nature portfolio

Peer Review Information

Journal: Nature Microbiology
Manuscript Title: NMICROBIOL-21081971D
Corresponding author name(s):
Daily longitudinal sampling of SARS-CoV-2 infection reveals substantial heterogeneity in infectiousness

Editorial Notes:

Redactions – published data	Parts of this Peer Review File have been redacted as indicated to remove third-party material.

Reviewer Comments & Decisions:

Decision Letter, initial version:

Dear Chris,

Thank you for your patience while your manuscript "Daily sampling of early SARS-CoV-2 infection reveals substantial heterogeneity in infectiousness and effects of the B.1.1.7 (Alpha) variant" was under peer review at Nature Microbiology. It has now been seen by our referees, whose expertise and comments you will find at the end of this email. In the light of their advice, we have decided that we cannot offer to publish your manuscript in Nature Microbiology.

From the reports, you will see that while they find your work of some potential interest, the referees raise concerns about the advance your findings represent over earlier work and the strength of the novel conclusions that can be drawn at this stage. In particular, both referee #2 and #3 mention the dependency of your results on the translation of qRT-PCR results into viral loads in Figure 1 and raise the concern that these results have a high variability. These data, and the code which we note is unavailable for readers or reviewers to assess, are the basis for the results reported and therefore we cannot proceed with your manuscript.

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I am sorry that we cannot be more positive on this occasion, but hope that you find the referees' comments helpful when preparing your paper for resubmission elsewhere.

Yours sincerely,

{redacted}

****
Reviewer Expertise:

Referee #1: Modelling, Infectious disease
Referee #2: Epidemiology, Respiratory viruses
Referee #3: Modelling, Infectious disease

Reviewers Comments:

Reviewer #1 (Remarks to the Author):

Understanding how infectiousness changes over the course of SARS-CoV-2 infection is really important for planning optimal control. This paper makes a very helpful contribution here.

The paper also provides insights into the contribution of between host variability in viral dynamics to differences in onward transmission.

Major comments

- Digital PCR-based calibration of the nasal PCR assay allowed Cn values to be converted to RNA copies / ml; Presumably similar was also done for the saliva PCR assay to allow Figure 2B to be plotted and associated analyses to be done? I couldn't see this in the main methods? It therefore

might be helpful to present the analysis in Figure 1 in terms of viral copies per ml rather than Ct / Cn values if these are not directly interchangeable? At the moment there are places in the text where Ct and Cn are used interchangeably, e.g. line 166, and plotting both on the same axis in figure 1 implies they are the same too? More discussion is needed on any differences in Ct/Cn/viral load arising from the efficiency of sample extraction and any dilution occurring during this process.

- I appreciate this is primarily a modelling study, but it might have been helpful to have some descriptive understanding of the number of days it took to reach peak viral loads and to decline to undetectable, this can be read off Figure 2B, but some text on this might be good too. The more detailed analysis is of model parameters, which given the models fitted I understand aren't the same as these slopes, although they do relate to them. These summary properties are important for implementing the findings here into policy. Adding days to peak and days to limit of detection as additional bar plots to Figure 2C and D might be useful?

- Given the wide spread of Ct values observed in community-based symptomatic and asymptomatic testing, with most tests in symptomatic individuals typically performed shortly after symptom onset, it is probably expected that peak viral loads vary in this current study? (I agree the current study provides much more data on dynamics though!) Following in from this, how much of variability in infectiousness would you assign to variation in viral load vs variation in infectiousness per viral load (Figure 3D)?

- is antigen rather than PCR a better assay of infectiousness when can't do viral culture? Figure 1C might suggest this, and if this could be related back to Figure 3D this would be interesting. It might be emphasised further that antigen rather than PCR based tests might be better assays of infectiousness?

- a fuller description of the limitations of the study would be helpful

Other comments

Abstract
- on first reading it isn't clear that slower growth rates for Alpha should result in enhanced transmission

Line 134 - define FIA

Figure 1A
- it would help to have all panels share the same x-axis to compare dynamics over time, e.g. -3 to +12 noting that not all points are plotted for [-6,-4] for participant 473107
- worth adding to legend what the viral culture was of, i.e. nasal swab

Figure 1B
- y axis label is Ct or Cn value?

Line 142 - "no detectable infectious virus in nasal samples" - does this mean culture negative?

Line 186 - might be helpful to have sense of effect size rather than just "statistically significant"

Figure 2B
- would "Fitted" be a better legend label than "Simulation", accepting the model fit may be arrived at by simulation
- same point as above about consistent x-axis values facilitating comparison of trajectories

Line 253 - I'm not expert in the specific models fitted, so will leave that to other reviewers to comment, however it might help readers to give some simple conceptual explanation in the results text that what is being reported for the expansion rate is not just the slope of the rise in Figure 2B, but a modelled concept, and similarly how the death rate corresponds to the decline
- a minor point here and elsewhere but are the units actually log10(viral load)/day rather than /day?

Reviewer #2 (Remarks to the Author):

In this manuscript, Ke et al. identified 60 mild COVID-19 adult cases who were early in the infection from a routine campus surveillance testing program which tested the saliva of students and employees indiscriminately every 2-4 days, and collected data on the Ct/Cn values from RTqPCR on their mid-turbinate nasal swabs (Cn) and saliva samples (Ct) respectively, as well as positive/negative viral culture (infectivity) results on nasal swabs using Vero-TMPRSS2 cells, daily for 14 days (Figure 1). The analyses were mainly divided into four parts:

Part A - First, they translated the Ct/Cn values to viral genome load based on linear regression on 4 nasal samples with Cn values and viral (genome) load empirically measured (the section "Viral genome load calibration" and Table A1 in the Supporting Text).

Part B - Then, they fit five different within-host mechanistic models (TCL/ RC/ VPR/ IEC/ Combined RC-IEC) to these derived/ translated viral load data, identified the best model and parameter combinations based on lowest AIC score to generate population parameter estimates for nasal swab and saliva respectively (Table S4), while evaluating possible between-individual heterogeneity due to age or virus strains. With the identified models (nasal: RC; saliva: IEC), they predicted viral load trajectories for nasal swab/ saliva and non-B1.1.7/B1.1.7 strains separately. They concluded that (Figure 2):
(1) (initial) virus growth rate in nasal swab was faster than in saliva,
(2) virus growth rate of B.1.1.7 strains was lower than non-B.1.1.7 strains in saliva,
(3) post-peak virus clearance was more rapid in nasal swab than saliva,
(4) significant correlation between age and effectiveness of anti-viral immune response in rendering target cells refractory to infection for nasal swabs but not in saliva, and
(5) viral load peaked one day earlier in saliva than in nasal samples in most individuals.

Part C - Separately, they modelled the probability of virus culture being positive based on the translated viral load for nasal swabs and the observed virus culture (positive/ negative) data using

three alternative models (linear/ power-law/ saturation) that may describe the relationship between translated viral load and infectious viral load, and selected the saturation model which has the lowest AIC score (Table S7). They estimated (Figure 3):
(1) a significant but weak correlation between virus culture positive and age,
(2) infectious virus shedding increased sharply when viral load > 10^5-10^6 copies/ml,
(3) substantial between-individual heterogeneity in infectious virus shedding that was not explained by viral (genome) load and suggested the heterogeneity may explain superspreading (i.e. heterogeneity in secondary transmission rates), and
(4) significant correlation between age and total infectiousness (infectious viral load * duration).

Part D - Lastly, based on the observed Ct values and the predicted viral genome or infectious virus trajectories from the above selected models, the authors concluded that (Figure 4):
(1) the viral genome shedding dynamics and the total infectiousness was indistinguishable between the B.1.1.7 and non-B1.1.7 strains in nasal swabs, but
(2) a slower rise in pre-peak viral load for B1.1.7 than non-B1.1.7 strains which may suggest a longer pre-peak shedding for B1.1.7 strains.

As demonstrated above, the validity of the results for parts B – D depend significantly on part A, i.e. whether the viral genome load that was used in most of the analyses was correctly translated from the measured the Ct/Cn values. Referring to page 6 of the Supporting Text, a simple linear regression was used for the translation only based on 4 observed viral load data. Moreover, only the sample with lowest Ct value (i.e. highest viral load) were measured in triplicate, but it is known that measured viral genome load or Ct values vary substantially between test (and even between replicates in the same test) for low viral load/ approaching the lower limit of detection of the assay. The issues with interpreting Ct values of RT-qPCR as viral load directly without a standard curve have been discussed by others (https://pubmed.ncbi.nlm.nih.gov/32562539/). One of the co-authors' recent paper also suggested a weaker correlation between Ct values and viral culture (https://pubmed.ncbi.nlm.nih.gov/33479756/)?

I do think that this is a very comprehensive dataset on viral genome shedding and viral culture on mild/ asymptomatic COVID-19 cases during early phase of infection. Given the concern above, is it possible for the authors to either (1) perform the quantification with standard curve on more samples (if all is not possible) for better translation and add more detailed description on the method of quantification (currently only "we first performed experiments on 4 nasal samples to measure the Cn values and quantify their viral genome load" was described), or (2) revise the manuscript substantially to base the analyses (as well as conclusions) on Ct values instead?

Other comments below:
(1) There is minimal discussion on the limitations of the study/ analyses – please add.
(2) line 133 – What does "Cn" value for nasal samples refer to?
(3) line 166 – Should "Ct" be "Cn" here?
(4) line 323 – What is the biological basis/ mechanism for the saturation model, i.e. amount of infectious virus would be capped at a certain threshold of viral genome copies and no longer increase even if more viral genome copies are available?
(5) Figure 1 – In the legend, please confirm "Note the Y axes are reversed in panel (A) and (B)" is

correct.
(6) Figure S2 – I believe it would be interesting for the readers to describe the viral culture positivity profile for asymptomatic infectees (e.g. individual # 445534, 449507). Surprisingly while there were some asymptomatic individuals with viral culture positivity detected, it seems none of the 60 individuals have viral culture positivity identified during pre-symptomatic phase/ minimal number of individuals were enrolled while presymptomatic, when it is expected a substantial number has viral shedding during presymptomatic phase? Would there be any potential bias in the symptom reporting?
(7) Supporting Text Page 6 section "Viral genome load calibration" – Why was a linear regression used – any evidence?

