

The role of viral interaction in household transmission of symptomatic influenza and respiratory syncytial virus

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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 looks at the impact of co-infection with respiratory viruses on the transmission potential, and also looks at the impact of susceptibility once already infected. The data used has great potential, and the authors use interesting and relevant methodology to add evidence to this very valuable topic. However, some modelling choices are unclear and could potentially be improved. In addition the work (including its limitations) should be better placed in context.

Overall comments

- Model equations should be available in order to evaluate the paper properly. In addition, the code should be made publicly available. (I am aware that the authors say it can be requested from the author, however it is standard practice to make the code publicly available, such as on GitHub. It is required for appropriate review of the work.) The authors don't even state what software the analysis was conducted in, or what fitting mechanism was used.
- The study finds no effect of having an influenza vaccine on influenza, yet an effect on RSV. The authors discuss only the aspect of RSV. Yet the finding that it has no impact on influenza is very surprising (and worrying) and should be discussed. Does this imply there is some deeper flaw with the model? Or are the authors really suggesting that the effectiveness of influenza vaccination is 0%? Or is there some reason why it would be different in this study population?
- Due to the nature of the study, only symptomatic individuals are included in the study. This seems like a major flaw, especially in the case of RSV where adults are often asymptomatic (I think estimates are around 40%). This makes it quite likely that the index case is not the real index, and that some transmissions within the household are not recorded. What are the implications of this? The authors mention this briefly in the discussion, but I think further discussion is needed. Ideally some further testing could be done on this to reduce the impact of the issue. Maybe the authors could run a sensitivity analysis including only contacts that are very young / old and therefore more likely to show symptoms? I'm not sure that is the best suggestion (and would depend on the sample size), but it would be nice to explore the impact of asymptomatic infections further.

Methods

- Why do the authors include the time window beyond the first 14 days of infection within a household as part of the "incidence rate ratio"? I would imagine infections that occur from a secondary case are not relevant to the transmission potential from the primary case?
- Just two age groups seems extremely crude, especially when considering the infection dynamics of RSV and flu. Why did the authors not do a more finegrained approach? (For instance age could instead be treated as a continuous variable, without changing the number of parameters in the model)
- What is the logic behind implementing the individual analysis with the four categories as the main predictor, rather than using two predictors (i.e. index case singly or co-infected? And exposed person already infected with another virus?). The four categories makes it hard to interpret (and also halves the data available for predicting each category, I think....). Particularly, it seems that one aspect (index case co-infection) is testing transmissibility, whereas the other (exposed person already infected) is testing susceptibility.
- The authors state that the individuals are considered vaccinated if they received a flu vaccine 14 days or more before the household exposure. I assume there is also a time-limit in terms of how long ago they received the vaccine? (presumably same season? How do they determine a season?)

Results

- It would be nice to see a spread of the duration between time from primary infection identification to the infection of secondary cases (to complement the very nice Tables 1-3!). This would help the reader understand if we're talking mostly about secondary cases or tertiary.
- It would also be nice to see (potentially in the supplement) some more analysis of the model. For instance, what proportion of the variation is seen between households (e.g. what is the ICC for the model?)

Discussion

- "To our knowledge, the first study to explicitly examines virus-virus associations in the context of respiratory virus transmission". This paper does similar <https://bmcmmedicine.biomedcentral.com/articles/10.1186/s12916-023-03100-5> (although more focused on susceptibility rather than transmission, and different methods etc.) I think this paper should be mentioned in the discussion as it addresses many of the limitations the authors mention regarding previous work done.
- Why do the authors hypothesise they see different results for the different viruses, particularly flu A and B. Wouldn't you expect the results to be the most similar for flu A and B, rather than fluA and RSV?

Abstract

- Only the results for IAV and RSV are mentioned, not IAB.

Introduction

- The intro is very "light" on what previous research exists, and could be expanded further.

Reviewer #2

(Remarks to the Author)

Short summary

The aim of this study was to determine the effect of coinfection versus single infection IAV, IBV or RSV on household transmission. Both infectivity (ie transmission from index case to household members) and susceptibility (risk of infection for household member) were assessed separately. This was done applying a household-level analysis and an individual-level analysis, respectively. The study revealed that the risk of RSV and IAV transmission is lower if the index case has a coinfection with another virus compared to index cases infected with only IAV or RSV. By contrast, household members infected with another virus had a higher risk of acquiring IAV and RSV than household members who were not infected with another virus. The estimates for the IBV analyses were in the same direction as the estimates for IAV and RSV, but not significant.

Overall, the paper is very clearly written and provides valuable, and unique novel insights into the epidemiological effects of viral coinfections. The topic is certainly of interest to the audience of Nature Communications.

Major comments

The study design included respiratory sampling and PCR testing conditional on the presence of acute respiratory illness. Asymptomatic household members were not sampled and there were no additional sample timepoints apart from the onset of illness. This design will certainly results in case underascertainment, as viral infections may occur without symptoms, or with minimal symptoms not meeting the ARI case definition. For the research question of this study, this is particularly relevant if it leads to differential misclassification of the index case; i.e. an infected household member with coinfection has a higher likelihood of being ascertained than a single infected household member, because coinfection is associated with more symptoms. A single infected person could be the true index case but is not detected and a subsequent coinfecting household member is then classified as the index case. The single infected true index case is no longer at risk of infection, but this is not accounted for in the estimation of secondary attack rate. Consequently, this leads to an underestimation of household transmission for coinfecting index cases. This may be an alternative explanation for the observed differences in attack rate from a single versus coinfecting index case. The authors should address this in more detail. Could they think of additional analyses to explore this potential source of case ascertainment bias? For instance, did they observe differences in symptom severity between single versus coinfecting subjects?

