

## Estimation of viral kinetics model parameters in young and aged SARS-CoV-2 infected macaques

Thalia Rodriguez and Hana M. Dobrovolny

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### Review timeline

Original submission: 5 January 2021  
Revised submission: 28 July 2021  
Final acceptance: 25 October 2021

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

## Review History

### RSOS-202345.R0 (Original submission)

#### Review form: Reviewer 1

**Is the manuscript scientifically sound in its present form?**

Yes

**Are the interpretations and conclusions justified by the results?**

No

**Is the language acceptable?**

Yes

**Do you have any ethical concerns with this paper?**

No

**Have you any concerns about statistical analyses in this paper?**

Yes

**Recommendation?**

Major revision is needed (please make suggestions in comments)

**Comments to the Author(s)**

Please see the review report (Appendix A).

**Decision letter (RSOS-202345.R0)**

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Dear Dr Dobrovolny

The Editors assigned to your paper RSOS-202345 "Mathematical modeling finds differences in viral kinetics in nasal passages, but not in the throat, of young and aged SARS-CoV-2-infected macaques" have now received comments from reviewers and would like you to revise the paper in accordance with the reviewer comments and any comments from the Editors. Please note this decision does not guarantee eventual acceptance.

We invite you to respond to the comments supplied below and revise your manuscript. Below the referees' and Editors' comments (where applicable) we provide additional requirements. Final acceptance of your manuscript is dependent on these requirements being met. We provide guidance below to help you prepare your revision.

We do not generally allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Editors, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available, we may invite new reviewers.

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Thank you for submitting your manuscript to Royal Society Open Science and we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Kind regards,

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Reviewer comments to Author:

Reviewer: 1

Comments to the Author(s)

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## Author's Response to Decision Letter for (RSOS-202345.R0)

See Appendix B.

## RSOS-202345.R1 (Revision)

### Review form: Reviewer 1

**Is the manuscript scientifically sound in its present form?**

Yes

**Are the interpretations and conclusions justified by the results?**

Yes

**Is the language acceptable?**

Yes

**Do you have any ethical concerns with this paper?**

No

**Have you any concerns about statistical analyses in this paper?**

Yes

**Recommendation?**

Accept as is

**Comments to the Author(s)**

I appreciate the authors for working on the revision in detail. The authors have adequately addressed all the comments I had. I recommend to accept this revised manuscript for publication in Royal Society Open Science.

### Decision letter (RSOS-202345.R1)

We hope you are keeping well at this difficult and unusual time. We continue to value your support of the journal in these challenging circumstances. If Royal Society Open Science can assist you at all, please don't hesitate to let us know at the email address below.

Dear Dr Dobrovolny,

It is a pleasure to accept your manuscript entitled "Estimation of viral kinetics model parameters in young and aged SARS-CoV-2 infected macaques" in its current form for publication in Royal Society Open Science. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.

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The proof of your paper will be available for review using the Royal Society online proofing system and you will receive details of how to access this in the near future from our production office ([openscience\\_proofs@royalsociety.org](mailto:openscience_proofs@royalsociety.org)). We aim to maintain rapid times to publication after acceptance of your manuscript and we would ask you to please contact both the production office and editorial office if you are likely to be away from e-mail contact to minimise delays to publication. If you are going to be away, please nominate a co-author (if available) to manage the proofing process, and ensure they are copied into your email to the journal.

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Thank you for your fine contribution. On behalf of the Editors of Royal Society Open Science, we look forward to your continued contributions to the Journal.

Kind regards,  
Royal Society Open Science Editorial Office  
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[openscience@royalsociety.org](mailto:openscience@royalsociety.org)

on behalf of Dr Shigui Ruan (Associate Editor) and Glenn Webb (Subject Editor)  
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Reviewer comments to Author:

Reviewer: 1

Comments to the Author(s)

I appreciate the authors for working on the revision in detail. The authors have adequately addressed all the comments I had. I recommend to accept this revised manuscript for publication in Royal Society Open Science.

