Evaluating reduced effectiveness after repeat influenza vaccination while accounting for confounding by recent infection and within-season waning

Qifang Bi, Barbra A. Dickerman, Huong Q. McLean, Emily T. Martin, Manjusha Gaglani, Karen J. Wernli, Balasubramani Goundappa, Brendan Flannery, Marc Lipsitch*, Sarah Cobey* , the US Flu Vaccine Effectiveness Network Investigators **

**Study group team members are listed in the acknowledgements.

1 Abstract

Background. Studies have reported that prior-season influenza vaccination is associated with higher risk of clinical influenza infection among vaccinees in a given season. Understanding the underlying causes requires consideration of within-season waning and recent infection.

Methods. Using the US Flu Vaccine Effectiveness (VE) Network data over 8 influenza seasons (2011-2012 to 2018-2019), we estimated the effect of prior-season vaccination on the odds of clinical infection in a given season, after accounting for waning vaccine protection using regression methods. We adjusted for potential confounding by recent clinical infection using inverse-probability weighting. We investigated theoretically whether unmeasured subclinical infection in the prior season, which is more likely in the non-repeat vaccinees, could explain the repeat vaccination effect.

Results. Repeat vaccinees vaccinated earlier in a season by one week. After accounting for waning VE, repeat vaccinees were still more likely to test positive for influenza A(H3N2) (OR=1.11, 95% CI:1.02-1.21) but not for influenza B (OR=1.03, 95% CI:0.89-1.18) or A(H1N1) (OR=1.03, 95% CI:0.90-1.19) compared to those vaccinated in the given season only. Recent clinical infection with the homologous (sub)type protected against clinical infection with A(H3N2) or B. Individuals with clinical infection in one season had 1.11 (95% CI:1.03-1.19) times the odds of switching vaccination status in the following season. Adjusting for recent clinical infections did not strongly influence the estimated effect of prior-season vaccination. Adjusting for subclinical infection could theoretically attenuate this effect.

Conclusion. Waning protection and recent clinical infection were insufficient to explain observed reduced VE in repeat vaccinees with a test-negative design.

Keywords. influenza; vaccine; waning vaccine protection; infection history; test negative design

2 Introduction

Many national health authorities and the World Health Organization recommend annual influenza vaccination of persons at high risk, with some countries recommending universal vaccination [1,2]. Despite these recommendations, vaccination coverage usually remains significantly lower than desired [3,4]. Variation in influenza immunization policy and vaccine hesitancy can be partially attributed to difficulties in estimating the direct effectiveness and indirect benefits of influenza vaccination accurately [2].

A controlled study over four decades ago first raised questions about repeated annual influenza vaccination, reporting that vaccination in an earlier season indirectly affected the risk of infection in the current season [5,6]. It was not until a test-negative study in Canada [7], a vaccine trial in Hong Kong [8]

and a household-based study in the United States [9] independently investigated vaccine effectiveness (VE) and immunogenicity among repeat vaccinees and non-repeat vaccinees in the 2009/10 and 2010/11 seasons that the phenomenon was investigated routinely [10–16]. Since then, reduced VE against A(H3N2) among repeat vaccinees compared with non-repeat vaccinees was observed during the 2010/11 and 2012/13 seasons in the United States [9,13,17], the 2014-2015 season in Canada and Europe [14,15], and the 2018/19 season against certain subclades of A/H3N2 in Europe [18]. Such effects have been less often reported for A(H1N1) and type B [10,11], which are comparatively less prevalent, though pooled analyses have shown this effect for type B [11].

