

# Multiplex serology reveals age-specific immunodynamics of respiratory pathogens in the wake of the COVID-19 pandemic

Corresponding Author: Ms Samantha Bents

**This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.**

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

This study investigates the dynamics of immunity to respiratory viruses in different age groups using serological data and mathematical models. The authors frame this investigation in the context of the SARS-CoV-2 pandemic, when non-pharmaceutical interventions effectively eliminated transmission of many other respiratory viruses. The interruption of transmission provides an opportunity to investigate immune waning in the absence of repeated infections, which complicate the inference of waning rates in other contexts. In addition, the “immune debt” incurred during this period might explain observed changes in the timing, intensity and age distribution of infections once NPIs were revoked.

To investigate immune waning in different age groups, the authors first consider changes in cross-sectional antibody levels between the Summer of 2020 and the Summer of 2021 in Seattle. They find substantial immune waning for several viruses, but unfortunately no samples representative of antibody levels prior to the NPIs were available for adults. For adults, the authors have paired samples bracketing a period when some viruses had already rebounded. (Paired samples are not available for children, though a third cross-sectional sample is available for children post-rebound.) The authors use a statistical model flexible enough to handle these different data types to estimate age-specific waning rates and the magnitude of antibody boosts induced by infection. They complement this analysis by fitting the model to a second dataset of antibody titers to influenza in a South African longitudinal cohort. The results suggest that boosts are stronger and waning rates are faster in children compared with adults. This finding motivates the development of mechanistic models to test if faster waning rates in children can explain differences in influenza epidemics before and after the accumulation of immune debt driven by NPIs targeting SARS-CoV-2. The authors conclude that they can and discuss the implications.

These are important questions that haven't been answered yet, and the data and models presented here are certainly useful for doing so, but I have significant concerns about the central conclusion that waning rates are faster in children compared with adults. I worry that age-specific differences in exposure risk might lead to similar patterns even if waning rates were similar between children and adults. These differences were, as far as I can tell, not allowed in the models, and I think doing so would be necessary to support the conclusion about differences in waning. I also wonder if the stronger boost estimated in children is truly an age-related difference or simply a consequence of the fact that boosts tend to be higher in people with lower pre-exposure titers, regardless of age.

Major comments

1. Could a higher infection risk in children, if unaccounted for in the models, spuriously lead to faster waning rates compared with adults? Here's the intuition: children might have a higher baseline exposure risk or a higher chance of infection per infectious contact (as acknowledged by the authors in the Discussion, when they cite refs 28-33). If children have higher attack rates, a cross-sectional sample of children will on average include more people with a recent infection than a similar sample of adults, so more children than adults will be waning toward some stable long-term titer. If we had paired pre-NPI and “immune-trough” cross-sectional samples for both children and adults, that alone might lead to a stronger drop in geometric mean titer in children, even if, individually, an infected adult and an infected child wane at the same rate. We don't have that direct comparison with the available data in this study, but something similar might happen in the models. As far as I can tell, the serosolver-based model assumes a time-varying attack rate that is shared across all ages. For the mechanistic model I wasn't sure: I see that the authors accounted for age-specific contact rates, but are they proportional or absolute (do kids make more contacts overall than adults? Or do different age groups simply allocate the same number of contacts

differently across age groups? It's important to give a precise definition of  $M_{ij}$ ).

Because faster waning in children is a central conclusion of the study, I think this issue needs to be addressed. Perhaps this is what the authors had in mind when they explained the exclusion of children 5-10 in the cross-sectional data from the model fits (ll. 575-577), but I couldn't follow. (On a related note, why were children 10-18 in South Africa excluded from the fits?)

2. Is the higher boost observed in children simply due to the antibody ceiling effect? The authors describe the antibody ceiling effect as the stabilization of antibody titers with age (ll. 110-112). I'm more familiar with the term being used to describe smaller fold-changes upon infection/vaccination in people with higher pre-exposure antibody levels (e.g. PMIDs: 35199825, 30971703) (which would lead to that stabilization over time). If children have lower pre-infection titers on average due to having fewer past exposures, that might lead to stronger boosts compared with adults. Importantly, we might not see a difference in boost size between children and adults if the model explicitly adjusted for baseline titers. It doesn't, as far as I can tell. I don't think the authors necessarily have to make that change, but it's important to acknowledge that the reported difference in boost size between children and adults might not be related to age itself.

Minor

3. I had a hard time following the serosolver model results when reading the main text. The description in the methods is great, but it would help to give some intuition in the main text. This is a fairly complicated analysis combining different types of data (cross-sectional and longitudinal), and I had to pause and spend some time thinking about how the model would be able to discern higher boosting and waning rates in children.

4. The decline in antibody levels in children is very clear in Fig 1c but hard to see in Fig. 1b. Would a boxplot be better in Fig. 2b? (Ideally with the actual points overlaid, but it might be too many in this case.). Also, the legend has been cut off in Fig 1a.

5. ll. 317-318. Did the immune debt really "disappear" in children after circulation rebounded in 2022? Was that quantified anywhere?

6. ll. 582-583: "The model was fit to two samples per individual in the South African population to maximize sample size". What does this mean? How does this increase sample size?

7. l 596: Should say "in a given time period  $j$ " (currently we don't know what  $j$  is).

