Shared peaks, divergent kinetics: influenza subtype temporal phenotypes in a contemporary human challenge panel

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INTRODUCTION & OBJECTIVES

Influenza virus infections remain a significant disease burden globally causing high levels of acute respiratory infections, morbidity and mortality. Challenge studies (also known as CHIM studies) have previously played an important role in the development of respiratory virus vaccines and treatments. They continue to be a powerful tool to deliver rapid efficacy signals. Here we compare the disease trajectories of a panel of three contemporary influenza challenge viruses, each of a different subtype.

CHALLENGE VIRUS PANEL

Three influenza challenge viruses were produced. Two influenza A viruses were produced, one H3N2 and one H1N1, both initiated from the isolation of the virus from clinical samples of community acquired infection. In addition, an influenza B virus was produced via a reverse genetics seed virus. All viruses were isolated and passaged in a qualified GMP cell line and manufactured under GMP conditions (see **Figure 1**).

Properties of the 3 influenza GMP challenge stocks produced

A/France/759/2021 H1N1
- Clinical sample from late December 2021
- GMP wild-type strain
- Clade B (6B.1A.5a.1)

A/England/7763/2022 H3N2
- Clinical sample from September 2022
- GMP wild-type strain
- Clade G1.3 (3C.2a1b.2a)

B/Connecticut/01/2021
- Reverse genetics produced to 100% sequence match of 2022 WHO Candidate Vaccine Viruses (CVV)s
- GMP wild-type strain
- Victoria lineage

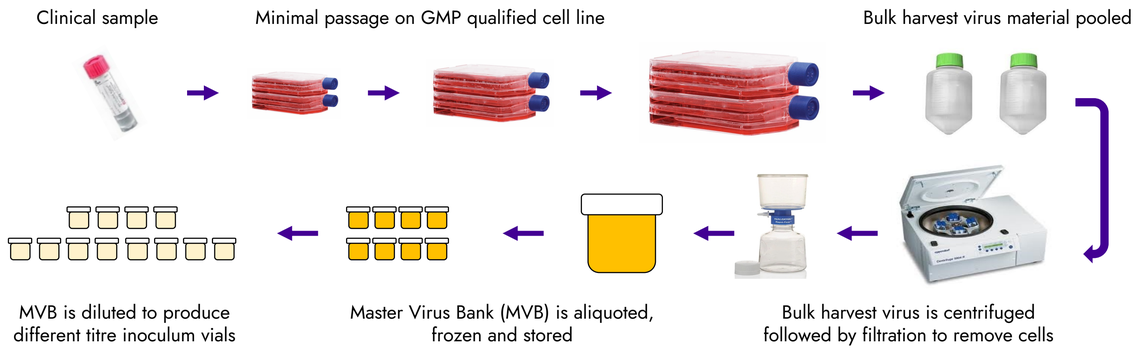
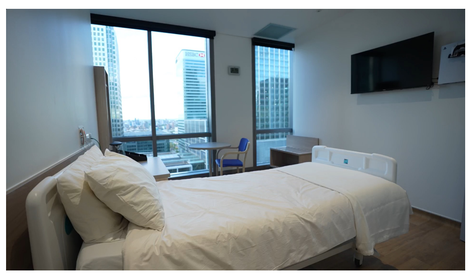


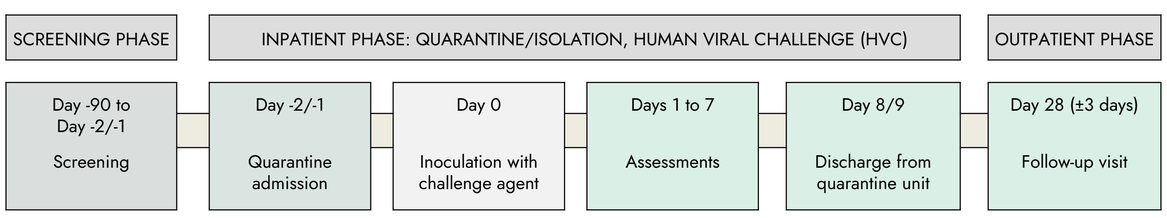
Figure 1: End to end process for the GMP Challenge virus batch production and subsequent inoculum vial filling.

