinars on key pandemic-related topics were held for the public, with leading WHO experts directly responding to questions.

As a forum for researchers to explain their work, and media and health professionals, and decision makers with access to accurate science, a global Science Translation network is being established. Members of this network will be institutes and organizations.

Result

Over 77 EPI-WIN updates have been developed on topics of public health relevance. Qualitative interviews reveal that these are shared with and used by national government officials, partners, key non-governmental organizations. These are further cascaded to the provincial level, where they allow easy access for local officials about the latest guidance and recommendations. Almost 200 EPI-WIN webinars have enabled WHO experts to directly explain the science to the public. The Science Translation network will provide resources and foster symbiotic engagement of different groups, leading to richer translation of science during emergencies.

Conclusion

For effective pandemic management, evidence-informed public actions are needed. This requires access and understanding of scientific information. Science distilled , through regular EPI-WIN updates and webinars, has led to updated evidence being accessed at local level. A multi-disciplinary global Science Translation Network will enable rich cross-sectoral learnings about the science during pandemics and development of a rich repository of resources.

Poster Reception III

Michal Kral - AOXI0567

Experimental Validation of Luteolin Derivatives as Influenza Endonuclease Inhibitors

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Background

Influenza viruses cause illness in a variety of species. Due to its high virulence and mutation rate, the influenza virus remains a major threat to public health. Multiple viable targets for drug development exist within the virus particle. Influenza RNA-polymerase is a heterotrimeric enzyme composed of subunits PA, PB1 and PB2. The PA subunit part of the influenza polymerase features endonuclease activity and is a target for anti-influenza therapeutics development, including the FDA-approved drug Xofluza. A general feature of endonuclease inhibitors is their ability to chelate Mg2+ or Mn2+ ions located in the enzyme's catalytic site.

Method

An AlphaScreen assay for screening PA-Nter inhibitors was developed based on the amplified luminescent proximity assay system and used to determine the inhibitory potency of the synthesized compounds. Results from the AlphaScreen assay were subsequently confirmed by a gel-based endonuclease assay, based on monitoring of the endonuclease-catalyzed cleavage of a single-stranded DNA plasmid in the presence of an inhibitor.

Result

Here, we report results from a subsequent investigation exploring structural changes at the C-7 and C-8 positions of luteolin. Experimental IC50 values were determined by AlphaScreen technology and subsequently confirmed by the gel-based inhibitory assay.

Conclusion

Our results indicate that substitutions at the C-8 yield the most potent inhibitors, with their effectivity comparable to the luteolin. However, C-7 derivates caused the inhibitory potency to decrease by an order of magnitude. We have solved the crystal structures of the wild type and I38T influenza endonuclease in complex with bound orientin at 1.9 Å and 2.2 Å resolution.

Poster Reception III

Waleed Aljabr - AOXI0666

Developing rapid molecular epidemiology and sequencing of influenza A virus infection

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Background

Influenza virus presents one of the world's biggest threats in terms of epidemic and pandemic potential. The threat to Saudi Arabia is particularly concerning due to the large numbers of migrant workers and the large influx of Pilgrims. This was illustrated with the 2009 swine origin H1N1 influenza A virus outbreak. Since the outbreak was recognised as pandemic in April 2009, the number of laboratory- confirmed cases in Saudi Arabia (as of 30th December 2009) was 15,850 and 124 deaths. The aim of this project was propose to use the latest deep sequencing approaches and unique bioinformatics approaches to; analyse historical samples to better validate current diagnostics, and improve the bioinformatics pipelines, to identify unknown pathogens that caused undiagnosed acute febrile illness.

Method

This research is based on in depth RNA sequencing of samples taken from patients. Samples from patients with acute febrile illness was compared to those from controls. The resulting sequence data was characterised using bioinformatics mounted on the Galaxy platform. The raw sequence was generated on the same by MinION.

Result

The data shows the genomes consensus for the genome assemblies. Also, the result demonstrates snps of amino acid in all genes, alignment of PB1-F2 genes and finally we can see there are two stop codons in all PB1-F2 genes.

Conclusion

These present a unique and unprecedented resource for studying infectious disease, especially respiratory diseases which are most likely to spread when large, diverse populations come into close contact.

Poster Reception III

Alex Mann - AOXI0665

Immunomodulators and treatment of disease in the influenza human challenge model

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Background

Human challenge studies using wild-type viruses replicate what is seen in the field in the same populations, and as such they have been used to aid vaccine and drug development for decades. Viral load and symptom reduction are typical key endpoints for direct acting antivirals. However, more recently immunomodulators have been developed that treat the disease processes and they look to establish mechanism of action in a human challenge model and focus on achieving reduction of both symptoms and disease biomarkers. Defining relevant endpoints can be important in establishing confidence of mechanism of action in infected humans.

Method

Twenty-seven eligible healthy subjects were pre-screened for moderately low or no antibodies to the challenge virus (≤20HAIs) and after admission to hVIVO's quarantine facility were inoculated with Influenza A/Perth/16/2009 wild type H3N2 virus. Once admitted to quarantine subjects were monitored 24/7 for safety, with frequent sampling for viral shedding and assessments of disease including subject self-reported symptom diary cards, temperature, nasal discharge, differential blood cell counts and immune markers. Detailed time-course sampling of blood was taken for a range of over 80 cytokines and chemokines as well as for transcriptomic microarray assays.

Result

Thirteen of twenty-seven subjects became infected (48%). Endpoints assessed included peak and AUC viral loads by PCR and culture, incidence of symptomatic disease, febrile illness, symptom type and severity. The microarray and cytokine/chemokine biomarker data were assessed in relation to established phenotype groups (e.g., febrile) as well as with data driven grouping and will be presented, in particular in relation to biomarker/inflammatory responses to infection and related to severity of disease.

Conclusion

Wild type influenza challenge viruses given to volunteers induces disease profiles that are similar to that seen in community infections with the more severe of which, being translatable, are important endpoints to include for assessment of immunomodulators in treating disease. These moderately severe groups show significant upregulation in inflammatory and other biomarkers that may be used to assist in establishing mechanism of action within infected humans.

Poster Reception III

Vasiliy Mishin - AOXI0669

A streamlined cell culture-based assay (IRINA) to monitor susceptibility to recommended influenza antivirals

Vasiliy Mishin[1], Daniel Flanigan[3], Anton Chesnokov[1], Mira Patel[1], Anwar Abd Elal[2], Ha Nguyen[1], David Wentworth[1], Larisa Gubareva[1]

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Background

Antivirals of two classes are recommended for the control of influenza, neuraminidase (NA) inhibitors (NAIs) and polymerase inhibitor baloxavir (BXA). NAI-susceptibility is monitored using NA inhibition (NI) assays, while Focus Reduction Assays (FRA) and the single-cycle replication assay high content imaging-based neutralization test (HINT) are used for BXA-susceptibility testing. To facilitate the implementation of BXA-susceptibility testing by surveillance laboratories, we developed a streamlined version of HINT. In this assay, named IRINA (Influenza Replication Inhibition Neuraminidase-based Assay), the cumbersome immunostaining-imaging is replaced with measuring NA activity. Notably, in this assay, the enzyme activity of nascent NA on the cell surface is measured. Susceptibility to BXA and NAIs was assessed using this approach.

Method

For BXA-susceptibility, HINT setup was used, where MDCK-SIAT1 cell suspension was added to virus mixed with serially diluted BXA. At 24hpi, cell supernatant was removed, NA substrate MUNANA was added to the monolayer, the reaction was stopped after 1h, NA activity was measured, and EC50 was calculated. To assess NAI susceptibility, cells infected with virus (without BXA) for 24h were used as the source of NA activity. After removing supernatant, serially diluted NAI was added to the monolayer for 1h, prior to addition of MUNANA. NA activity readouts were used to determine IC50. For validation, a reference virus panel and seasonal viruses from 2018-2021 were used. HINT and conventional NI assay were run for comparison.

Result

NA activity generated by nascent NA molecules on cells infected with normalized virus inoculum (300-3000 infectious particles) was ample, in a linear range, and strongly correlated ($r \geq 0.972$) with the infected cell population. Notably, BXA EC50s determined using IRINA and HINT differed by <2-fold. NA activity of reference viruses with known infectivity was used to determine dilutions for test viruses based on their NA activity. Using this approach, BXA EC50s were determined for over 100 seasonal viruses and were very similar (<2-fold) to the EC50s determined using HINT. IRINA-based testing of viruses with NAIs (oseltamivir, zanamivir, peramivir, and laninamivir) resulted in IC50s that were 1.5-4.6-fold higher than those determined using the conventional NI assay. Nevertheless, viruses with reduced inhibition were readily detectable when IC50 fold-increases were calculated.

Conclusion

We demonstrated that IRINA, a streamlined version of HINT that does not require immunostaining and a cell imager, can be used to assess susceptibility of influenza viruses to BXA and NAIs. Thus, offering a valuable tool to strengthen virological surveillance.

Poster Reception III

Huizhi Gao - AOXI0670

Measuring facilitators and barriers of the intention to newly approved vaccines among Chinese adults

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Background

Adults may have a higher risk of suffering from certain diseases nowadays. Vaccination is one of the most convenient preventive measures. The Chinese FDA has approved several vaccines in recent years, including HPV, COVID-19, Shingles and influenza vaccines. However, the attitude of and intention to take these newly approved vaccines among adults in China remain unknown.

