NERVTAG paper

Note on growth rate of SARS-CoV-2 B.1.1.7

Authors: Peter Horby, Cariad Evans, Catherine Huntley, Muge Cevik, Wendy Barclay, Julian Hiscox, John Edmunds, Neil Ferguson, Andrew Hayward, Maria Zambon.

SAGE meeting date: 22 April 2021

Summary

1. It is almost certain that the epidemiological growth rate of B.1.1.7 is higher than older variants in circulation. The biological mechanism(s) for this higher growth rate remains unclear. This paper reviews possible underlying epidemiological and biological mechanisms.

2. Compared to earlier variants, B.1.1.7 shows an increase in secondary attack rate (high confidence) but there is no evidence of a difference in serial interval (moderate confidence). It is, therefore, highly likely that the higher growth rate of B.1.1.7 compared to older variants is a function of an increased risk of transmission per contact (increased infectiousness or transmissibility) rather than a decrease in the interval between successive cases in a chain of transmission (reduced serial interval).

3. The biological processes that underlie virus transmission can be broken down into: (i) duration and level of virus replication in donor, (ii) emission/shedding of the virus from the donor into the environment including the air, (iii) survival of infectious virus in the environment, (iv) infectious dose, which is related to attachment and entry of virus into cells and, (v) evasion of innate or adaptive immunity.

4. It is likely that B.1.1.7 infection is associated with lower Ct values, suggesting higher viral RNA load, than ‘wild type’ infection (moderate confidence). However, lower Ct values do not necessarily equate to an increase in infectious virus. Lower Ct values may indicate faster replication, a change in the relative expression of a particular gene target, or a change in replication kinetics.

5. The replication kinetics of B.1.1.7 are similar to that of other lineages when grown independently in vitro (moderate confidence).

6. However, competitive replication assays in primary human airway epithelial cells or cell culture support a slight advantage in B.1.1.7 over other SARS-CoV-2 variants (low confidence).

7. Data are inconclusive on whether the duration of viral shedding is different in B.1.1.7 compared to other variants.

8. Data are insufficient to reach any conclusion on differences in the emission of viral variants into the environment by infected individuals.

9. Data suggest that the environmental survival of B.1.1.7 is not meaningfully different from other variants (moderate confidence).

10. It is likely that individual mutations present in the B.1.1.7 variant increase binding affinity (N501Y) and efficiency of cell entry (moderate confidence). This may mean that the average infectious dose is lower for B.1.1.7 than other variants (low confidence).

11. There is a moderate decrease in the ability of sera from first wave infections and vaccination to neutralize B.1.1.7. This may slightly reduce the protective effectiveness of prior infection or vaccines but does not abrogate protection.

12. B.1.1.7 may be less susceptible to interferon responses and this means the average infectious dose required to cause an infection may be lower (low confidence).

13. Overall, the most likely explanations for the competitive advantage of B1.1.7 over older virus variants are:

    a) A lower average infectious dose required to initiate infection (low confidence).

    b) Increased shedding of infectious virus, inferred from lower Ct values (low confidence).

Background

14. There is substantial evidence that the B.1.1.7 variant has a fitness advantage over earlier variants of SARS-CoV-2 and it has quickly replaced other variants to become the dominant variant in much of the UK. [1, 2, 3, 4] Samples with S-gene target failure (SGTF; a proxy for B.1.1.7) have predominated since mid-December 2020 and comprised 99.2% of cases in the week commencing 24 March 2021. [5]

15. This paper reviews possible epidemiological and biological mechanisms underlying the observed transmission advantage of the B.1.1.7 variant.

Potential epidemiological mechanisms for transmission advantage

16. An increase in the observed growth rate of a particular strain could be due to an increase in the risk of infection per contact (increased infectiousness) or a decrease in the interval between successive cases in a chain of transmission (reduced serial interval).

17. There is good evidence that B.1.1.7 is associated with an increase in secondary attack rates compared to earlier variants in circulation in the UK.

    a) PHE analysis of secondary attack rates using data from NHS Test and Trace found an increase in the secondary attack rates of around 30%-50% across most age groups, consistent across all regions. Between 30 November 2020 and 10 January 2021, 1,364,301 cases were reported; 35,597 (2.6%) had genomic sequencing data, and 18,160 of those with sequencing data (51%) had variant B.1.1.7. The secondary attack rate during this period was 11.3% for all index cases; 12.9% among those whose index case was confirmed B.1.1.7 by genomic sequencing and 9.7% among those whose index case was confirmed wild type by genomic sequencing. [3]

    b) Preliminary analysis of the ATACCC (household and non-household transmission) and INSTINCT (household transmission) studies estimated secondary attack rates in contacts of both SGTF and WT index cases. In both studies, the secondary attack rate was non-significantly higher for contacts of SGTF index cases than contacts of WT index cases. Comparison of secondary attack rates in household and non-household contacts showed a larger difference between B.1.1.7 variants and non-B.1.1.7 variants outside the household than inside the household, though findings were not significant in either group. [6]