Reviewer #3 (Remarks to the Author):

Ke and colleagues report a study in which those positive on a screening test for SARS-CoV-2 RNA, or contacts of those positive, underwent nasal and salivary sampling and semi-quantitative PCR for SARS-CoV-2, plus viral culture. They report substantial variability between hosts, plus compartmental differences within hosts, in the observed dynamics. They conclude that infectiousness is likely to vary substantially between hosts, with implications for the advent of superspreading events. They also conclude that saliva samples may give a better indication of infectiousness than nasal samples. They report little observed variation between original wild-type and B.1.1.7 variant infections.

I have been asked to focus in my review on epidemiology and modelling aspects of the work, although have commented on other areas where I feel I have sufficient expertise.

The study provides evidence supportive of the idea of clinically significant heterogeneity between individuals when infectiousness is considered. Owing to the inability to exclude confounders completely in the real-world setting, the evidence is not unequivocal. The authors have done an admirable job of attempting to bring to readers' attention the key characteristics of the study population that may affect external validity, plus where limitations to the study may be found, in a way that helps highlight what this kind of study can and cannot determine. As it stands, the study yields some unnecessary difficulties in allowing reproduction of analysis, and misses some methodological details that are important in understanding the utility of results. My feeling is that there are some aspects of methodology that as presented at present give the appearance of impairing the utility of the study: some of these can be fixed, but I think at least one definitely cannot (use of quantitative standards on the original PCRs). I think at least some of these issues may be seen as inevitably arising from attempting to study data generated in a real-world setting during an evolving public health situation, when the study was being run as an adjunct to a health protection programme. I think such attempts add value in terms of our understanding of an evolving public health situation, but it is especially important because of that situation to highlight to the casual reader all the caveats that result: my feeling is that the authors have clearly tried to do this openly, but there are some additional caveats below that need working into the text.

Notes on particular points follow:

1. Code availability. The authors state that code cannot be supplied as per an institutional policy. This statement directly contravenes a clear editorial policy, and ultimately it is the decision of the editorial team as to whether it is appropriate to make an exception, although I do not see why in this case LANL ought to expect different treatment from a company attempting to protect its commercial interests. The inclusion of code is not simply a nicety to permit further scientific analysis and discourse: there are aspects of the manuscript (indicated below) where I am unable to understand quite how the authors have derived a result from their description, and if the code were present I would have been able to inspect it to fill in a gap. Additionally, the authors have fitted a number of regression models by sequential changes of terms, and it may be more straightforward for some readers to follow what has been done and why in code form rather than in the supplementary tables. If the authors are unable to supply code, and the editorial team is of the view this is not ipso facto a fatal issue, then the authors must be prepared to be held to a far more stringent standard in the description of methodology and output than might otherwise be the case.

2. The manuscript as a whole would benefit from greater care in distinguishing between quantitative and semi-quantitative statements. It is important to remember that without a fixed, defined denominator, PCR can at best be semi-quantitative. From human subjects, the only fluids where PCR results can be considered truly quantitative are blood and cerebrospinal fluid, and in practice QC requirements mean that reliable quantitative results can only be obtained for blood. As an example of the kind of issue to which I am referring, the authors refer on page 11 to viral genome loads in copies/mL. Such units are meaningless unless all PCRs have been run with quantitative standards (it appears from the Supporting Text as if the authors have assumed the same reaction efficiency as in a single calibration assay, without including QCs for calibration in other assays). In addition, the number of copies per mL in a nasal swab depends not only upon the exact swabbing technique, but the volume of viral transport medium used, which the authors do not state. The issue of VTM volume further raises an issue of external validity of results, especially in a pandemic setting where clinical laboratories have had to change VTM suppliers because of supply limitations.

3. The authors report semiquantitative outputs from the two assays used as single Ct or Cn values. However neither assay is a simplex assay (the Thermo assay has RdRp, S and N targets, and the Alinity assay has RdRp and N targets), so one would expect there to be multiple values reported for the assays.

4. The authors state that Cn is not equal to Ct on page 3 of the manuscript, but then refer to Ct values for nasal samples on page 5 of the manuscript. What is the definition of Cn? I am unable to find a definition for Cn in manufacturer instructions, and indeed I am unable to find even a statement that the quality control for the assay guarantees reproducibility of Cn within any level of tolerability. Bearing in mind that a cornerstone of the conclusions of this paper is comparison of one manufacturer's Cn with another's Ct, this would be very helpful. (Indeed, bearing in mind the automated decisions being made in proprietary assays to derive a threshold against which Ct is measured, even direct comparison of Ct between one assay and another carries certain assumptions.)

5. Even without considering the Cn/Ct issue, there are a few further potential confounders when

comparing Ct values between different assays and targets that should be addressed - I am not sure whether this is possible and this may pose risk to the reliability of the conclusion comparing nasal and salivary samples. Firstly, because we are comparing targets at different positions within a member of the Nidovirales, there may be a significant differential contribution from sgRNA abundances to the different targets. Depending upon the target for which semiquantitative output is being reported, this may on its own yield a different temporal profile of semiquantitative results, especially if one sampling method acquired more sgRNA than another.

6. Secondly, immune responses targeted at different loci within the virus may differ, and in general interference by immune effector molecules (either as direct neutralisation or as assay interference) may differ between different assays of different targets at different times. This should at least be acknowledged as a potential limitation (an alternative explanation for the claim that temporal dynamics differ between compartments), highlighting the need for further work beyond this manuscript to remove this potential confounder.

7. There is a helpfully detailed description of cohort demographics on page 3 of the manuscript. It would be helpful for greater understanding of within-host dynamics to have a statement of any information collected about prior infection or immunisation (although the timing of data collection is likely to have preceded mass immunisation, it is unclear to me whether any participants may have been involved in vaccine trials, for example).

8. On page 6 of the manuscript, it is noted that four patients' data are simply excluded because "viral genome loads were very low". It is admirable that the authors have been up front about this; however, this appears to be a post hoc adjustment and there are no clear criteria or justifications for this decision. Bearing in mind that the main message of the manuscript seems to be that profiles in individuals are highly variable, removing data in this way seems odd. From a brief look at the plots of individual data, one might guess that the authors expect that they have caught these individuals at the end of their shedding period, and that this breaks a paradigm of setting "peak viral load" as day 0. If so, it may help to be more explicit about this choice, and the limitations associated with it.

9. In the supporting text, section 2, it should be noted that a number of assumptions are supported by a self-citation in preprint form (as an aside, the citation is given incorrectly!). I have not been able to check these assumptions in full, as effectively this would require reviewing another manuscript. If this is under review or in press, the authors should consider updating the reference; if it is not under review, the authors should consider whether it is most appropriate to cite a preprint as separate from this work in this case - whilst I see no problem in general with citing others' preprints, especially in a rapidly moving field, I think there is a difference between referencing others' work in this way - itself a form of peer review - and referencing one's own work in this way, which risks generating a piece of the scientific record whose foundations have not been subject to the same scrutiny.

10. In the supporting text, section 2, I am unable to reproduce equation S7, either by hand or by writing my own code. With the simplifying assumption, the system of ODEs becomes three-dimensional, and the eigenvalues corresponding to the exponents of the standard solutions are the solutions of an irreducible cubic characteristic equation. Whilst the level of detail given in the text

would be adequate were there code to which to refer for assistance, without such code I cannot see what is going wrong (either in the manuscript or in my own working): my solution is different and much less easy to simplify.

11. The number of possible parameters is large in comparison with the patient sample size, and this should be viewed as a limitation of the work. It is unclear from the description of the methodology the authors have given (and because code is not included it is not possible to inspect elsewhere) whether the authors have used a first-order or second-order Akaike Information Criterion; a second-order criterion would seem more appropriate in the contexts for which it has been used in this manuscript, and some of the scores are sufficiently close that this may change the conclusions regarding model fit.

12. In the supporting text, section 3, I presume "N1" and "N2" refer to the CDC primer/probe sets or target regions, rather than separate genes (there is one nucleocapsid gene in SARS-CoV-2)?

13. I am unclear in this section what experiments were performed to quantify viral genome loads. I am also unclear from table A1 which rows represent replicate experiments on the same sample, and which rows represent distinct samples.

14. There appears to be no attempt at calculating an error estimate in the linear regression in this section.

15. The section is labelled in a way that implies that it allows quantification of a viral load from Ct or Cn values, which the main text has stated to differ, but the calibration only relates to Cn values on one assay, and appears to assume that the reaction efficiency is the same for two different targets, which it need not be (i.e. it is unclear why a regression has been chosen that does not have a term allowing for different targets, or alternatively why there are not separate regressions). What about targets outside the N gene? With regard to the other assay, it does not seem sufficient to state that "the calibration curve for Ct values measured from saliva sample [sic] is not available to us". If the authors wish to make statements about quantitative load (notwithstanding that this is not especially meaningful; see note 2 above), then there must be some form of calibration. This would usually be performed by having quantification standards on each PCR that is run, as reaction conditions may vary. The kind of calibration they have attempted for the Cn values would seem to be the bare minimum, albeit that it would be below the accepted standard of reproducibility for a clinical setting, let alone a research setting. Given that there is a straightforward relationship between Ct or Cn values and what the authors call "viral load", I think the solution to this issue with lowest risk of misleading the casual reader would be throughout the manuscript to keep everything in terms of Ct or Cn, and to talk about number of cycles, or at most "amount of virus/virus genome".

16. The authors should justify why they expect the lack of RNA extraction step to lead to an underestimation of viral load by a constant amount - is there any evidence to support this?

17. There are three typographical errors on p.6 of the supporting text ("formular", "RTqPRC", "saliva sample"). (There are a few other typographical errors in the supporting text, and it would benefit from use of a spell checker.)

18. Part 4 of the supporting text again begins with a self-citation of a preprint (and with the same form of incorrect referencing) - please see note 9 for general comments on this.

19. Does part 4 of the supporting text refer only to nasal PCR? From surrounding context, I think this is the case, but this is not clearly stated to be so, and I am unclear if so why salivary PCR, which has been considered before, is not also considered here.

20. The methodology of part 4 describes why it is not appropriate to generate a single model associating what the authors have calculated as "viral RNA load" (effectively Ct or similar) with "infectious virus shed" (culture positivity). Would the authors agree that sampling heterogeneity is an alternative explanation for some of the observed variation (e.g. depth of sampling in the nose may change the association between amount of genetic material and amount of viable virus)? Is it possible to derive from these results a clinically useful single-PCR prediction for the probability of an individual having produced an infectious sample? (If not, why not?)

Decision Letter, first revision:

Dear Chris,

Thank you for your letter asking us to reconsider our decision on your Article entitled "Daily sampling of early SARS-CoV-2 infection reveals substantial heterogeneity in infectiousness and effects of the B.1.1.7 (Alpha) variant". After careful consideration we have decided that we would be willing to consider a revised version of your manuscript.