All coinfections are pooled, but the risk of transmission may be dependent on the co-infecting virus. There are a high number of HRV/HEV and CoV infections. The authors should consider to include a subgroup analyses per coinfecting virus, as this would yield additional insight into the relative importance of certain coinfections.

Minor comments

#90 How long was follow-up and what was the mean number of illness events per household? This is interesting to know since households could have multiple ARI episodes that were included in the analyses.

#117 Specify the panel of viruses.

#130 Describe the definition of a co-infection more clearly. It is not immediately clear that all viruses tested for are included in the definition and not just a combination of IAV, IBV and RSV.

#147 How were co-index cases handled in the analyses? A sensitivity analysis excluding all co-index cases should be included.

#151 Please provide an explanation why only IAV, IBV and RSV were analysed since data of more viruses is available.

161 What is the rationale for choosing a Poisson for the analysis? I wonder if the assumptions for a Poisson distribution are met in this case. Can the authors explain? An alternative option is a GEE model. (see for example: Verberk, et al. Transmission of SARS-CoV-2 within households: a remote prospective cohort study in European countries. European journal of epidemiology, 37(5), 549–561. <https://doi.org/10.1007/s10654-022-00870-9>)

#214 Authors should include in their table an overview of the time between symptom onset and sampling for single infected

and coinfecting subjects. This could be another source of case ascertainment bias. If differences are present, this should be addressed in the discussion.

#408 It is shortly mentioned that no distinction can be made between new and persistent infections. How can this influence the results, please address in more detail in the discussion.

The limitations section should address the fact that the study was only able to look at symptomatic viral infections, and that the observed effects may not necessarily be generalizable to all virus infections, including asymptomatic ones (and that may be a significant fraction of all viral infections).

Reviewer #3

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #4

(Remarks to the Author)

In the manuscript by Ibiebele et al. the authors analyze the impact of co-infection on the capacity of respiratory viruses to be transmitted within a household. This is a very important topic especially given the limited knowledge the field has about the effect of viral interaction at the transmission level. Due to the common co-circulation of various respiratory viruses whose seasonality coincides in many instances, studies such as the one presented are of significance to design successful prevention strategies. However, there are multiple limitations and weaknesses in the study presented, leading me to suggest major revisions in order to consider this study for publication. The main weakness of the study is the very limited scope, biological justification, and significance of the findings presented. There is also a significant lack of in-depth analyses to gather more evidence to support the authors' conclusions. The finding of reduced transmission of IAV and RSV associated with co-infection is not thoroughly tested and it's only based on the presented model with not further support. Additionally, there are multiple technical problems and lack of validation that contribute to the limitations of the study. Here are some of the major weaknesses I found in the manuscript:

- There are some important methodological problems with the current study such as the definition of an index case purely based on symptom onset. It has been shown in multiple studies with respiratory viruses that this might not be accurate or at least it might lead to some of these cases being falsely tagged as index cases. Given the limited number of co-infections in the cohort, this might be an important bias. Additionally, the models do not include any control for seasonality or overall positivity rate. In periods of high viral circulation, secondary cases might be in fact infections caused outside of the household, especially given the long period allowed for a secondary case. Some validation by molecular methods to confirm transmission within households, at least for a representative proportion of the cases, would have been needed to prove accuracy in the assessment of secondary cases.
- The grouping of RSV cases in the same category is problematic due to the significant differences between RSV A and B. These differences include high genetic divergence, different seasonal patterns, and possibly differences in outcomes. This concern could also be applied to different IAV subtypes that circulated in the seasons studied here.
- The manuscript does not indicate how many of the samples correspond to 2020 where all tests were self-administered. Not to mention the tremendous bias the early stages of SARS-CoV-2 pandemic would have introduced.
- How would these models account for possible asymptomatic index cases? Did the authors test a model with any correction factor that could account for this?
- Why time since symptom onset or time since index case positivity were not included at least in the sensitivity analyses?
- Given that the study is purely based on data modeling, shouldn't the authors have tested other models? Maybe testing models that usually fit better biological data such as negative binomial or zero-inflated models would work better.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have addressed my concerns from the first round of review. However, some of the key parts (e.g. the equations) and some sensitivity analyses (e.g. using continuous age) appear to only have been addressed in the response to reviewer and have not been added to the article itself or the appendix (as far as I can see).

Reviewer #2

(Remarks to the Author)

Major comments

We appreciate the efforts that have been made by the authors to address our comments. We are, however, still concerned

that the limitations of the study design that ignores asymptomatic infections, are not sufficiently addressed. The authors have added an additional analysis excluding person-time from the model but in our opinion, this does not solve the issue. There is still substantial potential for misclassification of both index and secondary cases in the absence of virus testing in participants not meeting the ARI case definition. This may have caused bias in effect estimates in either direction and the investigators are unable to further investigate this. Authors should therefore acknowledge that their findings only apply to symptomatic index cases and to transmission resulting in symptomatic disease. Findings could be different when asymptomatic and mildly symptomatic infections are considered. We propose the authors acknowledge this by further narrowing their aim to study the effect of symptomatic single versus co-infection on transmission. This should also be clear from the title: "the role of viral interaction in household transmission of symptomatic influenza and respiratory 2 syncytial virus"

Furthermore, we agree with the authors claim that studies related to the effect of coinfection on symptomatology have shown mixed results. Next, they argue that community studies have shown no difference and cite Galanti et al. for this. There are however also community studies that did find a difference between single and coinfection on illness severity. For instance, Sarna et al. found higher disease severity among coinfections in children <2 years who were sampled every week. Case ascertainment bias is therefore a valid concern in this study, which should be properly acknowledged.

Minor comments

#260 The statement that virus-virus interactions may depend on specific combinations of viruses is not supported by the cited reference.

#521 The term exposed contact is confusing since it might be read as household members that are exposed to the virus of interest. Alternative: non-IAV/IBV/RSV infected household member.