18. Data are limited but do not show a difference in serial intervals between B.1.1.7 and non-B.1.1.7 variants.

    a) In the ATACCC study of community contacts, comparison of B.1.1.7 infector-infectee pairs (N=22) and non-VOC pairs (N=29) shows no difference in serial intervals (median 2 days), based on onset of any of cough, fever, anosmia or aguesia. [6]

b) The Virus Watch community cohort study compared the serial intervals between COVID-19 positive cases in B.1.1.7 hotspot areas and non-hotspot areas. Hotspots were identified using regional-level pillar 2 data, where a hotspot was defined as a week in a particular region where SGTF was observed in >75% of PCR tests. For hotspot illnesses, the mean and median serial interval among potential household transmission pairs was 5 and 4 days respectively. For non-hotspots the mean and median serial intervals were 3.8 and 3 days respectively. These differences were not statistically significant. [7]

19. In summary, compared to earlier variants, B.1.1.7 shows an increase in secondary attack rate (high confidence) but no difference in serial interval (moderate confidence). It is, therefore, highly likely that the higher growth rates of B.1.1.7 are a function of an increased risk of transmission per contact (increased infectiousness) rather than a decrease in the interval between successive cases in a chain of transmission (reduced serial interval).

Potential biological mechanisms for transmission advantage

20. The biological processes that underlie virus transmission can be broken down into:

    I. Virus replication in the infected donor – duration and level.

    II. Emission/shedding of virus from the donor into the environment, including the air.

    III. Survival of infectious virus in the environment.

    IV. The amount of virus required to successfully initiate infection in a recipient, or infectious dose.

    V. Evasion of innate or adaptive immunity.

21. The B.1.1.7 variant has 23 mutations across the SARS-CoV-2 genome, in the Spike as well as other genes, that may affect replication and the capacity to antagonise the innate immune system. It is not yet known which individual or constellation of mutations contribute to increased transmissibility.

I) Replication and shedding of virus in an infected donor

22. The replication and shedding of virus in an infected donor, also known as viral dynamics, may confer a transmission advantage if there is higher viral load or longer duration of infectivity, leading to increased opportunity for infection of others.

i) Evidence regarding Ct values

23. Cycle threshold (Ct) value can be used to infer the relative abundance of RNA detected and therefore used as a surrogate for viral load. Ct values may vary due to changes in RNA abundance, but variation in gene targets and assays may also produce variation in results.

24. Several analyses have suggested that B.1.1.7 (as indicated by SGTF) had lower median Ct values than non-SGTF cases. However, these studies may be biased by epidemiological trends (the rapid growth in B.1.1.7 cases that occurred following introduction of this variant). A summary of studies comparing Ct values is below.

    a) Nose and throat swabs from 28th September 2020 to 2nd January 2021 in the UK's national COVID-19 Infection Survey (CIS), a representative sample of households with longitudinal follow-up, were tested by RT-PCR for three genes (N, S and ORF1ab). By January 2021, median Ct values were similar in SGTF and triple-gene positives in most regions/countries, suggesting that an earlier observation that SGTF samples had lower Ct values was due to more SGTF being new infections rather than intrinsic biological differences affecting viral loads. [4]

    b) A second analysis using CIS data from 31st August 2020 to 7th February 2021 found that Ct value distributions for ORF1ab+N+S positive tests and ORF1ab+N positive tests are best represented by a mixture of 4 sub-distributions. Comparison of the lowest Ct sub-distribution between triple positive and double (ORF1ab+N) positive tests suggests that ORF1ab+N positive tests has a mean Ct value that is approximately 2 units lower. The lowest sub-distribution of Ct values for ORF1ab+N+S positive tests had a mean Ct of 17.5, compared with 15.6 for ORF1ab+N positive tests. Results were consistent across multiple sensitivity analyses. [8]

    c) A comparison of Pillar 2 Ct values for S-gene negative and positive samples from 8th of November 2020 to 7th February 2021 found that, where a Ct value threshold of <=30 is applied, median Ct values for S-gene negative samples were approximately 2 units lower than S-gene positive samples by February 2021. Separate comparison of median Ct values for the N-gene and ORF1ab show Ct values to be approximately 1 and 2 units lower, respectively. These differences were not seen in MS2 control Ct values. [9]

    d) Analysis of data from the Birmingham Turnkey Lighthouse lab showed that samples with S-gene dropout had significantly lower median Ct values of ORF1a (18.16 and 22.30, $p<0.0001$) and N-gene (19.39 and 23.16, $p<0.0001$) targets, compared to samples where S-gene was detected. Using external quality assessment data, the authors estimate that a Ct value of approximately 15-16 on their TaqPath test equates to a viral load of $1x10^6$ copies per mL. While approximately 35% of S-dropout samples lie below this Ct value, only 10% of S-positive samples do. [10]