Method

We conducted a questionnaire survey to understand the intention and attitude to take vaccines. Participants aged 18-65 were randomly allocated to receive a figure page with vaccine knowledge information or a blank page. Both participants and researchers were blinded during the survey. We used multiple linear regression models to investigate the facilitators and barriers associated with vaccination intention.

Result

1506 participants were included in this survey. The adjusted intention in the knowledge group slightly increased. Higher perceived severity, beneficial attitudes and vaccination history were significantly associated with a substantially higher intention, while waiting for other people's practices to make a decision was considered as a barrier.

Conclusion

Understanding the barriers and facilitators to uptake of a future vaccine can provide recommendations for the design of interventions aimed at maximizing public acceptance.

Poster Reception III

Hanan Al Kindi - AOXI0672

Oman's experience of integrated influenza, MERS-CoV and SARS-CoV-2 surveillance

Hanan Al Kindi[2], Ibtihal Wahaibi[2], Intisar Al Shukri[2], Adil Al Wahaibi[5], Badir Al Abri[5], Bader Al Rawahi[3], Fatma Al Yaqoobi[1], Amal A Manni[6], Seif Al Abri[4], Amina A Jardani[2]

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Background

The emergence of the SARS-CoV-2 (SCV2) pandemic has demonstrated the ability of non-influenza viruses to cause a global pandemic and highlighted the needs for broad and strengthened surveillance for non-influenza viruses with epidemic and pandemic potential. The influenza sentinel surveillance system provides a good platform for the integrated surveillance of multiple pathogens with epidemic and pandemic potential. Local settings must be considered when selecting the pathogen of interest for the integrated surveillance, for example in our region, MERS remains circulating and need to be included. Oman is subtropical and influenza is detected all year, but with seasonal activity in winter months. First MERS case was detected in 2013. Acute Respiratory Infection (NARI) surveillance Policy in Oman was implemented since 2017, in 2020 for Influenza and MERS, SCV2 was also integrated in NARI in 2020

Method

NARI uses molecular laboratory surveillance. It included three SARI sentinel sites and two ILI sites. Number of ILI sites increased to 13 in 2020. All SARI and ILI tested for Influenza and SCV2. In addition, all ICU centers in the country test for MERS, and Respiratory Virus Panel (RVP) with 23 targets. We present integrated laboratory surveillance for 3 seasons (from winter 2019-2020 to winter 2021- 2022). commercial kits were used for detection based on rt-PCR and point of care test (POCT) Multiplex system rt-PCR used from commercial providers (for RVP 23 target test) or inhouse CDC protocol (for influenza/SCV2). Screening for MERS by upE/ORF target with N target used for detection

Result

attached

Conclusion

Influenza activity was high in 19-20 and 20-22 season but very low in 20-22. Continuous circulation flu and SCV2 noticed recently, so public health action for both diseases are required. Sentinel sites result produced a mirror image of the disease activity using smaller samples for testing with less cost. Only one severe case of MERS was detected with secondary cases due to quick diagnosis. Continue integrated surveillance of the three target viruses is an important public health action.

Poster Reception III

Tsogt Mend - AOXI0673

Seasonal Influenza vaccine KAP among health care workers in Mongolia

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Background

In Mongolia, seasonal influenza vaccination (SIV) is recommended for healthcare workers (HCWs) and the majority of influenza vaccine doses purchased by Mongolian government is allocated to this specific risk group, because of their increased risk of exposure and transmission from and to patients, respectively. HCWs are also important drivers of SIV vaccine uptake, as they can communicate the importance and safety of annual SIV to their patients. Although SIV uptake among HCWs has been consistently high in Mongolia, uptake in other groups, such as pregnant women, remains low, a potential indication that HCWs may not be effectively advocating for, or are discouraging patients from, SIV. We evaluated influenza and SIV knowledge, attitude, practice (KAP) as well as drivers/barriers to HCW SIV recommendation in Mongolia during the 2019-2020 season.

Method

A cross-sectional KAP survey was administered to HCWs in randomly selected health facilities in six provinces of Mongolia as well as in Ulaanbaatar, the capital, from December 2019 to April 2020. The survey was adapted from the Partnership for Influenza Vaccine Introduction (PIVI) generic KAP survey tool, developed for PIVI countries. The survey included questions on demographic characteristics, SIV uptake, knowledge, and willingness to recommend SIV to patients.

Result

A total of 1053 HCWs participated in the study, of which, 360 (34.2%) were physicians, 344 (32.7%) nurses, and 349 (33.1%) other HCWs, such as front office staff. Most HCWs (84.8%) had received SIV during the current season, and 47% were vaccinated three or more times in the past five years. More than half (55.5%) of HCWs considered influenza illness a potentially severe disease and most (95.4%) were aware that influenza posed adverse potential consequences for themselves, their family and patients.

The main reason cited for receiving SIV was to prevent influenza infection, as reported by 42.8% of HCWs. Reasons for not accepting SIV included concerns related to: potential adverse events following immunization (9.5%) and contraindications (14.7%).

Many HCWs (89.3%) stated they were willing to recommend SIV to their patients; 23.1% cited the main reason to recommend was to reduce morbidity and 9.4% stated to protect HCWs and others.

Conclusion

The KAP study results suggest a majority of HCWs in Mongolia have positive attitudes towards SIV, are consistently being vaccinated annually, and are willing to recommend SIV to their patients. The discrepancy between HCW-reported willingness to recommend SIV and the low uptake in risk groups signals a need for regular trainings to strengthen HCW capacity to effectively communicate with patients on the importance and safety of SIV.

Poster Reception III

Tsogt Mend - AOXI0674

KAP among pregnant women regarding seasonal flu vaccine in Mongolia

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Background

Seasonal influenza is a major public health issue in Mongolia, responsible for about 370,000 cases of ILI and 50,000 hospitalizations annually. Ahead of the 2018-2019 influenza season, the Mongolian NITAG (National Immunization Technical Advisory Group) recommended prioritizing seasonal influenza vaccine (SIV) for pregnant women (PW), in addition to other risk groups. Targeting a 15% coverage, 12,000 doses of SIV were allocated for PW in 2018-2019, but uptake was low (5,063 doses). We aimed to assess the knowledge, attitudes, and practices (KAP) related to influenza and SIV, including possible determinants and barriers to SIV uptake among PW in Mongolia.

Method

This cross-sectional KAP study was conducted in five provinces in Mongolia and five districts of Ulaanbaatar, the capital city, during the 2019-2020 influenza season. The Partnership for Influenza Vaccine Introduction (PIVI) generic KAP survey instrument for PW was adapted to fit Mongolian context. PW 18 years old or older, in their second or third trimester, attending one of the study sites for routine antenatal care from October to May were eligible for an interview. Logistic regression methods were used to identify predictors of willingness to receive SIV during pregnancy. The results are expressed as odds ratios (OR) with 95% confidence intervals (CI). Data were analyzed using STATA ® software (version 14.0).

Result

Eight hundred PW, half from provinces and half from Ulaanbaatar completed the survey. Although most (91%) respondents reported hearing about influenza disease, only one third (35.6%) were aware of the severity of the disease and half (53.8%) knew that it was more dangerous for PW. Factors associated with willingness to receive SIV during pregnancy included having received at least one SIV dose in the past (OR = 1.68; CI 95% = 1.07-2.62), belief that SIV was safe (OR = 2.22; CI 95% = 1.67-2.97), and belief that SIV was effective (OR = 3.43; CI 95% = 2.67-4.39). Only 101 (12.7%) participants answered they were discouraged from getting vaccinated during pregnancy, and the sources included healthcare workers (23.8%), friends (27.7%), social media (21.0%), and family members (9.9%).

Conclusion

For PW, past vaccination experience, as well as perceived safety and effectiveness of SIV were the main drivers for willingness to receive SIV in pregnancy. Lack of knowledge on the severity of influenza disease, particularly specific to increased risk for complications in pregnancy, signaled a missed opportunity for targeted messaging to encourage vaccine uptake in pregnancy. The findings from this study will inform a concerted effort in Mongolia to implement risk communication strategies to promote SIV in PW and improve uptake.

Poster Reception III

Sviatoslav Onyshchuk - AOXI0677

Late delivery of vaccine supplies resulting in poor vaccination coverage during in the influenza epidemic season, Ukraine, 2020-2021

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Background

According to the Ministry of Health of Ukraine, since the beginning of the epidemic season 2020-2021, 196,688 people have been vaccinated against influenza, which is approximately 0.5% of the population of Ukraine; this is one of the lowest levels of vaccination coverage for the last 3 seasons.The purpose of this analysis is to determine the primary reasons for the current, low influenza vaccination rate.

Method

To achieve the goal, coverage results of influenza vaccines were retrospectively examined. Evaluation of vaccine supply was conducted based on the official documents of logistic chain of the PHC on the distribution of influenza vaccine received as humanitarian aid and letters from the regions on the receipt of vaccines. To evaluate possible reasons for vaccine hesitancy, we used data from surveillance of influenza during the epidemic season 2020-2021, and preliminary data from the epidemiological study, "Đ¡ross-sectional survey to assess the knowledge, attitude and practice of influenza vaccination among primary healthcare workers in Ukraine for the 2020-2021 epÑ–-season," (hereinafter referred to as the CAR survey) conducted jointly by the IES and the PHC.

