Research Square

Preprints are preliminary reports that have not undergone peer review. They should not be considered conclusive, used to inform clinical practice, or referenced by the media as validated information.

Safety, tolerability and viral kinetics during SARS-CoV-2 human challenge

Ben Killingley
Department of Infectious Diseases, University College London Hospital, London, UK

Alex Mann
hVIVO Services Ltd., London, UK https://orcid.org/0000-0002-3744-4604

Mariya Kalinova
hVIVO Services Ltd., London, UK

Alison Boyers
hVIVO Services Ltd., London, UK

Niluka Goonawardane
Department of Infectious Disease, Imperial College London, London, UK

Jie Zhou
Department of Infectious Disease, Imperial College London, London, UK

Kate Lindsell
UK Vaccine Taskforce, Department of Business, Energy and Industrial Strategy, London, UK

Samanjit S. Hare
Department of Radiology, Royal Free London NHS Foundation Trust, London, UK

Jonathan Brown
Department of Infectious Disease, Imperial College London, London, UK https://orcid.org/0000-0001-6849-3962

Rebeeca Frise
Department of Infectious Disease, Imperial College London, London, UK

Emma Smith
National Heart and Lung Institute, Imperial College London, London, UK

Claire Hopkins
ENT Department, Guy's and St Thomas' NHS Foundation Trust, London, UK

Nicolas Noulin
hVIVO Services Ltd., London, UK

Brandon Londt
hVIVO Services Ltd., London, UK

Tom Wilkinson
Faculty of Medicine and Institute for Life Sciences, University of Southampton, and NIHR Southampton Biomedical Research Centre, University Hospital Southampton, Southampton, UK

Stephen Harden

Department of Radiology, Southampton General Hospital, Southampton, UK

Helen McShane

Department of Paediatrics, University of Oxford, Oxford, UK https://orcid.org/0000-0002-2126-5142

Mark Baillet

S-cubed Biometrics, Abingdon, UK

Anthony Gilbert

UK Vaccine Taskforce, Department of Business, Energy and Industrial Strategy, London, UK

Michael Jacobs

Department of Infectious Diseases, Royal Free London NHS Foundation Trust, London, UK

Christine Charman

UK Vaccine Taskforce, Department of Business, Energy and Industrial Strategy, London, UK

Priya Mande

UK Vaccine Taskforce, Department of Business, Energy and Industrial Strategy, London, UK

Jonathan S. Nguyen-Van-Tam

Division of Epidemiology and Public Health, University of Nottingham School of Medicine, Nottingham, UK

Malcolm G. Semple

Health Protection Research Unit in Emerging and Zoonotic Infections, Institute of Infection, Veterinary and Ecological Sciences, Faculty of Health and Life Sciences, University of Liverpool, Liverpool; Respiratory Department, Alder Hey Children's Hospital, Liverpool, UK https://orcid.org/0000-0001-9700-0418

Robert C. Read

Faculty of Medicine and Institute for Life Sciences, University of Southampton, and NIHR Southampton Biomedical Research Centre, University Hospital Southampton, Southampton, UK https://orcid.org/0000-0002-4297-6728

Neil M. Ferguson

MRC Centre for Global Infectious Disease Analysis, School of Public Health, Imperial College London, London, UK https://orcid.org/0000-0002-1154-8093

Peter J. Openshaw

National Heart and Lung Institute, Imperial College London, London, UK https://orcid.org/0000-0002-7220-2555

Garth Rapeport

National Heart and Lung Institute, Imperial College London, London, UK

Wendy S. Barclay

Department of Infectious Disease, Imperial College London, London, UK https://orcid.org/0000-0002-3948-0895

Andrew P. Catchpole

hVIVO Services Ltd., London, UK

Christopher Chiu (c.chiu@imperial.ac.uk)

Imperial College London https://orcid.org/0000-0003-0914-920X

Article

Keywords:

Posted Date: February 1st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1121993/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

To establish a novel SARS-CoV-2 human challenge model, 36 volunteers aged 18-29 years without evidence of previous infection or vaccination were inoculated with 10 $TCID_{50}$ of a wild-type virus (SARS-CoV-2/human/GBR/484861/2020) intranasally. Two participants were excluded from per protocol analysis due to seroconversion between screening and inoculation. Eighteen (~53%) became infected, with viral load (VL) rising steeply and peaking at ~5 days post-inoculation. Virus was first detected in the throat but rose to significantly higher levels in the nose, peaking at ~8.87 log_{10} copies/ml (median, 95% CI [8.41,9.53). Viable virus was recoverable from the nose up to ~10 days post-inoculation, on average. There were no serious adverse events. Mild-to-moderate symptoms were reported by 16 (89%) infected individuals, beginning 2-4 days post-inoculation. Anosmia/dysosmia developed more gradually in 12 (67%) participants. No quantitative correlation was noted between VL and symptoms, with high VLs even in asymptomatic infection, followed by the development of serum spike-specific and neutralising antibodies. However, lateral flow results were strongly associated with viable virus and modelling showed that twice-weekly rapid tests could diagnose infection before 70-80% of viable virus had been generated. Thus, in this first SARS-CoV-2 human challenge study, no serious safety signals were detected and the detailed characteristics of early infection and their public health implications were shown. ClinicalTrials.gov identifier: NCT04865237.

Introduction

Coronavirus disease 2019 (COVID-19) is a complex clinical syndrome caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Despite extensive research into severe disease of hospitalised patients[1], and many large studies leading to approval of vaccines and antivirals[2–4], the global spread of SARS-CoV-2 continues and is indeed accelerating in many regions. Infections are typically mild or asymptomatic in younger people but these likely drive community transmission[5] and the detailed time-course of infection and infectivity in this context has not been fully elucidated[6,7]. Deliberate human infection of low-risk volunteers enables the exact longitudinal measurement of viral kinetics, immunological responses, transmission dynamics and duration of infectious shedding after a fixed dose of well-characterised virus. Under these tightly controlled conditions, host factors leading to differences in clinical outcome can be tested and robustly inferred. While human infection challenge has been attempted during previous pandemics[8], none have been successfully established and no recent reports of coronavirus (including SARS-CoV-2) human challenge exist.

Experimental challenge with human pathogens requires careful ethical scrutiny and regulation but can deliver unparalleled information that can inform clinical policy and refinement of infection control measures, enabling the rapid evaluation of vaccines, therapeutics, and diagnostics. Invaluable information can be obtained by such studies in small numbers of participants under highly regulated and controlled settings, leading to wider societal benefits that offset the personal risks undertaken by the volunteers[9]. Recognising the potential benefits of SARS-CoV-2 human challenge, the World Health

Organization convened working groups early during the COVID-19 pandemic to consider the necessary ethical and practical frameworks[10]. The pros and cons of human infection challenge studies have been extensively reviewed elsewhere[11], but the key considerations underlying these studies during an active pandemic were to balance scientific and public health benefits with ensuring any risks to study participants (known and as yet uncertain) were minimised and managed.

The unique strengths of SARS-CoV-2 human challenge are its ability to standardise the viral inoculum, study conditions and exact timing of exposure, thus controlling for factors that unavoidably confound natural infection studies. This contrasts with even the most well-controlled field trials, including household contact studies. There, the viral quasi-species, inoculum dose, timing and conditions of exposure are unknown, and contacts are only identified following diagnosis of the index case, at which time secondary exposure has almost always already occurred[12], thus missing the early phase of infection. SARS-CoV-2 human challenge studies therefore fill a gap in the understanding of early factors involved in susceptibility to infection that cannot be addressed in other ways. With continuing infection and re-infection with SARS-CoV-2, controlled translational studies such as human infection challenge that inform public health strategy as well as accelerate access to more, and improved, interventions through more robust investigation of pathogenesis, correlates of protection and early proof-of-concept efficacy testing, remain a priority that justifies the ethics of this approach.

Here, we report results from the first volunteers inoculated with SARS-CoV-2 in a human challenge study, demonstrating the feasibility of deliberate infection with SARS-CoV-2, with no evidence of serious safety signals in these carefully selected healthy volunteers, and providing novel insights into the early dynamics of infection.

Results

SARS-CoV-2 human challenge causes rapid onset of upper respiratory tract infection with high peak viral loads.