8.  $S_i$  is shown as a subscript in eqs. 3 and 4. Looks like a typo.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)

# Manuscript: Multiplex serology reveals age-specific immunodynamics of respiratory pathogens in the wake of the COVID-19 pandemic

1. General Comments:

The manuscript describes the immunity dynamic and the circulation of respiratory viruses during and after the COVID-19 pandemic, to explain the higher burden of viral respiratory infections in children right after the pandemic period. To assess the seroprevalence of antibodies against the respiratory viruses was used a validated multiplex electrochemiluminescence immunoassay (MSD V-PLEX assay). Data from serological survey at US was linked with South Africa serological data and compared with the results obtained using the classical assay (hemagglutination inhibition assay) to detect the antibodies against influenza. The authors used the serological data and epidemiological data to model the immunodynamics of circulating respiratory viruses, the transmission. The modelling also included the age specific antibody dynamics to explain the age specific seroprevalence and respiratory viruses circulation. The authors conclude that was observed a greater antibody boosting and quicker waning in children under 5 yo compared to adults in two populations (USA and South Africa). The inclusion of age-specific serological data was important to anticipate the healthcare encounters.

In general, the manuscript is well written and the methods used are well described. The reviewer consider that seroepidemiological studies are of great value for the knowledge of respiratory viruses dynamics and epidemiology, and are critical to accurate transmission models.

I do, however consider that some revisions and clarifications should be done to the manuscript in order to consider it for publication in Nature Communications.

Comments:

1. In line 188 of the introduction, when is written ". Elevated serum antibody concentration levels can be indicative of a recent pathogen disease exposure" please consider also to include the antibodies acquired by vaccination, especially when talking

about influenza and SARS-CoV-2 in a period of high vaccination coverage.

2. I would suggest to detail in more detail the rationale to include data from South Africa in this study, and the comparability of the expected results in both countries.

3. The described assay used to test sera collected in US, the "V-PLEX COVID-19 Respiratory Panel 3 IgG Kit" is compared with the classical Hemagglutination inhibition classical assay used in South Africa. It should be described how widely is used the seroassay and the comparability with other studies.

4. In table I of Supplementary Material, I suggest to add the description of the number of sera considered in the South Africa study for the age groups >18y.

5. In the Methods section, line 512, I would advise to add the criteria and case definition to the selection of children tested by PCR.

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)

Thank you for the opportunity to review this paper. Given Nature Communications is quite explicit in their reviewer instructions, I used them as a template for comments.

1. What are the noteworthy results? Yes, highlighting age specific antibody kinetics and the importance of taking them into account to understand future risk of disease is noteworthy.

2. Will the work be of significance to the field and related fields? How does it compare to the established literature? If the work is not original, please provide relevant references. The work builds on previous literature, which has also identified the age effect, but the uniqueness of looking at the data post-pandemic to draw out this conclusion is a nice natural experiment.

3. Does the work support the conclusions and claims, or is additional evidence needed? Yes

4. Are there any flaws in the data analysis, interpretation and conclusions? Do these prohibit publication or require revision? No

5. Is the methodology sound? Does the work meet the expected standards in your field? Yes, assuming it is done correctly, for which I have some questions below.

6. Is there enough detail provided in the methods for the work to be reproduced? No. Specifically the methods explaining the approaches to generate Figure 2. See below.

Questions related to result from Figure 2:

As I understand it, the Seattle children (<11yo) data is cross-sectional for each of the three time periods, and there is no infection or vaccination history. Is that correct? If so, can you provide more context for how the model was fit to cross-sectional data but is described as fit to individual antibody level for a given time t.

On a related note, some of the Seattle adult (274/579) was repeat measurements, but not all (line 489). This is basically the same question as above; for repeat data estimating  $Y(i,a,p,t)$  is sensible, but for the 205 individuals with only one measurement, how was this model fit?

The SA data included paired samples, paired by what characteristic? The SA was longitudinal, which better matches the described model. But the methods also state only two time points (line 582) were used instead of three available (line 540).

Can you please show sersolver model fitting diagnostics - convergence plots of goodness of fit plots?

How different did the <5 yr old Seattle model output diagnostics look compared to the 5-11 yr old Seattle model output diagnostics given that it sounds like the model didn't converge for the 5-11 yr old model.

In the github repository, the overview file in the "sersolver\_model" suggests that the King\_County\_Sersolver.R can be run by using the simulated serological simulated\_hcov\_hku1\_pediatric\_serology.csv. But, I was unable to run the code b/c of missing data. The simulated\_hcov\_hku1\_pediatric\_serology.csv is never called in the .R file as suggested via "Line 68" in the overview file. Can you revise the github code/data/overview instructions as needed so it is easier for readers to engage with the repository?

Also, the overview of the code calls for antigenic\_map\_quarters.csv, what is the purpose of this data file? Was an antigenic map incorporated into the model fitting process?

(Remarks on code availability)

I was not able to run the code without error. See comments above.

In the github repository, the overview file in the "sersolver\_model" suggests that the King\_County\_Sersolver.R can be run

by using the simulated serological simulated\_hcov\_hku1\_pediatric\_serology.csv. But, I was unable to run the code b/c of missing data. The simulated\_hcov\_hku1\_pediatric\_serology.csv is never called in the .R file as suggested via "Line 68" in the overview file. Can you revise the github code/data/overview instructions as needed so it is easier for readers to engage with the repository?