25. The above studies relied on Pillar 2 samples tested with the TaqPath assay, used by Lighthouse laboratories. The Taqpath assay has 4 targets (N, ORF1ab, S, and MS2) which operate in the same well, and there is a possibility that the absence of the S-gene target in VOC samples could lead to alteration in the biochemical equilibrium of the PCR-reaction, and thus lower Ct values for the remaining targets. However, evidence to support that differences in Ct values are not due to assay artefact includes:

    a) Evidence that the base PCR reaction of the S gene occurs in SGTF samples, [11] and an absence of a comparable decrease in Ct values in the MS2 control of the TaqPath assay amongst Pillar 2 samples from 8th of November 2020 to 7th February 2021. [9]

    b) A lack of evidence to suggest an altered amplification curve or change in fluorescence signal values as determined by the AI software used by the Lighthouse laboratories to read results. [11]

26. There is also consistency of the finding of lower Ct values in the B.1.1.7 variant across other assays and other gene targets, and using alternative surrogates to infer correlates of viral load.

    a) A Public Health Wales study of 849 Pillar 1 samples obtained between 21 December 2020 and 26 January 2021 showed that Ct values, as obtained by the PerkinElmer assay and normalised against the endogenous control, were lower in SGTF samples than non-SGTF samples (as determined by the TaqPath assay). [12]

    b) A cohort study of patients admitted to hospital and testing positive for SARS-CoV-2 by PCR between 9 November and 20 December 2020 found that Ct values, as measured by an in-house N-gene PCR assay, were significantly lower in B.1.1.7 samples (n=27) than in non-B.1.1.7 samples (n=38) with a mean Ct of 28.8 [SD 4.7] and 32.0 [4.8], respectively (p=0.0085). [13]

    c) Comparison of SARS-CoV-2 positive clinical samples from B.1.1.7 (n=879) and B.1.177 (the dominant lineage in the UK prior to the introduction of B.1.1.7) infections (n=943) shows a significant increase in normalised subgenomic (sg) RNA expression profiles, independent of genomic reads, E gene cycle threshold, and days since onset at sampling. A significantly decreased E gene median Ct (in house diagnostic assay; B.1.177 median=25, B.1.1.7 median=23) and significantly greater relative light units RLU; Hologic Panther platform; B.1.177 median=1121, B.1.1.7 median = 1177) were also found in B.1.1.7 infections compared to B.1.177 infections. [14]

        i. Additionally, this paper observes a 16-fold increased expression in sgRNA containing ORF9b, which has been shown to modulate immune responses through interferon antagonism and recommends further experimental investigation to determine its role in transmissibility (see section V). [14]

27. The observation of an increased detection of sgRNA, specifically in relation to the N gene target, may offer some explanation for lower Ct values in B.1.1.7 observed in the other data sets presented using N gene target assays.

28. In summary, it is likely that B.1.1.7 infection is associated with lower Ct values, suggesting higher viral RNA load, than 'wild type' infection [moderate confidence].

29. While this indicates an increased abundance of RNA in these samples, it does not necessarily equate to an increase in infectious virus. Lower Ct values may indicate faster replication of B.1.1.7 in cells, a change in the relative expression of a particular gene target, or a change in replication kinetics. These findings may also be biased by the rapid growth in B.1.1.7 cases relative to other variants.

ii) Replication kinetics in vitro

30. In vitro studies have been performed to compare the replication of B.1.1.7 virus to other variants.

31. This was tested by comparing the growth of B.1.1.7 to other UK variants isolated over the course of the outbreak and to the South African (B.1.351) and Australian ('Victoria', obtained from near the start of the pandemic) variants. Three cell lines were used, VeroE6, Vero H/SLAM and ACE2-A549. The data indicate that the growth of B.1.1.7. in these three cell lines was no different to other isolates, some of which reached higher titres (see below). This suggests that the increased viral load in clinical samples associated with B.1.1.7 is not due to the virus replicating faster. This was in contrast to the South African variant that did have higher growth kinetics than most variants. [15]

32. A comparison of the replication kinetics of B.1.1.7, B.1.117.19, and B.1.258 isolates in primary human epithelial airway (HAE) cells showed no difference in E gene copies/mL (as quantified by qPCR) at 24, 48, and 72 hours post-infection. In Vero cells, B.1.1.7 isolates had significantly lower E gene copies/mL than B.1.258 and B.1.117.19 isolates at 24- and 48- hours post-infection. At 72 hours, there was no longer a significant difference between qPCR results for B.1.1.7 229, B.1.117.19, and B.1.258 isolates; but for B.1.1.7 111 isolates, qPCR results remained significantly lower than for B.1.258. [16]