Thirty-six healthy volunteers aged 18-29 years old were enrolled according to protocol-defined inclusion/exclusion criteria. Screening included assessments for known risk factors for severe COVID-19, including co-morbidities, low or high body mass index, abnormal safety blood tests, spirometry and chest radiography (Figure 1a & Protocol). The protocol had been given a favourable opinion by the UK Health Research Authority – Ad Hoc Specialist Ethics Committee (reference: 20/UK/2001 and 20/UK/0002). Written informed consent was obtained from all volunteers prior to screening and study enrolment. The study was overseen by a Trial Steering Committee (TSC) with advice from an independent Data and Safety Monitoring Committee (DSMB). The study was discussed with the Medicines and Healthcare products Regulatory Agency; since no medicinal product was being investigated, the study was deemed not a clinical trial according to UK regulations. As such, a EudraCT number was not assigned and the clinical study was registered with clinicaltrials.gov (identifier: NCT04865237). All participants were seronegative at screening by Quotient MosaiQ antibody microarray test and had no history of SARS-CoV-

2 vaccination or infection. However, two participants seroconverted between screening and inoculation, resulting in 34 individuals in the per protocol analysis.

As this human challenge model was developed during the ongoing pandemic, with no directly comparable safety data and incomplete understanding of long-term effects following COVID-19, an adaptive protocol was designed with stepwise progression to ensure maximal risk mitigation during the early stages and progression only as data on the clinical features of human SARS-CoV-2 challenge was acquired. Following extensive screening, participants were admitted to individual negative pressure rooms in an in-patient quarantine unit, with 24-hour medical monitoring and access to higher level clinical support. At admission and before inoculation, volunteers were screened for coincidental respiratory infection using the Biofire FilmArray. Initial cohorts comprised 3 sentinel individuals followed by 7 additional participants. As per protocol, these first 10 challenged participants were assigned to receive pre-emptive remdesivir once two consecutive twelve-hourly nose or throat swabs showed quantifiable SARS-CoV-2 detection by PCR, with the aim of mitigating any unexpected risk of progression to more severe disease. Following review by the DSMB and TSC, pre-emptive remdesivir was deemed unnecessary and target recruitment of a further 30 individuals under the same conditions but without remdesivir was advised. A further sentinel cohort of 3 individuals was then challenged, with no pre-emptive remdesivir given. This was followed by 3 more groups of 7, 7 and 9 individuals, following exclusion of 4 volunteers shortly before virus inoculation due to detection of other respiratory viruses. Once pre-emptive remdesivir was no longer used, clinical severity criteria (i.e. persistent fever, persistent tachycardia, persistent severe cough, greater than mild CT imaging changes or SaO2 $\leq$94%) were defined for triggering of rescue treatment with monoclonal antibodies (Regeneron), but no such treatment was ultimately required. Participants were quarantined for at least 14 days post-inoculation and until they met virological discharge criteria (see Online Methods), with planned follow-up for 1 year to assess for prolonged symptoms, including smell disturbance and neurological dysfunction.

All participants were inoculated with 10 $TCID_{50}$ of SARS-CoV-2/human/GBR/484861/2020 (a D614G-containing pre-alpha wild-type virus; Genbank Accession number OM294022) by intranasal drops (Figure 1b). Eighteen participants (53% according to the per protocol analysis, [95% CI [35,70]) subsequently developed PCR-confirmed infection. This infection rate met the protocol-specified target of 50-70% and there was therefore no further dose escalation. Demographics between infected participants and those who remained uninfected were similar (Table 1).

Table 1
Participant baseline physical and demographic characteristics, selected clinical features and adverse events.

Group	Total	Infected (sero-negative)	Uninfected (sero-negative)	Uninfected (sero-positive)
	(n=36)	(n=18)	(n=16)	(n=2)
Characteristic				
Age (years)				
Mean (SD)	21.8 (2.9)	22.2 (2.9)	20.8 (2.2)	26.5 (3.5)
Min, Max	18, 29	18, 27	18, 25	25, 29
Gender, n (%)				
Male	26 (72)	12 (67)	14 (88)	0
Female	10 (28)	6 (33)	2 (12)	2 (100)
Race, n (%)				
White or Caucasian	33 (92)	17 (94)	14 (88)	2 (100)
Mixed Ethnicity	3 (8)	1 (6)	2 (12)	0
BMI (kg/m^2)				
Mean (Range, SD)	23.2 (19.6-29.7, 2.6)	22.8 (19.9-26.4, 2.2)	23.4 (19.6-29.7, 3.0)	25.2 (23.3-27.1, 2.7)
Symptoms				
Report of any symptoms on 2 consecutive days, n (%)	22 (61)	17 (94)	5 (31)	0

*One participant was naturally infected with SARS-CoV-2 between discharge from quarantine and day 28 post-inoculation (p.i.), so is excluded. Their neutralising antibody titre at day 28 was 472 and their spike-specific IgG was 536.6.

**One subject reported a runny nose that was hindering smell and quickly resolved, 1 subject. reported partial smell loss having had ‘natural’ COVID 2 weeks before. Neither subject had a significant change in UPSIT compared to baseline

Subject reported smell disturbance only after performing the UPSIT, score was not significantly different to baseline

Group	Total	Infected (sero-negative)	Uninfected (sero-negative)	Uninfected (sero-positive)
	(n=36)	(n=18)	(n=16)	(n=2)
Fever				
>37.8^{0}C, n (%)		7 (39)	0	0
C-Reactive Protein				
CRP > 5mg/L, n (%)		5 (28)	0	0
Antibody Titres at 28 days p.i.				
Neutralising antibody titre (median)		863.5 (IQR 403)	Undetectable*	167.5
Spike-specific IgG titre (ELU/mL, median)		1549 (IQR 1865)	Undetectable*	178
Adverse events				
Any serious adverse event		0	0	0
Clinically significant adverse events thought to be associated with viral infection that occurred or worsened during the observation period				
Smell disturbance				
During Quarantine		12	0	0
Day 28		11	2**	1#
Day 90		4	0	0
Day 180		5	0	0

*One participant was naturally infected with SARS-CoV-2 between discharge from quarantine and day 28 post-inoculation (p.i.), so is excluded. Their neutralising antibody titre at day 28 was 472 and their spike-specific IgG was 536.6.

**One subject reported a runny nose that was hindering smell and quickly resolved, 1 subject. reported partial smell loss having had 'natural' COVID 2 weeks before. Neither subject had a significant change in UPSIT compared to baseline

Subject reported smell disturbance only after performing the UPSIT, score was not significantly different to baseline

Group	Total	Infected (sero-negative)	Uninfected (sero-negative)	Uninfected (sero-positive)
	(n=36)	(n=18)	(n=16)	(n=2)
Low white cell count (<2.0 x10^9/L)		1	0	0
Low lymphocytes (<0.75 x10^9/L)		9	0	0
Low neutrophils (<1.0 x10^9/L)		3	0	0
Low neutrophils (<0.5 x10^9/L)		1	0	0
Epididymal discomfort		1	0	0

*One participant was naturally infected with SARS-CoV-2 between discharge from quarantine and day 28 post-inoculation (p.i.), so is excluded. Their neutralising antibody titre at day 28 was 472 and their spike-specific IgG was 536.6.

**One subject reported a runny nose that was hindering smell and quickly resolved, 1 subject. reported partial smell loss having had 'natural' COVID 2 weeks before. Neither subject had a significant change in UPSIT compared to baseline

Subject reported smell disturbance only after performing the UPSIT, score was not significantly different to baseline

In the 18 infected individuals, viral shedding by qPCR became quantifiable in throat swabs from 40 hours (median, 95% CI [40,52]) (~1.67 days) post-inoculation, significantly earlier than in the nose (p=0.0225, where initial viral quantifiable detection occurred at 58 hours (95% CI [40,76]) (~2.4 days) post-inoculation (Figure 2a & 2b). This was initially closely paralleled by viable virus measured by focus forming assay (FFA), which was also quantifiably detected earlier in the throat than in the nose (p=0.0058, Figure 2b). Viral loads (VL) increased rapidly thereafter, with qPCR peaking in the throat at 112 hours (95% CI [76,160]) (~4.7 days) post-inoculation and later at 148 hours (95% CI [112,184]) (~6.2 days) post-inoculation in the nose (Figure 2a & 2c). However, at its peak, VL was significantly higher in nasal samples at 8.87 (95% CI [8.41,9.53]) log10 copies/mL and 3.9 (95% CI [3.34,4.42]) log10 FFU/mL than in the throat at 7.65 (95% CI [7.39,8.24]) log10 copies/mL and 2.92 (95% CI [2.68,3.56]) log10 FFU/mL (p<0.0001 for qRT-PCR and p=0.0024 for FFA, Figure 2d).

In both nose and throat, viral detection continued at high levels for several days and high cumulative VLs by area under the curve (AUC) were therefore seen, particularly in the nose (median 9.03, 95% CI [8.65,9.43] copies/mL by qPCR)(Figure 2e). In all infected participants, quantifiable virus by qPCR was still present at day 14 post-inoculation which necessitated prolonged quarantine of up to 5 extra days until qPCR Ct values had fallen to <33.5 in two consecutive nasal and throat swabs (as per protocol-defined discharge criteria). At these later timepoints, VLs by qRT-PCR were more erratic, with low level qPCR positivity remaining in 15/18 (83%) at discharge. At day 28 post-inoculation 6/18 (33%) remained

qPCR positive in the nose and 2/18 (11%) in the throat but by day 90 all participants were qPCR negative. Of the participants not meeting infection criteria and deemed uninfected, low level non-consecutive viral detections were observed only by qPCR in the nose of 3 participants and throat of 6 participants (Extended Figure 1a & 1b).