#578 A sensitivity analysis was performed where the risk window was 2-14 days instead of 1-14 days. How was a household member that tested positive at day 1 handled?

Galanti, M., Birger, R., Ud-Dean, M., Filip, I., Morita, H., Comito, D., Anthony, S., Freyer, G. A., Ibrahim, S., Lane, B., Matienzo, N., Ligon, C., Rabadan, R., Shittu, A., Tagne, E., & Shaman, J. (2019). Rates of asymptomatic respiratory virus infection across age groups. *Epidemiology and infection*, 147, e176. <https://doi.org/10.1017/S0950268819000505>

Sarna, M., Lambert, S. B., Sloots, T. P., Whiley, D. M., Alsaleh, A., Mhango, L., Bialasiewicz, S., Wang, D., Nissen, M. D., Grimwood, K., & Ware, R. S. (2018). Viruses causing lower respiratory symptoms in young children: findings from the ORCHID birth cohort. *Thorax*, 73(10), 969–979. <https://doi.org/10.1136/thoraxjnl-2017-210233>

Reviewer #3

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #4

(Remarks to the Author)

The authors have thoroughly answered and improved the manuscript. Currently, I think this much improved manuscript is ready for publication.

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Authors' Response to *Nature Communications* Review Comments

Comments from the authors: We would like to sincerely thank the reviewers for taking the time to review our work. We believe that edits made as a result of their feedback have strengthened the manuscript. The abstract, tables and figures, and section ordering have been edited to comply with Nature Communications formatting guidelines. Minor edits have been made to make certain language more clear and consistent throughout the manuscript (e.g., using the term “exposed” and “at risk” rather than “susceptible”). References have been added throughout. Edits can be viewed in the manuscript through track changes. Please note that the Methods section has been moved under the Discussion section, per Nature Communications formatting requirements. As such, the track changes format has made it difficult to view edits in this section, so edits made to the Methods section have been highlighted yellow. We have responded to specific reviewer comments below.

Comments from the Editors and Reviewers:

Reviewer #1

This study looks at the impact of co-infection with respiratory viruses on the transmission potential, and also looks at the impact of susceptibility once already infected. The data used has great potential, and the authors use interesting and relevant methodology to add evidence to this very valuable topic. However, some modelling choices are unclear and could potentially be improved. In addition the work (including it's limitations) should be better placed in context.

Response: We thank you for taking the time to provide detailed feedback. In particular, thank you for pointing out the lack of clarity around modeling choices and insufficient discussion of study limitations. We have made several edits to the manuscript to address these concerns. Point-by-point responses to specific feedback are included below.

1. Model equations should be available in order to evaluate the paper properly. In addition, the code should be made publicly available. (I am aware that the authors say it can be requested from the author, however it is standard practice to make the code publicly available, such as on GitHub. It is required for appropriate review of the work.) The authors don't even state what software the analysis was conducted in, or what fitting mechanism was used.

Response: Thank you for pointing out these omissions. A link to the Github in which the code is housed has been added under the “Code Availability” section on page 14 line 853. The statistical software has been added to the methods section on page 12 line 779-781 with the R package and model fitting mechanism used. Model equations have been provided below:

Model equation 1: estimating the household risk of virus transmission within households (j) with coinfecting index cases while accounting for total person-time at risk. Coinfection versus single infection among index cases was the primary predictor (X_1), and the outcome, μ_j , represents the expected number of virus transmissions for household j . This model was adjusted for household size (X_2) and age (<18 and ≥ 18 years) of the index case (X_3). Random intercepts (b_{0j}) were included to account for household clustering.

$$\ln(\mu_j) = \beta_0 + \beta_1 X_{1j} + \beta_2 X_{2j} + \beta_3 X_{3j} + b_{0j} + \ln(\text{person days at risk}) \quad (1)$$

Model equation 2: estimating an individual's (i) risk of infection when multiple viruses circulated simultaneously within their household (j). The main predictor (X_1), was a four-category variable with different combinations of exposure to a coinfecting index case and infection with a virus other than the primary virus of interest. Random intercepts were included to account for household clustering (b_{0j}). The outcome was acquisition of the virus of interest (Y). The age (<18 and ≥ 18 years) of the index case was included as a household-level covariate (X_2). Individual-level covariates included sex (X_3), age group (0-5, 6-11, 12-17, 18-49, and 50+) (X_4), and vaccination status.

$$\text{Logit}(\text{Pr}(Y_{ij})) = \beta_0 + \beta_1 X_{1ij} + \beta_2 X_{2j} + \beta_3 X_{3ij} + \beta_4 X_{4ij} + \beta_5 X_{5ij} + b_{0j} \quad (2)$$

2. The study finds no effect of having an influenza vaccine on influenza, yet an effect on RSV. The authors discuss only the aspect of RSV. Yet the finding that it has no impact on influenza is very surprising (and worrying) and should be discussed. Does this imply there is some deeper flaw with the model? Or are the authors really suggesting that the effectiveness of influenza vaccination is 0%? Or is there some reason why it would be different in this study population?

Response: Our models are not intended to estimate vaccine effectiveness. The confidence intervals for these estimates are quite wide and contain 1. From this, we would not conclude that the vaccine effectiveness was 0%. Further, while out of scope of this paper, vaccine effectiveness has been estimated to be lower within households compared to community exposures, as household exposures tend to involve close proximity for long durations of time. For example, a study by Ohmit et al. (2013) analyzed data from the HIVE cohort and observed no evidence of vaccine effectiveness in preventing within-household transmission of influenza once the virus was introduced into the household (<https://academic.oup.com/cid/article/56/10/1363/404283>). Another paper by Malosh et al. (2021) evaluated direct, indirect, and total vaccine effectiveness within the HIVE cohort, and the 95% confidence interval for their adjusted estimate of direct vaccine effectiveness for adults included '0' (<https://academic.oup.com/cid/article/73/7/1248/6265275>). We do not find this finding to be completely surprising, and we do not believe that this indicates a deeper flaw with our models. Still, the purpose for including vaccination status in our models is to control for potential confounding, not to estimate vaccine effectiveness. We have added a sentence to the discussion to clarify this on page 7, lines 502-503.