33. Another comparison of the replication kinetics of SARS-CoV-2 mutants encoding the individual N501Y spike substitution, the full complement of spike mutations present in B.1.1.7, and the wild type, found that there were no major differences among the three strains in Calu-3 cells. In Vero cells, samples collected 12-48 hours post infection showed that the B.1.1.7 and N501Y mutants consistently replicated to higher titres than the wild type, as measured using infectious plaque assays. In HAE cultures, the N501Y and B.1.1.7 mutants replicated significantly faster in early stages of infection, as measured by PFU. However, when measured in RNA copies, there was little difference. [17]

34. Comparison of the replication kinetics of B.1.1.7 and an ancestral European strain (D614G) in a human reconstituted bronchial epithelium and in vitro (VeroE6/TMPRSS2 and Caco-2 cell lines) showed no major differences. Putting the two viruses into competition in a human reconstituted bronchial epithelium showed that the B.1.1.7 outcompeted the ancestral strain, suggesting more efficient replication. [18]

35. In summary, the replication kinetics of B.1.1.7 are similar to that of other lineages when grown independently in vitro (moderate confidence). However, in competition with other variants, B.1.1.7 does appear to have a replication advantage (low confidence).

iii) Duration of viral shedding

36. Duration of viral shedding is another key determinant of transmissibility. PCR positivity does not equate to infectiousness, as it can detect viral particles for a significant period of time, and individuals may continue to test positive by PCR when this is no longer clinically significant. Our recommended definition of infectiousness is duration of detectable viable virus by viral culture, and currently no data are available on this for the B.1.1.7 variant. At time of writing, data on duration of viral shedding is limited and based on very small sample sizes.

37. A longitudinal study of 65 individuals undergoing daily surveillance PCR testing, including 7 infected with the B.1.1.7 variant, provides weak evidence that B.1.1.7 may cause longer duration of infection but with similar peak viral concentration compared to non-B.1.1.7 variants. [19] In this study, sample size is very small, and confidence in findings is low.

    a) The mean overall duration of infection (proliferation plus clearance) was 13.3 days (90% credible interval 10.1-16.5) in individuals with the B.1.1.7 variant, compared to 8.2 (90% credible interval 6.5-9.7) in individuals with the non-B.1.1.7 variant.

    b) The peak viral concentration in individuals infected with B.1.1.7 was 19.0 Ct [90% credible interval 15.8-22.0] compared to 20.2 Ct [19.0- 21.4] for individuals infected with non-B.1.1.7. This converts to 8.5$\log_{10}$ RNA copies/ml 64 [7.6, 9.4] for B.1.1.7 and 8.2$\log_{10}$ RNA copies/ml [7.8, 8.5] for non-B.1.1.7 variants, showing no significant difference.

    c) Mathematical modelling estimated the mean viral trajectories and despite the authors only commenting on prolonged viral estimates with 90% credible intervals, they do not discuss that the model also demonstrates earlier viral detection estimates for B1.1.7.

38. The ATACCC study also assessed viral shedding by daily swabbing in B.1.1.7 cases (n=27) and non-B.1.1.7 cases (n=50) over a minimum 14-day period (and up to 20 days). Using PCR as a measure of viral shedding, the profile of shedding in B.1.1.7 cases was very similar to non-B.1.1.7 cases. There was no evidence of prolonged viral shedding or increased magnitude of shedding in cases with the B.1.1.7 variant. Day of peak Ct, peak Ct value, and duration of PCR positivity were not significantly different in B.1.1.7 and non-B.1.1.7 infections. [6]

39. In summary, data are inconclusive on whether the duration of viral shedding is different in B.1.1.7 compared to other variants.

II) Emission of virus from the donor into the environment

40. The tissue distribution of virus replication may play a role in increased transmissibility. If confirmed in vivo, increased lung tropism could augment aerosol production and increased upper airway tropism could increase transmission depending on the density of ACE2 and TMPRSS2 expression.

41. A hamster model suggests improved replication of mutants bearing the N501Y mutation in the upper airway. [17]

42. The emission of virus may also be affected by symptom profiles. Available data suggest that there is no significant difference in the course of illness or symptom severity due to B.1.1.7 compared to illness caused by other variants. The Virus Watch community cohort study compared the type and timing of symptoms in B.1.1.7 hotspot (regional weeks where SGTF in >75% of Pillar 2 PCR swabs) and non-hotspot (regional weeks where SGTF in <25% of Pillar 2 PCR swabs) areas, and found that in areas where over 75% of PCR positive infections showed SGTF (B.1.1.7 hotspot), symptoms were less likely to be severe, and illness duration was generally shorter but met the case definition faster although this was a statistically non-significant finding. [7]

43. At time of writing, data are insufficient to reach any conclusions on difference in the emission of viral variants into the environment by infected individuals.