When re-submitting your study, please make sure that your codes are available for our reviewers to be evaluated.

Along with your revised manuscript, you should also submit a separate point-by-point response to all of the concerns raised by the referees, in each case describing what changes have been made to the manuscript or, alternatively, if no action has been taken, providing a compelling argument for why that is the case. If we feel that a substantial attempt has been made to address the referees' comments, this response will be sent back to the referees - along with the revised manuscript - so that they can judge whether their concerns have been addressed satisfactorily or otherwise.

I should stress, however, that we would be reluctant to trouble our referees again unless we thought that their comments had been addressed in full.

When revising your paper:

- ensure it complies with our format requirements for Articles as set out in our guide to authors at https://www.nature.com/nmicrobiol/content

- state in a cover note the length of the text, methods and legends; the number of references and the number of display items.

Please ensure that all correspondence is marked with your Nature Microbiology reference number in the subject line.

Please use the following link to submit your revised manuscript:

{redacted}

We hope to receive your revised paper within four weeks. If you cannot send it within this time, please let us know so that we can close your file. In this event, we will still be happy to reconsider your paper at a later date so long as nothing similar has been accepted for publication at Nature Microbiology or published elsewhere in the meantime. Should you miss the four-week deadline and your paper is eventually published, the received date will be that of the revised, not the original, version.

I would appreciate it if you could tell me if you think you will be able to submit a revised manuscript, and also the likely timescale.

I look forward to hearing from you soon.

Yours sincerely,

{redacted}

Author Rebuttal, first revision:

Reviewer #1 (Remarks to the Author):

Understanding how infectiousness changes over the course of SARS-CoV-2 infection is really important for planning optimal control. This paper makes a very helpful contribution here.

The paper also provides insights into the contribution of between host variability in viral dynamics to differences in onward transmission.

Major comments

- Digital PCR-based calibration of the nasal PCR assay allowed Cn values to be converted to RNA copies / ml; Presumably similar was also done for the saliva PCR assay to allow Figure 2B to be plotted and associated analyses to be done? I couldn't see this in the main methods? It therefore might be helpful to present the analysis in Figure 1 in terms of viral copies per ml rather than Ct / Cn values if these are not directly interchangeable? At the moment there are places in the text where Ct and Cn are used interchangeably, e.g. line 166, and plotting both on the same axis in figure 1 implies they are the same too? More discussion is needed on any differences in Ct/Cn/viral load arising from the efficiency of sample extraction and any dilution occurring during this process.

The reviewer raises several valid points here. Unfortunately, we were unable to obtain digital PCR based calibration data for the saliva PCR assay. We did obtain demonstrating the efficiency of the saliva PCR assay (i.e. the relationship between change in template copy number and change in Ct value) as well as the molecular limit of detection, which have been included in the supplemental material. These data indicate that while there may be some uncertainty in the estimates of absolute viral genome copy numbers in the saliva, the PCR assay used is able to accurately measure relative changes in saliva viral genome load over time, thus allowing us to describe the rates of both rise and fall in saliva viral genome load.

We prefer to present the data in figure 1 as Ct/CN values as this is the least processed form of the data and represents the metric that most people are familiar with. We have addressed places in the text where Ct and CN values are used interchangeably and have added text addressing how Ct/Cn/viral load may vary due to assay/sample details as the reviewer suggested (Lines: 482-5).

- I appreciate this is primarily a modelling study, but it might have been helpful to have some descriptive understanding of the number of days it took to reach peak viral loads and to decline to undetectable, this can be read off Figure 2B, but some text on this might be good too. The more detailed analysis is of model parameters, which given the models fitted I understand aren't the same as these slopes, although they do relate to them. These summary properties are important for implementing the findings here into policy. Adding days to peak and days to limit of detection as additional bar plots to Figure 2C and D might be useful?

These are great suggestions. We have included estimates of these key metrics in figure 2.

- Given the wide spread of Ct values observed in community-based symptomatic and asymptomatic testing, with most tests in symptomatic individuals typically performed shortly after symptom onset, it is probably expected that peak viral loads vary in this current study? (I agree the current study provides much more data on dynamics though!) Following in from this, how much of variability in infectiousness would you assign to variation in viral load vs variation in infectiousness per viral load (Figure 3D)?

Yes, it is expected that the measured peak viral loads vary in our study. We believe this variation reflects the true variation in peak viral loads (rather than variations in measured peak viral load due to variations in the days post symptom onset, as likely the case in other studies that were based on clinical or hospital-based testing). This is because of the frequent testing regime we employed and the fact that the first data points are typically not the data points with the highest viral load, indicating that we began daily sampling before the peak viral load. Overall, being able to accurately capture the level of variation in the viral load trajectories (including the peak viral load) is one of the major strengths of our study.

The heterogeneity in infectiousness arises from a complex nonlinear combination of heterogeneity in time-varying viral genome load and heterogeneity in infectiousness relative to viral load. As you pointed out, Fig. 3D represents one way to quantify the heterogeneity in infectiousness per viral load. However, it is not possible (to our knowledge) to formally quantify the relative contributions of these two sources of variation to the overall heterogeneity, because viral load varies with time in each individual, and thus the two contributing factors cannot be easily disentangled (as in linear models).

Nonetheless, to partly address your question, we calculated the variation in infectiousness arising from the variation in viral load assuming the relationship between viral load and infectious virus load is the same across all individuals (i.e. the parameter values of the saturation model are set to be the same (at the population estimates) across all individuals. See the two panels below for a comparison between the distribution of infectiousness shown in the main text (left) and the distribution of infectiousness assuming non individual variation in the parameters in the saturation model.

{REDACTED}

The variance of the distribution are 384.0 and 175.9 for the plots on the left and on the right, respectively. This indicates a large fraction of heterogeneity is driven by the heterogeneity in the genome viral load dynamics across individuals.

- is antigen rather than PCR a better assay of infectiousness when can't do viral culture? Figure 1C might suggest this, and if this could be related back to Figure 3D this would be interesting. It might

be emphasised further that antigen rather than PCR based tests might be better assays of infectiousness?

The reviewer raises an important question. Antigen test positivity is strongly associated with culture positivity in our data; however, the sensitivity and specificity of antigen test in predicting culture positivity is not strong enough to be used as a measure of culture positivity in a public health policy setting in our opinion. In addition, thoroughly evaluating the use of antigen tests or PCR tests as a surrogate for culture positivity to make robust conclusions requires in-depth analysis and comparisons across multiple platforms/assays. We feel that it is beyond the scope and focus of this manuscript.

- a fuller description of the limitations of the study would be helpful

Fair point, we have expanded the discussion of study limitations (Lines: 477-90).

Other comments

Abstract
- on first reading it isn't clear that slower growth rates for Alpha should result in enhanced transmission

In the revised manuscript, we have incorporated data on the timing of the last negative test result from study participants. After re-running our analyses with these data included, the difference in growth rate in saliva for Alpha and non-Alpha viruses is no longer significant. The text has been modified to reflect this.

Line 134 - define FIA

Done.

Figure 1A
- it would help to have all panels share the same x-axis to compare dynamics over time, e.g. -3 to +12 noting that not all points are plotted for [-6,-4] for participant 473107
- worth adding to legend what the viral culture was of, i.e. nasal swab

Excellent suggestions, these changes have been made

Figure 1B
- y axis label is Ct or Cn value?

Both, this has been fixed

Line 142 - "no detectable infectious virus in nasal samples" - does this mean culture negative?

Yes

Line 186 - might be helpful to have sense of effect size rather than just "statistically significant" Figure 2B

- would "Fitted" be a better legend label than "Simulation", accepting the model fit may be arrived at by simulation
Good point, this has been done.

- same point as above about consistent x-axis values facilitating comparison of trajectories Good

point, this has been fixed.

Line 253 - I'm not expert in the specific models fitted, so will leave that to other reviewers to comment, however it might help readers to give some simple conceptual explanation in the results text that what is being reported for the expansion rate is not just the slope of the rise in Figure 2B, but a modelled concept, and similarly how the death rate corresponds to the decline
- a minor point here and elsewhere but are the units actually log10(viral load)/day rather than /day?

The growth rate, r, is the slope of the viral load rise (on a log scale) before peak viral load. The rate of viral load decline after peak viral load is mostly determined by the death rate of productively infected cells (although other factors, such as the availability of target cells, also have an impact though to a lesser extent). We added descriptions of how the viral decline is related to our estimated parameter values in the text (see Lines 269-283 in the revised manuscript).

The unit of the estimated rate parameter values is /day, because the model describes how the viral load, rather than log10 viral load, changes (exponentially) per day (see the ODE models).

Reviewer #2 (Remarks to the Author):

In this manuscript, Ke et al. identified 60 mild COVID-19 adult cases who were early in the infection from a routine campus surveillance testing program which tested the saliva of students and employees indiscriminately every 2-4 days, and collected data on the Ct/Cn values from RTqPCR on their mid-turbinate nasal swabs (Cn) and saliva samples (Ct) respectively, as well as positive/negative viral culture (infectivity) results on nasal swabs using Vero-TMPRSS2 cells, daily for 14 days (Figure 1). The analyses were mainly divided into four parts:

Part A - First, they translated the Ct/Cn values to viral genome load based on linear regression on 4 nasal samples with Cn values and viral (genome) load empirically measured (the section “Viral genome load calibration” and Table A1 in the Supporting Text).

Part B - Then, they fit five different within-host mechanistic models (TCL/ RC/ VPR/ IEC/ Combined RC-IEC) to these derived/ translated viral load data, identified the best model and parameter combinations based on lowest AIC score to generate population parameter estimates for nasal swab and saliva respectively (Table S4), while evaluating possible between-individual heterogeneity due to age or virus strains. With the identified models (nasal: RC; saliva: IEC), they predicted viral load trajectories for nasal swab/ saliva and non-B1.1.7/B1.1.7 strains separately. They concluded that (Figure 2):

(1) (initial) virus growth rate in nasal swab was faster than in saliva,

(2) virus growth rate of B.1.1.7 strains was lower than non-B.1.1.7 strains in saliva,

(3) post-peak virus clearance was more rapid in nasal swab than saliva,

(4) significant correlation between age and effectiveness of anti-viral immune response in rendering target cells refractory to infection for nasal swabs but not in saliva, and

(5) viral load peaked one day earlier in saliva than in nasal samples in most individuals.