Test-negative studies conducted in healthcare settings have become the standard way to evaluate vaccine protection, including in repeat vaccinees. A test-negative design estimates VE by comparing vaccination coverage in persons with a medically-attended acute respiratory illness who test positive for influenza with those who test negative [19]. Several factors that may bias estimates of repeat vaccination effects in observational studies, including those using test-negative design, have not been accounted for. Vaccine-induced protection against influenza virus infection wanes within a season [20–28][29–34]. As a result, the vaccine protection estimated among otherwise similar groups of vaccinees may differ if the timing of vaccination is not taken into account. If repeat vaccinees tend to vaccinate substantially earlier in a season, waning protection could make the risk of infection among repeat vaccinees appear higher than that among non-repeat vaccinees.

Infection in past seasons may provide immune protection against infection in the current season. The infection block hypothesis [6,35–37] suggests that prior vaccinations block opportunities to experience highly immunogenic clinical influenza virus infections, which can lead to more cross-reactive and durable immune responses than vaccine-induced immunity, especially when circulating viruses differ from vaccine strains [38,39]. If true, the infection-block hypothesis could explain increased risk of infection among repeat vaccinees compared to non-repeat vaccinees, because repeat vaccinees are less likely to have experienced clinical or subclinical infections in the previous season than non-repeat vaccinees due to protection from prior vaccinations, and therefore, repeat vaccinees may have less immune protection at the start of an influenza season than non-repeat vaccinees. This might lead to a higher incidence of clinical influenza virus infection in the current season among repeat than among non-repeat vaccinees. The difference in risk between the two groups can be further amplified if recent infection, which is expected to be more likely in non-repeat vaccinees, improves vaccine immunogenicity and thus vaccine-induced protection [38], as has also been recently observed for SARS-CoV-2 [40].

In epidemiologic terms [41], under the infection-block hypothesis, infection in the previous season is a mediator between vaccination in the previous season and a clinical infection outcome in the current season. When we estimate the effect of repeated vaccination, as a mediator, infection in the previous season does not on its own introduce a source of bias. However, if infection in the previous season influences the decision to vaccinate in the current season as well as the probability of clinical infection in the current season, then it is also a confounder that can bias the estimated effect of repeated vaccination on clinical infection. Because infection in the previous season may be both a mediator and a confounder, appropriately adjusting for it requires an approach that can handle this treatment-confounder feedback, such as inverse-probability weighting [42].

We defined the repeat vaccination effect as the causal effect of the prior-season vaccination on odds of clinical PCR-confirmed influenza infection among individuals vaccinated in the current season. We designed analyses of the repeat vaccination effect that account for waning and the confounder-mediator status of prior-season clinical infection, and then conducted a theoretical analysis to consider the possibility that prior-season subclinical infection could explain the repeat vaccination effect. We chose to study the repeat vaccination effect defined as the relative odds of clinical infection because it is conceptually simpler than the observation of reduced VE from repeat vaccination. Our definition compares risks in two groups: those with vaccination in the previous and current seasons (repeat) vs. current season only (non-repeat). In contrast, the VE comparisons require risk estimates for those vaccinated only in the previous season and those vaccinated in neither season: they assess a ratio of risk (or odds) ratios, which is more complex and thus harder to interpret.

To assess the potential influence of within-season waning of vaccine-induced protection on the repeat vaccination effect, we determined whether the timing of vaccination differed between repeat and non-repeat vaccinees using data collected from the five US Flu VE Network sites and eight influenza

seasons, from the 2011-2012 season through the 2018-2019 season. We then assessed the effect of repeated vaccination after accounting for intra-season waning of vaccine protection (results in Section 4.1).

To assess whether *clinical* infection in the prior-season may have biased our estimate of the effect of repeated vaccination by acting as a confounder, we performed a second set of analyses. Using surveillance and enrollment data from the Marshfield Clinic Health System (MCHS), one of the US Flu VE Network sites, we assessed whether non-repeat vaccinees were enriched with individuals who had experienced recent clinical influenza virus infection compared with repeat vaccinees, i.e., we examined whether the decision to vaccinate depended on recently confirmed influenza virus infection. We then adjusted for confirmed clinical infection history using inverse-probability weighting (results in Section 4.2).