3. Due to the nature of the study, only symptomatic individuals are included in the study. This seems like a major flaw, especially in the case of RSV where adults are often asymptomatic (I think estimates are around 40%). This makes it quite likely that the index case is not the real index, and that some transmissions within the household are not recorded. What are the implications of this? The authors mention this briefly in the discussion, but I think further discussion is needed. Ideally some further testing could be done on this to reduce the impact of the issue. Maybe the authors could run a sensitivity analysis including only contacts that are very young / old and therefore more likely to show symptoms? I'm not sure that is the best suggestion (and would depend on the sample size), but it would be nice to explore the impact of asymptomatic infections further.

Response: We agree that the exclusion of asymptomatic cases is a limitation of the underlying cohort study. We performed age-stratified sensitivity analyses for IAV and RSV to the smallest level of granularity that still allowed for model convergence (Supplementary Tables 4-6). We were unable to do so for IBV due to sample size constraints. Please note that we added another

table that presents results from the RSV analysis stratified by ages 0-5 and 6+ (Supplementary Table 6). The sample size for older adults is not large enough to perform any of these analyses exclusively among this age group. We also conducted an additional sensitivity analysis where we excluded person-time at risk in the household-level analyses, eliminating concern that missed asymptomatic cases were included in the denominator/pool of susceptible individuals. Instead, the number of transmissions per household was compared between those with coinfecting and singly infected cases, adjusted for household size. In this sensitivity analysis, having a coinfecting index case remained associated with a reduced likelihood of virus transmission (Supplementary Table 12). We have added two paragraphs to the discussion section to address this concern regarding missed asymptomatic cases in detail: pages 8-9 lines 541-598.

4. Why do the authors include the time window beyond the first 14 days of infection within a household as part of the “incidence rate ratio”? I would imagine infections that occur from a secondary case are not relevant to the transmission potential from the primary case?

Response: We chose to characterize the entirety of each household illness event and therefore would prefer to examine each generation of illness transmission, rather than only including the first generation. While the index case may not be the individual transmitting the virus of interest to susceptible household members beyond 14 days, having an early case that was coinfecting remains a key attribute of that household illness event, indicating cocirculation of multiple viruses within the household. Additionally, households with index and secondary cases may have had susceptible household members who tested positive for a different virus beyond 14 days after the index case’s onset, but within 14 days of the secondary case’s onset. These household members would remain susceptible to the acquisition of the virus of interest from the secondary household member, and we are interested in whether infection with another virus may either block or facilitate infection with the virus of interest. Stopping the analysis 14 days following the index case’s illness onset would preclude us from examining these potential transmissions. We have added the mean and range serial intervals for each virus to Table 1; they are as follows: IAV 3.6 days (1-18), IBV 4.0 days (1-18), and RSV 4.5 days (1-14). We have added a box plot that illustrates the distribution of serial intervals (Figure 2). We also added a sentence describing the percentage of index/secondary pairs with serial intervals ≤ 5 days (page 3 lines 249-251). Due to the relatively short mean serial intervals, we believe that our analyses mostly captured secondary cases rather than tertiary cases.

5. Just two age groups seems extremely crude, especially when considering the infection dynamics of RSV and flu. Why did the authors not do a more finegrained approach? (For instance age could instead be treated as a continuous variable, without changing the number of parameters in the model)

Response: We believe you are referring to the “age of the index case <18 vs. ≥ 18 ” variable. We ran all of our models (household- and individual-level for all three viruses of interest) using a continuous variable for age of the index case and received very similar estimates and confidence intervals for our main predictors. We also compared model performance using AIC and BIC for each analysis, comparing the model with age as a continuous variable versus the one with age as a dichotomous variable. For all but two models (4/6) the AIC and BIC were quite similar. For the IAV household-level Poisson mixed effects models, the model that included age as a continuous variable had lower AIC and BIC than the model with age as a dichotomous variable. On the other hand, for the household-level RSV analysis, the AIC and BIC were lower for the model that included age as a dichotomous variable. We have included these results in the table below. We would like to maintain consistency by using the same age variable in all

models. We have chosen to include the dichotomous variable to improve interpretability (e.g., “having an index case under the age of 18 was associated with an increased risk of transmission...”).

Analysis	Age Variable	AIC/BIC	IRR (95% CI) for main predictor (exposure to coinfecting index case)
Household-level IAV	Dichotomous	1033.832/1055.345	0.44 (0.29—0.66)
Household-level IAV	Continuous	1026.964/1048.477	0.41 (0.27—0.63)
Household-level RSV	Dichotomous	531.812/551.380	0.51 (0.30—0.86)
Household-level RSV	Continuous	534.874/554.442	0.51 (0.31—0.85)

6. What is the logic behind implementing the individual analysis with the four categories as the main predictor, rather than using two predictors (i.e. index case singly or co-infected? And exposed person already infected with another virus?). The four categories makes it hard to interpret (and also halves the data available for predicting each category, I think....). Particularly, it seems that one aspect (index case co-infection) is testing transmissibility, whereas the other (exposed person already infected) is testing susceptibility.