Part C - Separately, they modelled the probability of virus culture being positive based on the translated viral load for nasal swabs and the observed virus culture (positive/ negative) data using three alternative models (linear/ power-law/ saturation) that may describe the relationship between translated viral load and infectious viral load, and selected the saturation model which has the lowest AIC score (Table S7). They estimated (Figure 3):
(1) a significant but weak correlation between virus culture positive and age,
(2) infectious virus shedding increased sharply when viral load > 10^5-10^6 copies/ml,
(3) substantial between-individual heterogeneity in infectious virus shedding that was not explained by viral (genome) load and suggested the heterogeneity may explain superspreading (i.e. heterogeneity in secondary transmission rates), and
(4) significant correlation between age and total infectiousness (infectious viral load * duration).

Part D - Lastly, based on the observed Ct values and the predicted viral genome or infectious virus trajectories from the above selected models, the authors concluded that (Figure 4):
(1) the viral genome shedding dynamics and the total infectiousness was indistinguishable between the B.1.1.7 and non-B1.1.7 strains in nasal swabs, but
(2) a slower rise in pre-peak viral load for B1.1.7 than non-B1.1.7 strains which may suggest a longer pre-peak shedding for B1.1.7 strains.

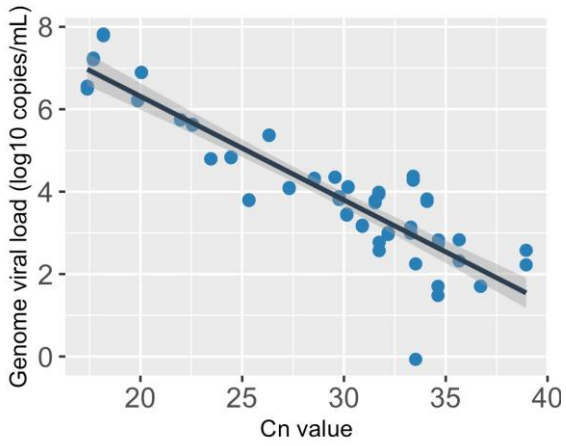
As demonstrated above, the validity of the results for parts B – D depend significantly on part A, i.e. whether the viral genome load that was used in most of the analyses was correctly translated from the measured the Ct/Cn values. Referring to page 6 of the Supporting Text, a simple linear regression was used for the translation only based on 4 observed viral load data. Moreover, only the sample with lowest Ct value (i.e. highest viral load) were measured in triplicate, but it is known that measured viral genome load or Ct values vary substantially between test (and even between replicates in the same test) for low viral load/ approaching the lower limit of detection of the assay. The issues with interpreting Ct values of RT-qPCR as viral load directly without a standard curve have been discussed by others (https://pubmed.ncbi.nlm.nih.gov/32562539/). One of the co-authors' recent paper also suggested a weaker correlation between Ct values and viral culture (https://pubmed.ncbi.nlm.nih.gov/33479756/)?

I do think that this is a very comprehensive dataset on viral genome shedding and viral culture on mild/ asymptomatic COVID-19 cases during early phase of infection. Given the concern above, is it possible for the authors to either (1) perform the quantification with standard curve on more samples (if all is not possible) for better translation and add more detailed description on the method of quantification (currently only "we first performed experiments on 4 nasal samples to measure the Cn values and quantify their viral genome load" was described), or (2) revise the manuscript

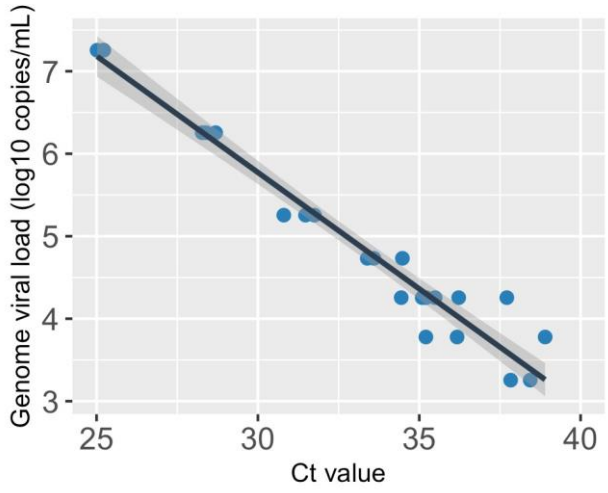
substantially to base the analyses (as well as conclusions) on Ct values instead?

The reviewer raises a very fair critique of our data. Per the reviewer's suggestion, we used digital PCR to measure viral genome loads for 62 samples with CN values ranging from 17 to 40. As shown in the figure below (which is included in the revised supplement), the log10 genome viral loads are linearly related to the CN values from RT-PCR (as seen in other studies, e.g. Kissler et al. PLOS Biology, (2021) 19, e3001333 and Han et al. Lancet Inf. Dis (2021) 21, 165). We estimate the intercept and the slope are 11.35 and -0.25, respectively. Note that this curve is similar to the curve we used in the original manuscript. In the revised manuscript, we used the new calibration curve to

translate CN values to genome viral loads in the nasal compartment, and revised the modeling part throughout. The qualitative conclusions remain the same as in the original manuscript.



In the revised manuscript, we also used a new set of data from the diagnostic lab that ran the saliva RTqPCR assays to translate the Ct values from the saliva samples to genome viral loads. In this new dataset, 0.9mL saliva was spiked with 0.1mL of viral RNA at a known concentration of 1.8x10^8 RNA copies/mL. 10-fold serial dilutions were performed until the final concentration was at 1.8x10^3 RNA copies/mL. Additionally, spiked saliva samples with final concentration of 5.4x10^4, 6.0x10^3, and 3.0x10^3 RNA copies/mL were prepared. Ct values were measured for these samples using the saliva qRT-PCR assay used throughout the study. We fitted a linear regression to the dataset. As shown in the figure below, the linear regression line describes the data well. The intercept and the slope of the regression are 14.24 and -0.28, respectively.



We used the new regression line to translate Ct values to genome viral loads in the saliva compartment, and revised the modeling part throughout.

Other comments below:

(1) There is minimal discussion on the limitations of the study/ analyses – please add.

As indicated above, we have expanded the discussion of the study limitations (Lines: 477-90).

(2) line 133 – What does “Cn” value for nasal samples refer to?

The CN value is an alternative metric for quantifying signal within a qPCR reaction that is meant to minimize user-to-user variation. It represents the cycle number at which point the rate of increase in PCR signal is maximal. In practical terms, it can be used interchangeably with Ct values in quantitative analysis of target concentration. We have added an explanation of the distinction between Ct and CN values, as well as a reference defining this metric and its use through comparison with Ct values (Lines: 134-7).

(3) line 166 – Should “Ct” be “Cn” here?

Yes, thanks for catching this.

(4) line 323 – What is the biological basis/ mechanism for the saturation model, i.e. amount of infectious virus would be capped at a certain threshold of viral genome copies and no longer increase even if more viral genome copies are available?

This is a great question, one that we do not have a definitive answer for, unfortunately. The phenomenon described here was also observed with another {REDACTED} Answering this question requires experiments, such as measuring immune responses or infectious viral titers of the samples that are beyond the scope and experimental capacity of the study. That said, we have several plausible potential explanations. First, the onset of the neutralizing antibody response may render some viral particles uninfectious during or after viral load peak. Second, the saturation may be due to a limitation of the cell culture assay. In this case, we would underestimate the true infectiousness of the individuals who experienced high viral loads. If true, the heterogeneity in infectiousness at the population level would be greater than we have estimated. However, as we showed in {REDACTED} the difference between the predictions of the saturation model and the power-law model, i.e the underestimation of the saturation model, would not be substantial.

(5) Figure 1 – In the legend, please confirm “Note the Y axes are reversed in panel (A) and (B)” is correct.

Thanks for catching this, it has been corrected.

(6) Figure S2 – I believe it would be interesting for the readers to describe the viral culture positivity profile for asymptomatic infectees (e.g. individual # 445534, 449507). Surprisingly while there were some asymptomatic individuals with viral culture positivity detected, it seems none of the 60 individuals have viral culture positivity identified during pre-symptomatic phase/ minimal number of individuals were enrolled while presymptomatic, when it is expected a substantial number has viral shedding during presymptomatic phase? Would there be any potential bias in the symptom reporting?

Yes, we absolutely expect some bias in the symptom reporting because all study participants are enrolling after either receiving a positive test result or being notified that they have been exposed to a known positive. For this reason, we have not emphasized analyses of the symptom data

(7) Supporting Text Page 6 section “Viral genome load calibration” – Why was a linear regression used – any evidence?

As we showed in the figure above, the use of a linear regression is fully justified. Other studies also used linear regressions (see Kissler et al. PLOS Biology, (2021) 19, e3001333 and Han et al. Lancet Inf. Dis (2021) 21, 165).

Reviewer #3 (Remarks to the Author):

Ke and colleagues report a study in which those positive on a screening test for SARS-CoV-2 RNA, or contacts of those positive, underwent nasal and salivary sampling and semi-quantitative PCR for SARS-CoV-2, plus viral culture. They report substantial variability between hosts, plus compartmental differences within hosts, in the observed dynamics. They conclude that infectiousness is likely to vary substantially between hosts, with implications for the advent of superspreading events. They also conclude that saliva samples may give a better indication of infectiousness than nasal samples. They report little observed variation between original wild-type and B.1.1.7 variant infections.

I have been asked to focus in my review on epidemiology and modelling aspects of the work, although have commented on other areas where I feel I have sufficient expertise.

The study provides evidence supportive of the idea of clinically significant heterogeneity between individuals when infectiousness is considered. Owing to the inability to exclude confounders completely in the real-world setting, the evidence is not unequivocal. The authors have done an admirable job of attempting to bring to readers' attention the key characteristics of the study population that may affect external validity, plus where limitations to the study may be found, in a way that helps highlight what this kind of study can and cannot determine. As it stands, the study yields some unnecessary difficulties in allowing reproduction of analysis, and misses some methodological details that are important in understanding the utility of results. My feeling is that there are some aspects of methodology that as presented at present give the appearance of impairing the utility of the study: some of these can be fixed, but I think at least one definitely cannot (use of
quantitative standards on the original PCRs). I think at least some of these issues may be seen as inevitably arising from attempting to study data generated in a real-world setting during an evolving public health situation, when the study was being run as an adjunct to a health protection programme. I think such attempts add value in terms of our understanding of an evolving public

health situation, but it is especially important because of that situation to highlight to the casual reader all the caveats that result: my feeling is that the authors have clearly tried to do this openly, but there are some additional caveats below that need working into the text.