CHALLENGE MODEL CONDUCT

Human virus challenge studies were conducted to characterise the three challenge strains: A/France/21 H1N1, A/England/22 H3N2 and B/Connecticut/21. Each study involved the recruitment and screening of 18–55-year-old healthy participants. Potential participants underwent an initial screening phase, including medical history and serology – all participants, to be eligible, had to have low (<10) or non-detectable (NDA) serum hemagglutination inhibition (HAI) titres. The serosuitable participants with low pre-existing immunity were inoculated intranasally on study day 0.

Participants were monitored 24/7 within hVIVO's quarantine unit for up to 9 days post viral inoculation. Regular assessments were performed including: ECG, bloods (haematology and biochemistry), nasopharyngeal swabs (NPS) for virology, vital signs and spirometry for safety. Immunological samples including serum were collected for immunological assessments. Participants returned for a Day 28 follow up visit and discharge from the study.





Symptoms Please report the highest level of symptoms you have experienced since completing the last diary card (if applicable), including any symptoms you currently have (tick ONE in each row)	I have NO symptoms	Just noticeable	It's clearly bothersome from time-to-time, but it doesn't interfere with me doing my normal daily activities	It's quite bothersome most or all of the time, and it stops me from participating in activities	Symptoms at rest
Runny Nose	☐0	☐1	☐2	☐3	
Stuffy Nose	☐0	☐1	☐2	☐3	
Sneezing	☐0	☐1	☐2	☐3	
Sore Throat	☐0	☐1	☐2	☐3	
Earache	☐0	☐1	☐2	☐3	
Malaise/Tiredness	☐0	☐1	☐2	☐3	
Headache	☐0	☐1	☐2	☐3	
Muscle and/or Joint Ache	☐0	☐1	☐2	☐3	
Chilliness/Feverishness	☐0	☐1	☐2	☐3	
Cough	☐0	☐1	☐2	☐3	
Chest Tightness	☐0	☐1	☐2	☐3	
Shortness of Breath	☐0	☐1	☐2	☐3	☐4
Wheeze	☐0	☐1	☐2	☐3	☐4

Figure 2: Self-reported participant symptom diary card

To define robust endpoints for use of the viruses in subsequent product efficacy testing, data and samples were collected from inoculated volunteers for up to 9 days as follows:

Viral load endpoints
- NPS: 2 x daily for viral load assessments by RT-qPCR and culture

Symptom endpoints
- Self-reported symptom diary card (**Figure 2**): 3 x daily
- Vital signs, including temperature: 3 x daily
- Mucus weights via used tissue collection: daily

Safety endpoints
- Physician assessments: daily
- Safety blood assessments: 3 x in quarantine with additional directed by PI, as required.

CLINICAL OUTCOME SUMMARY

Inoculation with all virus doses was demonstrated to be safe and well tolerated with no Serious Adverse Events (SAE)s or AEs of concern. All influenza strains elicited high infection rates (**Figure 3**) and robust, symptomatic disease curves (**Figure 4**). Infection rates were summarised by a range of different definitions: "infected" – lab-confirmed qPCR positivity on multiple days, "moderate-severe" infection – infected plus presence of grade 2 symptoms, "febrile" – infected plus observed febrile illness. Each virus displayed a unique disease profile, with A/France causing the fastest onset of infection.