Result

According to preliminary results of the CAR study, 17% of surveyed health care workers were not vaccinated against influenza due to the lack of a vaccine. According to influenza surveillance, two influenza vaccine shipments arrived influenza spread intensity period in October and February during the 2020-2021 season. The first batch of flu vaccine was received at the national level at the beginning of December 2020 and during the first week of December, this vaccine was distributed to 25 regions of the country. The vaccination campaign began in the middle of February 2021. However, the next batch of flu vaccine was not received until November 2021. While this second batch of flu vaccine was quickly distributed during first week of November to all regions and the vaccination campaign began in the middle of November, this gap in delivery may have caused the overall lower coverage in the 2020-2021 influenza season, resulting in a coverage of 15.9% among health care workers.

Conclusion

Our study shows that delayed vaccine supply and distribution and late conduct campaigns were two of the main reasons for poor vaccination coverage. We recommend ensuring timely vaccine distribution to the regional level by delivering the vaccine supply to the national level at least one month before the reported influenza spread intensity period and intensify administration of vaccine after they received.

Poster Reception III

Maggie H Wang - AOXI0678

Rapid prediction of COVID-19 vaccine effectiveness against new genetic variants of SARS-CoV-2 by genome analysis

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Background

Timely evaluation of the protective effects of COVID-19 vaccines against SARS-CoV-2 variants of concern (VOC) is urgently needed to inform pandemic control planning.

Method

Based on 78 vaccine efficacy or effectiveness (VE) data from 49 studies, and 1,984,241 SARS-CoV-2 sequences collected from 31 regions, we analyzed the relationship between genetic distance (GD) of circulating viruses against the vaccine strain and VE against symptomatic infection.

Result

We found that the GD of the receptor binding domain of the SARS-CoV-2 Spike protein is highly predictive of vaccine protection and accounted for 86.3% (p-value = 0.038) of the VE change in a vaccine platform-based mixed-effects model and 87.9% (p-value = 0.006) in a manufacturer-based model. We applied the VE-GD model to predict protection mediated by existing vaccines against new genetic variants and validated the results by published real world and clinical trial data, finding high concordance of predicted VEs with observed VEs. We estimated the VE against the Delta variant to be 82.8% (95% prediction interval: 68.7 - 96.0) using the mRNA vaccine platform, closely matching the reported VE of 83.0% from an observational study. Among the four sub-lineages of Omicron, the predicted VEs varied between 11.9% to 33.3%, with the highest VE predicted against BA.1, and the lowest against BA.2, using the mRNA vaccine platform.

Conclusion

The VE-GD framework enables predictions of vaccine protection in real time, and offers a rapid evaluation method against novel variants that may inform vaccine deployment and public health responses.

Poster Reception III

Yuki Furuse - AOXI0680

RNA modifications on viral genome and viral transcripts of influenza A virus

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Background

Recent studies about the transcriptome-wide presence of RNA modifications, known as the epitranscriptome, have revealed their importance in many cellular functions. Nevertheless, we understand little about RNA modifications in viral RNA, especially for negative-strand RNA viruses.

Method

A549 cell lines were infected with the PR8 strain of influenza A virus. RNA was extracted 16 hours post infection. After the fragmentation of RNA molecules, RNA immunoprecipitation (RIP) using antibodies for RNA modification, including m1A, m6A, ac4C, m5C, m7G, inosine, and pseudouridine, was performed. Following RNA-seq experiments determined RIP-enriched regions in the viral genome and viral transcripts. In addition, the effect of host's factors for RNA modification on viral growth was investigated through a re-analysis of screening assays from published studies.

Result

We provide a catalog of RNA modifications on RNA derived from influenza A virus. Possible regions with RNA modifications were found in both the positive and negative strands of viral RNA. Analyses of published data found that the expression levels of host's factors for RNA modifications were affected by viral infection, and some of the host factors likely have a proviral effect.

Conclusion

The present study suggests the presence of a variety of RNA modifications in RNA derived from influenza A virus. We also showed how viral infection affects the host's RNA modification factors and vice versa. RNA modification is a novel aspect of host-virus interactions, possibly leading to a discovery of previously unrecognized viral pathogenicity mechanisms.

Poster Reception III

Jin Il Kim - AOXI0681

Phylodynamics and genome-wide investigation of host adapted mutations of the hemagglutinin of H5Nx viruses

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Background

Highly pathogenic avian influenza H5 subtype viruses have caused human deaths since 1997. Accumulated mutations in hemagglutinin (HA) and genetic reassortment between the HA and neuraminidase (NA) might influence the genetic and antigenic diversity of H5Nx viruses and their perpetuation in nature.

Method

739 HA sequences was selected (based on the subtype, year of isolation, region) and was aligned using MAFFT (v7.419). Then, the HA phylogenetic tree was reconstructed using a time-resolved Bayesian inference method implemented in BEAST (v1.10.4).

Result

In the inferred HA phylogenetic tree, H5N6 and H5N8 HAs mainly constituted distinct genetic subgroups within clade 2.3.4.4, whereas H5N1 HA accounted mainly for clade 2.3.2.1. Of note, amino acid mutations around the receptor binding site (A131T, S133L, S137A, D187N, K193N, and R222Q) appeared to have been engraved across most H5Nx HAs. Especially, residues 133 and 193 were likely to have been positively selected in the HAs of clades 2.3.4.1 to 2.3.4.4, and the clade 2.3.2.1 HAs harbored key amino acid mutations at residues 158, 159, and 193. Moreover, ΔE130, I155T, and a novel N-linked glycosylation at residue 128 were observed only in human-isolated H5N6 HAs, which might be associated with viral host adaptation. These all might indicate independent molecular evolutionary pathways of respective H5Nx and H5N1 HAs and effects of NA subtypes on HA genetic diversity changes.

Conclusion

Given the molecular changes observed in the H5Nx HAs and genetic shift mechanism of influenza viruses, we may continuously confront novel variants of H5Nx viruses. Our results emphasize the necessity for early identification of HA mutations and functional assessment of H5Nx genetic variants in terms of pandemic preparedness.

Poster Reception III

Jin Il Kim - AOXI0682

Molecular evolution and recombination of SARS-CoV-2 in South Korea

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Background

SARS-CoV-2 has caused COVID-19 pandemic. During the pandemic, multiple genetic variants of SARS-CoV-2 have emerged, and variants of concerns caused unprecedented socioeconomic chaos also in South Korea.

Method

Complete genomic sequences of SARS-CoV-2 isolated in South Korea between January 2020 and April 2022 were collected from the EpiCoV database of Global Initiative on Sharing All Influenza Data. The 10,553 sequences were then aligned using MAFFT (v7.419) and edited in AliView (v1.27). The positions of the open reading frames (ORFs) and reported deletions were confirmed and each gene fragment was concatenated from 3' end to 5' end using SeaView. Maximum-likelihood (ML) phylogenetic trees (with or without the Omicron variants) were reconstructed using IQ-TREE (v.2.2.0, COVID-19 release) with 1,000 bootstrap and dating root-to-tip option. We then regressed root-to-tip genetic divergence against the sampling dates to investigate the temporal signal and evolutionary rates of our dataset using TempEst (v1.5.3). Potential recombination events of SARS-CoV-2 were traced using the same complete genome set used for the phylogenetic analysis by recombinant detection program 4 (RDP4).

Result

Of D614G-harboring SARS-CoV-2 genomic sequences isolated in South Korea, the complete genomes of clades B.1.619.1 (G) and B.1.1.7 (GRY, Alpha) appeared to be closely related in the phylogenetic tree. With Omicron variants (GRA, BA.1 and BA.2), however, the spikes of B.1.1.7, which appeared to be ancestors of the Omicron spikes, exhibited completely different evolutionary pathways from those of B.1.619.1. Similarly, clades B.1.617.2 (GK, Delta) and B.1.427/B.1.429 (GH, Epsilon) also exhibited phylogenetic incongruence between the complete and spike genes. Analysis of natural selection profiles in the spike proteins detected amino acid determinants (157 and 452 in Delta; 346 in Omicron BA.1; and 417 in Beta, Gamma, Delta, and Omicron), which might suggest their roles in the clade diversification of SARS-CoV-2. Of note, 11 putative genomic recombinants out of 15 detected appeared to retain N-terminal domain (n = 10) or receptor binding domain (n = 1) regions of the spike proteins originated from other SARS-CoV-2 donors.

Conclusion

Considered together, our results suggest molecular evolution of SARS-CoV-2 should be closely monitored by focusing on the effects of spike gene mutations on viral characteristics because viral immune evasion from vaccine-guided and natural immunities and genomic recombination strongly affect the emergence of novel SARS-CoV-2 variants.

Poster Reception III

Eun-Kyoung Lee - AOXI0683

Genetic and pathogenic characterization of High Pathogenicity Avian Influenza virus A(H5N8) clade 2.3.4.4, South Korea, 2014-2016

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Background

The H5N8 subtype of Gs/Gd high pathogenicity avian influenza viruses (HPAIVs) belonging to the subclade 2.3.4.4 was initially identified in domestic ducks from eastern China in 2010 and novel reassortant H5N8 HPAIVs were detected in live poultry markets in eastern China in late 2013. Subsequently, reassortant H5N8 HPAIVs were introduced into South Korea and Japan in early 2014. In late 2014, several countries in Europe and North America experienced an invasion of HPAI H5Nx viruses. The H5N8 HPAI outbreak in South Korea started in January 2014 and lasted for 28 months. The present study investigated the genetic and pathogenic characteristics of the viruses in South Korea during 2014-2016.