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## Appendix A

**Review Report:** Manuscript “**Mathematical modeling finds differences in viral kinetics in nasal passages, but not in the throat, of young and aged SARS-CoV-2-infected macaques**” by *Rodriguez and Dobrovolny*.

In this manuscript, authors used a basic viral dynamic model to study viral kinetics in nasal passages and throats of young and aged SARS-CoV-2 infected macaques. Based on the parameters estimated from the data fitting to the model, the authors claim that the difference in viral kinetics occur in nasal passages, but not in the throat of young and aged animals. Furthermore, they simulated the model to observe viral dynamics for different treatment timing. While the study of viral dynamics of SARS-CoV-2 within a host, including young and aged hosts, is very important, the analysis performed in this study is limited to make assertive claim that the authors have made. I have some concerns which need to be addressed before accepting for publication. My major concerns are:

- Only one model is used ignoring all other possible mechanism. Justify why this model is the most suitable: can basic model without having eclipse phase describe the data? Is it okay to absolutely ignore the immune responses?
- There are number of animals in both groups in the experiment, but only one data set for each group is considered for data fitting. Is it average?. At least, the standard errors should be indicated if the distinction to be made between the groups.
- The claim based on one data set in each group is hard to justify, especially when there are extremely limited viral data points above the detection limit. For example, you have only 3-5 data points above the limit of detection in young animals. It is in fact not clear or not mentioned how many data points were actually considered for fitting. The data points considered, including those below the limit of detection, might highly affect the parameter estimates.
- The nature of data clearly shows that the data in nasal and throat of young animals are almost the same (1-2 days to reach peak 4-5 days to reach below the limit of detection) and also the data in nasal and throat of aged animals are almost the same (4-5 days to reach peak 10-15 days to reach below the limit of detection). Therefore, I am not fully convinced that your claim on having the different dynamics in nasal passages, but the same dynamics in throat in young and aged animals. The different or the same values of parameters obtained between animals could be due to fitting error. For example, you observed  $\delta$  in throat of young animal to be lower making it similar to aged animal. It could have been because the solution curve was pulled towards the later data points, causing poor fitting; note that almost all the data points were missed in your fitting in throat of young animals (Fig 1). It may be possible to improve. It is known that post peak decay is higher for higher  $\delta$ . My main point is: it is very hard to claim this without further thorough analysis.
- The parameters estimated is based on one fixed set of values for  $k$  and  $c$ , which may vary from animal to animal (especially they may also be different for those two groups). It is important to do analysis on: does the result remain the same for different  $k$  and  $c$  values? What if they are also estimated as it is quite possible that they are different for young and aged animals?
- The statistical significance observed is misleading because these were not obtained based on number of subjects. It is based on data generated through bootstrapping from the models with distinct parameters. So, they are not independent animals data but the data generated from the same parameters with variations. Such claims are misleading.
- Is it enough to measure effectiveness of antiviral therapy, just based on infection duration? What about the level of viral load (peak, etc.)?



- What could be the biological reason to have the intended effect (longer infection duration) of treatment?
- Please explain how you obtained the expression of  $t_{inf}$ . It may not be obvious for many readers.
- Why  $T(0) = 1$  (page 15)? Was it scaled?
- Manuscript needs to be read carefully as it is written with error in number of places. For example:
  - Where is the dashed blue lines in Fig 1? It was pointed out in the figure caption.
  - It is mentioned young and aged “mice” in Figure 4. Mice?

## Appendix B

# Response to Reviewers for: Estimation of viral kinetics model parameters in young and aged SARS-CoV-2 infected macaques

Thalia Rodriguez and Hana M. Dobrovolny

July 23, 2021

Below, we respond to the reviewers' comments and provide a clear description and exact location for any change made to the manuscript to address their comments. We would like to thank the reviewers for their comments.