In contrast, viable virus was detectable by FFA for a more limited duration: 156 hours (median, 95% CI [120,192]) (6.5 days) in the nose and in the throat for 150 hours (95% CI [132,180]) (6.25 days; Figure 2f). The average time post-inoculation to clearance of viable virus was 244 hours (95% CI [208, 256]) or 10.2 days from the nose and 208 hours (95% CI [172,244]) or 8.7 days from the throat (Figure 2g). VLs by qPCR and FFA were significantly correlated in both nose and throat (Extended Figure 3a & 3b). Although there was a striking degree of concordance between the shape and magnitude of individuals' VL curves (Figure 2a) and between VLs in the nose and throat (Figure 2i), greater inter-individual variability was observed in timing of VL between nose and throat (Extended Figure 4). Despite relatively high levels of late qPCR detection, the latest that viable virus could be detected was day 12 post-inoculation in the nose and day 11 in the throat (Figure 2g). In contrast, swabs by qPCR that became undetectable in quarantine during the resolution phase first occurred at 352 hours (95% CI [340,364]) (~14.6 days) in the nose and 340 hours (95% CI [304,352]) (~14.7 days) in the throat although some later continued to fluctuate around the limits of quantification and detection (Figure 2h).

Of the first 10 participants prospectively assigned to receive pre-emptive remdesivir on PCR-confirmed infection, 6 became infected. No apparent differences were seen in VL by qPCR (Extended Figure 2a) or FFA (Extended Figure 2b) between remdesivir-treated and untreated infected individuals and cumulative virus (AUC) was similar (Extended Figure 2c). While there was an apparent trend towards lower mean nasal VL during the treatment period and delayed VL peak in the 6 remdesivir-treated individuals (Extended Figure 2d), this was not observed in the throat, primarily driven by one individual and was not statistically significant. With no significant differences between remdesivir-treated and untreated participants, infected individuals were therefore analysed together.

Thus, following SARS-CoV-2 human challenge, viral shedding begins within 2 days of exposure, rapidly reaching high levels with viable virus detectable up to 12 days post-inoculation, and significantly higher VL in the nose than the throat despite its later onset.

Serum neutralising antibodies are mounted rapidly following SARS-CoV-2 challenge infection

The rapid onset of infection was reflected in serum antibody responses. No increase in serum antibodies by microneutralisation or anti-spike protein IgG ELISA was observed in those deemed uninfected, even where isolated viral detections had occurred, except for one participant who acquired natural COVID-19 after discharge from quarantine (Figure 3a & 3b). In contrast, serum antibodies were generated in all infected participants with neutralising antibody titres of 425 (median, IQR 269) at 14 days post-inoculation and a further rise to 863.5 (IQR 403) at 28 days (Figure 3a). A slower rise was seen in spike

protein-binding IgG measured by ELISA, with a median increase to 192.5 (IQR 393.1) ELU/mL at day 14 followed by an increment by day 28 to 1549 (IQR 1865) ELU/mL (Figure 3b). Of note, in the two participants who seroconverted between screening and inoculation, both neutralising and S protein binding antibodies were detectable at admission to the quarantine unit on day -2 pre-inoculation. Both individuals were excluded from the per protocol infection rate analysis but remained uninfected, with no change in their serum antibody levels post-inoculation.

SARS-CoV-2 human challenge infection causes mild disease with no evidence of serious safety signals

Following infection, symptoms by self-reported diary (Supplementary Table 1) became apparent from 2-4 days post-inoculation (Figure 4a) when symptoms started diverging from challenged but uninfected individuals, who reported both fewer and milder symptoms with no consistent pattern (Figure 4a and Extended Figure 1c). Symptom scores exhibited greater variability than VLs, with inconsistent onset and peak cumulative daily scores ranging from 0 to 29. Symptoms were most frequent in the upper respiratory tract and included nasal stuffiness, rhinitis, sneezing and sore throat (Figure 4b, 4c and Extended Figure 5). Systemic symptoms of headache, muscle/joint aches, malaise and feverishness were also recorded. There was no difference in symptoms between remdesivir-treated and untreated individuals (Extended Figure 6). All symptoms were mild-to-moderate, with peak symptoms (at 112 hours post-inoculation (95% CI [88,208]) aligning closely with peak VL in the nose, which was significantly later than peak VL in the throat by FFA (88 hours, 95% CI [76,112], p=0.0114) (Figure 4d, Extended Figure 4). However, despite the temporal association between nasal VL and symptoms, there was no correlation between the amount of viral shedding by qPCR or FFA and symptom score AUC (Figure 4e & 4f).

Seven participants (39% of infected) had temperatures of >37.8°C. Otherwise there were no notable disturbances in any clinical assessments, including daily spirometry and thoracic CT scans. No serious adverse events were reported and no criteria for commencing rescue therapy were met. A total of 18 adverse events deemed probably or possibly related to virus infection were largely due to transient and non-clinically significant leukopenia and neutropenia, and mild muco-cutaneous abnormalities during the quarantine period (Table 1 and Supplementary Table 2).

SARS-CoV-2 human challenge infection commonly causes smell disturbance

To assess the degree and kinetics of smell disturbance, University of Pennsylvania Smell Identification Tests (UPSITs) were conducted. No smell disturbance was observed during quarantine in uninfected individuals (Extended Figure 1d). However, 12 infected participants (67%) reported some degree of smell disturbance. While other symptoms peaked with nasal VLs, the nadir of UPSIT scores was 6-7 days later (Figure 4a, Extended Figure 4). Complete smell loss (anosmia) occurred in 9 individuals (50%), but most experienced rapid improvement before day 28. Although at day 28 some smell disturbance was still reported by 11 participants (61%), by day 180 this number had fallen to 5. Of these, only one individual still had measurable smell impairment at 180 days post-inoculation, although this was improving both

subjectively and objectively (UPSIT at baseline=31, day 11=9, day 28=11, day 90=17, day 180=23). Two of the remaining reported mild parosmia and two had mild reduction in smell subjectively (although UPSIT scores had normalised). Six individuals received smell training advice, including 2 who also received treatment with short courses of oral and intranasal steroids.

Anosmia is therefore a common feature of human SARS-CoV-2 challenge that generally onsets several days later than viral shedding and resolves quickly in most individuals. Together, these findings indicate that human SARS-CoV-2 challenge at this inoculum dose has low risk of causing severe symptoms in healthy young adults but leads to large amounts of nasopharyngeal virus even in the absence of respiratory or systemic disease.

Antigen testing by lateral flow assay is strongly associated with virus detection by quantitative culture

Lateral flow assay (LFA) rapid antigen tests are commonly used to identify potentially infectious people in the community but their usefulness in early infection is unknown. To test the performance of LFA over the entire course of infection, antigen testing was performed using the same morning nose and throat swab samples assessed for VL. None of the uninfected participants had a positive LFA test at any time, whereas all infected individuals had positive LFA for ≥2 days (Extended Figure 7). Despite earlier viral detection in the throat by other methods, median time to first detection by daily LFA tests was the same in nose and throat at 4 days (range 2-8) post-inoculation (Figure 5a). This was on average 24-48 hours after first qPCR positivity (Figure 5b) and within 24 hours of FFA (Figure 5c). Of note, in 9 of 18 infected individuals, viable virus became detectable by FFA one or more day before the first positive LFA. Towards the end of infection, the last LFA detection mainly occurred 24-72 hours after viable virus detection had ceased.

To assess the relationship between VL and probability of a positive LFA, logistic regression models were fitted using generalised estimating equations to control for repeated within-participant assessments. Log_{10} VL was a significant predictor ($P<2x10^{-5}$) of LFA positivity with an odds ratio of 5.01 (95% CI [2.93,8.57]) when predicting LFA from FFA in nose (Figure 5e). Area under the receiver operating characteristic curves (AUROC) were high at 0.96 for nasal qPCR, and 0.89 for throat qPCR (Extended Figure 8a) but lower for FFA, particularly in the throat (AUC 0.69). To test longitudinal performance as infection progressed, the sensitivity and specificity of LFA when compared with qPCR and FFA were calculated for each day post-exposure (Figure 5f). With both tests and anatomical sites, sensitivity of LFA was limited at the beginning and end of acute illness. However, from ~4 days post-inoculation, LFA demonstrated high sensitivity as a surrogate for qPCR or FFA-positivity. Overall, LFA was highly specific although some “false positives” were observed in relation to FFA (but not qPCR).