Notes on particular points follow:

1. Code availability. The authors state that code cannot be supplied as per an institutional policy. This statement directly contravenes a clear editorial policy, and ultimately it is the decision of the editorial team as to whether it is appropriate to make an exception, although I do not see why in this case LANL ought to expect different treatment from a company attempting to protect its commercial interests. The inclusion of code is not simply a nicety to permit further scientific analysis and discourse: there are aspects of the manuscript (indicated below) where I am unable to understand

quite how the authors have derived a result from their description, and if the code were present I would have been able to inspect it to fill in a gap. Additionally, the authors have fitted a number of regression models by sequential changes of terms, and it may be more straightforward for some readers to follow what has been done and why in code form rather than in the supplementary tables. If the authors are unable to supply code, and the editorial team is of the view this is not ipso facto a fatal issue, then the authors must be prepared to be held to a far more stringent standard in the description of methodology and output than might otherwise be the case.

As indicated in the original submission, we were constrained by LANL policy on code sharing. We have regenerated all necessary code in a way that can be shared publicly. Thus we are including all code used along with this resubmission, and will post our code on GitHub prior to publication for open public access.

2. The manuscript as a whole would benefit from greater care in distinguishing between quantitative and semi-quantitative statements. It is important to remember that without a fixed, defined denominator, PCR can at best be semi-quantitative. From human subjects, the only fluids where PCR results can be considered truly quantitative are blood and cerebrospinal fluid, and in practice QC requirements mean that reliable quantitative results can only be obtained for blood. As an example of the kind of issue to which I am referring, the authors refer on page 11 to viral genome loads in copies/mL. Such units are meaningless unless all PCRs have been run with quantitative standards (it appears from the Supporting Text as if the authors have assumed the same reaction efficiency as in a single calibration assay, without including QCs for calibration in other assays). In addition, the number of copies per mL in a nasal swab depends not only upon the exact swabbing technique, but the volume of viral transport medium used, which the authors do not state. The issue of VTM volume further raises an issue of external validity of results, especially in a pandemic setting where clinical laboratories have had to change VTM suppliers because of supply limitations.

The reviewer raised an important caveat in interpreting PCR results. Indeed, the PCR results obtained from nasal swabs and saliva samples won't be as reliable as results from blood or cerebrospinal fluid. A large extent of noise exists in the data due to collection process or measurement errors as the reviewer pointed out. However, this type of data represents the most quantitative data available for quantifying viral kinetics for respiratory infections. The raw Ct values or the translated viral loads were frequently used in quantifying influenza infection previously (Pawelek et al. PLoS Comp. Biol. 8(6), e1002588) and more recently SARS-CoV-2 infection (Néant, et al. PNAS 118(8); Goyal, et al. Science advances, 6(47), eabc7112; Kissler et al. PLOS Biology, 19, e3001333). These studies have shed light on important infection mechanisms of within-host viral infection and the modeling results have been used to understand viral pathogenesis, effectiveness of testing strategies and predict the effectiveness of antivirals, etc. Therefore, we believe that this study provides an important dataset (and the best quantitative dataset available) for a quantitative

understanding of SARS-CoV-2 infection. We strongly believe that analyzing and modeling this dataset will lead to meaningful and important conclusions. In the revised manuscript, we revised the wording in the manuscript to better distinguish between quantitative and semi-quantitative statements.

More importantly, as indicated above, we have added additional data to address the relationships between our PCR results and the viral RNA copy numbers in our samples (see above in our response to Reviewer 2 or the revised supplementary text). These data are based on calibration assays that were not performed at the same time as the original assay runs, but all PCR data in the study were generated using EUA diagnostic assays performed in CLIA certified laboratories. EUA approval for

both of these assays required empirical validation of both assay efficiency and between-run consistency. In addition, we have data from the laboratory that performed all RTqPCR assays on nasal swabs in this study that demonstrates no significant differences in CN values obtained for control nasal swab samples assessed on different runs performed on different days, as shown in the figure below :

{REDACTED}

As with all clinical assays, there is the potential for noise arising from variation in sample collection technique. To minimize this, all sample collections were remotely observed by trained health care workers who verified that the sample was collected correctly. In addition, VTM volume and supplier and collection swabs were all consistent throughout the course of the study. This detail has been added to the methods.

In addition to our effort to minimize noise arising from sample collection/measurements, we specifically designed our modeling approach to make robust conclusions despite this noise. First, we fit our models to data collected across the individuals in the cohort together using a non-linear mixed effect modeling approach (explained in more detail in our response to Point 11 below). This approach is a powerful statistical approach to minimize and detect systematic noises in the dataset. In addition, by fitting models to all data together, we will be able to minimize the issue of model non-identifiability arising from noisy datasets. This makes predictions and conclusions more robust. Second, the parameters used to make conclusions in the study in the model depend only on the rates of increase or decline in viral load over time. This dependency means that only the relative changes in the viral load or Ct values impact on the estimation of those parameter values (i.e. the absolute values do not matter). As we have shown in the newly added experimental results, the assay efficiency for amplying viral genomes are consistent across days of study and across different individual samples for both the nasal and the saliva samples. Therefore, although the absolute number or concentration of the viral genomes may be semi-quantitative, this does not impact on the model estimation.

3. The authors report semiquantitative outputs from the two assays used as single Ct or Cn values. However neither assay is a simplex assay (the Thermo assay has RdRp, S and N targets, and the Alinity assay has RdRp and N targets), so one would expect there to be multiple values reported for the assays.

All values collected for the Thermo assay are included in the raw data table provided and are highly correlated. For simplicity's sake we only presented the Ct values from a single target. None of the conclusions would change if we used Ct values from either of the other two targets. The Alinity assay only reports the CN value from a single target; however, the reliability of this readout to quantify

target RNA copy #s is empirically demonstrated in our calibration curve data set that we have added as part of the revision.

4. The authors state that Cn is not equal to Ct on page 3 of the manuscript, but then refer to Ct values for nasal samples on page 5 of the manuscript. What is the definition of Cn? I am unable to find a definition for Cn in manufacturer instructions, and indeed I am unable to find even a statement that the quality control for the assay guarantees reproducibility of Cn within any level of tolerability. Bearing in mind that a cornerstone of the conclusions of this paper is comparison of one manufacturer's Cn with another's Ct, this would be very helpful. (Indeed, bearing in mind the automated decisions being made in proprietary assays to derive a threshold against which Ct is measured, even direct comparison of Ct between one assay and another carries certain assumptions.)

As indicated above, we have included a more in-depth description of the definition of CN relative to Ct values, along with a reference that defines the distinction in more detail (Lines: 134-7). It is important to note that none of our conclusions are based on direct comparisons of CN values in the nasal swab samples with Ct values from the saliva PCR because we are independently modeling dynamics within each compartment. None of the conclusions of the paper depend upon comparing one manufacturer's CN value with another's Ct, as the reviewer suggests. The closest we come to that is the comparison of peak shedding times between saliva and nasal swab samples in Fig. 2F. Timing of peak viral load in each compartment is determined relative to other samples within that compartment and thus is not subject to issues arising from comparing across assays.

5. Even without considering the Cn/Ct issue, there are a few further potential confounders when comparing Ct values between different assays and targets that should be addressed - I am not sure whether this is possible and this may pose risk to the reliability of the conclusion comparing nasal and salivary samples. Firstly, because we are comparing targets at different positions within a member of the Nidovirales, there may be a significant differential contribution from sgRNA abundances to the different targets. Depending upon the target for which semiquantitative output is being reported, this may on its own yield a different temporal profile of semiquantitative results, especially if one sampling method acquired more sgRNA than another.

The issue of comparing between assays is addressed in the response to the point above. As for issues comparing between targets within a single assay, we find a high degree of correlation in Ct values between different targets within the same assay, suggesting that our results are not skewed by variation in sgRNA levels between ORFs. Nevertheless, we have indicated in our discussion of study limitations that the PCR assays we use may pick up non-genomic viral RNAs (Lines: 484-5).

6. Secondly, immune responses targeted at different loci within the virus may differ, and in general interference by immune effector molecules (either as direct neutralisation or as assay interference) may

differ between different assays of different targets at different times. This should at least be acknowledged as a potential limitation (an alternative explanation for the claim that temporal dynamics differ between compartments), highlighting the need for further work beyond this manuscript to remove this potential confounder.

This point is not entirely clear. No major mammalian immune defense mechanism would be expected to have differential effects on detection of the different PCR targets. SARS-CoV-2 is a non-segmented virus and neutralization of virions or clearance of infected cells will have equivalent effects on molar abundance of all PCR targets.

7. There is a helpfully detailed description of cohort demographics on page 3 of the manuscript. It would be helpful for greater understanding of within-host dynamics to have a statement of any information collected about prior infection or immunisation (although the timing of data collection is likely to have preceded mass immunisation, it is unclear to me whether any participants may have been involved in vaccine trials, for example).

Apologies for the lack of clarity on this. We did collect information on prior infection and vaccination and none of the participants included in this paper had been vaccinated or were aware of any prior infection. This has been noted in the revised version.

8. On page 6 of the manuscript, it is noted that four patients' data are simply excluded because "viral genome loads were very low". It is admirable that the authors have been up front about this; however, this appears to be a post hoc adjustment and there are no clear criteria or justifications for this decision. Bearing in mind that the main message of the manuscript seems to be that profiles in individuals are highly variable, removing data in this way seems odd. From a brief look at the plots of individual data, one might guess that the authors expect that they have caught these individuals at the end of their shedding period, and that this breaks a paradigm of setting "peak viral load" as day 0. If so, it may help to be more explicit about this choice, and the limitations associated with it.

Yes, the reason for excluding these individuals is that the Ct or CN values are consistently high (i.e. the viral genome loads are low) during the study period. This is an indication that the infections have long passed the peak viral load period. From a statistical point of view, adding in data from these individuals won't add any more information or increase statistical power in inferring the early dynamics of SARS-CoV-2 infection. Indeed, we included data from these individuals in our parameter estimation, and found no substantial difference in the estimates of the parameter values and the conclusions. We decided to exclude these individuals because if we include them, the reported individual estimates of parameter values for these individuals will be unreliable/meaningless. We added the justification in the revised manuscript in lines 207-214.

9. In the supporting text, section 2, it should be noted that a number of assumptions are supported by a self-citation in preprint form (as an aside, the citation is given incorrectly!). I have not been able to check these assumptions in full, as effectively this would require reviewing another manuscript. If this is under review or in press, the authors should consider updating the reference; if it is not under review, the authors should consider whether it is most appropriate to cite a preprint as separate from this work in this case - whilst I see no problem in general with citing others' preprints, especially in a rapidly moving field, I think there is a difference between referencing others' work in this way - itself a form of peer review - and referencing one's own work in this way, which risks generating a piece of the scientific record whose foundations have not been subject to the same scrutiny.