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have fully addressed my main concerns by showing that age-specific differences in waning are not an artifact of age-specific differences in exposure. I commend them on the interesting paper and thorough revisions.

I only have a couple of suggestions:

1. The authors added a paragraph in the Discussion to address potential confounding by age-specific differences in exposure (405-420). I think the paragraph sells the analysis short. Lines 409-410 say that a difference in recent exposure "leads to faster estimated waning rates". That's only true if differences in the time since prior infections aren't properly adjusted for, which the authors do as they mention later in the paragraph. So I think it should say something like "which could lead to faster estimated waning rates if differences in the time since recent infection aren't accounted for". Similarly in line 412 ("leading to slower waning wane estimates [if age-specific difference in exposure aren't accounted for]").

2. Thanks for clarifying that  $M_{ij}$  is absolute and not proportional, but I'd still give its precise definition and units (beyond just saying it "describes the contact matrix between individuals in age groups  $j$  and  $i$ "). For instance, is it "contacts per person per day"? Is it an index? Etc.

(Remarks on code availability)

The code is well documented and seems clean and well written. I have not performed an in-depth review. I was able to run one the scripts (King\_County\_Serosolver.R)

Reviewer #3

(Remarks to the Author)

The authors have made thorough and appropriate revisions in response to the reviewers' comments. Thank you. I have no further comments.

(Remarks on code availability)

The code is available on github.

The analysis is separated into two phases, 1) influenza transmission model and 2) serosolver model for individual-level serology data. 1. influenza\_model: R code required to generate age-structured SEIRS influenza transmission model and produce relevant figures. 2. serosolver\_model: R code required to produce estimates of boosting and waning rates from South Africa and King County individual-level serological data. Simulated data provided to run the serosolver model for one antigen for King Country serological data. Data available upon request to run the serosolver model for S. Africa data.

Two "Overview files", available for each phase, and a README file were provided with enough instruction for installing and running the analysis. I was able to run the code for the influenza model, the serosolver model for King County using simulated data. I was not able to run the code for the serosolver model for S. Africa as this data is only available upon request.

**Open Access** This Peer Review File is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

In cases where reviewers are anonymous, credit should be given to 'Anonymous Referee' and the source.

The images or other third party material in this Peer Review File are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0/>

*Multiplex serology reveals age-specific immunodynamics of respiratory pathogens in the wake of the COVID-19 pandemic: Reviewer Response*

**Reviewer #1 (Remarks to the Author):**

This study investigates the dynamics of immunity to respiratory viruses in different age groups using serological data and mathematical models. The authors frame this investigation in the context of the SARS-CoV-2 pandemic, when non-pharmaceutical interventions effectively eliminated transmission of many other respiratory viruses. The interruption of transmission provides an opportunity to investigate immune waning in the absence of repeated infections, which complicate the inference of waning rates in other contexts. In addition, the “immune debt” incurred during this period might explain observed changes in the timing, intensity and age distribution of infections once NPIs were revoked.

To investigate immune waning in different age groups, the authors first consider changes in cross-sectional antibody levels between the Summer of 2020 and the Summer of 2021 in Seattle. They find substantial immune waning for several viruses, but unfortunately no samples representative of antibody levels prior to the NPIs were available for adults. For adults, the authors have paired samples bracketing a period when some viruses had already rebounded. (Paired samples are not available for children, though a third cross-sectional sample is available for children post-rebound.) The authors use a statistical model flexible enough to handle these different data types to estimate age-specific waning rates and the magnitude of antibody boosts induced by infection. They complement this analysis by fitting the model to a second dataset of antibody titers to influenza in a South African longitudinal cohort. The results suggest that boosts are stronger and waning rates are faster in children compared with adults. This finding motivates the development of mechanistic models to test if faster waning rates in children can explain differences in influenza epidemics before and after the accumulation of immune debt driven by NPIs targeting SARS-CoV-2. The authors conclude that they can and discuss the implications.

These are important questions that haven't been answered yet, and the data and models presented here are certainly useful for doing so, but I have significant concerns about the central conclusion that waning rates are faster in children compared with adults. I worry that age-specific differences in exposure risk might lead to similar patterns even if waning rates were similar between children and adults. These differences were, as far as I can tell, not allowed in the models, and I think doing so would be necessary to support the conclusion about differences in waning. I also wonder if the stronger boost estimated in children is truly an age-related difference or simply a consequence of the fact that boosts tend to be higher in people with lower pre-exposure titers, regardless of age.

**Major comments**

1. Could a higher infection risk in children, if unaccounted for in the models, spuriously lead to faster waning rates compared with adults? Here's the intuition: children might have a higher

baseline exposure risk or a higher chance of infection per infectious contact (as acknowledged by the authors in the Discussion, when they cite refs 28-33). If children have higher attack rates, a cross-sectional sample of children will on average include more people with a recent infection than a similar sample of adults, so more children than adults will be waning toward some stable long-term titer. If we had paired pre-NPI and “immune-trough” cross-sectional samples for both children and adults, that alone might lead to a stronger drop in geometric mean titer in children, even if, individually, an infected adult and an infected child wane at the same rate. We don’t have that direct comparison with the available data in this study, but something similar might happen in the models. As far as I can tell, the serosolver-based model assumes a time-varying attack rate that is shared across all ages. For the mechanistic model I wasn’t sure: I see that the authors accounted for age-specific contact rates, but are they proportional or absolute (do kids make more contacts overall than adults? Or do different age groups simply allocate the same number of contacts differently across age groups? It’s important to give a precise definition of  $M_{ij}$ ).