Where asymptomatic/pre-symptomatic LFA testing programmes exist, testing is usually recommended twice weekly. To model the differential impact of LFA testing frequency that incorporate viral dynamics throughout infection, the mean proportion of VL AUC that had yet to occur (and might be responsible for

transmission if undiagnosed) by the time of a first positive LFA test with testing cadences of 1-7 days was modelled. For both FFA (Figure 5g) and qPCR (Extended Figure 8b), infection would be recognised at or before >90% of the VL AUC had occurred if testing was daily. As the period between tests increased, the proportion of VL AUC declined with twice-weekly testing capturing 70-80% of virus and weekly testing still exceeding 50% if nose and throat swabs were combined. Thus, LFA positivity is strongly associated with culturable virus and therefore contagiousness and can be highly effective as a trigger for interventions to interrupt transmission.

Discussion

We here report the virological and clinical results from the first SARS-CoV-2 human challenge study. With a low inoculum dose of 10 $TCID_{50}$, robust viral replication was observed in 53% of seronegative participants. After an incubation period of <2 days, VLs escalated rapidly, peaking at high levels and continuing for over a week. Symptoms were present in 89% of infected individuals but, despite high VLs, were consistently mild-to-moderate, transient and predominantly confined to the upper respiratory tract. Anosmia/dysosmia was common, occurred later than other symptoms and resolved without treatment in most participants within 90 days. In those with residual smell disturbance, their sense of smell steadily improved during the follow-up period, consistent with the good long-term prognosis seen in community cases[13]. There was no evidence of pulmonary disease in infected participants based on clinical and radiological assessments.

The natural infectious dose of SARS-CoV-2 is unknown but based on *in vitro* and preclinical models, the virus is understood to be highly infectious[14–16] and well-adapted to rapid and high-titre replication in human respiratory mucosa[17]. Early in the pandemic, a WHO Advisory Group published expert consensus guidelines recommending a starting dose of 10^2 $TCID_{50}$[10]. Here, based on *in vitro* data of high viral replication in primary human airway epithelial cells, we started with a tenfold lower dose of 10 $TCID_{50}$ (equivalent to 55 FFU) and found it sufficient to meet the 50-70% target infection rate. With prospective household contact studies having similarly shown high secondary attack rates of ~38%[12], this suggests that the model can recapitulate higher exposure than naturally-acquired infection events. In contrast, experimental infections of non-human primates have used 1,000-10,000 times more virus, with intratracheal or combined upper/lower airway administration, which results in markedly different kinetics to those observed during human infection[18,19]. In human challenge studies with other respiratory viruses such as influenza viruses and RSV, inoculum doses are typically also much higher at 10^4-10^6 $TCID_{50}$ since all volunteers have been exposed multiple times throughout life to those viruses, with pre-existing immunity reducing susceptibility and resulting in substantially lower peak viral loads at 10^3-10^4 copies/mL by PCR[20,21]. Thus, neither animal models nor human data from other viral infections were helpful in estimating the optimal SARS-CoV-2 inoculum dose.

Although some studies have measured the response to SARS-CoV-2 infection longitudinally in humans[22–24], none can capture host features at the time of virus exposure, the early events prior to

symptom onset, or the detailed course of infection that can be shown by experimental challenge. Whilst the incubation period from the estimated time of natural exposure to perceived symptom onset has previously been estimated as ~5 days[25,26], this best aligns with peak symptoms and is longer than the true incubation period. With close questioning, symptoms were found to be associated with viral shedding within 2 to 4 days of inoculation but did not peak until day 4-5. Thus, virus was first detected (first in the throat, then the nose) ~2 days before peak symptoms and increased steeply to achieve a sustained peak, in many cases before peak symptoms were reached, consistent with modelling data indicating that up to 44% of transmissions occur before symptoms are noted[6]. Anosmia was a later symptom, potentially explained by the proposed mechanism whereby only ACE2- and TMPRSS2-expressing supporting cells rather than neurones themselves are directly infected, leading to delayed secondary olfactory dysfunction[27].

Pre-emptive remdesivir was administered to the first 6 infected participants as risk mitigation during early model development as trial data had suggested efficacy in shortening time to recovery in hospitalised patients[28]. However, no statistically significant effect on viral load or symptoms was detectable in this small cohort. Field data have questioned the effectiveness of remdesivir in the hospitalised patient setting[29] but antiviral treatment is commonly more effective early in the course of infection. This study was not designed nor powered to assess the efficacy of early treatment with remdesivir so this remains to be tested, but such prospective human challenge studies would be well placed to answer the question of antiviral efficacy, with treatment commenced at different times relative to virus exposure.

A key unresolved question for public health has been whether transmission is less likely to occur during asymptomatic/mild infection compared to more severe disease. Some studies have shown a correlation between disease severity and extent of viral shedding[30,31], but others have not[32]. Overall, peak VLs reported in natural infection (~10^5-10^8 copies/mL) are lower than those observed in this study[6,33–36] However, these are invariably sampled at the time of case ascertainment and, where longitudinal samples have been taken, these indicate that patients are already in the downward phase of the VL curve[24]. It is therefore likely that most samples miss the peak of viral shedding. With virus present at significantly higher titres in the nose than the throat, these data provide clear evidence that emphasises the critical importance of wearing face coverings over the nose as well as mouth. Furthermore, our data clearly show that SARS-CoV-2 viral shedding occurs at high levels irrespective of symptom severity, thus explaining the high transmissibility of this infection and emphasising that symptom severity cannot be considered a surrogate for transmission risk in this disease. This remains relevant with the widespread transmission of the Delta and Omicron variants, where antigenic divergence along with waning vaccine-induced immunity lead to VL during breakthrough infection at comparably high levels to those in seronegative individuals[12,37].

Despite the relatively small sample size, limited variation between infected study participants and longitudinal analysis permits several conclusions of public health importance. Detailed viral kinetics show that some individuals still shed culturable virus at 12 days post-inoculation (i.e. up to 10 days after

symptom onset) and, on average, viable virus was still detectable 10 days post-inoculation (up to 8 days after symptom onset). These data therefore support the isolation periods of 10 days post-symptom onset advocated in many guidelines to minimise onward transmission[38]. High levels of asymptomatic/pauci-symptomatic viral load also highlight the potential positive impact of routine asymptomatic testing programmes that attempt to diagnose infection in the community so that infection control measures such as self-isolation can be implemented to interrupt transmission. In several jurisdictions, these rely on rapid antigen tests, with recent re-analysis of cross-sectional LFA validation data having suggested that sensitivity for infectious virus may be higher than previously estimated at ~80%[39]. Reassuringly, longitudinal LFA data following SARS-CoV-2 challenge also strongly predicted culturable virus aside from the very earliest time-points where sensitivity was lower. In addition, LFA was highly reliable in predicting the disappearance of viable virus and therefore also underpin “test to release” strategies, which are increasingly being used to shorten the period of self-isolation. While positive LFA results were occasionally seen with negative FFA results (causing a reduction in specificity in relation to the viable virus assay), there were no false positives when comparing LFA to qPCR, implying the relatively lower sensitivity of viral culture rather than false positivity of LFA. Although some uncertainty remains in directly extrapolating these data to the community where self-swabbing and more concentrated samples may alter sensitivity, these results support their continued use for identifying those most likely to be infectious. Our modelling also suggests that this strategy remains effective even if imperfectly implemented, with routine testing as little as every 7 days able to interrupt more than half the virus still to be shed by an individual, if acted upon.

Although these first-in-human data do not preclude rare adverse events that can only be detected in larger-scale studies, our results indicate that human challenge with SARS-CoV-2 is consistent with natural infection in healthy young adults, having caused no serious unexpected consequences and therefore supporting further development and expansion. This first report focuses on safety, tolerability and virological responses, but the uniquely controlled nature of the model will also enable robust identification of host factors present at the time of inoculation and associated with protection in those individuals who resisted infection. Analysis of local and systemic immune markers (including potentially cross-reactive antibodies, T cells and soluble mediators) from this SARS-CoV-2 human challenge study that may explain these differences in susceptibility are therefore ongoing. In addition, with the feasibility of this approach having been demonstrated using a prototypic wild-type strain, further challenge studies are now underway in which previously infected and vaccinated volunteers will be challenged with escalating inoculum doses and/or viral variants to investigate the interplay between virus and host factors that influence clinical outcome. Together, these studies will thus optimise the platform for rapid evaluation of vaccines, antivirals and diagnostics by generating efficacy data early during clinical development and avoiding the uncertainties of studies that require ongoing community transmission.