Method

The present study analyzed the complete genome sequences of H5N8 HPAIVs identified in 388 (of 393 total cases) poultry farms and 53 (of 58 total cases) wild bird cases during the 2014-2016 outbreak in South Korea. Maximum-likelihood (ML) phylogenies were generated using the RAxML method. A genetic subgroup was defined as a monophyletic cluster of sequences with high bootstrap support (>70%). Animal experiments were to evaluate the infectivity, transmissibility, and pathogenicity of the first detected viruses (index virus) of three subgroups (C1, C2, and C4) of H5N8 HPAIVs in specific pathogen free (SPF) chickens.

Result

A ML phylogenetic analysis showed that the C0 subgroup had diverged into multiple subgroups within South Korea. The detection of four distinct subgroups, C1, C2, C4, and C5, diverging from C0 viruses in the phylogenetic tree suggests that each subgroup had evolved independently within South Korea during 2014-2016 without any reassortment. The virus, initially introduced into the western part of South Korea, which contains large populations of domestic ducks, was subsequently disseminated into other regions throughout the country. The representative viruses of subgroup C1, C2, and C4 showed significant pathobiological differences in SPF chickens. H1731 (C1) had a longer Mean death time (MDT), significantly lower viral titers in tissues, and lower transmissibility in chickens compared with H2102 (C2) and H1924 (C4). The virulence of H1731 in chickens was also lower than that of the buan2 (the index virus of C0).

Conclusion

AIV circulation allows viruses to adapt to particular species, affecting their virulence and host specificity via genetic evolution. It needed continued genomic surveillance and pathobiological characterization of HPAIV in birds. Enhanced biosecurity in poultry farms should be implemented to prevent the introduction, maintenance, and spread of HPAIV.

Poster Reception III

Nga Ha - AOXI0684

Influenza Detected Through Routine Medical Care Reported From Selected Hospitals Vietnam, 2019-2021

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Background

Influenza was infrequently reported worldwide to the WHO's Global Influenza Surveillance and Response System from 2019-2021, including from the two National Influenza Centers (NICs) in Vietnam. Specimens collected as part of sentinel outpatient influenza-like illness (ILI) surveillance in Vietnam have also declined in part due to the COVID-19 pandemic but also because the national ILI guideline has not been updated. There are a number of large hospitals in Vietnam that routinely test for influenza; however, these hospitals' data may not have been shared with government public health reporting systems.

Method

To explore development of a new hospital-based influenza surveillance project, a questionnaire was sent to a convenience sample of 21 hospitals within Vietnam that included both large national referral hospitals as well as smaller provincial ones; none participate in the national influenza surveillance system. The survey collected information about testing data for influenza from 2019 to 2021. The influenza surveillance data from was also collected from the WHO GISRS system where two NICs, National Institute of Hygiene and Epidemiology (NIHE) and Pasteur Institute Ho Chi Minh (PI HCM), are requested to submit weekly testing data from ILI surveillance sites.

Result

Twenty-one hospitals (100%) responded to the questionnaire, nineteen (90%) provided both the number of specimens tested and number positive for influenza. Influenza testing was not routine in two of the 21 hospitals responding (9.5%)). Among the 19 laboratories that performed influenza tests, 2 (9.5%) performed only Real-time RT-PCR testing, 9 (47 %) performed only rapid tests, and 8 (42%) can perform both but over 95% were tested using rapid tests. The total number of specimens tested per month by hospital ranged from 80 to 9,777 in northern region hospitals, while the range went from 0 to 68 in southern region hospitals; per hospital testing remained stable during the 3 years. Median monthly percent positive for the country in 2019 was 23.9% (range: 5.1 - 46.5), 0.89% (0.1 - 32.1) in 2020, and 1.2% (0.2 - 21.0) in 2021. From May to December 2021, 0.5% (1225/229424) influenza specimens were positive from Northern hospitals, 3.5% (16/454) from Southern regions, and 0% (0/248) from both NICs.

Conclusion

Hospitals in Vietnam are currently testing a significant number of patients for influenza, primarily through the use of rapid tests, and represent an untapped potential for hospital based surveillance.

Poster Reception III

Brenda Coleman - AOXI0692

Risk factors for contracting COVID-19 in Canadian healthcare personnel, prior to and during the omicron wave

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Background

The ongoing COVID-19 pandemic continues to cause illness and death across the world. Determining factors associated with infection may identify risks of transmission for everyone, including healthcare personnel (HCP).

Method

A test-negative interim analysis of Canadian HCP taking part in a cohort study from June 2020 to July 2022. Multivariable generalized estimating equations were used to determine factors associated with SARS-CoV-2 infection prior to as compared to during the omicron wave. Estimates were adjusted for days in the study, age, gender, and province using Poisson regression models with exchangeable correlations and robust variance estimation of incidence rate ratios (IRR).

Result

Among 2540 participating HCP, 942 positive tests (by PCR or rapid antigen) were reported; 92 prior to and 850 during the omicron wave. 1085 participants provided 1956 test results prior to Dec 2021 while 1510 provided 3150 test results afterwards.

Factors significantly associated with COVID-19 prior to the omicron wave included exposure to a positive adult (IRR 9.14) or child (IRR 4.40) household contact, or to a friend (IRR 3.22), patient (IRR 2.13), or co-worker (IRR 1.74) who had tested positive in the previous 14 days, as well as participating in four or more different group activities (with 5 or more people in each) in the previous 14 days (IRR 2.38). Two doses of a vaccine against SARS-CoV-2 was protective (IRR 0.30) compared with 0 or 1 dose.

Factors significantly associated with a positive test during the omicron wave included exposure to a positive adult (IRR 1.84) or child (IRR 2.32) household contact or a friend (IRR 1.25) who had tested positive previous 14 days, participating in three or more group activities in the previous 14 days (IRR 1.26), and current tobacco smoking (IRR 1.56). Working in an office without coworkers or clients was protective (IRR 0.70) as was receipt of two or three doses of vaccine against COVID-19 (IRR 0.48 & 0.38, respectively) compared with 0 or 1 dose.

Conclusion

HCP who were exposed to people known to be infected with SARS-CoV-2 were at higher risk of infection before and during the omicron wave, with household exposures having the highest IRR. Two or three doses of COVID-19 vaccine reduced the risk of infection compared with fewer/no doses. During the omicron wave, social activities such as smoking, group activities, and working in close proximity with other people were also associated with a significant increase in the incidence of infection.

Poster Reception III

Elif Alyanak - AOXI0693

Continued Decline in US Routine Adult and Adolescent Influenza Vaccination, 2019 through 2021

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Background

During the COVID-19 pandemic, routine healthcare delivery methods were disrupted due to office closures and social distancing measures. Originally intended to mitigate the transmission of COVID-19, interruptions to in-person healthcare utilization led to a sharp decline in immunization rates, increasing the risk for preventable disease such as influenza. Since 2010, flu vaccination has been routinely recommended by national immunization technical advisory groups (NITAGs) annually for all individuals aged 6 months and older. Previous analyses observed declines in flu vaccine administration across most ages, and as the pandemic continues, it is critical to understand whether reduced rates continue or have begun to recover towards pre-pandemic levels.

Method

Avalere analyzed changes in flu immunization administration claims in individuals ≥7 years of age using 2019-2021 pre-adjudicated medical and pharmaceutical claims for populations in Medicaid managed care, Medicare Advantage, and commercial health plans. Data were accessed from the Inovalon MORE2 Registry®, a large scale multi-payer dataset of 338 million+ de-identified patients. Using Current Procedural Terminology (CPT) and Healthcare Common Procedure Coding System (HCPCS) codes, claims and enrollment data from 2019, 2020, and 2021 were used to calculate the percentage of the population with an influenza vaccination. The percent change in claims was assessed by comparing the difference in claims between the months of 2019 (pre-pandemic baseline) and the corresponding months of subsequent years.

Result

Based on the claims assessment, the decline of annual influenza vaccination observed in 2020 persisted into 2021 across all markets and age groups (Table 1). The greatest continuous decline over the study period was observed among the commercially insured adolescent population where year-over-year decline was associated with an 8% decrease in claims. While vaccination increased among the Medicare Advantage population in 2020 (42% vs. 36% in 2019), claims substantially halved in 2021 (21%).

Conclusion

This analysis suggests lapses continue in routine care for vaccine-preventable diseases, specifically flu vaccination, across all ages. The reduction in flu vaccination claims continued, even through a period of increased immunization communication and disease mitigation strategies. Given overlap of the 2022 influenza season with the ongoing COVID-19 pandemic, stakeholders continue to consider how to encourage immunization recovery and reduce vaccine fatigue or hesitation.

Poster Reception III

John Youhanna - AOXI0694

Cell-based influenza vaccines that are identical to clinical isolates offer improved protection against influenza with demonstrated increased effectiveness

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Background

Vaccination has been demonstrated to offer the best protection against infectious disease, including influenza. Traditional influenza vaccines have been manufactured in embryonated chicken eggs and while useful the virus undergoes egg adaptation that can alter the effectiveness of such vaccine.

Method

For at least seven influenza seasons a fully mammalian developed influenza vaccine has been available. Unlike other vaccines, sequence analysis of the viruses selected for inclusion in this cell vaccine (n= 15 of both hemagglutinin and neuraminidase) demonstrated that no mutations occur in the antigenic sites of either hemagglutinin or neuraminidase indicating that "cell" adaptation does not occur.