Response: We considered including two separate predictors, as this reviewer has suggested. The 4-category predictor explicitly allows for interactive effects between “exposure to a coinfecting index case” and “susceptible contact infected with a different virus”, whereas using two predictors (index case coinfection and susceptible contact coinfection) would assume that the risk is averaged within those predictors (e.g. that coinfection affects the risk for a susceptible contact equally whether or not the index case is coinfecting.) Instead, category 4 captures those susceptible household members who were infected with other viruses and were also exposed to coinfecting index cases and allows this effect to vary from those exposed to singly infected index cases. Therefore, we believe the 4-category predictor is more appropriate for our research question.

7. The authors state that the individuals are considered vaccinated if they received a flu vaccine 14 days or more before the household exposure. I assume there is also a time-limit in terms of how long ago they received the vaccine? (presumably same season? How do they determine a season?)

Response: Thank you for this clarification. We considered whether the influenza vaccine was received at least 14 days prior to the illness event but within the same season. The HIVE study season ran seasonally from October to May in 2010-2014, and beginning the following year, the study year has run year-round. A study season starts on July 1 of one year and runs through June 30 of the following year. Receipt of the influenza vaccine was assessed each study year. This has been clarified on page 13 lines 807-808.

8. It would be nice to see a spread of the duration between time from primary infection identification to the infection of secondary cases(to complement the very nice Tables 1-3!).

This would help the reader understand if we're talking mostly about secondary cases or tertiary.

Response: Thank you for this suggestion. We have added the means and ranges for the serial intervals to a newly created Table 1. We have also included bar plots to illustrate the distribution of serial intervals for each virus (Figure 2).

9. It would also be nice to see (potentially in the supplement) some more analysis of the model. For instance, what proportion of the variation is seen between households (e.g. what is the ICC for the model?)

Response: ICC's have been added to the captions in Tables 5 and 6.

10. - "To our knowledge, the first study to explicitly examines virus-virus associations in the context of respiratory virus transmission". This paper does similar <https://bmcmmedicine.biomedcentral.com/articles/10.1186/s12916-023-03100-5> (although more focused on susceptibility rather than transmission, and different methods etc.) I think this paper should be mentioned in the discussion as it addresses many of the limitations the authors mention regarding previous work done.

Response: Thank you for directing us to this paper by Waterlow et al. (2023). Indeed, that study focuses on the impacts of viral interaction on susceptibility rather than observing specific transmission events documented between individuals, where the coinfection status of the probable index case is explicitly known, but their results do support our finding of a positive association between infection with a different virus and acquisition of influenza/RSV. We have added this reference to the introduction on page 1 lines 102-104 and the discussion on page 6 lines 442-445 and page 7 lines 485-488.

11. - Why do the authors hypothesise they see different results for the different viruses, particularly flu A and B. Wouldn't you expect the results to be the most similar for flu A and B, rather than fluA and RSV?

Response: We realize that the way we discussed IBV throughout the paper was slightly confusing and may have led readers to believe the direction of associations differed for IBV compared to IAV and RSV. The estimates were generally in the same direction but were not significant and confidence intervals were quite wide, possibly due to sample size constraints. Please note that the confidence intervals for IAV and IBV largely overlap and that most IAV point estimates were included in the IBV confidence intervals. The one exception to this was in the individual-level analysis, category 4, in which the point estimate for IAV was 1.33 (95% CI 0.64-2.75) was narrowly outside of the 95% CI for IBV (1.36-21.37). Therefore, we would not state that our results were definitively different between IAV and IBV.

12. Abstract - Only the results for IAV and RSV are mentioned, not IAB.

Response: We added a sentence about IBV to the abstract – lines 29-30.

13. - The intro is very "light" on what previous research exists, and could be expanded further.

Response: We added several references to the introduction and throughout the manuscript to more comprehensively capture existing literature.

Reviewer #2

1. The study design included respiratory sampling and PCR testing conditional on the presence of acute respiratory illness. Asymptomatic household members were not sampled and there were no additional sample timepoints apart from the onset of illness. This design will certainly results in case underascertainment, as viral infections may occur without symptoms, or with minimal symptoms not meeting the ARI case definition. For the research question of this study, this is particularly relevant if it leads to differential misclassification of the index case; i.e. an infected household member with coinfection has a higher likelihood of being ascertained than a single infected household member, because coinfection is associated with more symptoms. A single infected person could be the true index case but is not detected and a subsequent coinfecting household member is then classified as the index case. The single infected true index case is no longer at risk of infection, but this is not accounted for in the estimation of secondary attack rate. Consequently, this leads to an underestimation of household transmission for coinfecting index cases. This may be an alternative explanation for the observed differences in attack rate from a single versus coinfecting index case. The authors should address this in more detail. Could they think of additional analyses to explore this potential source of case ascertainment bias? For instance, did they observe differences in symptom severity between single versus coinfecting subjects?

Response: We thank you for taking the time to provide detailed feedback. We have made edits throughout our manuscript to address these comments and have provided point-by-point responses below.

Not capturing asymptomatic cases is certainly a limitation of this study that could have led to misclassification of index cases and missed identification of household transmissions. We are unable to effectively compare symptom severity, as all cases were required to have at least two qualifying symptoms in order to be tested, and it would be difficult to grade disease severity due to lack of variability. The potential bias you raised—specifically related to differential misclassification—would be true if coinfecting household members are more likely to be tested than singly infected household members. Studies related to coinfection symptomatology have had mixed results. Several community-based studies have shown no difference in the likelihood or severity of symptoms between coinfecting and singly infected cases (www.cambridge.org/core/journals/epidemiology-and-infection/article/rates-of-asymptomatic-respiratory-virus-infection-across-age-groups/D8E75BDC5B16AEC88DB57CD9B291BB37 and <https://academic.oup.com/jid/article/207/6/982/899260>). If there truly is no consistent difference in the presence or severity of symptoms between singly infected and coinfecting cases, we would not expect the absence of asymptomatic infections to lead to differential misclassification. Still, in order to investigate the potential impacts of the exclusion of asymptomatic cases, we conducted several sensitivity analyses. First, we stratified our IAV and RSV individual-level analyses by the age of the susceptible individuals. Several studies suggest that young children are less likely to be asymptomatic when infected with respiratory viruses compared to other age groups. Therefore, we would expect the estimates in age group 0-5 to be less impacted by misclassification due to missing asymptomatic cases. In these analyses, the observed associations between predictors and acquisition of IAV/RSV were generally in the same direction as in the unstratified analysis. To account for the possibility of having missed a true index case who was then incorrectly included in the pool of susceptible individuals, we conducted another sensitivity analysis where we excluded person-time at risk from the household-level analyses and instead focused only on comparing the number of symptomatic secondary cases within households with coinfecting versus singly infected index cases. We still included the total number of household members as a covariate, but this was to adjust for