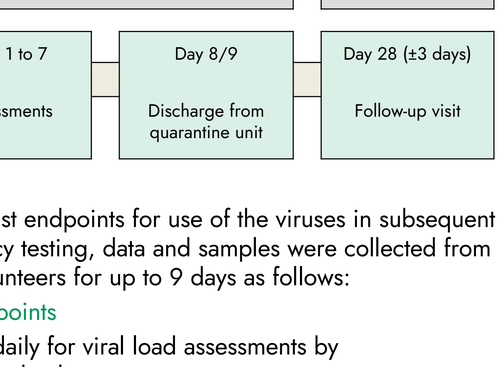


Figure 3: clinical Infection rates observed

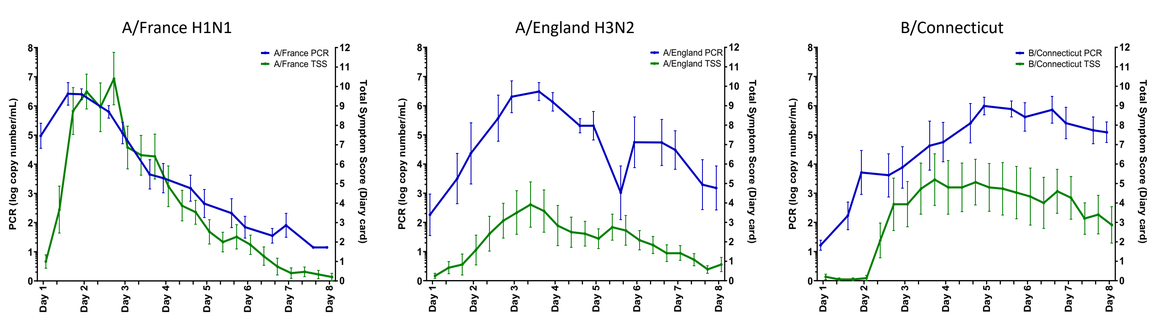


Figure 4: Disease trajectories observed with each virus, as measured by both qPCR viral load and total symptom score (TSS)

ADDITIONAL DATA ANALYSIS

Disease severity was further explored by comparing the viral load area under the curve (AUC), peak viral load and Peak symptom score (**Figure 5**). Robust disease was observed with all viruses. The AUC range was broadly similar between the viruses with higher mean AUCs for B/Connecticut and A/England. Mean Peak viral load was between 7 and 8 log10copies /mL for each of the viruses. Peak symptom score was generally higher with A/France with only one asymptomatic individual.

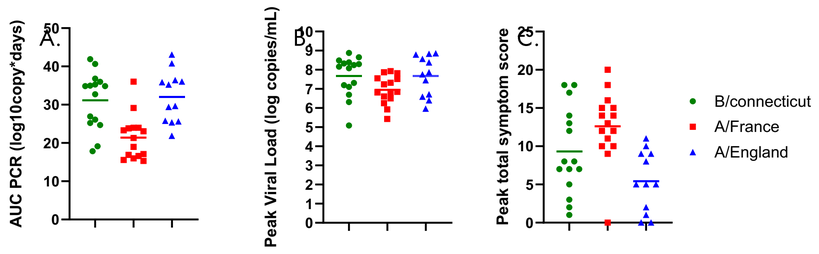


Figure 5: Disease severity comparisons of infected participants. A. AUC PCR, B. Peak viral load, C. Peak total symptom score (TSS). Each symbol represents one infected participant. Lines shown are group mean indicators