Result

The development of this mammalian-based vaccine system that incorporates both haemagglutinin and neuraminidase ensures that the significant protective antigens are identical to the circulating strains in both amino acid sequence and glycosylation pattern.

Conclusion

The consistency of sequence match to the circulating strain is reflected in real world effectiveness data demonstrating the benefit of cell-based vaccines to protect against influenza.

Poster Reception III

Guy De Bruyn - AOXI0695

Heterologous and Homologous Boosting with a SARS-CoV-2 Recombinant Protein Vaccine with AS03 Adjuvant in Adults 18 Years of Age and Older

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Background

Current prototype booster vaccines extend protection against COVID-19 caused by emerging variants of concern, followed by rapid waning. Variant booster vaccines that provide broad antigenic coverage are needed. In a planned interim analysis, we evaluated the immunogenicity and safety of a prototype and two Beta variant-containing recombinant spike protein vaccines with AS03 adjuvant for heterologous and homologous boosting.

Method

VAT00002 is a multi-country Phase III trial. Eligible participants were 18 years and older and had received heterologous priming with a complete primary series of a prototype mRNA (Pfizer or Moderna) or adenovirus-vectored (Janssen or AstraZeneca) COVID-19 or adjuvanted protein (CoV2 preS dTM-AS03 (D614)) COVID-19 vaccine. The study interventions were a single injection of 5µg Sanofi-GSK (S-G) prototype recombinant spike protein vaccine [hereafter, MV(D614)], 5µg (B.1.351) monovalent [MV(Beta)] vaccine, or 2.5+2.5µg (D614+B.1.351) bivalent [BiV] booster vaccine. All formulations included AS03 adjuvant. The control group of SARS-CoV-2-naïve adults received a primary series [two doses of 10µg MV(D614) separated by 21 days]. All participants are followed for safety throughout the trial. Responses to the D614G, B.1.351 and Omicron BA.1 strains were evaluated in a validated lentivirus-based pseudovirus neutralization (PsVN) assay at day 1 and day 15.

Result

1285 participants were enrolled in MV(614) arms, 707 to MV(Beta) arms, and 625 to BiV arms. D614G PsVN GMTs post MV(D614)-boosting were similar across priming vaccines and substantially higher than following the MV(D614)-primary series. Post-booster B.1.351 GMTs for the MV(Beta) booster increased 34-fold and 75-fold from the baseline titer for pooled mRNA- and adenovirus-primed 18 - 55yr old participants, respectively, with D614G GMTs being raised by 13- and 31-fold baseline. Post-booster GMTs for the MV(Beta) booster were comparable to the BiV Beta-containing booster vaccine. Higher magnitude serum cross-neutralizing titers against BA.1 were elicited by the beta-containing vaccines than the MV(D614) booster. Response trends in adults 56+ yrs were similar. Solicited reactions were predominantly of mild-to-moderate intensity and of short duration. No safety concerns were identified during this study.

Conclusion

Sanofi-GSK recombinant spike protein MV(Beta) booster vaccine with AS03 adjuvant demonstrated an acceptable safety profile and provided high titers of cross-neutralizing antibodies across multiple variants, including Omicron. This Beta-variant booster is an attractive alternative for the Fall 2022 campaign.

Poster Reception III

Gustavo Dayan - AOXI0696

Efficacy of a bivalent SARS-CoV-2 Recombinant Protein Vaccine with AS03 adjuvant (CoV2 preS dTM-AS03 [D614 and B.1.351]) in adults 18 years of age and older

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Background

The global spread of highly transmissible SARS-CoV-2 variants of concern (VOC) necessitates the development of variant strain vaccines. Current COVID-19 vaccines based on the D614 strain Spike protein show modest levels of protection against new VOC. To combat the emergence of variant strains, Sanofi and GSK are developing a bivalent vaccine formulation including the ancestral D614 strain and a B.1.351 (Beta) strain.

Method

A parallel-group, Phase III, modified double-blind study was conducted in adults 18 years of age and older to assess the efficacy and safety of a bivalent recombinant protein vaccine (CoV2 preS dTM-AS03 [5 µg D614 + 5 µg B.1.351]) with AS03 adjuvant for prevention of COVID-19 [NCT04904549]. Participants were randomized to receive 2 injections of either the investigational vaccine or placebo in a 1:1 ratio, administered 21 days apart. The study was conducted in Colombia, Ghana, India, Kenya, Mexico, Nepal, Uganda and Ukraine. Participants were contacted at least once a week to detect COVID-19-like illness. The primary efficacy endpoint was the occurrence of symptomatic COVID-19 (SC). An interim analysis was conducted with cut-off date March 15, 2022. Vaccine efficacy (VE) results post-dose 2 (PD2) are presented.

Result

A total of 13002 participants were randomized in the study. The median follow-up duration PD2 was ~ 2 months. Most participants in the VE analyses were SARS-CoV-2 non-naïve and ~ 6% were SARS-CoV-2 naïve. VE against SC in all participants regardless of prior SARS-CoV-2 infection was 64.7% (95% CI: 46.6; 77.2) meeting the primary study endpoint. The VE against SC in non-naïve participants was 75.1% (95% CI: 56.3; 86.6) and 30.9% (95% CI: -39.3; 66.7) in naïve participants. Most cases were associated with BA.1 and BA.2 Omicron subvariants, while no BA.4 or BA.5 cases were observed. VE against SC caused by Omicron VOC was 72.5% (95% CI: 49.5; 86.0) in all participants and 93.9% (95% CI 75.9; 99.3) in non-naïve participants. Although we were not able to obtain sequencing results in ~ 44% of the cases, sensitivity analyses that considered these cases as caused by the Omicron variant showed a VE of 63.1% (95% CI: 43.9; 76.2) in all participants and 73.8% (95% CI 53.9; 85.9) in non-naïve participants. Few SC cases in adults ≥ 60 years, and few severe or hospitalized cases were observed. The vaccine was well tolerated and showed an acceptable safety profile.

Conclusion

This is the first clinical trial showing efficacy against the Omicron VOC. This study provides evidence that an adjuvanted recombinant protein vaccine containing a non-circulating Beta strain, in addition to the D614 prototype strain, can confer protection against variants not included in the vaccine.

Poster Reception III

Ju Hwan Jeong - AOXI0697

Combination therapy with nirmatrelvir and molnupiravir improves the survival of SARS-CoV-2 infected mice

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Background

As the SARS-CoV-2 pandemic remains uncontrolled owing to the continuous emergence of variants of concern, there is an immediate need to implement the most effective antiviral treatment strategies, especially for risk groups.

Method

Here, we evaluated the therapeutic potency of nirmatrelvir, remdesivir, and molnupiravir and their combinations in SARS-CoV-2-infected K18-hACE2 transgenic mice.

Result

Systemic treatment of mice with each drug (20 mg/kg) resulted in slightly enhanced antiviral efficacy and yielded an increased life expectancy of only about 20-40% survival. However, combination therapy with nirmatrelvir (20 mg/kg) and molnupiravir (20 mg/kg) in lethally infected mice showed profound inhibition of SARS-CoV-2 replication in both the lung and brain and synergistically improved survival times up to 80% compared to those with nirmatrelvir (P= 0.0001) and molnupiravir (P= 0.0001) administered alone.

This combination therapy effectively reduced clinical severity score, virus-induced tissue damage, and viral distribution compared to those in animals treated with these monotherapies. Furthermore, all these assessments associated with this combination were also significantly higher than that of mice receiving remdesivir monotherapy (P= 0.0001) and the nirmatrelvir (20 mg/kg) and remdesivir (20 mg/kg) combination (P= 0.0001), underscored the clinical significance of this combination. By contrast, the nirmatrelvir and remdesivir combination showed less antiviral efficacy, with lower survival compared to nirmatrelvir monotherapy, demonstrating the inefficient therapeutic effect of this combination.

Conclusion

The combination therapy with nirmatrelvir and molnupiravir contributes to alleviated morbidity and mortality, which can serve as a basis for the design of clinical studies of this combination in the treatment of COVID-19 patients.

Poster Reception III

Ra Mi Cha - AOXI0698

Pathogenesis of clade 2.3.4.4b H5N8 high pathogenicity avian influenza virus isolated from South Korea in 2020-2021

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Background

High pathogenicity avian influenza (HPAI) H5Nx viruses belong to clade 2.3.4.4. lineage caused outbreaks in worldwide. In South Korea, clade 2.3.4.4b H5N8 HPAI first detected in wild bird and caused 109 outbreaks in poultry farms along with 234 wild bird cases during 2020-2021 winter season. H5N8 HPAI viruses also revealed to have at least seven genotypes. First circulated virus was genetically closed to viruses reported in Europe, 2019-2020 and major H5N8 viruses circulated later showed a close genetic relationship with viruses detected in Europe during late 2020. In this study, we evaluated the pathogenicity and transmissibility of first isolated A/mandarin duck/Korea/H242/2020 H5N8 (H242/20(H5N8)) in chickens and ducks.

Method

Animal experiments performed with SPF chickens and AI-antibody free ducks to examine the pathogenicity of H242/20(H5N8) and compared with those of A/duck/Korea/HD1/2017(H5N6) (HD1/17(H5N6)), clade 2.3.4.4b H5N6 HPAI virus isolated during the 2017-2018 HPAI outbreak in Korea. Mean chicken lethal dose (mCLD50), Mean death time (MDT) and mean bird infectious dose (BID50) in ducks, were measured in virus inoculated group via intranasally route. To determine virus transmissibility, three naïve birds were co-housed eight hours after the inoculation. Virus shedding was examined by oropharyngeal (OP) and cloacal (CL) swabs collected for 2-5 days post inoculation (dpi) and viral replications also observed in internal organs at 3 dpi.