Finally, we theoretically assessed the plausibility of the infection block hypothesis and enhanced VE from recent *subclinical* infections as explanations for the repeat vaccination effect. Specifically, we evaluated what rates of prior subclinical infections and what level of infection-induced protection or VE enhancement in repeat and non-repeat vaccinees were consistent with the observed effect of repeated vaccination. We provide important justification for considering timing of vaccination, waning of VE, and infection history when estimating infection risk (results in Section 4.3).

3 Methods

3.1 Study setting and study population

During the study period, the US Flu VE Network consisted of five study sites in Wisconsin, Michigan, Washington, Pennsylvania, and Texas [9,12,13,17,21,27,43]. During each enrollment season, outpatients 6 months of age and older were eligible for recruitment if they presented with acute respiratory illness with symptom onset within the last 7 or 10 days depending on the Flu VE site. Each eligible patient completed an enrollment interview that included questions on specific time and location for plausibility and status of influenza vaccination in the study enrollment season (the current season), status of influenza vaccination in the season immediately preceding the study enrollment season (the previous season), demographic information, and underlying health conditions. Participants were tested for influenza by real-time reverse transcription polymerase chain reaction (rRT-PCR) assay. Influenza-positive samples were first typed and then A-sub- or B-lineage-typed. For simplicity throughout we refer to individuals with PCR-confirmed symptomatic influenza virus infection (including those medically attended and not attended) as having “clinical infection”. Influenza vaccination status was confirmed by reviewing immunization records and state registries. Repeat vaccinees are defined as individuals vaccinated in both the current season and the previous season. Non-repeat vaccinees are defined as individuals vaccinated in the current season only. Conditions that may increase the risk of complications attributable to severe influenza are defined as high-risk conditions. High-risk conditions were chronic pulmonary (including asthma), cardiovascular (excluding isolated hypertension), renal, hepatic, neurologic, hematologic, or metabolic disorders (including diabetes mellitus). Persons who were immunocompromised due to any cause, including but not limited to immunosuppression caused by medications or human immunodeficiency virus infection, are also considered to have a high-risk condition.

We analyzed data collected over 8 seasons (from the 2011-2012 through the 2018-2019 seasons) from all five sites. We defined vaccinated individuals as persons who were vaccinated 14 or more days before illness onset, for consistency with prior analyses. We excluded individuals who received more than one dose each season before symptom onset and were under 1 year of age at vaccination. We conducted sensitivity analyses that additionally excluded individuals vaccinated after the start of a season (i.e., after the first symptomatic case was detected at each site) to reduce potential differential depletion of susceptible individuals to influenza virus infection [20].

Data from the MCHS, the US Flu VE Network site in Wisconsin, were available over 12 seasons (from the 2007-2008 through the 2018-2019 seasons). Enrollment history and rRT-PCR testing history, which were not available from the other sites, were available at the MCHS starting from the 2007-2008 season. MCHS is the main outpatient and inpatient care provider for nearly all 61,000 residents in its

contiguous geographic area [44]. Analyses that involve adjustment for prior clinical infection were performed using data from MCHS only.

3.2 Statistical analyses

Causal framework: The effect of repeated vaccination can be conceptualized as a contrast of vaccination strategies sustained over two influenza seasons (the previous and the current season). That is, the repeat vaccine strategy involves receipt of an influenza vaccine in the previous season and current season, and the non-repeat vaccine strategy involves no receipt of an influenza vaccine in the previous season, but receipt of one in the current season. In Supplementary Section 3, we include a directed acyclic graph (Supplementary Figure 3.1) that encodes our assumptions about the underlying causal structure in this two-season framework, and we describe the potential confounding and selection bias [45]. However, due to data constraints, we simplified parts of the analyses to address questions under a single-season framework.