Declarations

Acknowledgments

We thank the study participants for their time and commitment. The authors gratefully acknowledge support from the UK Vaccine Taskforce of the Department of Business, Energy and Industrial Strategy of Her Majesty's Government (BEIS). We thank the following individuals and organizations for their invaluable contributions to development and implementation of the SARS-CoV-2 human challenge project: Arun Anandakumar, Kenneth Baillie, Sheila Casserly, Sue Cleiff, Celia Chu and the NIHR Clinical Research Network staff at The Royal Bolton Hospital, Kevin Fenton, Barry Flutter and the team at the Zayed Centre for Research at Great Ormond Street Hospital (ZRC at GOSH), Gabrielle Gainsborough, Fergus Gleason, Laura Grey, Rob Heyderman, Julia Hippisley-Cox, Martin Johnson, Kamlesh Khunti, Maria Mayer, Maya Moshe, Madeline Ocampo, Claire Skinner, Claire Steves, Pradeep Subudhi, Dave Thomas, Greg Towers, Helen Ward, Olivia Watson, Jie Zhou, the Royal Free London NHS Foundation Trust, Human Infection Challenge Network for Vaccine Development (HIC-Vac) and ISARIC4C Investigators (https://isaric4c.net/about/authors/). We are grateful to Gilead for providing remdesivir for the study. We thank the members of the independent DSMB (Stephen Gordon [chair], Peter Smith [chair], Anna Durbin, Fred Hayden, and David Dockrell) and Medical Oversight Committee/TSC (RCR [chair], Daniela Ferreira, HMcS, PO, AC) for their invaluable advice. CChi and PO are supported by the NIHR Imperial Biomedical Research Centre (BRC) award to Imperial College Healthcare NHS Trust and Imperial College London. PO is supported by an NIHR Senior Investigator Award (NIHR201385) and UKRI MRC CIC Award (MR/V028448/1). HMcS is supported by the NIHR Oxford Biomedical Research Centre (NIHR-BRC-1215-20008). RCR is supported by an NIHR Senior Investigator Award (NF-SI-0617-10010) and by the NIHR Southampton Biomedical Research Centre (IS-BRC-1215-20004). ISARIC4C is funded by the National Institute for Health Research (NIHR; award CO-CIN-01), the Medical Research Council (MRC; grant MC_PC_19059), the NIHR Health Protection Research Unit in Emerging and Zoonotic Infections at University of Liverpool in partnership with Public Health England (PHE), in collaboration with Liverpool School of Tropical Medicine and the University of Oxford (NIHR award 200907), Liverpool Experimental Cancer Medicine Centre provided infrastructure support for this research (grant reference C18616/A25153) and NIHR Health Protection Research Unit in Respiratory Infections (NIHR award 200927). NMF acknowledges funding from the NIHR Health Protection Research Unit in Modelling and Health Economics, the MRC Centre for Global Infectious Disease Analysis and the Jameel Institute. JSN-V-T is seconded to the Department of Health and Social Care, England (DHSC). The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, DHSC or BEIS.

References

1. Drake TM, Riad AM, Fairfield CJ, et al. Characterisation of in-hospital complications associated with COVID-19 using the ISARIC WHO Clinical Characterisation Protocol UK: a prospective, multicentre cohort study. Lancet 398, 223–237 (2021).
2. Voysey M, Clemens SAC, Madhi SA, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet 397, 99–111 (2021).

3. Thomas SJ, Moreira ED, Kitchin N, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine through 6 Months. N Engl J Med 385, 1761–1773 (2021).
4. Fischer W, Eron JJ, Holman W, et al. Molnupiravir, an Oral Antiviral Treatment for COVID-19. medRxiv. Jun 17 2021;doi:10.1101/2021.06.17.21258639
5. He J, Guo Y, Mao R, Zhang J. Proportion of asymptomatic coronavirus disease 2019: A systematic review and meta-analysis. J Med Virol 93, 820–830 (2021).
6. He X, Lau EHY, Wu P, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Medicine 26, 672–675 (2020).
7. Syangtan G, Bista S, Dawadi P, et al. Asymptomatic SARS-CoV-2 Carriers: A Systematic Review and Meta-Analysis. Front Public Health 8, 587374 (2020)
8. Rosenau M. Experiments to determine mode of spread of influenza. JAMA 73, 311–313 (1919).
9. Rapeport G, Smith E, Gilbert A, Catchpole A, McShane H, Chiu C. SARS-CoV-2 Human Challenge Studies - Establishing the Model during an Evolving Pandemic. N Engl J Med 385, 961–964 (2021).
10. Levine MM, Abdullah S, Arabi YM, et al. Viewpoint of a WHO Advisory Group Tasked to Consider Establishing a Closely-monitored Challenge Model of Coronavirus Disease 2019 (COVID-19) in Healthy Volunteers. Clin Infect Dis 72, 2035–2041(2021).
11. Deming ME, Michael NL, Robb M, Cohen MS, Neuzil KM. Accelerating Development of SARS-CoV-2 Vaccines - The Role for Controlled Human Infection Models. N Engl J Med 383, e63 (2020).
12. Singanayagam A, Hakki S, Dunning J, et al. Community transmission and viral load kinetics of the SARS-CoV-2 delta (B.1.617.2) variant in vaccinated and unvaccinated individuals in the UK: a prospective, longitudinal, cohort study. Lancet Infect Dis S1473-3099(21)00648–4 (2021).
13. Renaud M, Thibault C, Le Normand F, et al. Clinical Outcomes for Patients With Anosmia 1 Year After COVID-19 Diagnosis. JAMA Netw Open 4(6), e2115352 (2021).
14. Imai M, Iwatsuki-Horimoto K, Hatta M, et al. Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. Proc Natl Acad Sci USA 117(28), 16587–16595 (2020).
15. Sia SF, Yan LM, Chin AWH, et al. Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. Nature 583, 834–838 (2020).
16. Abdelnabi R, Boudewijns R, Foo CS, et al. Comparing infectivity and virulence of emerging SARS-CoV-2 variants in Syrian hamsters. EBioMedicine 68, 103403 (2021).
17. Hou YJ, Okuda K, Edwards CE, et al. SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract. Cell 182, 429–446 (2020).
18. Corbett KS, Flynn B, Foulds KE, et al. Evaluation of the mRNA-1273 Vaccine against SARS-CoV-2 in Nonhuman Primates. N Engl J Med 383, 1544–1555 (2020).
19. Williamson BN, Feldmann F, Schwarz B, et al. Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2. Nature 585, 273–276 (2020).

20. Paterson S, Kar S, Ung SK, et al. Innate-like Gene Expression of Lung-Resident Memory CD8. Am J Respir Crit Care Med 204, 826–841 (2021).
21. Jozwik A, Habibi MS, Paras A, et al. RSV-specific airway resident memory CD8+ T cells and differential disease severity after experimental human infection. Nat Commun 6, 10224 (2015).
22. Ke R, Zitzmann C, Ho DD, Ribeiro RM, Perelson AS. In vivo kinetics of SARS-CoV-2 infection and its relationship with a person's infectiousness. medRxiv Jun 2021;doi:10.1101/2021.06.26.21259581.
23. Kim MC, Cui C, Shin KR, et al. Duration of Culturable SARS-CoV-2 in Hospitalized Patients with Covid-19. N Engl J Med 384, 671–673 (2021).
24. Zou L, Ruan F, Huang M, et al. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. N Engl J Med 382, 1177–1179 (2020).
25. Yu P, Zhu J, Zhang Z, Han Y. A Familial Cluster of Infection Associated With the 2019 Novel Coronavirus Indicating Possible Person-to-Person Transmission During the Incubation Period. J Infect Dis 221, 1757–1761(2020).
26. Lauer SA, Grantz KH, Bi Q, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. Ann Intern Med 172, 577–582 (2020).
27. Butowt R, von Bartheld CS. Anosmia in COVID-19: Underlying Mechanisms and Assessment of an Olfactory Route to Brain Infection. Neuroscientist 27, 582–603 (2020).
28. Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the Treatment of Covid-19 - Final Report. N Engl J Med 383, 1813–1826 (2020).
29. Barratt-Due A, Olsen IC, Nezvalova-Henriksen K, et al. Evaluation of the Effects of Remdesivir and Hydroxychloroquine on Viral Clearance in COVID-19: A Randomized Trial. Ann Intern Med 174, 1261–1269 (2021).
30. Eberhardt KA, Meyer-Schwickerath C, Heger E, et al. RNAemia Corresponds to Disease Severity and Antibody Response in Hospitalized COVID-19 Patients. Viruses 12, 1045 (2020).
31. Fajnzylber J, Regan J, Coxen K, et al. SARS-CoV-2 viral load is associated with increased disease severity and mortality. Nat Commun 11, 5493 (2020).
32. Marks M, Millat-Martinez P, Ouchi D, et al. Transmission of COVID-19 in 282 clusters in Catalonia, Spain: a cohort study. Lancet Infect Dis 21, 629–636 (2021).
33. Munker D, Osterman A, Stubbe H, et al. Dynamics of SARS-CoV-2 shedding in the respiratory tract depends on the severity of disease in COVID-19 patients. Eur Respir J 58, 2002724 (2021).
34. Walsh KA, Jordan K, Clyne B, et al. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. J Infect 81, 357–371 (2020).
35. S B, AG LH, S Y, et al. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Viral Load in the Upper Respiratory Tract of Children and Adults With Early Acute Coronavirus Disease 2019 (COVID-19). Clin Infect Dis 73, 148–150 (2021).

36. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 581, 465–469 (2020).
37. Levine-Tiefenbrun M, Yelin I, Alapi H, et al. Viral loads of Delta-variant SARS-CoV-2 breakthrough infections after vaccination and booster with BNT162b2. Nature Medicine 27, 2108–2110 (2021)
38. Quilty BJ, Clifford S, Hellewell J, et al. Quarantine and testing strategies in contact tracing for SARS-CoV-2: a modelling study. Lancet Public Health 6, e175-e183 (2021).
39. Petersen I, Crozier A, Buchan I, Mina MJ, Bartlett JW. Recalibrating SARS-CoV-2 Antigen Rapid Lateral Flow Test Relative Sensitivity from Validation Studies to Absolute Sensitivity for Indicating Individuals Shedding Transmissible Virus. Clin Epidemiol 13, 935–940 (2021).

Figures

Figure 1

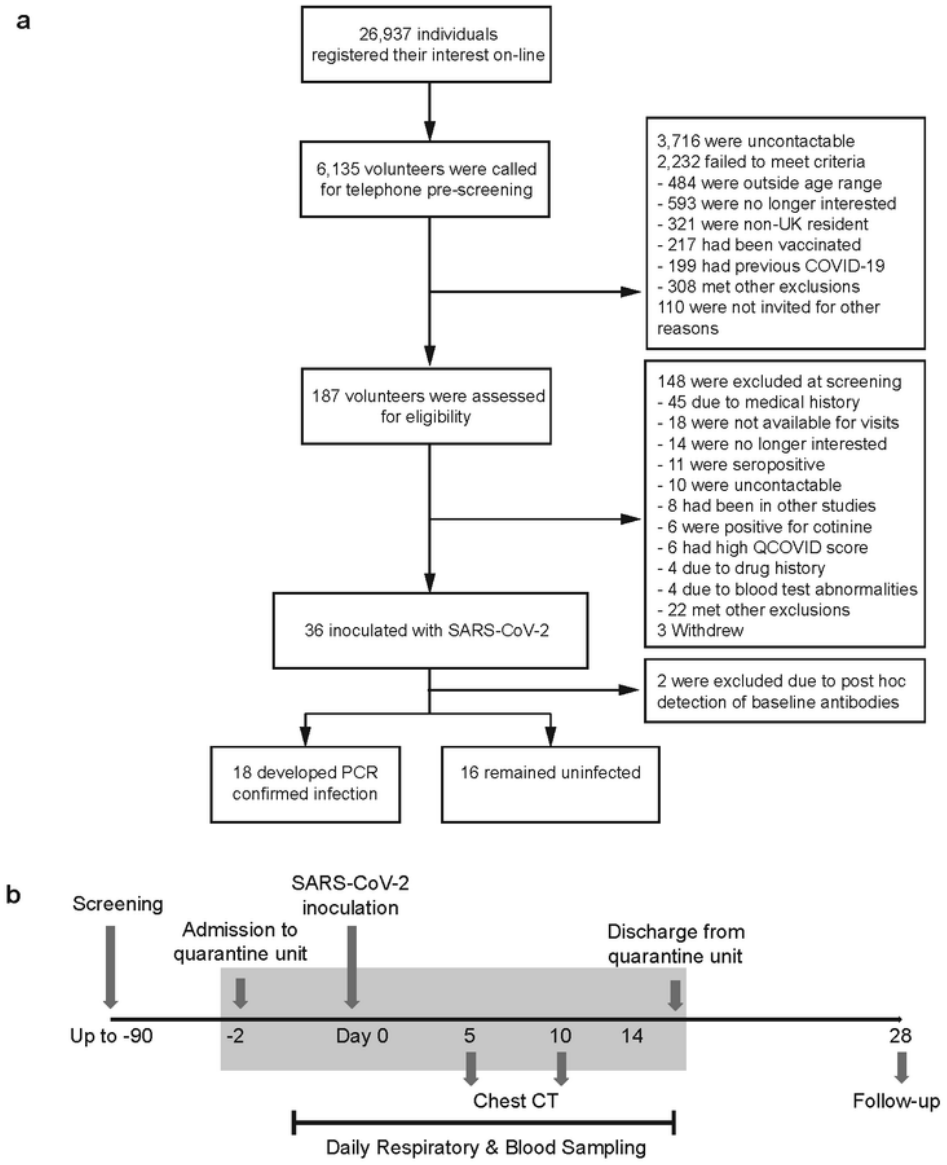

Figure 1: Screening, inoculation, assessments, and sampling. Healthy adult volunteers aged 18-29 years old were enrolled for SARS-CoV-2 challenge. (a) CONSORT diagram shows inclusions/exclusions and infection outcomes. (b) Diagram showing the clinical study design up to day 28 post-inoculation.

Figure 1

Screening, inoculation, assessments, and sampling. Healthy adult volunteers aged 18-29 years old were enrolled for SARS-CoV-2 challenge. (a) CONSORT diagram shows inclusions/exclusions and infection outcomes. (b) Diagram showing the clinical study design up to day 28 post-inoculation.

Figure 2

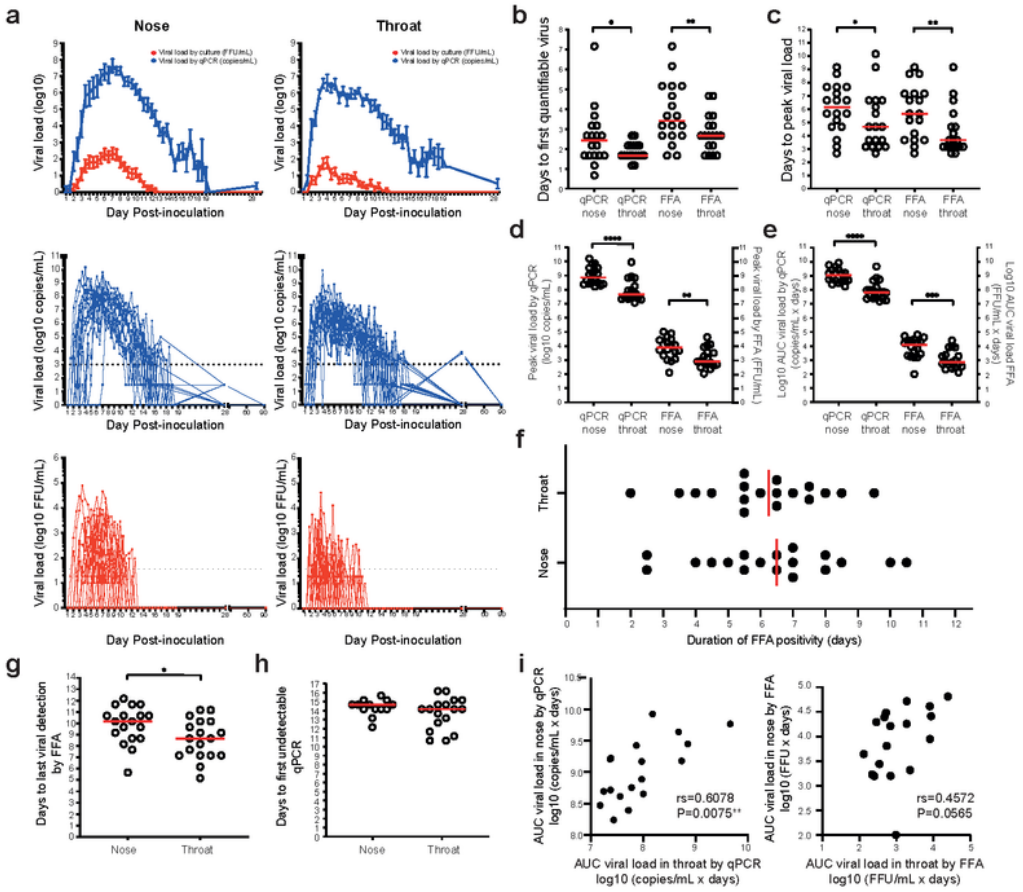

Figure 2: Viral shedding after a short incubation period peaks rapidly following human SARS-CoV-2 challenge. Healthy adult volunteers were challenged intranasally with SARS-CoV-2. In the 18 infected individuals, (a) VL in twice-daily nose and throat swab samples was measured by qPCR (blue) and focus-forming assay (FFA, red). Results are expressed as mean +/- S.E.M. Dotted lines represent the lower limit of quantification. (b) Median time to first quantifiable virus (c) peak VL are shown in red. (d) Peak and (e) cumulative (AUC) VL by qPCR and FFA in the nose and throat are compared. Wilcoxon matched-pairs signed rank tests are used to test significance. (f) Total duration of viral detection by FFA in nose and throat are shown. Medians are shown in red. (g) Median time to the last viral detection by FFA post-inoculation is shown in red. (h) Median time to the first undetectable VL by qPCR in the individuals who became undetectable while in quarantine is shown in red. (i) AUC VL by qPCR and FFA are correlated in nose vs. throat. Spearman's r and P values are shown. *P<0.05, **P<0.01, ***P<0.0001, ****P<0.0001.