This preprint is fully peer-reviewed and in press at {REDACTED}. We updated our reference list.

10. In the supporting text, section 2, I am unable to reproduce equation S7, either by hand or by writing my own code. With the simplifying assumption, the system of ODEs becomes three- dimensional, and the eigenvalues corresponding to the exponents of the standard solutions are the solutions of an irreducible cubic characteristic equation. Whilst the level of detail given in the text would be adequate were there code to which to refer for assistance, without such code I cannot see what is going wrong (either in the manuscript or in my own working): my solution is different and much less easy to simplify.

In the revised manuscript, we listed the full derivation for r in Eqn. S7 and S8 of the supplementary material. Note that a critical step for this derivation is to assume that the target cell remains constant, i.e. a reasonable assumption during the exponential viral load increase phase, because the numbers of infected cell (i.e. the decrease in the target cells) are orders of magnitude lower than the initial target cell number, and thus the change in the number of target cells is negligible. We also numerically checked the accuracy of the derivation.

11. The number of possible parameters is large in comparison with the patient sample size, and this should be viewed as a limitation of the work. It is unclear from the description of the methodology the authors have given (and because code is not included it is not possible to inspect elsewhere) whether the authors have used a first-order or second-order Akaike Information Criterion; a second- order criterion would seem more appropriate in the contexts for which it has been used in this manuscript, and some of the scores are sufficiently close that this may change the conclusions regarding model fit.

The numbers of fitted parameters (14 and 15 in the best models for the nasal and saliva samples, respectively) are very small compared to the number of data points (640 and 787 for the datasets from nasal and saliva samples, respectively). See Table S4 for all the estimated parameter values (including the means and standard deviations of the population parameter values). The low number of parameter values being fitted is because the model fitting was performed using a non-linear mixed effect modeling approach, i.e. a statistically appropriate and powerful approach to analyze longitudinal datasets from many individuals such as the one we analyze here. In this approach, a single model is fitted to the longitudinal data taken from all individuals. The parameters in this model are assumed to have both fixed effects and random effects, where the fixed effects are the value shared across all individuals and the random effects describing variations (around the fixed effects) of the parameters for each individual. In our fitting (as described in the Methods and Supplementary Material), the random effects are assumed to follow either a normal or a log-normal distribution in the population (a commonly assumed and in general empirically validated assumption). The individual parameters (Table S5) that are used to describe trajectories for each individual were derived from the estimated distributions of the population parameters (Table S4), instead of being estimated from data directly. Therefore, they are not counted in the calculation of AIC. For an overview and description of the statistical foundation of this approach, please see Chapter 5 of the book, 'Longitudinal Data Analysis, Fitzmaurice, G., Davidian, M., Verbeke, G., & Molenberghs, G. (Eds.). (2008). CRC press.' by Marie Davidian.

The AIC scores reported in the original manuscript are the AIC scores without correction. The AIC score with a correction for small sample sizes (AICc - I assume that is what you refer to as the 2nd order AIC) differs from the AIC score by (2k^2+2k)/(n-k-1). Because of the large number of data points (n) compared to the number of fitted parameters (k), the magnitude of the difference between AIC and AICc is very small. Noneless, we report the AICc scores in the revised manuscript.

12. In the supporting text, section 3, I presume "N1" and "N2" refer to the CDC primer/probe sets or target regions, rather than separate genes (there is one nucleocapsid gene in SARS-CoV-2)?

Correct, these are separate target regions, not genes.

13. I am unclear in this section what experiments were performed to quantify viral genome loads. I am also unclear from table A1 which rows represent replicate experiments on the same sample, and which rows represent distinct samples.

Each row is a distinct sample, sorry for the confusion.

14. There appears to be no attempt at calculating an error estimate in the linear regression in this section.

In the revised supplementary material of the manuscript, we showed the data and linear regressions in Table A1 and A2. The shaded areas around the regression lines show the standard error of the estimate. It is clear that the standard error is very small.

15. The section is labelled in a way that implies that it allows quantification of a viral load from Ct or Cn values, which the main text has stated to differ, but the calibration only relates to Cn values on one assay, and appears to assume that the reaction efficiency is the same for two different targets, which it need not be (i.e. it is unclear why a regression has been chosen that does not have a term allowing for different targets, or alternatively why there are not separate regressions). What about targets outside the N gene? With regard to the other assay, it does not seem sufficient to state that "the calibration curve for Ct values measured from saliva sample [sic] is not available to us". If the authors wish to make statements about quantitative load (notwithstanding that this is not especially meaningful; see note 2 above), then there must be some form of calibration. This would usually be performed by having quantification standards on each PCR that is run, as reaction conditions may vary. The kind of calibration they have attempted for the Cn values would seem to be the bare minimum, albeit that it would be below the accepted standard of reproducibility for a clinical setting, let alone a research setting. Given that there is a straightforward relationship between Ct or Cn values and what the authors call "viral load", I think the solution to this issue with lowest risk of misleading the casual reader would be throughout the manuscript to keep everything in terms of Ct or Cn, and to talk about number of cycles, or at most "amount of virus/virus genome".

The reviewer raises a fair point about the absence of equivalent calibration curve data for the saliva RTqPCR assay. Unfortunately, the diagnostic laboratory that ran the saliva RTqPCR assay used in this study is not equipped to perform a digital droplet PCR-based calibration as we performed for the nasal swab RTqPCR assay. Instead, in the revised supplement, we have included data that quantifies the efficiency of the saliva RTqPCR assay using serial dilutions of spiked in viral RNA. These data establish the quantitative relationship between viral genome copy number in a sample and the measured Ct value. Altogether, given that we clearly lay out how we are estimating viral genome loads from the experimental data in the manuscript, we feel that it is appropriate to refer to our genome load estimates rather than keep everything in terms of CN/Ct as the reviewer suggests.

16. The authors should justify why they expect the lack of RNA extraction step to lead to an

underestimation of viral load by a constant amount - is there any evidence to support this?

This claim is supported by the cited manuscript (still only available as a preprint) that details the development and validation of the saliva RTqPCR assay used in this study.

17. There are three typographical errors on p.6 of the supporting text ("formular", "RTqPRC", "saliva sample"). (There are a few other typographical errors in the supporting text, and it would benefit from use of a spell checker.)

Thank you for catching these, they have been corrected.

18. Part 4 of the supporting text again begins with a self-citation of a preprint (and with the same form of incorrect referencing) - please see note 9 for general comments on this.

The preprint is fully peer-reviewed and in press at {REDACTED}now. The reference list is updated.

19. Does part 4 of the supporting text refer only to nasal PCR? From surrounding context, I think this is the case, but this is not clearly stated to be so, and I am unclear if so why salivary PCR, which has been considered before, is not also considered here.

Yes, the reviewer is correct, this section only applies to the nasal PCR data, we have clarified this in the text. We did not consider the relationship between PCR and infectious virus shedding in saliva because we were unable to measure infectious virus shedding for comparison in the same way that we did for nasal samples.

20. The methodology of part 4 describes why it is not appropriate to generate a single model associating what the authors have calculated as "viral RNA load" (effectively Ct or similar) with "infectious virus shed" (culture positivity). Would the authors agree that sampling heterogeneity is an alternative explanation for some of the observed variation (e.g. depth of sampling in the nose may change the association between amount of genetic material and amount of viable virus)? Is it possible to derive from these results a clinically useful single-PCR prediction for the probability of an individual having produced an infectious sample? (If not, why not?)

We feel that the possibility raised by the reviewer is unlikely, given that both viral culture and PCR assays were run on the same sample. While there is no doubt some variability in efficiency of recovery due to sampling heterogeneity (discussed both above and in the manuscript), there is neither empirical data nor a clear biological explanation supporting the idea that the relationship between Ct values and the amount of infectious virus varies significantly over the region of the nasal passages accessed during

sample collection. As for the last question, we clearly describe in the paper (shown in figures 1B and 3) how the relationship between RTqPCR results and probability of infectious virus presence can be variable. It would certainly be possible for someone to derive a PCR-based prediction of infectious status probability but this is beyond the scope of this study.

Decision Letter,second revision:

Dear Chris,

Thank you for your email and the response to the reviewer's comments. I have read your responses and discussed them with the editorial team and we would like to ask you to submit a revised version of the manuscript incorporating the appropriate modifications. Please also address any other remaining comments.

Thank you for your patience while your manuscript "Daily sampling of early SARS-CoV-2 infection reveals substantial heterogeneity in infectiousness and effects of the B.1.1.7 (Alpha) variant" was under peer-review at Nature Microbiology. It has now been seen by 3 referees, whose expertise and comments you will find at the of this email. You will see from their comments below that while they find your work of interest, some important points are raised. We are very interested in the possibility of publishing your study in Nature Microbiology, but would like to consider your response to these concerns in the form of a revised manuscript before we make a final decision on publication.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

If you have not done so already please begin to revise your manuscript so that it conforms to our Article format instructions at http://www.nature.com/nmicrobiol/info/final-submission/

The usual length limit for a Nature Microbiology Article is six display items (figures or tables) and 3,000 words. We have some flexibility, and can allow a revised manuscript at 3,500 words, but please consider this a firm upper limit. There is a trade-off of ~250 words per display item, so if you need more space, you could move a Figure or Table to Supplementary Information.

Some reduction could be achieved by focusing any introductory material and moving it to the start of your opening 'bold' paragraph, whose function is to outline the background to your work, describe in a sentence your new observations, and explain your main conclusions. The discussion should also be limited. Methods should be described in a separate section following the discussion, we do not place a word limit on Methods.

Nature Microbiology titles should give a sense of the main new findings of a manuscript, and should not contain punctuation. Please keep in mind that we strongly discourage active verbs in titles, and that they should ideally fit within 90 characters each (including spaces).

We strongly support public availability of data. Please place the data used in your paper into a public data repository, if one exists, or alternatively, present the data as Source Data or Supplementary Information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. For some data types, deposition in a public repository is mandatory - more information on our data deposition policies and available repositories can be found at https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data.