Result

In chickens, mCLD50 of H242/20(H5N8) group showed approximately 10 times higher EID50 than those of HD1/17(H5N6). Virus inoculated chickens showed 100% mortality with the MDT of 4.3 days which are shorter than HD1/17(H5N6) group. H5N8 group survived longer with higher titer viral shedding via OP and CL route than H5N6 group. At 3dpi, both virus replication detected in all organs but H5N8 group showed much lower viral titer than H5N6 group. All ducks infected with either HPAI virus survived without clinical symptoms. In contact groups, the transmissibility of ducks (100%) are higher than chickens (33.3%). Virus inoculated ducks also showed a longer virus shedding period and a lower virus titer in the organs at 3dpi than chickens.

Conclusion

Our data showed that the pathogenesis of H242/20(H5N8) was less virulent than HD1/17(H5N6) in chicken. These results may indicate that the risk of viral contamination on farms may be increased by H242/20(H5N8). In addition, virus infected ducks survived without clinical signs and shed the virus longer with a higher transmission rate than chickens, suggesting that ducks may play an important role as a silent carrier in the fields. Therefore, the understanding of the pathogenic features of HPAI viruses is needed for the effective control measure of HPAI outbreaks in the field.

Poster Reception III

Wey Wen Lim - AOXI0699

Immunogenicity and durability of immune responses to mRNA and inactivated COVID-19 vaccines among healthcare workers in Hong Kong

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Background

COVID-19 is a potentially fatal acute respiratory disease that is still causing significant mortality and morbidity three years into the pandemic. Even though vaccines against the earlier strains of SARS-CoV-2 are now available, breakthrough infections can still occur due to waning circulating antibodies and immune escape by new variants. Since February 2021, healthcare workers in Hong Kong were prioritised to receive COVID-19 vaccination with the mRNA (BNT162b2) and inactivated (CoronaVac) vaccines. In this study, we assessed humoral immune responses to both vaccines in our healthcare worker cohort and investigated the association between these immune responses and vaccine effectiveness.

Method

We recruited HCWs from public and private healthcare institutions across Hong Kong and collected blood samples at enrolment and every 6 months from June 2020 to June 2022. We also collected post-vaccination blood samples from a subset of volunteers between 10 - 42 days after each dose of vaccine. Serological evidence of infection and immune responses to vaccination were assessed by measuring levels of SARS-CoV-2 binding antibodies by an enzyme-linked immunosorbent assay (ELISA) and SARS-CoV-2 neutralising antibodies by surrogate virus neutralization test (sVNT) and plaque reduction neutralization test (PRNT).

Result

We present here preliminary results on the immunogenicity of homologous and heterologous vaccination with one to three doses of mRNA or inactivated vaccines. Among the 1,736 HCWs enrolled in our cohort, 195 HCWs provided pre- and post-vaccination blood samples after each dose of either vaccine. Homologous vaccination with two doses of BNT162b2 elicited levels of neutralising antibodies (sVNT inhibition = 96.8%, range = 56.1%, 98.2%) comparable to those generated by natural infections in the first wave (sVNT inhibition = 84.0%, range = 32.9%, 93.8%). Similar levels were achieved with three doses of CoronaVac (sVNT inhibition = 91.3%, range = 87.4%, 95.8%) and heterologous vaccination with two doses of CoronaVac followed by a booster dose of BNT162b2 vaccine (sVNT inhibition = 97.1%, range = 85.8%, 97.7%). These antibody levels waned faster after second doses and slower after the third dose for both vaccines.

Conclusion

Both the BNT162b2 mRNA vaccine and inactivated CoronaVac vaccines can generate robust antibody responses comparable to that of natural infections. Observations from this study are consistent with existing evidence that three doses of the CoronaVac vaccine, or a heterologous boost with the BNT162b2 vaccine following two doses of the CoronaVac vaccine are required to achieve similar levels of neutralising antibodies in vaccinees who received two doses of the BNT162 mRNA vaccine.

Poster Reception III

Zoé Schmal - AOXI0700

Interrupting lipid metabolism serves as a promising strategy to inhibit SARS-CoV-2 replication in adipocytes

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Background

Obesity belongs to one of the major risk factors associated with fatal COVID-19. We and others have shown that adipose tissue, in addition to the lung, serves as a major organ where productive SARS-CoV-2 replication takes place. On one hand, overall increase in virus load hampers virus clearance and on the other hand, it disturbs organ and systemic lipid metabolism.

Method

In this study, we assessed whether lipid components might be required for SARS-CoV-2 replication in adipocytes by using inhibitors targeting lipid metabolism.

Result

Here, we show that cell treatment with THL (Tetrahydrolipstatin, a clinically approved lipase inhibitor) as well as Atorva (Atorvastatin, a clinically approved inhibitor of cholesterol synthesis) resulted in significant reduction of SARS-CoV-2 (WT, δ, o) replication in human mature adipocytes (hMSC-TERT20). In contrast, treatment of human lung cells with THL only resulted in modest inhibition of SARS-CoV-2 (WT, δ, o) - replication. Treatment of human lung cells with Atorva did not significantly impair SARS-CoV-2 (WT, δ, o) replication.

Conclusion

These findings suggest that clinically approved drugs interrupting lipid metabolism might serve as a promising strategy to reduce virus load in adipose tissue, thereby reducing overall disease burden in COVID-19 patients at high risk.

Poster Reception III

Yun Hee Baek - AOXI0701

A rapid method for generating infectious SARS-CoV-2 and variants using mutagenesis and circular polymerase extension cloning

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Background

The appearance of SARS-CoV-2 variants in late 2020 raised alarming global public health concerns. Despite continued scientific progress, the genetic profiles of these variants bring changes in viral properties that threaten vaccine efficacy. Thus, it is critically important to investigate the biologic profiles and significance of these evolving variants.

Method

In this study, we demonstrate the application of circular polymerase extension cloning (CPEC) to the generation of full-length clones of SARS-CoV-2. We report that, combined with a specific primer design scheme, this yields a simpler, uncomplicated, and versatile approach for engineering SARS-CoV-2 variants with high viral recovery efficiency.

Result

This new strategy for genomic engineering of SARS-CoV-2 variants was implemented and evaluated for its efficiency in generating point mutations (K417N, L452R, E484K, N501Y, D614G, P681H, P681R, Δ69-70, Δ157-158, E484K+N501Y, and Ins-38F) and multiple mutations (N501Y/D614G and E484K/N501Y/D614G), as well as a large truncation (ΔORF7A) and insertion (GFP).

Conclusion

The application of CPEC to mutagenesis also allows the inclusion of a confirmatory step prior to assembly and transfection. This method could be of value in the molecular characterization of emerging SARS-CoV-2 variants as well as the development and testing of vaccines, therapeutic antibodies, and antivirals.

Poster Reception III

Min-Chul Jung - AOXI0702

Triplicated Recombinant Proteins Composed of Conserved Sequence of Hemagglutinin Stem and Membrae 2 Ectodomain Induce Cross-Protection Against Homologous and Heterologous Influenza A Virus

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Background

Influenza viruses are classified into four species from A to D, and among them, influenza A virus(IAV) is the major case that infects humans and causes severe respiratory diseases. Due to the characteristics of genome and antigenic surface protein, various mutations occur frequently so immune escape becomes easy, which makes a burden on prevention of infection by vaccines. Current influenza vaccines are produced by predicting strains expected to be prevalent. Still, if predictions are missed or a new variation suddenly appears, the vaccine's effectiveness is significantly decreased. Therefore, developing a universal vaccine that can protect against various mutants is indispensable to solving these problems. Currently, multiple approaches are being developed for universal vaccines, such as mRNA, recombinant subunit proteins, virus-like particles, and viral vector vaccines. Several candidates have reached clinical trials, but none have been commercialized. Some candidates need to address safety issues, and even recombinant protein vaccines, which are recognized as the safest, often impede mass production due to protein stability and insolubility.

Method

Here, we designed a recombinant protein vaccine with short helix residues of hemagglutinin stem domain(HA2SH) and ectodomain of M2 protein(M2e) exposed on the outer surface of the virion and highly conserved that it can induce broad protection. Also, we developed the 3XHA2SH-M2e peptides that were repeated in triplicate to mimic the trimeric structure of hemagglutinin and enhance its immunogenicity. As a result, the vaccine composition was optimized and applied to the virus challenge test in mice.

Result

Our data showed the potential for the 3XHA2SH-M2e recombinant protein vaccine candidate. Through the purification process, it was confirmed that this vacccine candidate is soluble and stable so it can solve the problem of mass production with low cost. Finally, it showed high immunogenecity and ability to protect against various strains of influenza A virus in mice infection test.

Conclusion

Furthermore, we plan to prove the protective effect against various viruses and strengthen the effectiveness by introducing self-assembly nanoparticles.

Poster Reception III

Nicola Chiwandire - AOXI0703

Test-negative case-control study of SARS-CoV-2 vaccine effectiveness against SARS-CoV-2-associated hospitalisation in South Africa 2021 to 2022

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Background

COVID-19 real-world vaccine effectiveness (VE) studies conducted using the WHO-recommended test negative case-control design and leveraging established surveillance systems are limited in sub-Saharan Africa. We aimed to assess the effectiveness of the Pfizer (BNT162b2) and Johnson & Johnson (Ad26.COV2.S) vaccines against SARS-CoV-2-associated hospitalisation in individuals ≥18 years including by HIV status.