Figure 2

Viral shedding after a short incubation period peaks rapidly following human SARS-CoV-2 challenge. Healthy adult volunteers were challenged intranasally with SARS-CoV-2. In the 18 infected individuals, (a) VL in twice-daily nose and throat swab samples was measured by qPCR (blue) and focus-forming assay (FFA, red). Results are expressed as mean +/- S.E.M. Dotted lines represent the lower limit of quantification. (b) Median time to first quantifiable virus (c) peak VL are shown in red. (d) Peak and (e)

cumulative (AUC) VL by qPCR and FFA in the nose and throat are compared. Wilcoxon matched-pairs signed rank tests are used to test significance. (f) Total duration of viral detection by FFA in nose and throat are shown. Medians are shown in red. (g) Median time to the last viral detection by FFA post-inoculation is shown in red. (h) Median time to the first undetectable VL by qPCR in the individuals who became undetectable while in quarantine is shown in red. (i) AUC VL by qPCR and FFA are correlated in nose vs. throat. Spearman's r and P values are shown. *P<0.05, **P<0.01, ***P<0.0001, ****P<0.0001.

Figure 3

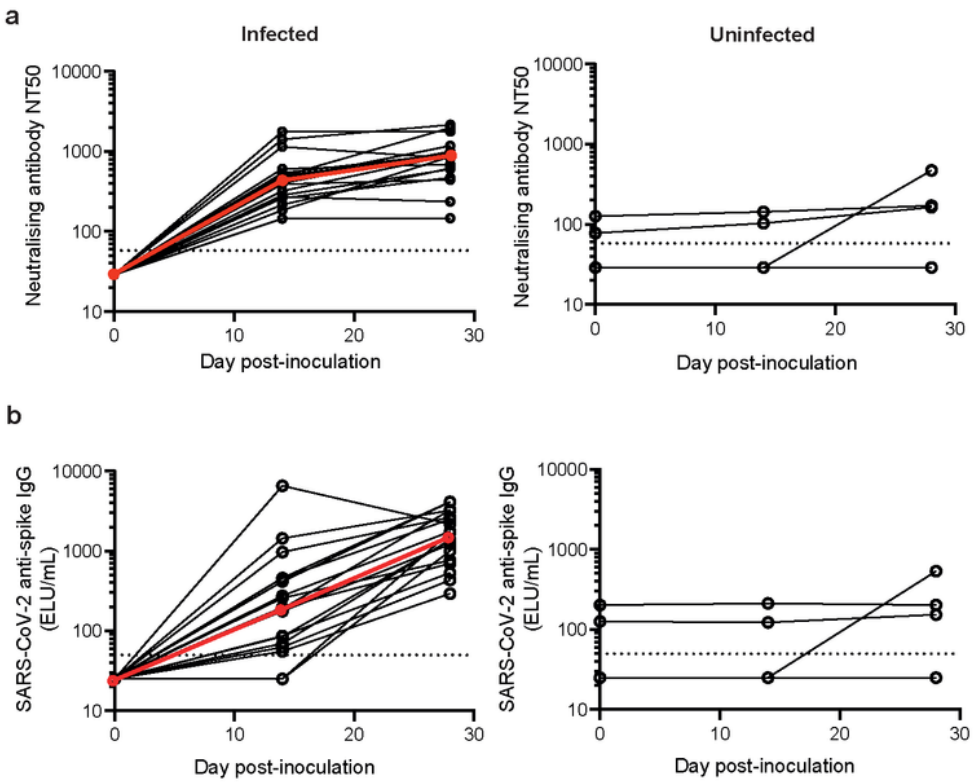

Figure 3: Neutralising antibodies are generated more rapidly than anti-spike protein IgG following human SARS-CoV-2 challenge infection. Serum was collected pre-inoculation and at days 14 and 28 after inoculation with SARS-CoV-2. (a) Serum neutralising antibodies were measured by microneutralisation assay in participants who became infected and those who remained uninfected. NT50 = 50% neutralising antibody titre. (b) Serum SARS-CoV-2 anti-spike IgG was measured by ELISA and expressed as ELISA laboratory units per mL (ELU/mL). Individual data points and median (red) are shown. The lower limit of detection (LLOD) of each assay is shown by the dotted line. Undetectable samples were assigned a value of half the LLOD.

Figure 3

Neutralising antibodies are generated more rapidly than anti-spike protein IgG following human SARS-CoV-2 challenge infection. Serum was collected pre-inoculation and at days 14 and 28 after inoculation with SARS-CoV-2. (a) Serum neutralising antibodies were measured by microneutralisation assay in participants who became infected and those who remained uninfected. NT50 = 50% neutralising antibody titre. (b) Serum SARS-CoV-2 anti-spike IgG was measured by ELISA and expressed as ELISA

laboratory units per mL (ELU/mL). Individual data points and median (red) are shown. The lower limit of detection (LLOD) of each assay is shown by the dotted line. Undetectable samples were assigned a value of half the LLOD.