household size rather than strictly the number of susceptible individuals. In this sensitivity analysis, having a coinfecting index case remained associated with a reduced likelihood of virus transmission (Supplementary Table 12). We have added two paragraphs to the discussion section to address this concern regarding missed asymptomatic cases in detail: pages 8-9 lines 541-598.

2. All coinfections are pooled, but the risk of transmission may be dependent on the co-infecting virus. There are a high number of HRV/HEV and CoV infections. The authors should consider to include a subgroup analyses per coinfecting virus, as this would yield additional insight into the relative importance of certain coinfections.

Response: We ran the analyses restricting to RV/EV as the coinfecting/cocirculating viruses, then again restricting to cCOV as the coinfecting/cocirculating viruses. These results have been added to the supplementary material (Supplementary Tables 6-9) and referenced in the results section on pages 5 lines 353-381 and discussion page 7 lines 467-477.

3. #90 How long was follow-up and what was the mean number of illness events per household? This is interesting to know since households could have multiple ARI episodes that were included in the analyses.

Response: From 2010-2014, households were followed from October to May. Starting in October 2014, households were followed year-round. We added the mean number of illness events per household to Table 1.

4. 117 Specify the panel of viruses.

Response: This has been added to the methods section on page 10 lines 648-650.

5. #130 Describe the definition of a co-infection more clearly. It is not immediately clear that all viruses tested for are included in the definition and not just a combination of IAV, IBV and RSV.

Response: This has been clarified on page 11 lines 732-734.

6. #147 How were co-index cases handled in the analyses? A sensitivity analysis excluding all co-index cases should be included.

Response: Co-index cases were not included in the analyses as at-risk household members. This has been clarified on page 12 lines 752-753.

7. #151 Please provide an explanation why only IAV, IBV and RSV were analysed since data of more viruses is available.

Response: For this analysis, influenza viruses and RSV were chosen as the primary viruses of interest due to their public health importance, including their potential for severe illness among vulnerable groups, and because they are targets of vaccination campaigns.

8. # 161 What is the rationale for choosing a Poisson for the analysis? I wonder if the assumptions for a Poisson distribution are met in this case. Can the authors explain? An alternative option is a GEE model. (see for example: Verberk, et al. Transmission of SARS-CoV-2 within households: a remote prospective cohort study in European

Response: Poisson mixed effects regression models were chosen for the household-level analyses because the outcome is a count (number of secondary cases within the household) divided by total person-time at risk for the household. We also did these analyses using Poisson GEE models, which generally produced similar results with wider confidence intervals for the IAV and RSV analyses. A comparison of the IRR and 95% CI between these two types of models is summarized in the table below. To maintain consistency across all viruses of interest, we decided to also use a mixed effects model for IBV, despite the slightly wider confidence interval compared to the GEE model. In addition to the estimates being more precise than those of the GEE models, mixed effects models are generally thought to be better able to account for clustering than GEE models. For these reasons, we have chosen to use Poisson mixed effects regression models for the household-level analyses.

Virus of Interest	Poisson Mixed Effects Model IRR (95% CI)	Poisson GEE Model IRR (95% CI)
IAV	0.44 (0.29—0.66)	0.54 (0.29—1.01)
IBV	0.85 (0.39—1.84)	0.84 (0.39—1.80)
RSV	0.51 (0.30—0.86)	0.50 (0.26—0.98)

9. #214 Authors should include in their table an overview of the time between symptom onset and sampling for single infected and coinfecting subjects. This could be another source of case ascertainment bias. If differences are present, this should be addressed in the discussion.

Response: We have created a new Table 1 that includes the mean (range) days from symptom onset to specimen collection for coinfecting and singly infected cases. For IAV the mean (range) were 2.5 (0-7) for coinfecting and 2.6 (0-10) for singly infected cases. For IBV, the mean (range) were 2.7 (0-8) for coinfecting cases and 2.6 (0-9) for singly infected cases. For RSV, the mean (range) were 2.9 (0-8) for coinfecting cases and 2.8 (0-9) for singly infected cases. We do not believe these values represent substantial differences between coinfecting and singly infected cases. Therefore, we do not believe this is a likely source of case ascertainment bias.

10. #408 It is shortly mentioned that no distinction can be made between new and persistent infections. How can this influence the results, please address in more detail in the discussion.

Response: We have expanded upon this in the limitations section, page 9 lines 600-617.

11. The limitations section should address the fact that the study was only able to look at symptomatic viral infections, and that the observed effects may not necessarily be generalizable to all virus infections, including asymptomatic ones (and that may be a significant fraction of all viral infections).

Response: We have added two paragraphs to the discussion section to address this concern regarding missed asymptomatic cases in detail: pages 8-9 lines 541-598.