## Reviewer 1

In this manuscript, authors used a basic viral dynamic model to study viral kinetics in nasal passages and throats of young and aged SARS-CoV-2 infected macaques. Based on the parameters estimated from the data fitting to the model, the authors claim that the difference in viral kinetics occur in nasal passages, but not in the throat of young and aged animals. Furthermore, they simulated the model to observe viral dynamics for different treatment timing. While the study of viral dynamics of SARS-CoV-2 within a host, including young and aged hosts, is very important, the analysis performed in this study is limited to make assertive claim that the authors have made. I have some concerns which need to be addressed before accepting for publication. My major concerns are:

**1.1 Only one model is used ignoring all other possible mechanism. Justify why this model is the most suitable: can basic model without having eclipse phase describe the data? Is it okay to absolutely ignore the immune responses?**

Note that a model without an eclipse phase will not have fewer free parameters since we have fixed the duration of the eclipse phase. That said, it is possible to fit the data with a simpler model without an eclipse phase, but we get best fits with higher SSRs for most of the data sets (see Table below), so we presented the results for the model with an eclipse phase in the manuscript.

Data Set	Eclipse SSR	No Eclipse SSR
Young Nasal	3.23	4.04
Aged Nasal	1.89	1.80
Young Throat	0.46	0.469
Aged Throat	2.35	2.70

We have added this information to the supplementary material.

The issue with more complex models is that they have more parameters that need to be estimated. As the reviewer later points out, the number of data points in each data set is small. While we can fit more complex models to the data and probably get lower SSRs, some of these parameters will not be identifiable, making comparisons of parameter estimates between data sets difficult. While the immune response is not explicitly included, the effect of the immune response is implicitly incorporated in the parameter value estimates since the immune response will change the average death rate of cells (via killing of infected cells by cytotoxic T lymphocytes) or could change the infection or production rates (via interferon).

**1.2 There are number of animals in both groups in the experiment, but only one data set for each group is considered for data fitting. Is it average? At least, the standard errors should be indicated if the distinction to be made between the groups.**

We do not have access to viral time courses in individual animals, only the averaged time courses presented in [14]. We have added the standard errors to Fig. 1.

**1.3 The claim based on one data set in each group is hard to justify, especially when there are extremely limited viral data points above the detection limit. For example, you have only 3-5 data points above the limit of detection in young animals. It is in fact not clear or not mentioned how many data points were actually considered for fitting. The data points considered, including those below the limit of detection, might highly affect the parameter estimates.**

It is common practice to combine multiple measurements into an average time course, for in vitro [11, 12], animal [6], and human challenge studies [2, 10], and make comparisons between the average time courses for the groups. A typical example of this is in determining treatment efficacy of antiviral compounds when researchers assess whether the antiviral shortens the duration of an infection. So while it is one data set in each group, they are not data sets based on measurements from single individuals, but are measurements averaged over several individuals.

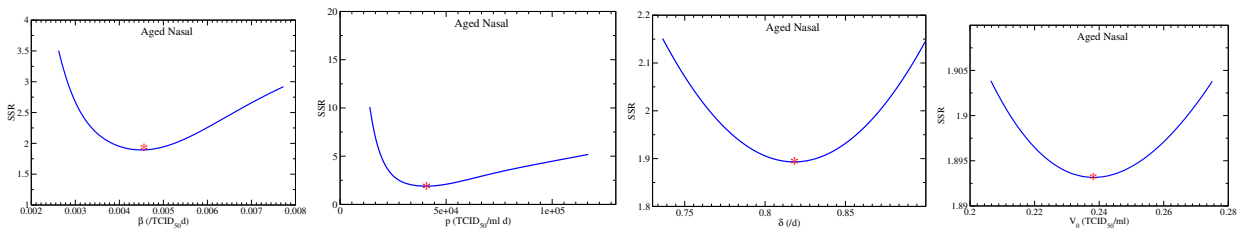
The data set with the smallest number of points above threshold is the time course of virus in the throat of young animals with 4 data points above threshold. The low number of data points above threshold is part of the reason we fixed some of the parameters — by fixing some data points that are not independently identifiable [16], we hopefully improve the identifiability of the parameters that are left. As mentioned in the Methods section, “for