Because faster waning in children is a central conclusion of the study, I think this issue needs to be addressed. Perhaps this is what the authors had in mind when they explained the exclusion of children 5-10 in the cross-sectional data from the model fits (ll. 575-577), but I couldn’t follow. (On a related note, why were children 10-18 in South Africa excluded from the fits?)

We thank the reviewer for raising this thoughtful point. We first want to acknowledge that differences in age-specific infection risk are addressed in both the serosolver modeling approach and the mechanistic model. Yet, this comment has prompted us to give this important question more thought. We have now included additional methodological clarification in the revised version and a deeper discussion of the meaning of our results, as detailed below.

We concur that the serosolver model is indeed detecting more infections in children, as expected due to higher infection risk, but our analyses suggest that differences in waning rates persist when these differences in attack rates are accounted for. In the serosolver model, we fitted the model to each age group separately, and thus the model estimates a time-varying attack rate for each age group independently and their respective serological data, and thus attack rates are not shared across age groups. The model also estimates individual-level infection histories, thus any differences in waning rates between groups account for differences in exposure. We have added text to clarify this in lines 645-646.

In the mechanistic model, children experience increased risk of infection through several mechanisms: 1) increased absolute contacts in  $M_{i,j}$  as you inferred (we clarified that  $M_{i,j}$  is an absolute number of contacts between ages  $i$  and  $j$  in lines 756-757) and 2) a higher proportion of susceptibles compared to other age groups simply because children are born naive and transition to their first infection after some time 3) via increased transmissibility,  $\omega_i$ , which applies a scaling factor on the transmission rate that translates to children under 5 years being 2x more infectious than older age groups (based on prior modeling work). This increased transmissibility, along with a high number of assortative contacts, fuels infections in children

under 5 yrs. Taken together, differences in age-specific infection risk have been addressed in the current methodology and we have added additional text to clarify these points.

Beyond this, we also used the serosolver model to further assess the extent to which age-specific infection rates could potentially bias waning estimates, in line with the reviewer's concerns. We set up an experiment where we *fixed* the prior on the infection rate to be the same between children and adults at 10% and then another where we set the infection rates to be 2x higher in children than adults (based on plausible upper bound differences from published serological studies (10.1016/j.vaccine.2018.04.063, 10.1186/s12879-014-0670-5, 10.1086/652647)). We tested our experiment with the influenza AH3 data from King County. We found that under the scenario where infection rates for children were 2x that of adults (mean attack rate over time = 20%), the waning rate was 0.19 (CrI: 0.14, 0.26), compared to 0.16 (CrI: 0.05, 0.27) for the scenario where we fixed the mean attack rate to be 10%. These estimates overlapped in their credible intervals and were significantly greater than the waning rate estimated for adults. These findings suggest quicker waning estimates for children are robust to a range of plausible attack rates. We added a comment about this sensitivity analysis in Supplementary Methods 1.

Lastly, we did not present the data for the 10-18 year olds in South Africa so the age groups could be compared directly with the available King County serology, which did not sample from children between 10-18 years old. We added a note in the methodology to this effect (lines 660-661). We additionally ran the model on these age groups and have now included the results in Supplementary Table 4. The results suggested that individuals aged 11-18 yo in South Africa exhibited antibody dynamics consistent with older individuals.

Lastly, we added a new paragraph in the discussion that addresses the impact of higher infection risk on antibody kinetics, clinical protection, and broader epidemiological patterns (lines 406-421):

*Longitudinal sero-surveys have demonstrated that antibody kinetics follow a two-phase pattern after pathogen exposure, including a sharp rise and decline over the first 2-3 months, followed by a slower decline that can last for years until the next exposure (35,36). Because young children—and more broadly, immunologically naive individuals—experience high infection rates, sero-surveys in these groups are more likely to capture recent exposures. This increases the likelihood of observing sharp antibody rises and declines, which in turn leads to faster estimated waning rates. In contrast, immunologically primed individuals such as adults are less frequently infected and thus more often captured during periods of slow antibody decline, leading to slower waning rate estimates. Importantly, both our serological analysis framework and our transmission model consider higher infection rates among younger individuals, and the observed age differences in antibody waning remain robust to a range of plausible attack rates (Supplementary Methods 1). We believe that these differences in waning are epidemiologically meaningful, regardless of the underlying exposure-risk mechanisms, as long as antibody titers correlate monotonically with protection against infection. Our serology analysis suggests that*

*faster waning in younger individuals may lead to quicker loss of clinical protection, which may influence the epidemiological dynamics of respiratory viruses.*

2. Is the higher boost observed in children simply due to the antibody ceiling effect? The authors describe the antibody ceiling effect as the stabilization of antibody titers with age (ll. 110-112). I'm more familiar with the term being used to describe smaller fold-changes upon infection/vaccination in people with higher pre-exposure antibody levels (e.g. PMIDs: 35199825, 30971703) (which would lead to that stabilization over time). If children have lower pre-infection titers on average due to having fewer past exposures, that might lead to stronger boosts compared with adults. Importantly, we might not see a difference in boost size between children and adults if the model explicitly adjusted for baseline titers. It doesn't, as far as I can tell. I don't think the authors necessarily have to make that change, but it's important to acknowledge that the reported difference in boost size between children and adults might not be related to age itself.