Please include a data availability statement as a separate section after Methods but before references, under the heading "Data Availability". This section should inform readers about the availability of the data used to support the conclusions of your study. This information includes accession codes to public repositories (data banks for protein, DNA or RNA sequences, microarray, proteomics data etc…), references to source data published alongside the paper, unique identifiers such as URLs to data repository entries, or data set DOIs, and any other statement about data availability. At a minimum, you should include the following statement: "The data that support the findings of this study are available from the corresponding author upon request", mentioning any restrictions on availability. If DOIs are provided, we also strongly encourage including these in the Reference list (authors, title, publisher (repository name), identifier, year). For more guidance on how to write this section please see:
http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf

To improve the accessibility of your paper to readers from other research areas, please pay particular attention to the wording of the paper's opening bold paragraph, which serves both as an introduction and as a brief, non-technical summary in about 150 words. If, however, you require one or two extra sentences to explain your work clearly, please include them even if the paragraph is over-length as a result. The opening paragraph should not contain references. Because scientists from other sub-disciplines will be interested in your results and their implications, it is important to explain essential but specialised terms concisely. We suggest you show your summary paragraph to colleagues in other fields to uncover any problematic concepts.

If your paper is accepted for publication, we will edit your display items electronically so they conform to our house style and will reproduce clearly in print. If necessary, we will re-size figures to fit single or double column width. If your figures contain several parts, the parts should form a neat rectangle when assembled. Choosing the right electronic format at this stage will speed up the processing of your paper and give the best possible results in print. We would like the figures to be supplied as vector files - EPS, PDF, AI or postscript (PS) file formats (not raster or bitmap files), preferably generated with vector-graphics software (Adobe Illustrator for example). Please try to ensure that all figures are non-flattened and fully editable. All images should be at least 300 dpi resolution (when figures are scaled to approximately the size that they are to be printed at) and in RGB colour format. Please do not submit Jpeg or flattened TIFF files. Please see also 'Guidelines for Electronic Submission of Figures' at the end of this letter for further detail.

Figure legends must provide a brief description of the figure and the symbols used, within 350 words, including definitions of any error bars employed in the figures.

When submitting the revised version of your manuscript, please pay close attention to our href="https://www.nature.com/nature-research/editorial-policies/image-integrity">Digital Image Integrity Guidelines. and to the following points below:

-- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures.
-- that control panels for gels and western blots are appropriately described as loading on sample processing controls
-- all images in the paper are checked for duplication of panels and for splicing of gel lanes.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

Please include a statement before the acknowledgements naming the author to whom correspondence and requests for materials should be addressed.

Finally, we require authors to include a statement of their individual contributions to the paper -- such as experimental work, project planning, data analysis, etc. -- immediately after the acknowledgements. The statement should be short, and refer to authors by their initials. For details please see the Authorship section of our joint Editorial policies at http://www.nature.com/authors/editorial_policies/authorship.html

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* ensure it complies with our format requirements for Letters as set out in our guide to authors at www.nature.com/nmicrobiol/info/gta/

* state in a cover note the length of the text, methods and legends; the number of references; number and estimated final size of figures and tables

* resubmit electronically if possible using the link below to access your home page:

{redacted}

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Please ensure that all correspondence is marked with your Nature Microbiology reference number in the subject line.

Nature Microbiology is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. This applies to primary research papers only. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit www.springernature.com/orcid.

We hope to receive your revised paper within three weeks. If you cannot send it within this time, please let us know.

We look forward to hearing from you soon.
{redacted}

****

Reviewer Expertise:

Referee #1:
Referee #2:
Referee #3:

Reviewers Comments:

Reviewer #1 (Remarks to the Author):

I'm content with the changes made and thank the authors for their full responses to my comments

Reviewer #2 (Remarks to the Author):

Thank you for your responses to my comments. I have no additional comments except that I would suggest to include your response to my enquires on #4 (biological basis for the saturation model) and #6 (potential bias in symptom reporting) in the main text of the revised manuscript.

Reviewer #3 (Remarks to the Author):

Thank you for this revised manuscript and accompanying rebuttal.

1. I am grateful to the authors for the pieces of code included with the revised submission. This gives me a better idea of what the authors are intending with the code, although I remain not entirely clear which of the models described in the supplementary information is actually implemented in the different sections of the code, i.e. where the different models are fitted and Akaike Information Criteria derived. It might be helpful to comment the code lightly to make explicit cross-reference to the manuscript. It remains impossible for me to run the code, because the code is heavily dependent on data structures that are not included with the revised submission, and because it is supplied in a form that does not allow me easily to determine package dependencies. Certainly in the case of the figures, it would be possible (if tedious) to reverse engineer the pertinent content of the data structures from the figures themselves, so there seems little reason not to make available such data. I am grateful to the authors for the reminder of the institutional restrictions under which they are working: I do not have much to add to my previous comment, save to reiterate that ultimately I see determination of whether the level of data availability presented here is adequate to be an editorial policy decision.

2. I am grateful to the authors for expanding on the derivation of original equation [S7] (equation [S8] in the revised version). I think there are two errors in the expanded derivation, one typographical, but one more critical. The typographical error is that to fit the given model, the entry in the first row, third column of the matrix in equation [S7] needs to have the opposite sign. The more critical error is that the characteristic equation with this sign error corrected is ckR_0\delta - (c+\lambda)(k\delta + k\lambda +\delta\lambda + \lambda^2)=0, whereas the value for \lambda the authors derive uses the equation ckR_0\delta - c(k\delta + k\lambda +\delta\lambda + \lambda^2)=0. (It is relatively straightforward to see that there must be _some_ problem, because the authors derive only two eigenvalues, without repetition, for a linearly independent three-dimensional system, for which the multiplicity of eigenvalues should be three.) The eigenvalues derived, and in consequence the derived initial viral growth rate, are therefore incorrect. I am unclear from the information available to me as to whether this derived term is being used in any further calculations or plots: if it is, these will need amending.

3. I remain concerned about the way in which a proxy for quantification is being reported as a quantitative value. I think it is important here to ensure we are not conflating two issues: (1) whether a proxy for quantification is useful as the best measure we have to get a handle on infection dynamics, and (2) what is the most scientifically appropriate way to report findings from this kind of assay. I think we are all agreed as to (1) - nobody is recommending outright rejection of the manuscript on these grounds! With regard to (2), we are dealing with three types of quantity: C_t, CN (which is not wholly equivalent to C_t: see figure 4B of Freeman et al BioTechniques 26(1999)112-125 for a quick graphical illustration of why not), and viral load. The issue is that these quantities are not wholly interchangeable, and it is potentially misleading to act as if they are. This is summarized succinctly in the last paragraph of the CID letter that reviewer 2 cites: "care must be taken not to directly interpret the raw Ct values from RT-PCR results as quantitation units of viral load, in the absence of an appropriate standard curve, to avoid misunderstanding when measuring viral load across different clinical samples or when comparing 2 nucleic acid testing approaches for SARS-CoV-2/COVID-19 detection." A more detailed description of the underlying chemistry that leads to this issue and its interaction with the mathematics is described in the Freeman et al paper. I am grateful

to the authors for the citations of different approaches taken to this in the literature. However, I think this is tangential for two reasons. Firstly, it is easy to get bogged down in a discussion of comparability between studies - e.g. whilst I enjoyed considering the applicability of a study on aspirated pony mucus to the current situation, I hope you will forgive me for omitting a full discussion of the similarities and differences! Secondly, the modelling of dynamics of assay results in molecular testing is highly cross-disciplinary, and even if there were a study directly comparable with the current situation, the fact that there has been a confluence of authors and reviewers whose regular specialisms do not quite appreciate the details of molecular assays sufficiently to get this aspect of the modelling spot on during an evolving emergency does not mean we should continue to propagate this misunderstanding. The key point here is that reporting C_t or CN values as quantitative values is only valid if quantitative standards have been run _in parallel, on the same assay, at the same time_ as clinical samples. (That is, the situation cannot be rescued by later experiments - those do not constitute "an appropriate standard curve" as described above.) There will of course be a relationship between these values and copy numbers of viral RNA in samples (and the residuals in the correlation should be constrained in an accredited laboratory running any controls and applying some form of Westgard rules), but applying a regression otherwise is misleading unless the additional uncertainties have been taken into account and reported. Applying a quantitative translation based on post hoc experiments is the equivalent of omitting a control on an experiment and then deciding to accept the experiment because the control run later passed. One reason that the accredited laboratories have _not_ reported viral loads in their assay outputs that have not used standards on the assay is that it is generally recognised that this would be poor practice, and would likely lead to loss of accreditation. I do not see why here the academic literature should hold itself to a lesser standard than the routine clinical practice from which this literature is derived. I would repeat that I am not questioning the utility of the analysis to get a handle on viral dynamics during infection, but I am strongly suggesting that reporting this analysis in terms of "viral load" at best could confuse, and at worst is inaccurate and misleading. At the very least, I would caution the authors that whilst it might generate more traction with a general audience to use the term "viral load" here, it will generate commensurate heat amongst specialists (and already has in the literature).

Thank you for the responses/clarifications in response to the other comments/queries.

Author Rebuttal, second revision:

Reviewer #1 (Remarks to the Author):

I'm content with the changes made and thank the authors for their full responses to my comments

Thank you again for your comments!

Reviewer #2 (Remarks to the Author):

Thank you for your responses to my comments. I have no additional comments except that I would suggest to include your response to my enquires on #4 (biological basis for the saturation model) and #6 (potential bias in symptom reporting) in the main text of the revised manuscript.

Thanks! We address the potential for bias in symptom reporting in lines 194-196.

Reviewer #3 (Remarks to the Author):

Thank you for this revised manuscript and accompanying rebuttal.

1. I am grateful to the authors for the pieces of code included with the revised submission. This gives me a better idea of what the authors are intending with the code, although I remain not entirely clear which of the models described in the supplementary information is actually implemented in the different sections of the code, i.e. where the different models are fitted and Akaike Information Criteria derived. It might be helpful to comment the code lightly to make explicit cross-reference to the manuscript. It remains impossible for me to run the code, because the code is heavily dependent on data structures that are not included with the revised submission, and because it is supplied in a form that does not allow me easily to determine package dependencies. Certainly in the case of the figures, it would be possible (if tedious) to reverse engineer the pertinent content of the data structures from the figures themselves, so there seems little reason not to make available such data. I am grateful to the authors for the reminder of the institutional restrictions under which they are working: I do not have much to add to my previous comment, save to reiterate that ultimately I see determination of whether the level of data availability presented here is adequate to be an editorial policy decision.

Apologies for these difficulties. We made the code publicly available via GitHub (https://github.com/BROOKELAB/Viral-dynamics-modeling) and are providing a raw data table that can easily be read into the code. We will also add comments to the code to indicate the connections to the relevant figures in the manuscript. We have indicated in the supplementary text sections describing the Refractory Cell Model and the Immune Effector Cell Model that these two models are the best models used for fitting nasal and saliva data, respectively.