III) Survival of infectious virus in the space between donor and recipient

44. A study was performed by PHE to compare the decay rates of B.1.1.7 and lineage B.1.351. on a stainless steel surface to that of an early English isolate. Each variant was dried onto stainless steel and held at 19C and 57%RH for 7 days. No significant difference to the decay rate of the B.1.1.7 was found for either variant. [20]

45. A comparison of four lineages, including B.1.1.7, shows that the stability of SARS-CoV-2 in aerosols does not vary greatly across several environmental conditions. [21]

46. The ancestral SARS-CoV-2 and the B.1.1.7 variant show comparable decay in infectivity within the first 5 minutes of aerosolisation, decaying to ~40 % of their starting viral titre. A similar loss of infectivity is seen at 40 % and 90 %RH over this timeframe. [22]

47. Comparison of the thermostability and infectivity of pseudotyped viruses showed that, following incubation across a range of temperatures, some of the point mutations found on the B.1.1.7 spike are associated with increased infectivity, when compared with the ancestral D614G strain. [23]

48. Data on survival of infectious virus in the space between the donor and the recipient are currently limited but do not provide any evidence that the environmental survival of the B.1.1.7 is meaningfully different from other variants (moderate confidence).

IV) Infectious dose

49. The amount of virus required to successfully initiate infection in a recipient can be influenced by the strength of binding of virus to its receptor, the efficiency of cell entry, and the ability of the virus the counter the host immune system. Together, these will determine the human infectious dose.

i) Binding affinity

50. Improved binding affinity may increase transmissibility by requiring lower infectious dose to initiate infection. Higher binding affinity is also correlated with better viral replication.

51. Change N501Y in the spike protein is thought to be a possible major determinant of the increased transmissibility. N501 is one of the key amino acids interacting with the ACE-2 receptor, and mutations that enhance affinity have been noted at this site. [24]

52. A binding assay using recombinant spike RBD and human ACE2 proteins showed an increase in RBD/ACE2 binding in mutants with an N501Y substitution. [17]

ii) Efficiency of cell entry

53. Pseudoviruses bearing ΔH69/ΔV70 (as in the B.1.1.7 spike) were found to have a two-fold higher infectivity over a single round of infection compared to wild-type, as measured by RLU per nU of RT activity. [25] Replacement of H69 and V70 residues in pseudovirus containing the B.1.1.7 spike was found to be associated with reduced infectivity and syncytia formation. [26] An in-vivo competition assay in a hamster model found that the deletion of codons 69-70 was associated with a fitness advantage. [17]

54. SARS-CoV-2 can transmit efficiently because it has a furin cleavage site in Spike that enables rapid entry into cells rich in TMPRSS2 protease, and evasion of endosomal IFITM proteins. [27, 28] Mutation in B.1.1.7 at P681H increases the efficiency of furin cleavage of Spike and likely enhances cell entry. It is pertinent that several other emerging variants also bear mutations close to the furin cleavage site. [29]

55. In summary, it is likely that individual mutations present in the B.1.1.7 variant increase binding affinity and efficiency of cell entry (moderate confidence). This may mean that the average infectious dose is lower for B.1.1.7 than other variants.

V) Evasion of innate or adaptive immunity

56. There is antigenic difference between B.1.1.7 and older viruses, but there is not sufficient to escape from natural or vaccine-acquired immunity. Studies of the ability of vaccine and convalescent sera to neutralise B.1.1.7 pseudovirus or pseudotyped particles expressing all mutations present in the variant have found either no effect, or a small reduction in neutralisation ability, the clinical significance of which is unclear.

57. The B.1.1.7 variant does, however, escape NTD antibodies. This may be more important in some individuals than others.

58. B.1.1.7 bears truncation of ORF8 and a 3 amino acid deletion in nsp6, a viral protein attributed with interferon antagonistic properties. Data are currently lacking to support a change in interferon induction by the B.1.1.7 variant.

59. Comparison of the potency of human interferons against several viral lineages found evidence of increased interferon resistance in the B.1.1.7 variant. B.1.1.7 showed evidence of resistance to both IFN-I and IFN-III when compared to ancestral strains. [30] The same pattern is being reported by other groups including Towers at UCL and Neil at Kings.

60. The clinical significance of the antigenic difference between B.1.1.7 and ancestral variants that has been shown in-vitro is small. Clinical estimates of vaccine efficacy against B.1.1.7 are similar to estimates against ancestral variants for both the AstraZeneca and Novovax vaccines [31, 32] Reinfection rates for B.1.1.7 remain low and are similar to those of previous strains. [33]

61. In summary:

    a) There is a moderate decrease in the ability of sera from first wave infections and vaccination to neutralize B.1.1.7. This may slightly reduce the protective effectiveness of prior infection or vaccines but does not abrogate protection.

    b) B.1.1.7 may be less susceptible to interferon responses and this means the average infectious dose required to cause an infection may be lower (low confidence).