We appreciate that the reviewer has raised this important point, and concur with the reviewer's comments. The antibody ceiling effect can manifest as both the stabilization of titers with age, and a smaller antibody rises upon exposure in individuals with high pre-existing titers than individuals with low pre-existing titers. We have updated lines 103-104 to clarify this more explicitly.

We fully agree with the reviewer that the size of the boost, along with the pace of waning, are not a feature of age but instead a feature of repeat exposure. We had alluded to this point in the discussion of the original manuscript but have now expanded on this point. Our results for SARS-CoV-2, in which adults experience a large boost closely mirroring the response children exhibited to the antigens of endemic respiratory pathogens, supports that the higher boost is likely driven more so by the number of prior exposures than age. We have added text to clarify this further in the discussion (lines 378-381). In the modeling framework, we make no assumption about what the extent of the boost means in terms of duration or magnitude of protection, and simply note that children tend to experience larger boosts, likely due to lower pre-existing titers. We also note that we implemented versions of the model where we estimated pre-existing titers in addition to boosting and waning parameters for each age group, and found that children experienced slightly higher boosts than adults to all antigens besides SARS-CoV-2. For example, for the influenza AH3 data from King County, we found that fitting baseline titers for children <5 yo led to an estimated antibody boost of 1.38 (95% CrI 0.93, 2.16) titers compared to 1.02 (95% CrI 0.85, 1.21) for adults.

Minor

3. I had a hard time following the serosolver model results when reading the main text. The description in the methods is great, but it would help to give some intuition in the main text. This is a fairly complicated analysis combining different types of data (cross-sectional and longitudinal), and I had to pause and spend some time thinking about how the model would be able to discern higher boosting and waning rates in children.

Thank you for raising our attention to this point. We used the serosolver model to jointly estimate individual-level infection histories, antibody boosting and antibody waning rates which were consistent with the various observed datasets. This is a multilevel model which generates predicted antibody kinetics over time as a function of inferred infection events, assuming that observed antibody titres are normally distributed around the model-predicted antibody level. Note that the same model framework can be fit to different datasets, as the underlying data-generating process is modelled in the same way, with differences in study design and assay being accounted for in the observation model. We have added an introductory paragraph to the beginning of the results to better clarify the purpose of including the various data sources and analyses (lines 135-153):

*To assess how immunological dynamics to endemic respiratory viruses changed during the COVID-19 pandemic, and how those changes in turn influenced transmission dynamics, we drew from multiple data sources. First, we combined virological surveillance and age-stratified serological data from a novel assay from King County, WA, to evaluate how pandemic disruptions impacted antibody dynamics across age groups. We then used the serosolver model to estimate age-specific antibody kinetics parameters such as boosting and waning rates across a range of respiratory pathogens (20). To test the generalizability of our findings across epidemiological contexts, we used the same model to analyze a pre-pandemic influenza serological study from South Africa, which used a more established assay (hemagglutinin inhibition assay) and provided multiple serological samples per person for all age groups. The multi-level serosolver model jointly estimates individual-level infection histories and antibody kinetics parameters and can be fit flexibly to a variety of datasets, with differences in study design and assay being accounted for in the model. This cross-context validation enabled us to distinguish age-related immunological patterns that persisted despite key differences in assay, vaccination rates, local exposure histories, and pandemic-driven disruptions. Finally, we used a mechanistic modeling framework calibrated to influenza epidemiology in King County, WA, to assess whether our insights on age-specific immunological dynamics could improve our understanding of the post-pandemic resurgence of influenza, which exhibited an atypical rebound.*

4. The decline in antibody levels in children is very clear in Fig 1c but hard to see in Fig. 1b. Would a boxplot be better in Fig. 2b? (Ideally with the actual points overlaid, but it might be too many in this case.). Also, the legend has been cut off in Fig 1a.

We have added a median line to Fig 2b to better show the declines in antibody levels and fixed the legend in Fig 1a. Thank you for suggesting these figure improvements. The figure appeared busy with individual points overlaid for all individuals but we added a figure demonstrating this in the Supplementary Fig. 1.

5. II. 317-318. Did the immune debt really “disappear” in children after circulation rebounded in 2022? Was that quantified anywhere?

We assessed this question in Supplementary Tables 2-3, which presented Kolmogorov-Smirnov statistical tests to compare mean antibody concentration levels in 2022 to 2020 baseline. Since 2020 reflects the antibody landscape after a typical respiratory season (2019-2020 winter season), we are testing any differences in 2022 titers with a baseline season. A lack of statistical difference would not rule out the null hypothesis, which is no difference in antibody titers between 2020 and 2022. In other words, if we find a non-significant difference in titers, it means that there is no statistical support for a residual immunity debt in 2022. We found that for the majority of antigens (aside from influenza in young children), there was no statistically significant difference between 2020 and 2022, suggesting that this may be the case (no support for a lingering immunity debt). We have now referenced this finding more explicitly and referenced these findings in Supplementary Tables 2-3 (line 191).