Reviewer #3:

1. There are some important methodological problems with the current study such as the definition of an index case purely based on symptom onset. It has been shown in

multiple studies with respiratory viruses that this might not be accurate or at least it might lead to some of these cases being falsely tagged as index cases. Given the limited number of co-infections in the cohort, this might be an important bias. Additionally, the models do not include any control for seasonality or overall positivity rate. In periods of high viral circulation, secondary cases might be in fact infections caused outside of the household, especially given the long period allowed for a secondary case. Some validation by molecular methods to confirm transmission within households, at least for a representative proportion of the cases, would have been needed to prove accuracy in the assessment of secondary cases.

Response: We thank you for taking the time to provide detailed feedback. We have made several edits to the manuscript to address these concerns and provide point-by-point responses to specific feedback below.

First, we will address the comment related to mistaking community acquisition for household transmission. This is a valid concern. There have been several studies that have utilized molecular testing to explore transmission of influenza viruses within the HIVE cohort. One study by Valesano et al. (2020) examined IBV transmission within HIVE households from 2010-2017. Of the household index-secondary case pairs that had high-quality sequencing data, all (15/15) were confirmed to represent true household transmissions. This paper can be accessed here: <https://journals.asm.org/doi/10.1128/jvi.01710-19>. Another study by McCrone et al. (2018) examined IAV transmission within HIVE households from 2010-2015. Of the household index-secondary pairs that had high-quality sequencing data, the authors determined that 90% (47/52) represented true household transmissions. This paper can be accessed here: <https://elifesciences.org/articles/35962>. Unfortunately, we do not have such data for RSV. Regardless, these data, along with the understanding that household exposures typically encompass the highest-risk exposures, lead us to believe that misclassification of transmission events may be minimal in our study and would be unlikely to severely impact our results. This has been addressed in further detail in the discussion (pages 9-10 lines 617-627).

2. The grouping of RSV cases in the same category is problematic due to the significant differences between RSV A and B. These differences include high genetic divergence, different seasonal patterns, and possibly differences in outcomes. This concern could also be applied to different IAV subtypes that circulated in the seasons studied here.

Response: Unfortunately, we do not have results that differentiate RSV A/B subgrouping, and therefore we are unable to separate these subtypes in our analysis. We added this as a limitation (page 10 lines 639-641). We did perform the household- and individual-level analyses for H3N2 and H1N1, and we have added these results to the supplementary material (Supplementary Tables 10-11). These are referenced in the manuscript on page 5 lines 383-387.

3. The manuscript does not indicate how many of the samples correspond to 2020 where all tests were self-administered. Not to mention the tremendous bias the early stages of SARS-CoV-2 pandemic would have introduced.

Response: We have added the number of samples that correspond to the period of self-collected swabs on page 11 lines 711-713. The last IAV case included in the analysis occurred on March 12, 2020; the last IBV case occurred on March 4, 2020; the last RSV case occurred on March 15, 2020. The Michigan stay-at-home order was enacted March 23, 2020. No cases included in the analysis occurred past the stay-at-home order date. A paper by Fine et al. (2023)

characterized viral circulation within the HIVE cohort during the first year of the pandemic and found no influenza or RSV circulation within the study population between April through September of 2020 (<https://onlinelibrary.wiley.com/doi/10.1111/irv.13106>). Therefore, we do not believe the SARS-CoV-2 pandemic led to tremendous bias in our study. For clarity, we have added more detail regarding this in the results section, page 3 lines 228-230.

4. How would these models account for possible asymptomatic index cases? Did the authors test a model with any correction factor that could account for this?

Response: It is difficult to predict the exact impact of missed asymptomatic cases in our analysis. Multiple community-based studies have identified no difference in the likelihood or severity of symptoms between coinfecting and singly infected cases. Still, in order to investigate the potential impacts of the exclusion of asymptomatic cases, we conducted several sensitivity analyses. First, we stratified our IAV and RSV individual-level analyses by the age of the susceptible individuals. Several studies suggest that young children are less likely to be asymptomatic when infected with respiratory viruses compared to other age groups. Therefore, we would expect the estimates in age group 0-5 to be less impacted by any misclassification due to missing asymptomatic cases. In these analyses, the observed associations between predictors and acquisition of IAV/RSV were generally in the same direction as in the unstratified analysis. To account for the possibility of having missed a true index case who is then incorrectly included in the pool of susceptible individuals, we conducted another sensitivity analysis where we excluded person-time at risk from the household-level analyses and instead focused only on the number of symptomatic secondary cases within each household, adjusting for total household size. In this sensitivity analysis, having a coinfecting index case remained associated with a reduced likelihood of virus transmission (Supplementary Table 12). We have added two paragraphs to the discussion section to address this concern regarding missed asymptomatic cases in detail: pages 8-9 lines 541-598.

5. Why time since symptom onset or time since index case positivity were not included at least in the sensitivity analyses?

Response: The log of the total person-time at risk was included in the household-level analyses. We defined this variable as the time from index case positivity to either symptom onset of secondary cases or the end of the at-risk period for non-secondary cases. We were not able to include person-time at risk in the individual-level analyses due to singular fit issues.

6. Given that the study is purely based on data modeling, shouldn't the authors have tested other models? Maybe testing models that usually fit better biological data such as negative binomial or zero-inflated models would work better.

Response: We tested a negative binomial model for the household-level analyses because there was overdispersion of the Poisson model for IAV with dispersion ratio of 0.999 using `dispersion_glmmer()`. Underdispersion was detected for the negative binomial, with a dispersion ratio of 0.939. Both models resulted in similar estimates: IRR 0.45 (95% CI: 0.29 – 0.68) for the negative binomial vs. IRR 0.44 (95% CI: 0.29 – 0.66) for the Poisson. We chose to stick with the Poisson because the dispersion ratio was closer to 1. We referenced this article: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6290908/>, which suggests a dispersion ratio <1.2 is acceptable for Poisson models. For the IBV and RSV analyses, no overdispersion was detected with the Poisson models, and therefore we chose to use the Poisson models. No zero-inflation was detected with the Poisson models for the IAV, IBV, and RSV analyses and

therefore, the use of zero-inflated models was not appropriate. A summary of the other models tested has been added as Supplementary Table 13.