Method

We conducted a test-negative case-control study to estimate the VE of the BNT162b2 and Ad26.COV2.S vaccines against SARS-CoV-2-associated hospitalisation in individuals enrolled in national pneumonia sentinel syndromic surveillance in South Africa who were vaccine-eligible, had complete vaccine histories and SARS-CoV-2 PCR results. Receiving one dose of Ad26.COV2.S vaccine or two doses of BNT162b2 vaccine ≥14 days before interview date was considered fully vaccinated. We determined VE in the entire study population and stratified by the Omicron and Delta variant periods, and by HIV status using multiple logistic regression adjusting for age, sex, race, month of admission, HIV infection status, previous self-reported SARS-CoV-2 infection, underlying condition and province.

Result

From April 2021 through April 2022, we enrolled 2,828 inpatients aged ≥18 years. 974 (34.4%) tested positive, 3.9% cases and 9.7% controls were fully BNT162b2 vaccinated and 3.2% cases and 5.1% controls fully Ad26.COV2.S vaccinated. BNT162b2 VE against SARS-CoV-2-associated hospitalisation over the entire, Delta, and Omicron periods was 47% (95% confidence interval [CI], 17%-64%), 92% (95% CI, 48%-99%), and 17% (95% CI, -48%-54%) respectively. Ad26.COV2.S VE against hospitalisation over the entire, Delta, and Omicron periods was -10% (95% CI, -83%-34%), 56% (95% CI, -115%-91%), and -17% (95% CI, -134%-42%) respectively. Among HIV uninfected adults, BNT162b2 VE against hospitalisation over the entire and Delta periods was 64% (95% CI, 35%-80%), and 86% (95% CI, 9%-98%) respectively. Whilst among adult people living with HIV (PLWH), BNT162b2 VE against hospitalisation over the entire and Delta periods was -1% (95% CI, -115%-52%), and 88% (95% CI, -249%-100%) respectively.

Conclusion

Two doses of BNT162b2 vaccine were effective against SARS-CoV-2-associated hospitalisation over the entire and Delta variant periods, overall, and for HIV uninfected adult inpatients. Limited sample size precluded evaluation of effectiveness of the Ad26.COV2.S vaccine and in PLWH. Other limitations included not controlling for waning immunity, or time since vaccination, and self-reported previous SARS-CoV-2 infection; however, these findings provide real-world estimates of the effectiveness of the SARS-CoV-2 vaccines in a sub-Saharan African context.

Poster Reception III

Mariam Mansouri - AOXI0704

Systems-oriented modelling methods in preventing and controlling emerging infectious diseases in the context of healthcare policy - A scoping review

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Background

Emerging infectious diseases (EIDs) arise and affect society in complex ways. Therefore, we conducted a scoping review to explore how systems-oriented simulation methods have been used to investigate and inform health care systems preparedness and response to EIDs.

Method

We used the Joanna Briggs Institute framework for scoping review. We included peer-reviewed articles published between January 2000 and March 2021. Pathogens of interest were chosen based on World Health Organisation's list of prioritised infectious diseases for research and development. We only included studies that explicitly simulated the interrelationship and/or dynamics between the system's elements.

Result

Our initial search yielded 9984 studies. After screening and assessment by two independent reviewers, 117 full-text articles were assessed for eligibility, and seven studies were included in a qualitative synthesis. The studies were published between 2009 and 2021. Most focused on sarbecoviruses and targeted healthcare policymakers and governments. Most of the studies incorporated classical epidemiological models alongside systems-oriented methods. The studies largely focussed on disease dynamics and the burden on human health, the economy and healthcare systems. The most frequently reported challenge related to the timeliness and quality of epidemiological and geographical data.

Conclusion

Systems dynamics approaches can help policymakers understand the elements of a complex system and thus offer potential solutions for preventing and controlling EIDs. Our review indicates that systems-oriented methods can be used alone or in combination with classical mathematical modelling. In the context of healthcare policy, systems methods can elucidate diseases dynamics and the impact of policy change on the number of new cases. Moreover, systems methods can be used to assess the agility and resilience of the healthcare system when facing the threat of an EID.

Poster Reception III

Nika Nemanichvili - AOXI0705

Tissue microarrays to visualize influenza D attachment to host receptors in the respiratory tract of farm animals

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Background

Binding of an influenza D virus to the host cell is the first step in determining the host and tissue tropism which ultimately drives viral pathogenicity. Tools to study virus-host interactions, and subsequent host specificity, tissue tropism, and pathogenesis, are limited. Several assays are available, including cell culture systems and glycan arrays, but many of these systems rely on an extensive knowledge of the receptor distribution within the studied animals and organs. The influenza D virus has a preference of 9-O-acetylated sialic acid receptors bound by the trimeric hemagglutinin-esterase fusion protein (HEF). 9-O-acetylated sialic acid receptors are expressed in various host species but knowledge of distribution is lacking in many animals. While cattle are the main reservoir for IDV, the viral genome has also been detected in domestic pigs. In addition, antibodies against IDV have been detected in other farm animals such as sheep, goats, and horses.

Method

We have developed a tissue microarray system using respiratory tissues from farm animals such as cattle, domestic pigs, goats, sheep and horses. With these tissue microarrays we aimed to determine the host and tissue tropism and potential differences between the two major influenza D clades, D/Oklahoma and D/660. We used recombinantly produced HEF proteins (HEF S57A) from the major clades D/Oklahoma (D/OK) and D/Oklahoma/660 (D/660) to study their host and tissue tropism through receptor binding. Protein histochemical staining of these farm animal tissue microarrays with the HEF proteins allowed us to visualize receptor these receptor bindings.

Result

All five tested farm animal species express host surface receptors for both the D/OK and the D/660 clade of IDV. While cattle expressed receptors in the entire respiratory tract except for the lungs, the binding of both clades was restricted to the upper respiratory tract in domestic pigs, sheep, goats, and horses. No differences in host or tissue tropism was discovered between D/OK and D/660 indicating that D/660 has the same potential for virus-host interactions as the much larger D/OK clade, while hemagglutination assays showed that D/OK has a 2-fold higher binding affinity than D/660 for receptors on red blood cells. The removal of O-acetylation from receptors via saponification treatment confirmed that receptor-binding of both clades was dependent on O-acetylated sialic acids.

Conclusion

We demonstrate that all five tested farm animal species express host surface receptors for both the D/OK and the D/660 clade of IDV. While cattle tissues expressed receptors in the entire respiratory tract except for the lungs, HEF binding was restricted to the nasal and pharyngeal epithelium in domestic pigs, sheep, goats, and horses.

Poster Reception III

Francis Amirtharaj - AOXI0707

Influenza surveillance system in United Arab Emirates: Past, Present and the Future

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Background

The United Arab Emirates (UAE) National Influenza Centre (NIC) inaugurated in 2019, actively participates in WHO GISRS program monitoring influenza and other respiratory viruses. During the COVID-19 pandemic, globally an overall decline in influenza surveillance and transmission due to public health initiatives including changes in the heath-seeking behavior. Here we discuss influenza trends in the UAE before and during pandemic and future initiatives to enhance influenza surveillance

Method

An epidemiological study in Sentinel sites was carried out from 2019-2022 of SARI and ILI cases. Routine laboratory confirmed influenza trends across government health-care facilities was used as a comparison. COVID-19 positivity rate in Sentinel samples was examined to assess influenza infection patterns. Epidemic thresholds for flu A and flu B incidence rates were assessed based on the compared stratified epidemic curves centered at seasonal midpoints

Result

Past

Sentinel Surveillance focused on ILI cases across 4 government outpatient clinics and SARI cases across 2 government hospitals. In UAE influenza season typically began in October with predominant transmission of flu A (H3N2 and H1N1-2009) and B-Victoria in 2019. Epidemic threshold was observed in November with 55% positivity rate (out of 283) and 43% in December 2019 (out 224) (Fig 1).

Present

During the first COVID-19 wave, sentinel surveillance was interrupted as health-care facilities became designated COVID-19 centres. At the end of 2020 until mid-2021 only one ILI site and one SARI were active. COVD-19 test was conducted for ILI and SARI cases and all samples in 2020 tested retrospectively. Efforts were made in 2021 to re-activate ILI Sentinel Surveillance by expanding case definition to Acute Respiratory Infection (ARI) and more SARI sites were integrated into the network. In both routine diagnostic testing and sentinel surveillance, clear decline of influenza was seen during the COVID-19 pandemic with minimal transmission. Interestingly, in UAE, influenza transmission started sooner in 2022, with the highest infection rates epi-week 23-27.

Future

Sentinel Surveillance program was enhanced with ongoing field visits, training of all stakeholders to achieve goal of collecting samples from a representative population to meet target set by the WHO (Fig 2).

Conclusion

Given the complexity and changing trend, an integrated sentinel surveillance system are projected to deliver quick and precise community-wide influenza surveillance.