6. ll. 582-583: “The model was fit to two samples per individual in the South African population to maximize sample size”. What does this mean? How does this increase sample size?

Thank you for raising this point. In the serosolver modeling framework, we specified that every individual had the same number of longitudinal samples. In the South Africa data, a smaller number of individuals had greater than 2 samples (i.e. 3-5), but if we only analyzed these individuals, we would have greatly decreased our sample size and thus inferential power. We have rephrased in lines 661-662: The model was fit to two samples per individual available in the South African dataset.

7. l 596: Should say “in a given time period  $j$ ” (currently we don’t know what  $j$  is).

We added this clarification of  $j$ , thank you.

8.  $S_i$  is shown as a subscript in eqs. 3 and 4. Looks like a typo.

This has been fixed, thank you.

## **Reviewer #2 (Remarks to the Author):**

# Manuscript: Multiplex serology reveals age-specific immunodynamics of respiratory pathogens in the wake of the COVID-19 pandemic

### **1. General Comments:**

The manuscript describes the immunity dynamic and the circulation of respiratory viruses during and after the COVID-19 pandemic, to explain the higher burden of viral respiratory infections in children right after the pandemic period. To assess the seroprevalence of antibodies against the respiratory viruses was used a validated multiplex electrochemiluminescence immunoassay (MSD V-PLEX assay). Data from serological survey at US was linked with South Africa serological data and compared with the results obtained using the classical assay (hemagglutination inhibition assay) to detect the antibodies against influenza. The authors used

the serological data and epidemiological data to model the immunodynamics of circulating respiratory viruses, the transmission. The modelling also included the age specific antibody dynamics to explain the age specific seroprevalence and respiratory viruses circulation. The authors conclude that was observed a greater antibody boosting and quicker waning in children under 5 yo compared to adults in two populations (USA and South Africa). The inclusion of age-specific serological data was important to anticipate the healthcare encounters. In general, the manuscript is well written and the methods used are well described. The reviewer consider that seroepidemiological studies are of great value for the knowledge of respiratory viruses dynamics and epidemiology, and are critical to accurate transmission models.

I do, however consider that some revisions and clarifications should be done to the manuscript in order to consider it for publication in Nature Communications.

Comments:

1. In line 188 of the introduction, when is written “. Elevated serum antibody concentration levels can be indicative of a recent pathogen disease exposure” please consider also to include the antibodies acquired by vaccination, especially when talking about influenza and SARS-CoV-2 in a period of high vaccination coverage.

We thank Reviewer 2 for their thoughtful comments and consideration of our manuscript. This is a critical point and we have added this clarification to line 100.

2. I would suggest to detail in more detail the rationale to include data from South Africa in this study, and the comparability of the expected results in both countries.

The inclusion of the data from South Africa served several purposes. First, it provided longitudinal pediatric samples, since only cross-sectional data was available for children from King County, WA, and longitudinal data enables for stronger identification of individual-level immunological dynamics. The South Africa context differs greatly from King County in that influenza vaccination is very rare and the samples were collected pre-pandemic, allowing us to assess whether the age-specific waning patterns observed in King County could be driven primarily by repeated vaccination or pandemic perturbations. We have now added an introductory paragraph to the beginning of the results to better clarify the purpose of including the South Africa data (lines 135-153).

3. The described assay used to test sera collected in US, the “V-PLEX COVID-19 Respiratory Panel 3 IgG Kit” is compared with the classical Hemagglutination inhibition classical assay used in South Africa. It should be described how widely is used the seroassay and the comparability with other studies.

Based on our PubMed searches, there are no published studies that directly compare the V-PLEX Respiratory Panel 3 IgG Kit to the more established Hemagglutinin Inhibition Assay. The V-PLEX Respiratory Panel 3 IgG Kit has been used increasingly since the pandemic, primarily to characterize antibody responses to SARS-CoV-2 vaccination and infection (e.g.

10.1016/j.jcv.2021.105050, 10.20411/pai.v9i2.715, 10.1093/jalm/jfab161). We noted this usage in the methodology.

4. In table I of Supplementary Material, I suggest to add the description of the number of sera considered in the South Africa study for the age groups >18y.

This information is provided in Table 1 of the main text.

5. In the Methods section, line 512, I would advise to add the criteria and case definition to the selection of children tested by PCR.

Thank you, we have added this clarification (lines 582-583).

### **Reviewer #3 (Remarks to the Author):**

Thank you for the opportunity to review this paper. Given Nature Communications is quite explicit in their reviewer instructions, I used them as a template for comments.

1. What are the noteworthy results? Yes, highlighting age specific antibody kinetics and the importance of taking them into account to understand future risk of disease is noteworthy.

2. Will the work be of significance to the field and related fields? How does it compare to the established literature? If the work is not original, please provide relevant references. The work builds on previous literature, which has also identified the age effect, but the uniqueness of looking at the data post-pandemic to draw out this conclusion is a nice natural experiment.

3. Does the work support the conclusions and claims, or is additional evidence needed? Yes

4. Are there any flaws in the data analysis, interpretation and conclusions? Do these prohibit publication or require revision? No

5. Is the methodology sound? Does the work meet the expected standards in your field? Yes, assuming it is done correctly, for which I have some questions below.