In the new submission, we also make publicly available all the files needed to run Monolix to perform non-linear mixed effect modeling of the viral genome load data (derived from the RTqPCR Ct/CN values). The AIC values are part of the output files from Monolix, i.e. the 'logLikelihood.txt' files in folders in the

directory 'MonolixFiles' (also included in the submission and made publicly available). Note that the reported AICc values were calculated from those AIC values.

2. I am grateful to the authors for expanding on the derivation of original equation [S7] (equation [S8] in the revised version). I think there are two errors in the expanded derivation, one typographical, but one more critical. The typographical error is that to fit the given model, the entry in the first row, third column of the matrix in equation [S7] needs to have the opposite sign. The more critical error is that the characteristic equation with this sign error corrected is ckR_0\delta - (c+\lambda)(k\delta + k\lambda +\delta\lambda + \lambda^2)=0, whereas the value for \lambda the authors derive uses the equation ckR_0\delta - c(k\delta + k\lambda +\delta\lambda + \lambda^2)=0. (It is relatively straightforward to see that there must be _some_ problem, because the authors derive only two eigenvalues, without repetition, for a linearly independent three-dimensional system, for which the multiplicity of eigenvalues should be three.) The eigenvalues derived, and in consequence the derived initial viral growth rate, are therefore incorrect. I am unclear from the information available to me as to whether this derived term is being used in any further calculations or plots: if it is, these will need amending.

Thank you very much for being thorough in reviewing our derivations and pointing out these errors! Yes, the sign in the expression is a typographical error. For the second error you pointed out, the formula for the initial exponential growth rate, r (Eqn. S8 in the previous version) is a good/correct approximation. However, we forgot to include a key assumption/simplification step in the derivation in the supplementary text (that allows us to reduce the ODE further into a two-dimensional system) which will certainly lead to confusion. Apologies!

The key step we forgot to include last time is the quasi-steady state assumption. Biologically, the dynamics of viruses are much quicker than the dynamics of protectively infected cells. For example, the rate of viral clearance is in the time scale of minutes and hours, whereas the death of productively infected cells is in days. This separation of time scale allows us to make the assumption that the viral population dynamics is in a quasi-steady state with the dynamics of infected cells, i.e. dV/dt aprrox.= 0. This assumption is well supported by experiments and is commonly used in the viral dynamic modeling literature (as cited in the revised supplementary text). With this assumption, the virus population is a constant ratio of the productively infected cell population, i.e. V=pi*I/c. This reduces the ODE system into a two-dimensional system with variables E and I, and the expression of the leading eigenvalue for

the Jacobian matrix is expressed as in the formula shown. We explicitly added this assumption and revised the derivations in the supplementary text.

Thanks again for your comments. These really help to improve the readability and rigor of our analyses.

3. I remain concerned about the way in which a proxy for quantification is being reported as a quantitative value. I think it is important here to ensure we are not conflating two issues: (1) whether a proxy for quantification is useful as the best measure we have to get a handle on infection dynamics, and (2) what is the most scientifically appropriate way to report findings from this kind of assay. I think we are all agreed as to (1) - nobody is recommending outright rejection of the manuscript on these grounds! With regard to (2), we are dealing with three types of quantity: C_t, CN (which is not wholly equivalent to C_t: see figure 4B of Freeman et al BioTechniques 26(1999)112-125 for a quick graphical illustration of why not), and viral load. The issue is that these quantities are not wholly interchangeable, and it is potentially misleading to act as if they are. This is summarized succinctly in the last paragraph of the CID letter that reviewer 2 cites: "care must be taken not to directly interpret the raw Ct values from RT-PCR results as quantitation units of viral load, in the absence of an appropriate standard curve, to avoid misunderstanding when measuring viral load across different clinical samples or when comparing 2 nucleic acid testing approaches for SARS-CoV-2/COVID-19 detection." A more detailed description of the underlying chemistry that leads to this issue and its interaction with the mathematics is described in the Freeman et al paper. I am grateful to the authors for the citations of different approaches taken to this in the literature. However, I think this is tangential for two reasons. Firstly, it is easy to get bogged down in a discussion of comparability between studies - e.g. whilst I enjoyed considering the applicability of a study on aspirated pony mucus to the current situation, I hope you will forgive me for omitting a full discussion of the similarities and differences! Secondly, the modelling of dynamics of assay results in molecular testing is highly cross-disciplinary, and even if there were a study directly comparable with the current situation, the fact that there has been a confluence of authors and reviewers whose regular specialisms do not quite appreciate the details of molecular assays sufficiently to get this aspect of the modelling spot on during an evolving emergency does not mean we should continue to propagate this misunderstanding. The key point here is that reporting C_t or CN values as quantitative values is only valid if quantitative standards have been run _in parallel, on the same assay, at the same time_ as clinical samples. (That is, the situation cannot be rescued by later experiments - those do not constitute "an appropriate standard curve" as described above.) There will of course be a relationship between these values and copy numbers of viral RNA in samples (and the residuals in the correlation should be constrained in an accredited laboratory running any controls and applying some

form of Westgard rules), but applying a regression otherwise is misleading unless the additional uncertainties have been taken into account and reported. Applying a quantitative translation based on post hoc experiments is the equivalent of omitting a control on an experiment and then deciding to accept the experiment because the control run later passed. One reason that the accredited laboratories have _not_ reported viral loads in their assay outputs that have not used standards on the assay is that it is generally recognised that this would be poor practice, and would likely lead to loss of accreditation. I do not see why here the academic literature should hold itself to a lesser standard than the routine clinical practice from which this literature is derived. I would repeat that I am not questioning the utility of the analysis to get a handle on viral dynamics during infection, but I am strongly suggesting that reporting this analysis in terms of "viral load" at best could confuse, and at worst is inaccurate and misleading. At the very least, I would caution the authors that whilst it might generate more traction with a general audience to use the term "viral load" here, it will generate commensurate heat amongst specialists (and already has in the literature).

We appreciate the reviewer's concerns here. To avoid confusion/misunderstanding, we have plotted the data in terms of Ct/CN value rather than "viral genome load". We have also updated the manuscript text to make clear that our analyses are based on Ct/CN values, not absolute measurements of viral genome load.

Furthermore, we clearly stated at the beginning of the supplementary text that we performed experiments to derive the calibration curve to translate Ct/CN values to viral genome load for the purpose of viral dynamic modeling only (because this kind of approach represents the best available approach for understanding SARS-CoV-2 viral dynamics).

Decision Letter,third revision:

Dear Chris,

Thank you for submitting your revised manuscript "Daily sampling of SARS-CoV-2 infection reveals substantial heterogeneity in infectiousness" (NMICROBIOL-21081971C). It has now been seen by two of the original referees and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Microbiology, pending minor revisions to comply with our editorial and formatting guidelines.

If the current version of your manuscript is in a PDF format, please email us a copy of the file in an

editable format (Microsoft Word or LaTex)-- we can not proceed with PDFs at this stage.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Microbiology Please do not hesitate to contact me if you have any questions.

Sincerely,

{redacted}

Reviewer #2 (Remarks to the Author):

Thank you for your responses to my earlier comments and I have no further comments.

Reviewer #3 (Remarks to the Author):

Thank you for the detailed responses to my further comments, which all seem appropriate to me.

Decision Letter,final checks:

Dear Chris,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Microbiology manuscript, "Daily sampling of SARS-CoV-2 infection reveals substantial heterogeneity in infectiousness" (NMICROBIOL-21081971C). Please carefully follow the step-by-step instructions provided in the attached file, and add a response in each row of the table to indicate the changes that you have made. Please also check and comment on any additional marked-up edits we have proposed within the text. Ensuring that each point is addressed will help to ensure that your revised manuscript can be swiftly handed over to our production team.

We would like to start working on your revised paper, with all of the requested files and forms, as soon as possible (preferably within two weeks). Please get in contact with us if you anticipate delays.

When you upload your final materials, please include a point-by-point response to any remaining reviewer comments.

If you have not done so already, please alert us to any related manuscripts from your group that are

under consideration or in press at other journals, or are being written up for submission to other journals (see: https://www.nature.com/nature-research/editorial-policies/plagiarism#policy-on-duplicate-publication for details).

In recognition of the time and expertise our reviewers provide to Nature Microbiology's editorial process, we would like to formally acknowledge their contribution to the external peer review of your manuscript entitled "Daily sampling of SARS-CoV-2 infection reveals substantial heterogeneity in infectiousness". For those reviewers who give their assent, we will be publishing their names alongside the published article.

Nature Microbiology offers a Transparent Peer Review option for new original research manuscripts submitted after December 1st, 2019. As part of this initiative, we encourage our authors to support increased transparency into the peer review process by agreeing to have the reviewer comments, author rebuttal letters, and editorial decision letters published as a Supplementary item. When you submit your final files please clearly state in your cover letter whether or not you would like to participate in this initiative. Please note that failure to state your preference will result in delays in accepting your manuscript for publication.

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As you prepare your final files we encourage you to consider whether you have any images or illustrations that may be appropriate for use on the cover of Nature Microbiology.

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Please submit your suggestions, clearly labeled, along with your final files. We'll be in touch if more information is needed.

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If you have any further questions, please feel free to contact me.

{redacted}

Reviewer #2:
Remarks to the Author:
Thank you for your responses to my earlier comments and I have no further comments.

Reviewer #3:

Remarks to the Author:
Thank you for the detailed responses to my further comments, which all seem appropriate to me.

Decision Letter,final checks:

Dear Chris,

I am pleased to accept your Article "Daily longitudinal sampling of SARS-CoV-2 infection reveals substantial heterogeneity in infectiousness" for publication in Nature Microbiology. Thank you for having chosen to submit your work to us and many congratulations.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Microbiology style. We look particularly carefully at the titles of all papers to ensure that they are relatively brief and understandable.

Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required. Once your paper has been scheduled for online publication, the Nature press office will be in touch to confirm the details.

After the grant of rights is completed, you will receive a link to your electronic proof via email with a request to make any corrections within 48 hours. If, when you receive your proof, you cannot meet this deadline, please inform us at rjsproduction@springernature.com immediately. You will not receive your proofs until the publishing agreement has been received through our system

Due to the importance of these deadlines, we ask you to please let us know now whether you will be difficult to contact over the next month. If this is the case, we ask you to provide us with the contact information (email, phone and fax) of someone who will be able to check the proofs on your behalf, and who will be available to address any last-minute problems.

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