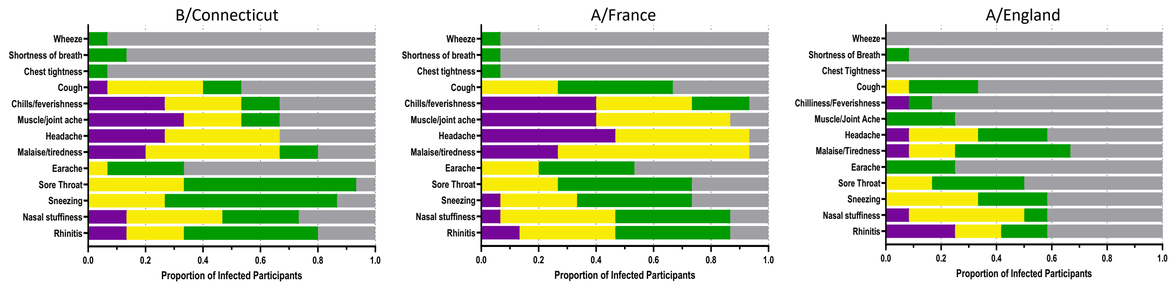


Figure 7: Maximum symptom severity exhibited by the study participants, expressed as the proportion of participants with each symptom severity grade. Each symptom on the diary card is represented separately. The 13 symptoms are grouped together for lower respiratory tract (LRT), systemic and upper respiratory tract (URT)symptoms. LRT symptoms: Wheeze, shortness of breath, chest tightness, cough; Systemic symptoms: chills/feverishness, muscle / joint ache, headache, malaise/ tiredness, earache; URT symptoms: Sore throat, sneezing, nasal stuffiness, Rhinitis. No symptoms; Grade 1: just noticeable; Grade 2: clearly bothersome time to time; Grade3: Quite bothersome most of the time

Comparing the maximum symptom grade recorded by the study participants for each of the three challenge viruses shows that greater than 90% of the infected participants were symptomatic for B/Connecticut and A/France viruses compared to 65% of those inoculated with A/England. URT symptoms were the highest severity scores within A/England infected participants whereas systemic symptoms were highest in the other two viruses. This is directly linked to the high febrile illness rates observed with B/Connecticut and A/France, with the febrile illness driving up the systemic symptom scores. As expected in healthy adult populations, LRT symptoms were rare and low grade if observed. All viruses exhibited robust symptoms, as demonstrated by the relatively high levels of grade 2 and 3 symptoms observed.

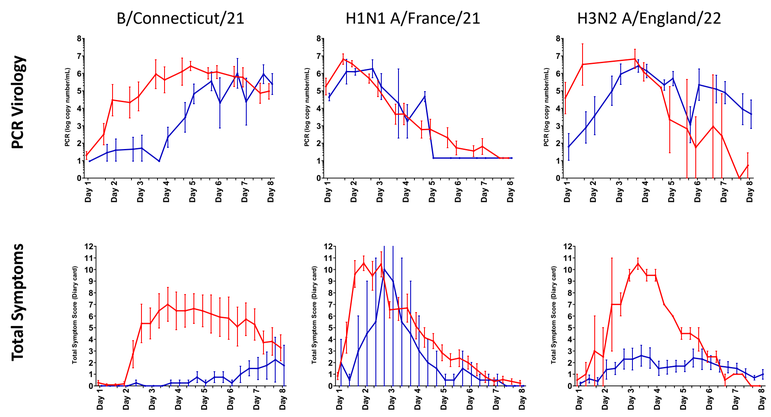


Figure 6: Comparison of viral load and total symptom score in febrile and afebrile infected participants showed greater magnitudes of virology and symptoms in febrile infected participants.

Febrile infected, red;
Afebrile infected, blue.

Separating participants who exhibited febrile illness from afebrile infections, illustrates that fever is a major driver of overall symptom severity (**Figure 6**). Furthermore, febrile infected participants tended to have faster onset of infection. Substantially lower symptoms were seen in general in afebrile individuals infected with B/Connecticut and A/England despite high viral load.

DISCUSSION & CONCLUSIONS

Our analysis of the data from three different influenza viruses has found that different viruses can elicit very different disease profiles. We suggest that this is likely to be strain-specific as opposed to influenza subtype-specific. The evidence for this is that prior influenza A challenge viruses have also shown different characteristics to the 3 strains studied here even though this study contains viruses that represent all the major subtypes in wide human circulation.

Typically challenge studies are used to test the efficacy of products that are strain-agnostic. Therefore, the availability of different challenge viruses with different disease characteristics provides the opportunity to choose the challenge virus that best facilitates the desired study design or clinical endpoint to be tested.

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