6. Is there enough detail provided in the methods for the work to be reproduced? No. Specifically the methods explaining the approaches to generate Figure 2. See below.

Questions related to result from Figure 2:

As I understand it, the Seattle children (<11yo) data is cross-sectional for each of the three time periods, and there is no infection or vaccination history. Is that correct? If so, can you provide more context for how the model was fit to cross-sectional data but is described as fit to individual antibody level for a given time t.

On a related note, some of the Seattle adult (274/579) was repeat measurements, but not all (line 489). This is basically the same question as above; for repeat data estimating  $Y(i,a,p,t)$  is sensible, but for the 205 individuals with only one measurement, how was this model fit?

We thank Reviewer 3 for their thoughtful comments and careful attention to our complex study design and model code. It is correct that there was no observed infection history available for the Seattle individuals, as these were residual samples from hospitals and blood donations available for further research. However for children <11 yo in Seattle, we had information on influenza vaccination history.

Although each child <11 yo in Seattle is sampled only once, the model is jointly inferring a latent history of past infection events for each individual based on their titer measurement at a given time period (e.g., from birth or from 2020 for SARS-CoV-2). This means that if an individual is missing a titer measurement at a given time point (which will be the case for cross-sectional data), the model infers the likely antibody response and whether the individual was infected or not at that time point. Thus, if an individual has a high antibody response at a given time relative to other measurements, the model will likely infer that an infection occurred, and the converse is true as well. From this latent history of past infections, the model is able to jointly infer population-level boosting and waning parameters.

We also acknowledge that it is certainly ideal to have longitudinal data to make inferences about antibody kinetics and this data format would have been preferred if it were available. This was the key motivation for analyzing the South Africa data in addition to the King County data, so that we could assess whether the observed patterns from the cross-sectional data were upheld in a longitudinal context. Our results from South Africa confirmed that the immunological patterns observed in the cross-sectional data from King County persisted in a longitudinal context. In response to this Reviewer and Reviewer 1, we have now added an introductory paragraph to the beginning of the results to better clarify the purpose of including the various data sources and analyses (lines 135-15).

The SA data included paired samples, paired by what characteristic? The SA was longitudinal, which better matches the described model. But the methods also state only two time points (line 582) were used instead of three available (line 540).

Thank you for pointing this out. The South Africa data was paired only in time, meaning that we used two samples for each individual. The data were not paired on any other characteristics. We have clarified this point in line 657-658.

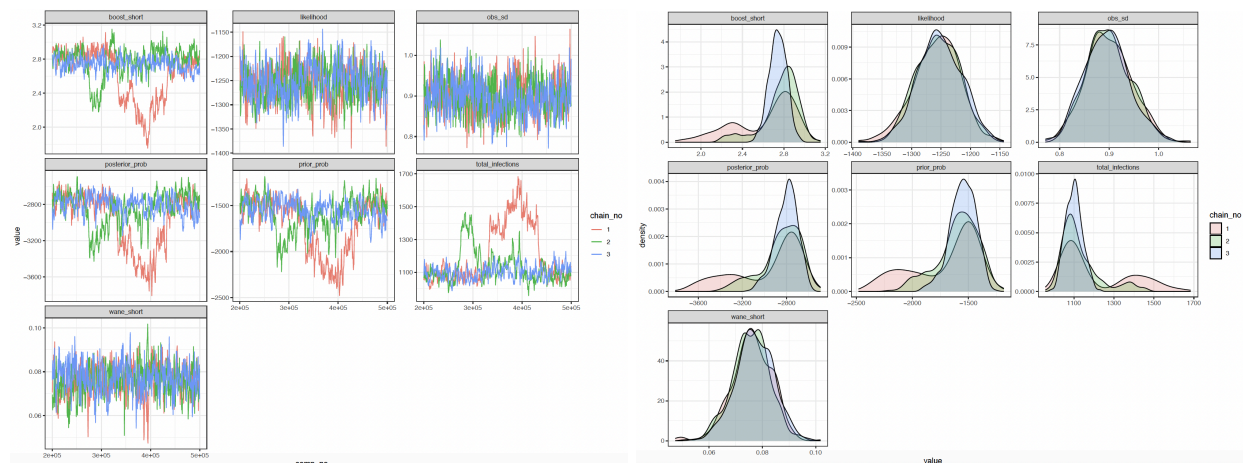
Can you please show serosolver model fitting diagnostics - convergence plots of goodness of fit plots?

We have added model diagnostic plots to the Supplementary Figs. 5 for antigens for one population from King County, WA, and South Africa that demonstrate 1) trace plots showing chain mixing and 2) posterior density plots for estimated parameters.

How different did the <5 yr old Seattle model output diagnostics look compared to the 5-11 yr old Seattle model output diagnostics given that it sounds like the model didn't converge for the 5-11 yr old model.

Below is an example of model output diagnostics (same plots as described above) for children 5-10 yo in King County against an influenza antigen, where  $\hat{R}$  was not  $< 1.01$  for all parameters estimated, and thus results were not presented in the main text. It was likely more difficult to achieve parameter identifiability (and thus model convergence) in this population because individual exposure risk is more varied in this age group compared to the <5 population, which is largely comprised of immunologically naive children experiencing primary infections.