Conclusions

62. Transmissibility is a complex characteristic, influenced by a multitude of factors. A number of epidemiological and biological mechanisms may be responsible.

63. Biological changes in transmissibility are often small and cannot be seen without large numbers of cases. More data is required to improve confidence on the assessment of biological mechanisms for a B.1.1.7 transmission advantage.

64. Overall, the most likely explanations for the competitive advantage of B1.1.7 over older virus variants are:

    a) Enhanced binding affinity and efficiency of cell entry cell (moderate confidence).

    b) Increased shedding of infectious virus, inferred from lower Ct values (low confidence).

65. Amplification of transmission is expected to be where the burden already exists - congregate living facilities, such as nursing homes, homeless shelters, detention centres, and university accommodation, as well as high risk occupational settings such as meat-packing plants, warehouses, manufacturing and food sectors may be especially vulnerable to variants with enhanced transmissibility. [34]

Recommendations

66. Regular sampling from patients with B.1.1.7 is required to understand viral load dynamics, including the duration of infection and timing of peak viral load. Further data will be available from ATACCC and INSTINCT studies in the near future.

67. Systematic monitoring of vaccine efficacy and potential immune escape cases by PHE is supported.

68. Assays for evaluating the biology of B.1.1.7 or any VOI/VOC should contain a diverse selection of isolates.

References:

[1] E. Volz, S. Mishra, M. Chand, J. C. Barrett, R. Johnson, L. Geidelberg, W. R. Hinsley, D. J. Laydon, G. Dabrera, A. O'Toole, R. Amato, M. Ragonnet-Cronin, I. Harrison, B. Jackson, C. V. Ariani, O. Boyd, N. J. Loman, J. T. McCrone, S. Goncalves, D. Jorgensen, R. Myers, V. Hill, D. K. Jackson, K. Gaythorpe, N. Groves, J. Sillitoe, D. P. Kwiatkowski, The COVID-19 Genomics UK (COG-UK) Consortium, S. Flaxman, O. Ratmann, S. Bhatt, S. Hopkins, A. Gandy, A. Rambaut and N. M. Ferguson, "Assessing transmissibility of SARS-CoV-2 lineage B.1.1.7 in England," *Nature,* 2021.

[2] N. G. Davies, S. Abbott, R. C. Barnard, C. I. Jarvis, A. J. Kucharski, J. D. Munday, C. A. Pearson, T. W. Russell, D. C. Tully, A. D. Washburne, T. Wenseleers, A. Gimma, W. Waites, K. L. Wong, K. van Zandvoort, J. D. Silverman, CMMID Covid-19 Working Group, COVID-19 Genomics UK (COG-UK) Consortium, K. Diaz-Ordaz, R. Keogh, R. M. Eggo, S. Funk, M. Jit, K. E. Atkins and W. J. Edmunds, "Estimated transmissibility and impact of SARS-CoV-2," *Science,* vol. 372, no. 6538, 2021.

[3] Public Health England, "Investigation of novel SARS-CoV-2 variant - Variant of Concern 202012/01: Technical Briefing 5," PHE, London, 2021.

[4] A. S. Walker, K.-D. Vihta, O. Gethings, E. Pritchard, J. Jones, T. House, I. Bell, J. I. Bell, J. N. Newton, J. Farrar, I. Diamond, R. Studley, E. Rourke, J. Hay, S. Hopkins, D. Crook, T. Peto, P. C. Matthews, D. W. Eyre, N. Stoesser, K. B. Pouwels and COVID-19 Infection Survey Team, "Increased infections, but not viral burden, with a new SARS-CoV-2 variant," *medRxiv [preprint],* 2021.

[5] Public Health England, "SARS-CoV-2 variants of concern and variants under investigation in England: Technical Briefing 8," Public Health England, London, 2021.

[6] ATACCC Study Team, "Preliminary analysis of ATACCC Data," 2021.

[7] Virus Watch Study Team, "Preliminary Analysis," 2021.

[8] S. Walker, "Comparison of Ct values in ORF1+N and triple positives 8 February 2021," *Unpublished analysis, presented to NERVTAG,* 2021.

[9] N. Ferguson, "Comparing Pillar 2 CT values for S-gene negative and positive samples," *Unpublished analysis, presented to NERVTAG,* 2021.