Accounting for within-season waning of vaccine protection: For the analyses in this section, we used data across the five sites in the US Flu VE Network. Because infection status in the previous season was not available across all sites in the Flu VE Network, we estimated relative odds of infection under a single-season framework and treated previous-season vaccination status as a pre-baseline variable (a component of the L node in Supplementary Figure 3.1).

We first determined whether the timing of vaccination in the current season differed between repeat and non-repeat vaccinees. We restricted this analysis to current-season vaccinees and fit a linear regression model with the dependent variable of calendar week of vaccination in the current season and the following independent variables: prior-season vaccination status, age group (0-4, 5-9, 10-19, 20-64, 65+), sex, presence of at least one documented high-risk condition in the prior season (referred to as comorbidity), and influenza season.

Using logistic regression models, we then estimated the relative odds of clinical infection among repeat vaccinees with reference to non-repeat vaccinees after adjusting for time of vaccination in the current season to account for the waning of vaccine protection (see methods in Supplementary Section 2). The study outcome is (sub)type-specific PCR-confirmed clinical infection. Independent variables are an indicator for having been vaccinated 2-9, 10-13, 14-17, 18-21, or over 21 weeks before symptom onset in the current season, a dichotomous indicator for having been vaccinated only in the prior season, a dichotomous indicator for having been vaccinated in both the current and the prior season, age group, sex, comorbidity, influenza season, study site, and calendar month of symptom onset. Using the same model, we assessed the waning of vaccine protection by comparing the relative odds of infection among individuals tested 2-9, 10-13, 14-17, 18-21, and ≥22 weeks after vaccination with respect to those not vaccinated in the current season.

Adjustment for clinical infection history Because MCHS was the only site that had linked previous study enrollment and infection history for participants, only data from MCHS could be used to assess the impact of clinical infection history. In this section, we estimated the effect of repeated vaccination after adjusting for clinical infection history under a two-season framework (Supplementary Section 3).

To determine how a clinical infection outcome in the current season is associated with clinical infection with the homologous (sub)types and separately with the heterologous (sub)types in any prior season (the $I_0 \rightarrow I_1$ edge in Supplementary Figure 3.1), we assessed the odds ratio of clinical infection in the current season among 3 groups of individuals with reference to those whose last detected clinical infection was 1-2 seasons before the current season. The three groups are individuals whose last detected clinical infection was 3-5 seasons before the current season, 6-9 seasons before the current season, as well as individuals who had no documented infection during the enrollment seasons (i.e., since the 2007-2008 season) indicating that their most recent prior clinical infection was reported ≥ 9 seasons before the current season, dated back to seasons outside of our study period, or that they had

no detected symptomatic infection. The reference group is individuals whose last detected clinical infection was 1-2 seasons before the current season. The other independent variables were sex, age category, comorbidity, calendar month, influenza season, and current- and previous-season vaccination status. We excluded from this analysis individuals enrolled during the 2009-2010 season and the 2009 Pandemic season due to the atypical circulation of virus and vaccine types.

We then assessed whether clinical influenza virus infections in the previous season influenced the decision to vaccinate in the current season using logistic regression models (the $I_0 \rightarrow V_1$ edge in Supplementary Figure 3.1). Only the vaccination status in the current season and the prior season were used because we wanted to use the same vaccination records that were collected by the other US Flu VE Network sites. The dependent variable is vaccination in the current season, and the independent variable is infection status of any (sub)type in the previous season. The model is stratified by vaccination status in the previous season. The model additionally adjusts for age group, sex, comorbidity, and an indicator of vaccination frequency (i.e., out of the three seasons immediately before the enrollment season, the number of seasons in which individuals were vaccinated). We excluded those who were vaccinated in the previous season after being clinically infected in that season. We assessed individuals' tendency to switch vaccination status after clinical infections by age group, separately among individuals vaccinated and unvaccinated in the previous season.