5-10 yo:



In the github repository, the overview file in the "serosolver\_model" suggests that the King\_County\_Serosolver.R can be run by using the simulated serological simulated\_hcov\_hku1\_pediatric\_serology.csv. But, I was unable to run the code b/c of missing data. The simulated\_hcov\_hku1\_pediatric\_serology.csv is never called in the .R file as suggested via "Line 68" in the overview file. Can you revise the github code/data/overview instructions as needed so it is easier for readers to engage with the repository?

Also, the overview of the code calls for antigenic\_map\_quarters.csv, what is the purpose of this data file? Was an antigenic map incorporated into the model fitting process?

The serosolver model can flexibly incorporate antigenic maps when there is data available on multiple lineages of a subtype (e.g. 10.1371/journal.pcbi.1007294, 10.1371/journal.pbio.3002864), yet this is an optional feature. Given that the serological data we analyzed only reported subtype information (e.g. AH3, AH1, B/Vic) that were not specific to specific lineages or strains, our analysis did not necessitate the use of an antigenic map. The modeling framework still requires an antigenic map input, but we set the function that uses it in

the inferential process to null. We have added this information to GitHub so it is clear that the antigenic map is not being used in this analysis, although it could be useful in subsequent analyses if more resolved lineage data were available. We have also added a comment in the methods section (lines 695-699).

Reviewer #3 (Remarks on code availability):

I was not able to run the code without error. See comments above.

In the github repository, the overview file in the "serosolver\_model" suggests that the King\_County\_Serosolver.R can be run by using the simulated serological simulated\_hcov\_hku1\_pediatric\_serology.csv. But, I was unable to run the code b/c of missing data. The simulated\_hcov\_hku1\_pediatric\_serology.csv is never called in the .R file as suggested via "Line 68" in the overview file. Can you revise the github code/data/overview instructions as needed so it is easier for readers to engage with the repository?

We appreciate that the reviewers detected this issue with the publicly available code. The file "King\_County\_Serosolver.R" in the "serosolver\_model" now correctly includes a line to load in a simulated serology dataset which preserves privacy but retains the main features of the original Seattle data (simulated\_hcov\_hku1\_pediatric\_serology.csv) and run the subsequent analysis. We have ensured that the code available on GitHub can now be run seamlessly and two independent scientists have successfully tested the revised code.

## REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have fully addressed my main concerns by showing that age-specific differences in waning are not an artifact of age-specific differences in exposure. I commend them on the interesting paper and thorough revisions.

We thank the reviewer for their positive comments and attention to the importance of accounting for age-specific differences in exposures when estimating antibody kinetics. We believe that highlighting the ways in which our analysis accounted for this in the discussion has strengthened the manuscript.

I only have a couple of suggestions:

1. The authors added a paragraph in the Discussion to address potential confounding by age-specific differences in exposure (405-420). I think the paragraph sells the analysis short. Lines 409-410 say that a difference in recent exposure "leads to faster estimated waning rates". That's only true if differences in the time since prior infections aren't properly adjusted for, which the authors do as they mention later in the paragraph. So I think it should say something like "which could lead to faster estimated waning rates if differences in the time since recent infection aren't accounted for". Similarly in line 412 ("leading to slower waning wane estimates [if age-specific difference in exposure aren't accounted for]").

We agree with this clarification and have updated the text in this paragraph in line with the reviewer's suggestions.

2. Thanks for clarifying that  $M_{ij}$  is absolute and not proportional, but I'd still give its precise definition and units (beyond just saying it "describes the contact matrix between individuals in age groups  $j$  and  $i$ "). For instance, is it "contacts per person per day"? Is it an index? Etc.

The contact matrix is measured in contacts per person per day, which we aggregated to the monthly scale to align with the model's temporal scale. We have clarified this detail in the methods section:

" $M_{ij}$  describes the contact matrix between individuals in age groups  $j$  and  $i$ , where  $M_{ij}$  is measured in contacts per day per person, and children experience a higher absolute number of contacts than adults. We used an expanded version of the contact matrix originally described by Mossong et al., which describes population mixing patterns for

several European countries and aggregated daily contacts to monthly to align with the model's temporal scale.”

Reviewer #1 (Remarks on code availability):

The code is well documented and seems clean and well written. I have not performed an in-depth review. I was able to run one the scripts (King\_County\_Serosolver.R)

We thank the reviewer for confirming that the King\_County\_Serosolver.R script runs seamlessly.

Reviewer #3 (Remarks to the Author):

The authors have made thorough and appropriate revisions in response to the reviewers' comments. Thank you. I have no further comments.

Reviewer #3 (Remarks on code availability):

The code is available on github.

The analysis is separated into two phases, 1) influenza transmission model and 2) serosolver model for individual-level serology data. 1. influenza\_model: R code required to generate age-structured SEIRS influenza transmission model and produce relevant figures. 2. serosolver\_model: R code required to produce estimates of boosting and waning rates from South Africa and King County individual-level serological data. Simulated data provided to run the serosolver model for one antigen for King Country serological data. Data available upon request to run the serosolver model for S. Africa data.

Two "Overview files", available for each phase, and a README file were provided with enough instruction for installing and running the analysis. I was able to run the code for the influenza model, the serosolver model for King County using simulated data. I was not able to run the code for the serosolver model for S. Africa as this data is only available upon request.

We thank the reviewer for their careful review of our analysis code and for verifying that our documentation is both understandable and functional.