[10] M. Kidd, A. Richter, A. Best, J. Mirza, B. Percival, M. Mayhew, O. Megram, F. Ashford, T. White, E. Moles-Garcia, L. Crawford, A. Bosworth, T. Plant and A. McNally, "S-variant SARS-CoV-2 is associated with significantly higher viral loads in samples tested by ThermoFisher TaqPath RT-QPCR.," *medRxiv [preprint],* 2020.

[11] P. Klapper, *Personal Communications,* 2021.

[12] A. Couzens, I. Murrell, L. Gifford, B. Johns, K. Pheasant, L. Turner, J. Evans and C. Moore, "Higher Relative Viral Load Excretion Determined by Normalised Threshold

Crossing Value in Acute Cases infected with the B.1.1.7 Lineage VOC 202012/01 (Using S gene target failure as a Proxy) When Compared to other Circulating Lineages in Wales," *medRxiv,* 2021.

[13] D. Frampton, T. Rampling, A. Cross, H. Bailey, J. Heaney, M. Byott, R. Scott, R. Sconza, J. Price, M. Margaritis, M. Bergstrom, M. J. Spyer, P. B. Miralhes, P. Grant, S. Kirk, C. Valerio, Z. Mangera, T. Prabhahar, J. moreno-Cuesta, N. Arulumaran, M. Singer, G. Y. Shin, E. Sanches, S. M. Paraskevopoulou, D. Pilay, R. A. McKendry, M. Mirfenderesky, C. F. Houlihan and E. Nastouli, "Genomic characteristics and clinical effect of the emergent SARS-CoV-2 B.1.1.7 lineage in London, UK: a whole-genome sequencing and hospital-based cohort study," *The Lancet Infectious Diseases,* 2021.

[14] M. D. Parker, B. B. Lindsey, D. R. Shah, S. Hsu, A. J. Keeley, D. G. Partridge, S. Leary, A. Cope, A. State, K. Johnson, N. Ali and et al, "Altered Subgenomic RNA Expression in SARS-CoV-2 B.1.1.7 Infections," *bioRxiv [preprint],* 2021.

[15] T. Prince, X. Dong, R. Penrice-Randal, N. Randle, C. Hartley, H. Goldswain, B. Jones, M. G. Semple, J. K. Baillie, P. J. Openshaw, L. Turtle, ISARIC4C Investigators, G. L. Hughes, E. R. Anderson, E. L. Patterson, J. Druce, G. Screaton, M. W. Carroll, J. P. Stewart and A. H. Julian, "Sequence analysis of SARS-CoV-2 in nasopharyngeal samples from patients with COVID-19 illustrates population variation and diverse phenotypes, placing the in vitro growth properties of B.1.1.7 and B.1.351 lineage viruses in context," *bioRxiv [preprint],* 2021.

[16] J. C. Brown, D. H. Goldhill, J. Zhou, T. P. Peacock, R. Frise, N. Goonawardane, L. Baillon, R. Kugathasan, A. L. Pinto, P. F. McKay, J. Hassard, M. Moshe, A. Singanayagam, T. Burgoyne, ATACCC Investigators, PHE Virology Consortium and W. S. Barclay, "Increased transmission of SARS-CoV-2 lineage B.1.1.7 (VOC 2020212/01) is not accounted for by a replicative advantage in primary airway cells or antibody escape," *bioRxiv [preprint],* 2021.

[17] Y. Liu, J. Liu, K. S. Plante, J. A. Plante, X. Xie, X. Zhang, Z. Ku, Z. An, D. Scharton, C. Schindewolf, V. D. Menachery, P.-Y. Shi and S. C. Weaver, "The N501Y spike substitution enhances SARS-CoV-2 transmission," *bioRxiv [preprint],* 2021.

[18] F. Touret, L. Luciani, C. Baronti, M. Cochin, J.-S. Driouich, M. Gilles, L. Thirion, A. Nougairede and X. de Lamballerie, "Replicative fitness SARS-CoV-2 20I/501Y.V1 variant 1 in a human reconstituted bronchial epithelium," *bioRxiv [preprint],* 2021.

[19] S. Kissler, J. R. Fauver, C. Mack, C. G. Tai, M. I. Breban and et al., "Densely sampled viral trajectories suggest longer duration of acute infection with B.1.1.7 variant relative to non-B.1.1.7 SARS-Cov-2," *Preprint,* 2021.

[20] A. Bennett, "Preliminary analysis," 2021.

[21] M. Schuit, J. Biryukov, K. Beck, J. Yolitz, J. Bohannon, W. Weaver, D. Miller, B. Holland, M. Krause, D. Freeburger, G. Williams, S. Wood, A. Graham, M. Rosovitz, A. Bazinet, A. Phillips, S. Lovett, K. Garcia, E. Abbott, V. Wahl, S. Ratnesar-Shumate and P. Dabisch, "The stability of an isolate of the SARS-CoV-2 B.1.1.7 lineage in aerosols is similar to three earlier isolates," *The Journal of Infectious Diseases,* 2021.