Figure 4

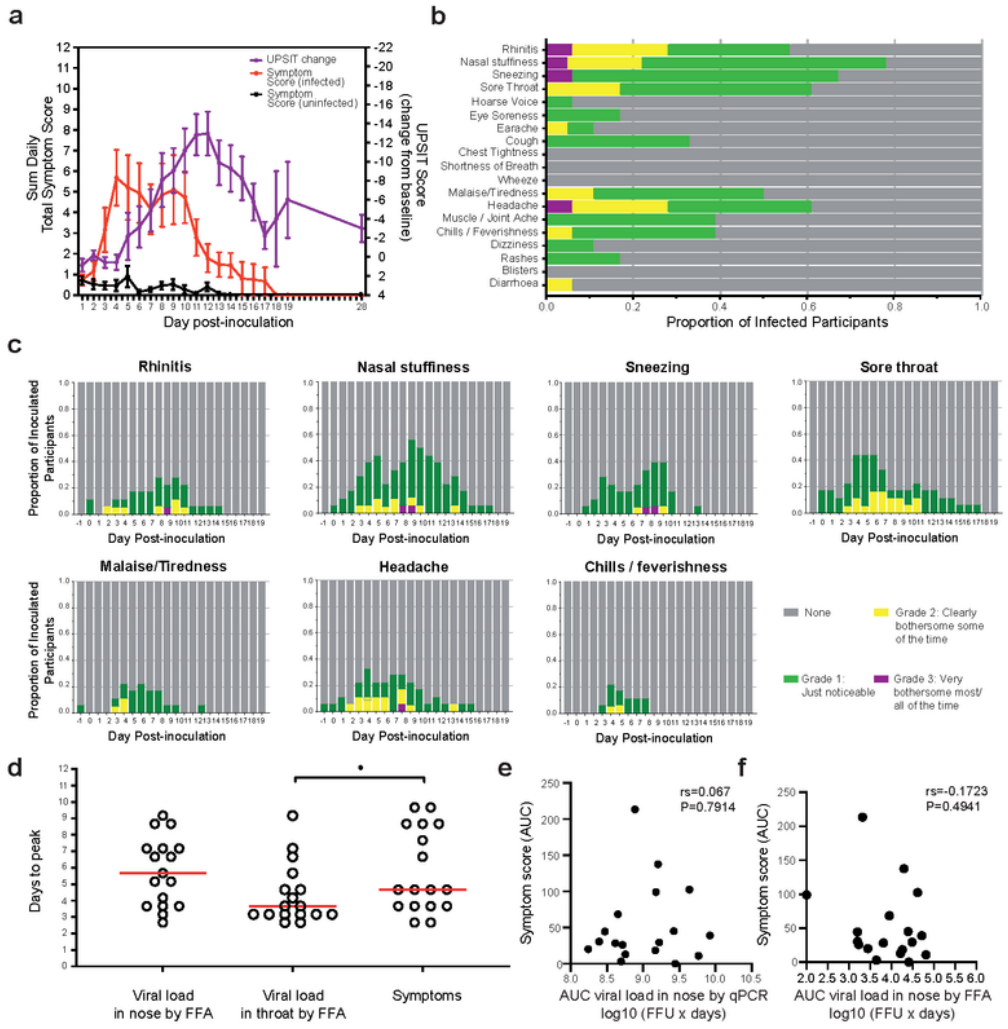

Figure 4: Human SARS-CoV-2 challenge induces mild symptoms that correlate with timing but not magnitude of viral load. Symptom scores were collected using self-reported symptom diaries 3 times daily from the 18 infected individuals. (a) Total symptom (red) and fall in UPSIT (purple) scores are shown for all infected participants. Total symptom scores for uninfected individuals are shown in black. Mean +/- S.E.M. symptom scores are shown. (b) The frequency and peak severity of symptoms of each type reported by infected participants over the course of the quarantine period are shown. (c) The frequency and severity of the 7 most commonly-reported symptoms are shown on each day during the quarantine period scored as follows: none (grey), grade 1 just noticeable (green), grade 2 clearly bothersome some of the time (yellow) and grade 3 very bothersome most/all of the time (purple. The day post-inoculation of peak VL in (d) nose and throat are compared with the day of highest reported symptom score. Medians are shown and Wilcoxon matched-pairs signed rank test used (*P<0.05). Area under the curve (AUC) of VL in the nose by (e) qPCR and (f) FFA is correlated with AUC of total symptom scores over the quarantine period. Spearman's r and p-values are shown.

Figure 4

Human SARS-CoV-2 challenge induces mild-moderate symptoms that correlate with timing but not magnitude of VL. Symptom scores were collected using self-reported symptom diaries 3 times daily from the 18 infected individuals. (a) Total symptom (red) and fall in UPSIT (purple) scores are shown for all infected participants. Total symptom scores for uninfected individuals are shown in black. Mean +/- S.E.M. symptom scores are shown. (b) The frequency and peak severity of symptoms of each type reported by infected participants over the course of the quarantine period are shown. (c) The frequency and severity of the 7 most commonly-reported symptoms are shown on each day during the quarantine period scored as follows: none (grey), grade 1 just noticeable (green), grade 2 clearly bothersome some of the time (yellow) and grade 3 very bothersome most/all of the time (purple). The day post-inoculation of peak VL in (d) nose and throat are compared with the day of highest reported symptom score. Medians are shown and Wilcoxon matched-pairs signed rank test used (*$P<0.05$). Area under the curve (AUC) of VL in the nose by (e) qPCR and (f) FFA is correlated with AUC of total symptom scores over the quarantine period. Spearman's r and p-values are shown.

Figure 5

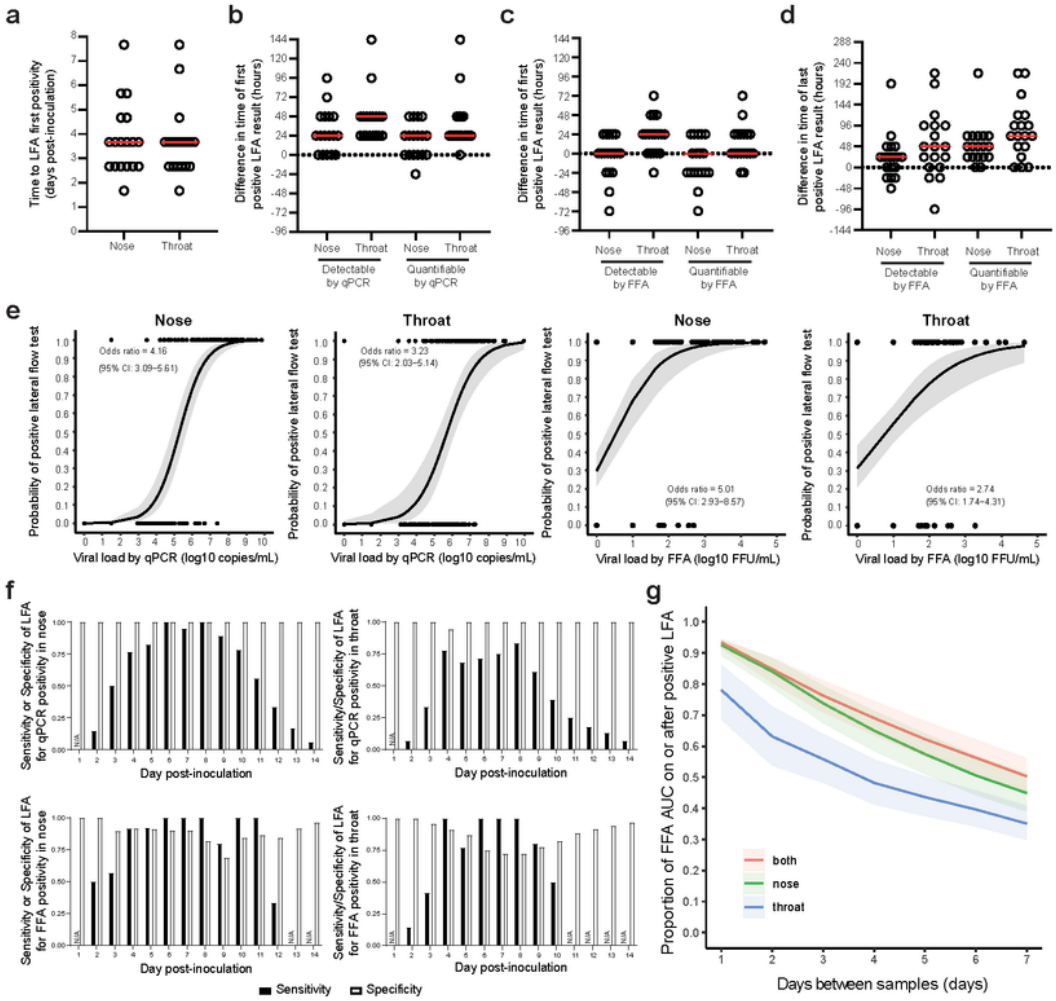

Figure 5: Rapid antigen testing by lateral flow accurately predicts infectious virus shedding. Nose and throat swab samples in viral transport medium were tested by lateral flow assay (LFA). (a) Time to first LFA positivity is shown. Median (red) difference in timing between LFA and nose or throat are shown for first (b) qPCR and (c) FFA detection and quantification. (d) Median (red) and individual number of days between the last detectable or quantifiable FFA result compared with LFA are shown. (e) Generalised estimating equations logistic regression showing odds ratios and 95% confidence intervals for lateral flow test positivity at viral loads by qPCR and FFA in the nose and throat. (f) Sensitivity (black) and specificity (white) of LFA in determining qPCR and FFA viral detection in the nose and throat over the course of challenge infection. N/A indicates where there were no true positives. (g) Impact of frequency of LFA testing on the proportion of viable virus shedding after LFA diagnosis from the nose (green), throat (blue) or both nose and throat (red).

Figure 5

Rapid antigen testing by lateral flow accurately predicts infectious virus shedding. Nose and throat swab samples in viral transport medium from the 18 infected individuals were tested by lateral flow assay (LFA). (a) Time to first LFA positivity is shown. Median (red) difference in timing between LFA and nose or throat are shown for first (b) qPCR and (c) FFA detection and quantification. (d) Median (red) and individual number of days between the last detectable or quantifiable FFA result compared with LFA are

shown. (e) Generalised estimating equations logistic regression showing odds ratios and 95% confidence intervals for lateral flow test positivity at VLs by qPCR and FFA in the nose and throat. (f) Sensitivity (black) and specificity (white) of LFA in determining qPCR and FFA viral detection in the nose and throat over the course of challenge infection. N/A indicates where there were no true positive results. (g) Impact of frequency of LFA testing on the proportion of viable virus shedding after LFA diagnosis from the nose (green), throat (blue) or combined nose and throat (red).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigE1UninfectedVLv2.pdf
- FigE2Remv1.pdf
- FigE3qPCRFFACorrelationv3.pdf
- FigE4aIndividualVLSxRemv2.pdf
- FigE4bIndividualVLSxNoRemv2.pdf
- FigE5aSxDetailed.pdf
- FigE5bSxDetailed.pdf
- FigE5cSxDetailed1.pdf
- FigE6SxRem.pdf
- FigE7aLFAFFAqPCR2.pdf
- FigE7bLFAFFAqPCR.pdf
- FigE8ROCImpactv2.pdf
- TableS1SymptomDiary.docx
- TableS2abnormalbloods.doc
- flatChiuepc1.pdf
- flatChiurs2.pdf