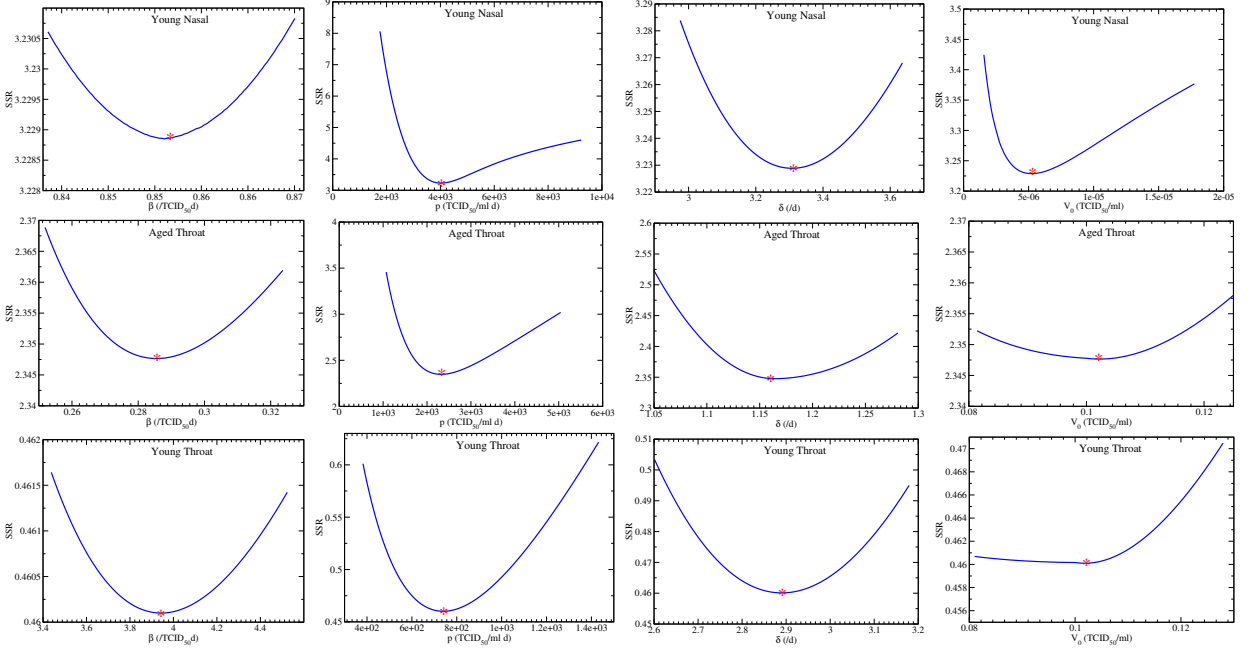
experimental data at the detection threshold, model estimates only contribute to the SSR if they are above the threshold.” So while all points are included in the fitting, the points at threshold near the end of the infection are taken into account while those at later days do not really contribute to the SSR.

**1.4 The nature of data clearly shows that the data in nasal and throat of young animals are almost the same (1-2 days to reach peak 4-5 days to reach below the limit of detection) and also the data in nasal and throat of aged animals are almost the same (4-5 days to reach peak 10-15 days to reach below the limit of detection). Therefore, I am not fully convinced that your claim on having the different dynamics in nasal passages, but the same dynamics in throat in young and aged animals. The different or the same values of parameters obtained between animals could be due to fitting error. For example, you observed  $\delta$  in throat of young animal to be lower making it similar to aged animal. It could have been because the solution curve was pulled towards the later data points, causing poor fitting; note that almost all the data points were missed in your fitting in throat of young animals (Fig 1). It may be possible to improve. It is known that post peak decay is higher for higher  $\delta$ . My main point is: it is very hard to claim this without further thorough analysis.**