[22] Reid Group, Bristol University, "Unpublished analysis," 2021.

[23] T. Tada, B. M. Dcosta, M. Samanovic-Golden, R. S. Herati, A. Cornelius, M. Mulligan and N. R. Landau, "Neutralization of viruses with European, South African, and United States SARS-CoV-2 variant spike proteins by convalescent sera and BNT162b2 mRNA vaccine-elicited antibodies," *bioRxiv[preprint],* 2021.

[24] T. N. Starr, A. J. Greaney, S. K. Hilton and et al., "Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding," *Cell,* pp. 1295-1310.e20, 2020.

[25] S. A. Kemp, D. A. Collier, R. P. Datir, I. A. Ferreira, S. Gayed, A. Jahun, M. Hosmillo, C. Rees-Spear and et al., “SARS-CoV-2 evolution during treatment of chronic infection,” *Nature Online,* 2021.

[26] S. A. Kemp, B. Meng, I. A. Ferreira, R. Datir, W. T. Harvey, G. Papa, S. Lytras, D. A. Collier, A. Mohamed, G. Gallo, N. Thakur, The COVID-19 Genomics UK (COG-UK) Consortium, A. M. Carabelli, J. C. Kenyon, A. M. Lever, A. De Marco, C. Saliba, K. Culap, E. Cameroni, L. Piccoli, D. Corti, L. C. James, D. Bailey, D. L. Robertson and R. K. Gupta, “Recurrent emergence and transmission of a SARS-CoV-2 spike dletion H69/V70,” *biorxiv [preprint].*

[27] T. P. Peacock, D. H. Goldhill, J. Zhou, L. Baillon, R. Frise, O. C. Swann, R. Kugathasan, R. Penn, J. Brown, R. Sanchez-David, L. Braga, M. Kavanagh Williamson, J. A. Hassard, E. Staller, B. Hanley, M. Osborn, M. Giacca, A. D. Davidson, D. A. Matthews and W. S. Barclay, “The furin cleavage site of SARS-CoV-2 spike protein is a key determinant for transmission due to enhanced replication in airway cells,” *bioRxiv [preprint],* 2020.

[28] H. Winstone, M. J. Lista, A. C. Reid, C. Bouton, S. Pickering, R. P. Galao, C. Kerridge, K. J. Doores, C. Swanson and S. J. Neil, “The polybasic cleavage site in the SARS-CoV-2 spike modulates viral sensitivity to Type I interferon and IFITM2,” *ASM Journal of Virology,* vol. Online, 2021.

[29] E. B. Hodcroft, D. B. Domman, D. J. Snyder, K. Oguntuyo, M. Van Diest, K. H. Densmore, K. C. Schwalm, J. Femling, J. L. Carroll, R. S. Scott, M. M. Whyte, M. D. Edwards, N. C. Hull, C. G. Kevil, J. A. Vanchiere, B. Lee, D. L. Dinwiddie, V. S. Cooper and J. P. Kamil, “Emergence in late 2020 of multiple lineages of SARS-CoV-2 Spike protein variants affecting amino acid position 677,” *MedRxiv [preprint],* 2021.

[30] K. Guo, B. S. Barrett, K. L. Mickens, K. J. Hasenkrug and M. L. Santiago, “Interferon Resistance of Emerging SARS-CoV-2 Variants,” *bioRxiv [preprint],* 2021.

[31] K. R. Emary, T. Golubchik, P. K. Aley, C. V. Ariani, B. Angus, S. Bibi, B. Blane, D. Bonsall, P. Cicconi, S. Charlton, E. A. Clutterbuck, A. M. Collins, T. Cox, T. C. Darton, C. Dold and at al., “Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 variant of concern 202012/01 (B.1.1.7): an exploratory analysis of a randomised controlled trial,” *The Lancet,* vol. 397, no. 10282, 2021.

[32] Novovax, “Novavax COVID-19 Vaccine Demonstrates 89.3% Efficacy in UK Phase 3 Trial: Press release,” 2021.

[33] M. S. Graham, C. H. Sudre, A. May, M. Antonelli, B. Murray, T. Varsavsky, K. Klaser, L. S. Canas, E. Molteni, M. Modat, D. A. Drew, L. H. Nguyen, L. Polidori, S. Selvachandran, C. Hu, J. Capdevila, The COG-UK Consortium, A. Hammers, A. T. Chan, J. Wolf, T. Spector, C. Steves and S. Ourselin, “Changes in symptomatology, re-infection and transmissibility associated with SARS-CoV-2 variant B.1.1.7: an ecological study,” *The Lancet,* 2021.

[34] NERVTAG/EMG, “SARS-COV-2: Transmission Routes and Environments, 22 October 2020,” 2021.