The authors would like to, once again, sincerely thank the reviewers for their time and effort in reviewing this manuscript.

Reviewer #1 (Remarks to the Author):

The authors have addressed my concerns from the first round of review. However, some of the key parts (e.g. the equations) and some sensitivity analyses (e.g. using continuous age) appear to only have been addressed in the response to reviewer and have not been added to the article itself or the appendix (as far as I can see).

Response: We thank you for taking the time to review this manuscript and provide valuable feedback. The information you requested has been added to the supplementary material. The rationale for not using a continuous age variable can be found in Table 13, and the model equations can be found in Table 14.

Reviewer #2 (Remarks to the Author):

Major comments

We appreciate the efforts that have been made by the authors to address our comments. We are, however, still concerned that the limitations of the study design that ignores asymptomatic infections, are not sufficiently addressed. The authors have added an additional analysis excluding person-time from the model but in our opinion, this does not solve the issue. There is still substantial potential for misclassification of both index and secondary cases in the absence of virus testing in participants not meeting the ARI case definition. This may have caused bias in effect estimates in either direction and the investigators are unable to further investigate this. Authors should therefore acknowledge that their findings only apply to symptomatic index cases and to transmission resulting in symptomatic disease. Findings could be different when asymptomatic and mildly symptomatic infections are considered. We propose the authors acknowledge this by further narrowing their aim to study the effect of symptomatic single versus co-infection on transmission. This should also be clear from the title: "the role of viral interaction in household transmission of symptomatic influenza and respiratory 2 syncytial virus"

Response: We have clarified that we only considered symptomatic infections in the title and abstract. In the discussion (page 9, lines 414-415), we stated that our findings may only apply to symptomatic infections.

Furthermore, we agree with the authors claim that studies related to the effect of coinfection on symptomatology have shown mixed results. Next, they argue that community studies have shown no difference and cite Galanti et al. for this. There are however also community studies that did find a difference between single and coinfection on illness severity. For instance, Sarna et al. found higher disease severity among coinfections in children <2 years who were sampled every week. Case

ascertainment bias is therefore a valid concern in this study, which should be properly acknowledged.

Response: We agree with the reviewers' assessment that some community studies may suggest greater severity associated with coinfections versus single virus infections. Our intent was to highlight the fact that there has been no consistent association between symptomatology and coinfection versus single virus infection identified in the literature. We edited the sentence on page 9, line 390 to clarify that we are referring to symptomatology more broadly—that is, the presence or absence of symptoms as well as symptom severity. In the study by Sarna et al., specifically in the analysis evaluating the presence of acute respiratory infection symptoms versus asymptomatic infections, the risk ratios and attributable fraction in the exposed for “any virus” in the single virus analysis are similar to those for codetections, with overlapping confidence intervals. In the analysis examining lower respiratory tract infections, the risk ratio and attributable fraction in the exposed are higher for infections involving codetections compared to those involving “any [single] virus.” From this we would conclude that in this study population, while coinfections may have been associated with a greater risk of lower respiratory tract infections, the presence or absence of any ARI symptoms was similar between infections involving codetections and single virus detections. Therefore, we do not believe that the findings from this study refute our statement that the results of community-based studies suggest that coinfecting cases would not necessarily have been more likely to meet the ARI case definition required for testing than singly infected cases. We thank you for directing us to this study, and we have added it as a reference (page 9, lines 403-407). Additionally, we did acknowledge that case ascertainment bias could have biased our findings (page 8, lines 344-347). We also edited the beginning of the paragraph starting on page 8, line 360 to clarify that this entire paragraph refers to potential bias related to the exclusion of asymptomatic cases.

Minor comments

#260 The statement that virus-virus interactions may depend on specific combinations of viruses is not supported by the cited reference.

Response: We switched the cited reference for this statement (page 6, line 274).

#521 The term exposed contact is confusing since it might be read as household members that are exposed to the virus of interest. Alternative: non-IAV/IBV/RSV infected household member.

Response: We changed the language to “household contact” as a result of this feedback. We previously considered using the suggested language; however, we do not feel that it is accurate to say “non-IAV/IBV/RSV infected household member” since asymptomatic individuals were not tested.

#578 A sensitivity analysis was performed where the risk window was 2-14 days instead of 1-14 days. How was a household member that tested positive at day 1 handled?

Response: These were re-characterized as co-index cases rather than susceptible household contacts. This has been clarified in the methods section (page 13, lines 594-595).

Galanti, M., Birger, R., Ud-Dean, M., Filip, I., Morita, H., Comito, D., Anthony, S., Freyer, G. A., Ibrahim, S., Lane, B., Matienzo, N., Ligon, C., Rabadan, R., Shittu, A., Tagne, E., & Shaman, J. (2019). Rates of asymptomatic respiratory virus infection across age groups. *Epidemiology and infection*, 147, e176. <https://doi.org/10.1017/S0950268819000505>

Sarna, M., Lambert, S. B., Sloots, T. P., Whitley, D. M., Alsaleh, A., Mhango, L., Bialasiewicz, S., Wang, D., Nissen, M. D., Grimwood, K., & Ware, R. S. (2018). Viruses causing lower respiratory symptoms in young children: findings from the ORChID birth cohort. *Thorax*, 73(10), 969–979. <https://doi.org/10.1136/thoraxjnl-2017-210233>

Reviewer #3 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response: We thank you for taking the time to review and provide valuable feedback.

Reviewer #4 (Remarks to the Author):

The authors have thoroughly answered and improved the manuscript. Currently, I think this much improved manuscript is ready for publication.

Response: We thank you for your time and for providing valuable feedback that improved this manuscript.