We disagree with the assessment of the reviewer that the viral time courses are similar in the nose and throat of both young and aged animals. We agree that the time courses in the nose and throat of young animals have similar time of peak and time to resolution. In aged animals, however, the viral titer time course appears to peak sooner and resolve sooner in the throat of aged animals — this appears to be what the model fits are capturing. That said, we have gone back and re-fit all the data sets using several different algorithms available as options in `scipy.minimize`. This has allowed us to find slightly better fits for all data sets, with the largest decrease in SSR occurring in the fit to the young throat data where the best fit curve now goes through all the earlier time points, but misses the measurement at  $t = 6$  d. This has led to additional statistically significant differences — with  $R_0$  in the nose now also being statistically different between young and old, as well as  $\beta$  and  $\delta$  being different in the throat. Consequently, we have changed the title and modified the manuscript accordingly.

As an additional assessment of whether we have the best fit, we examined the likelihood profiles for all the estimated parameters (shown below and included in the supplemental material).

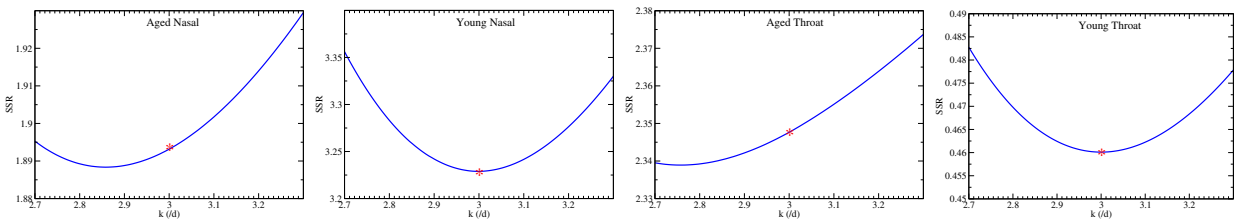




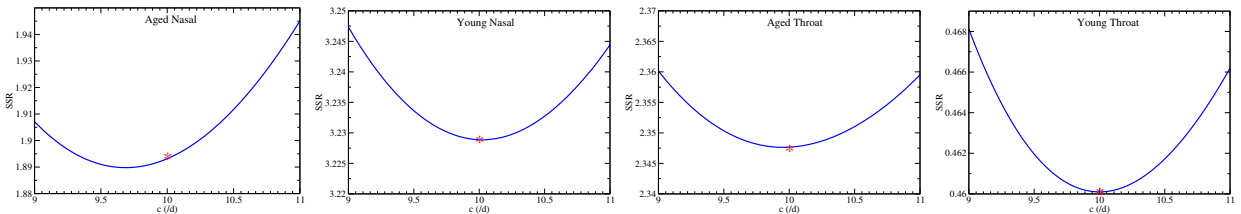
In all cases, the profile has a local minimum, with our best fit estimate falling at the minimum.

### 1.5 The parameters estimated is based on one fixed set of values for $k$ and $c$ , which may vary from animal to animal (especially they may also be different for those two groups). It is important to do analysis on: does the result remain the same for different $k$ and $c$ values? What if they are also estimated as it is quite possible that they are different for young and aged animals?

We agree with the reviewer that it is possible that  $k$  and  $c$  are different for young and aged animals. However, with just viral titer data, only one of  $\delta$ ,  $k$  or  $c$  is identifiable for the target cell limited model used here [16]. In fact, when all three of these parameters are allowed to vary, the parameter estimation often assigns them the same value when using this model to fit viral titer data only [1, 3, 13]. Since the data is limited, as the reviewer points out, and these parameters are not independently identifiable, we chose to fix them, as has been done in other recent COVID modeling studies [7, 8]. To assess the effect of the chosen values of  $k$  and  $c$ , we examine the likelihood profiles for these variables. The likelihood profiles for  $k$  are shown below.