Poster Reception III

Jeanette Tingstedt - AOXI0708

Differential recognition of influenza A virus H1N1 neuraminidase by vaccine-induced antibodies in pigs and ferrets

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Background

The enzymatic activity of influenza neuraminidase (NA) mediates release of virus progeny from infected cells and augment virus entry by cleaving sialylated glycoproteins in mucus. There is growing interest in targeting these functions during the viral life cycle through vaccination, which warrants a clear understanding of NA-specific immunity. Our polyvalent influenza DNA vaccine candidate encodes seven pandemic influenza proteins that includes, among others, the NA from the H1N1 influenza virus A/California/7/09. The DNA vaccine proved protective against homologous and heterologous influenza virus challenges in relevant animal models. To understand the underlying mechanisms of protection, we here define the functionality of vaccine induced NA-specific antibodies and identify antigenic sites of the vaccine-homologous NA protein in both vaccinated pigs and ferrets.

Method

Animals were vaccinated with the influenza DNA vaccine candidate encoding selected surface and internal proteins of pandemic H1N1 and H3N2 influenza viruses. Sera obtained pre-vaccination, post-vaccination and post-challenge with H1N1 influenza virus A/California/7/09 were analyzed for NA inhibition against recombinant H7N1CA09 (NIBRG-127) virus and possible antigenic sites were identified using linear and conformational peptide microarrays covering the entire NA protein of A/California/04/2009(H1N1)pdm09.

Result

NA-specific antibodies, induced by the influenza DNA vaccine candidate, inhibited the enzymatic function of NA of the pandemic H1N1 virus (A/California/7/09) presented on a recombinant H7N1 virus. These antibodies targeted critical sites of NA such as the enzymatic site, second sialic binding site and framework residues, as shown by high-resolution epitope mapping. We observed a species-specific and differential epitope recognition in vaccinated pigs and ferrets. In addition, we identified new possible antigenic sites that potentially block the catalytic activity of NA. In particular, one epitope recognized solely in pigs and ferrets that possess antibody-mediated NA inhibition could be a key antigenic site affecting NA function, despite not containing any residues from the above-mentioned critical sites.

Conclusion

The findings show that the influenza DNA vaccine candidate induces NA-specific antibodies that target known critical sites and new potential antigenic sites of NA, inhibiting the catalytic activity.

Poster Reception III

Mathis Funk - AOXI0709

TRANSIENT STEM-LOOP RNA STRUCTURES AND PURINE-RICH SEQUENCES AT THE H5 HEMAGGLUTININ CLEAVAGE SITE DRIVE DUPLICATIONS BY THE INFLUENZA POLYMERASE: A POTENTIAL EXPLANATION OF HIGHLY PATHOGENIC AVIAN INFLUENZA GENESIS

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Background

Highly pathogenic influenza viruses (HPAIV) can emerge from H5 and H7 low pathogenic avian influenza viruses (LPAIV), causing severe disease with mortality rates up to 100%. The conversion from LPAIV to HPAIV occurs via the acquisition of a multi-basic cleavage site in the hemagglutinin (HA) gene, often due to nucleotide insertions at the HA cleavage site (HACS) via stuttering/backtracking of the influenza virus RNA-dependent RNA polymerase (RdRp). Yet, the exact molecular mechanism underlying this phenomenon remains unknown. We and others have suggested previously that conserved RNA structures surrounding the HACS might play a role. Here we further hypothesize that transient RNA structures forming around the RdRp during replication of purine-rich regions lead to insertions at the HACS.

Method

A virus-free influenza replication system was used in order to detect all insertions without constraints at the protein level or selection biases. Experiments were performed with and without influenza RdRp and a circular next-generation sequencing approach was developed to accurately discriminate insertions due to the influenza RdRp from background insertions, allowing detection of rare insertions with high confidence. Mutant HAs with modified RNA structures or sequences were designed using an in-house bio-informatic tool predicting transient RNA structures, and their impact on insertion pattern and frequency was assessed to test the proposed hypothesis.

Result

No insertions were detected when using an H5 HA with consensus LPAIV HACS. When using a mutant H5 HA with more adenines at the HACS, shown to be insertion-prone in serial passaging experiments, insertions were detected. The observed insertions fell into two categories: insertions of multiple different nucleotides, due to backtracking and duplication of neighboring sequences, or repeated insertions of the same nucleotide, due to RdRp stuttering. Destabilization of predicted transient RNA structures led to a five-fold reduction of duplications. When the insertion-prone H5 HACS sequence was placed in another HA region where transient RNA structures were not predicted to form, a low level of repeated insertions was observed, but duplications were almost completely abolished. After mutating this region to enable formation of transient enclosing RNA structures similar to those predicted at the HACS, a three-fold increase in duplications was observed.

Conclusion

Our results show that insertions by the influenza RdRp due to duplications are the result of the presence of insertion-prone RNA sequences and the formation of transient RNA stem-loop structures. The interplay of these two factors might explain why HPAIV-yielding insertions are a rare phenomenon restricted to only two HA subtypes.

Poster Reception III

Paula Couto - AOXI0710

Regional experience on influenza, SARS-CoV-2 RSV, and ORV sustainable surveillance and lessons from COVID-19 pandemic

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Background

Influenza and other respiratory viruses (ORV) in the Americas are monitored by WHO sentinel surveillance through SARINET regional network integrating COVID-19 since 2020. PAHO was the first WHO region to have integrated surveillance, COVID-19 was then incorporated. Lessons learned from COVID-19 pandemic on strengthening the integrated regional influenza/ORV surveillance towards sustainability in the Americas were assessed.

Method

Qualitative-quantitative analysis of 2020-2022 period was carried out focusing on surveillance capacity; technical cooperation experiences and official documents on surveillance, preparedness, readiness and response.

Result

COVID-19 pandemic presented challenges and opportunities for integrated surveillance. Since 2010, 32 countries periodically shared Flunet/FluID data and 25 countries integrated SARS-CoV-2 into sentinel surveillance guaranteeing sustainability of COVID 19 trends monitoring. SARS-CoV-2 integration into sentinel SARI/ILI surveillance allowed monitoring of influenza, RSV and SARS-CoV-2; rapid assessment of transmission and seasonality with detection of changes in the epidemiological patterns of circulating viruses; monitoring of genetic characteristics of viruses strains; assessment of vaccine effectiveness and impact; assessment of burden of disease, and detection of unusual respiratory events.

High-priority surveillance objectives were identified based on existing surveillance systems:

- Prioritized strengthened surveillance for early detection and risk assessment, monitoring epidemiological situation as well as the impact on human health and systems (enhanced ILI surveillance, assess ruptures, ensure system flexibility for pandemic monitoring).

- Sustainable regional enhancements and innovations from the pandemic include leveraging the existing SARINET/REVELAC vaccine effectiveness network; leveraged information systems (PAHOFlu) to allow for seasonal/pandemic monitoring; advances in molecular epidemiology with CDC SARS-CoV-2 and Influenza multiplex assay implementation and genomic sequencing (COVIGEN regional network); and integrated into PAHO Flu.

Conclusion

COVID-19 pandemic highlighted the need to address sustainability of other future enhancements and innovations. The role of sentinel surveillance network is key to monitor disease transmissibility and severity (with integration of genomic surveillance component) and inform control and prevention strategies. Ensuring leveraged overarching coordination mechanisms to enhance sustainable influenza and ORV surveillance during the COVID-19 pandemic to inform prevention and control plans and policies are key ahead of influenza and ORV seasons with adaptation to concurrent COVID-19 emergency.

Poster Reception III

Alfredo Bruno - AOXI0712

Implementation of Systematic and Routine Genomic Surveillance at the National Influenza Centre of Ecuador

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Background

Since the confirmation of the COVID-19 pandemic, the scientific world has seen the importance of having a genomic surveillance system that contributes to the response to health questions. With the presence of the new coronavirus, Global Surveillance has generated a large number of SARS-CoV-2 genetic sequences and related metadata to support the response to the COVID-19 pandemic. For this purpose, NIC-Ecuador Implemented the first systematic and routine genomic surveillance operational plan in Ecuador in order to increase the percentage of sequencing by EW, number of shared sequences of the Country in the GISAID Platform, identify circulating variants of SARS-CoV-2, therefore the laboratory, carried out the selection process of samples to be sequenced according to the criteria established in the Operational Plan for Systematic and Routine Genomic Surveillance of SARS-CoV-2, through the use of next generation sequencing technology, with the use of the COVIDSeq kit and the MiSeq ILLUMINA equipment

Method

The methodology to be used is divided into several phases: first, develop the strategic planning of the plan second, Identified institutions in the country with massive sequencing capacity for SARS-CoV-2; socialized the plan; to organize technical meetings, organize working groups and constant reporting of variants detected through official channels, all of each step with Ministry of Public Health.

Result

With the implementation of these genomic surveillance strategy had been increased national sequencing capacity and NIC had a median of 9.27 % of sequenced samples per week, with a minimum value of 0.02% and a maximum value of 55.56%. Laboratoty sequenced 3973 samples in the period of this study, it can be seen a co-circulation of the Alpha, Gamma and Delta and other variants between 10 to 46 EW of 2021. The predominant variant was Delta with 1162 samples representing 29.25% of the cases; Delta was found from 25 EW 2021 to 5 EW 2022, followed by the Omicron variant with 1087 samples represented by 27.36%, and the period of circulation for Omicron was from 49 EW, predominantly from 51 EW.

Conclusion

It is important to mention that genomic surveillance should be strengthened and resources and experienced personnel should be maintained to ensure the sustainability of surveillance and provide scientific information to national health authorities for decision making in benefit of the public health of Ecuadorians and to contribute to GISRS and IHR.