We see that even though  $k$  was not free to vary during the minimization process, the fixed values are near the minimal SSR. The likelihood profiles for  $c$  are shown below.



These are also near the minimal SSR values.

As an additional check, we re-fit the data assuming values of  $k = 1/d$  or  $k = 5/d$  and  $c = 5/d$  or  $c = 20/d$ . As shown in the table below, in most cases, the SSR is lowest for the fixed values of  $k = 3/d$  and  $c = 10/d$ ; we have highlighted fits with lower SSR in bold.

New fixed value	Data set	SSR
$k = 1$ d	Aged Nasal	<b>1.59</b>
	Young Nasal	4.24
	Aged Throat	2.43
	Young Throat	1.23
$k = 5$ d	Aged Nasal	1.96
	Young Nasal	<b>3.23</b>
	Aged Throat	2.41
	Young Throat	0.460
$c = 5$ d	Aged Nasal	<b>1.74</b>
	Young Nasal	3.23
	Aged Throat	<b>2.33</b>
	Young Throat	0.460
$c = 20$ d	Aged Nasal	2.04
	Young Nasal	3.22
	Aged Throat	2.35
	Young Throat	0.469

While there are a some lower SSRs, there is no particular value of  $k$  or  $c$  that improves fits for all the data sets. This assessment of the choice of  $k$  and  $c$  is now also included in the supplementary material.

**1.6 The statistical significance observed is misleading because these were not obtained based on number of subjects. It is based on data generated through bootstrapping from the models with distinct parameters. So, they are not independent animals data but the data generated from the same parameters with variations. Such claims are misleading.**

This type of statistical comparison has been used in other studies [11, 12, 15] to compare parameter estimates for different viral infection conditions. The basic idea is the same as

when statistically comparing measurements from different animal cohorts. In the case of multiple animals, the error in the parameter values is estimated using animal-to-animal variation. We do not have measurements from multiple animals, so we need a different method for estimating the error in our parameter values — bootstrapping is a common technique for estimating error in parameter values determined via model fitting [4, 5]. For this reason, we do not believe the results are misleading. However, we have gone through the manuscript and added text to clarify that the statistical analysis is not based on animal to animal variation and have added the following to the discussion section “Statistical comparisons were performed on parameter distributions estimated through bootstrapping, a technique used to determine variability in model parameter estimates, so do not reflect parameter variability due to animal-to-animal variation.”

**1.7 Is it enough to measure effectiveness of antiviral therapy, just based on infection duration? What about the level of viral load(peak,etc.)?**

We have added the plots for viral peak to Fig. 4.

**1.8 What could be the biological reason to have the intended effect (longer infection duration) of treatment?**

If the dosage of antiviral is not sufficient to suppress production enough to suppress the infection, then reducing the production rate means that the amount of virus within the host increases, but slowly. If the viral load surpasses the threshold of detection, but is increasing slowly, the viral titer will remain above the threshold for a longer period of time. This lengthening of infection duration is combined with a lower viral titer peak (as now shown in the manuscript), so the overall effect on disease severity is not entirely clear. We have included these comments in the manuscript.

**1.9 Please explain how you obtained the expression of  $t_{\text{inf}}$ . It may not be obvious for many readers.**

A derivation of  $t_{\text{inf}}$  is found in [9]. We now point the reader to that reference in the text.

**1.10 Why  $T(0) = 1$  (page 15)? Was it scaled?**

We are using units of relative cell number for the cells. We have noted this in the text.

**1.11 Manuscript needs to be read carefully as it is written with error in number of places. For example: Where is the dashed blue lines in Fig 1? It was pointed out in the figure caption. It is mentioned young and aged “mice” in Figure 4. Mice?**

We have corrected both of these.

## References

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