Next, we estimated the effect of repeated vaccination after adjusting for the clinical infection status of any (sub)type in the previous season. Because clinical infection status in the previous season is both a confounder and a mediator of the joint effect of current- and previous-season vaccination status on infection in the current season, to handle this treatment-confounder feedback, we used inverse-probability weighting to account for clinical infection status of any (sub)type in the previous season (see Supplementary Section 3 for detailed methods). Weights are calculated based on the inverse of each individual's probability of being vaccinated in each season, given their previous vaccination status, infection status in the prior season, influenza season, and baseline covariates (i.e., sex, age group, comorbidities). These inverse-probability weights are then "stabilized" using the probabilities of being vaccinated given vaccination history, influenza season, and the above-mentioned baseline covariates (but not infection status). To account for the possible influence of the timing and intensity of influenza epidemics each season on the timing of vaccination, we excluded individuals who were vaccinated after the start of a season (i.e., individuals who were vaccinated after the first 5% of PCR-confirmed cases presented symptoms in each season) under the assumption that the decision to vaccinate early in a season is not correlated with risk factors for infection (e.g., age and comorbidities). We accounted for waning vaccine protection in the main analyses. We omitted it in sensitivity analyses for consistency with a typical longitudinal cohort analysis of a 2-season period, which would not condition on timing of vaccination as a post-baseline variable in the weighted outcome model. For the main analyses, individuals who were not clinically infected in the previous season consisted of those who did not enroll in the previous season, those who were eligible but refused enrollment, and those who were enrolled but tested negative for influenza. We excluded those who were eligible but refused enrollment in the previous season as a sensitivity analysis [46].

Impact of subclinical infection To understand how subclinical infection history not detected by the US Flu VE Network may impact the effect of repeated vaccination, we evaluated the proportion of repeat and non-repeat vaccinees who would have had to have been subclinically infected in the previous season to reproduce the observed effect of repeated vaccination. Fundamentally, we aimed to assess the impact of misclassification of infection status in the previous season (the I_0 node in Supplementary Figure 1) using a single-season framework. To achieve this objective, we built a theoretical model and created a pseudo population of repeat and non-repeat vaccinees with various infection statuses in the previous seasons (see Supplemental Section 4 for detailed methods). We then made assumptions about protection conferred by clinical and subclinical infection in the prior season against future infection in the various groups. That is, we assumed clinical infection in the previous season confers perfect protection against clinical infection in the current season, whereas subclinical infection in the previous season confers partial protection in the current season. We also assumed vaccination status in the previous season does not affect the odds of infection in the current season. Based on estimates from studies in the US [47], we assumed a 1% clinical attack rate among vaccinated individuals (denoted by a) and 2% among unvaccinated individuals (denoted by x in Supplementary Section 4). We also assumed a 1.5% current-season clinical attack rate among vaccinees not infected in the previous

season (denoted by $\gamma\theta$). We performed a sensitivity analysis using estimates representing a high incidence setting [47][48]. We illustrated how the results are consistent with both the infection block hypothesis and the hypothesis of enhanced vaccine immunogenicity post-infection under different interpretations of the same parameters.

Data and relevant code are available at https://github.com/cobeylab/FluVE_repeatvac_public.

4 Results

Between the 2011-2012 and 2018-2019 seasons, individuals enrolled in the US Flu VE Network contributed 61,943 visits, of which 55,728 (90.0%) met the inclusion criteria of our analyses. Of those included in our analyses, 50.2% (27,986/55,728) of visits were by individuals who had received one dose of the current seasonal influenza vaccine ≥14 days prior to illness onset date (Supplementary Figure 1.1). Among those vaccinated ≥14 days prior to illness onset, 73.7% (20,630/27,986) of visits were by individuals who were vaccinated at least once in the previous season, and who we refer to as repeat vaccinees (Supplementary Table 1.1).

Surveillance data from the MCHS were available between the 2007-2008 season and the 2018-2019 season. In total, 16,378 individuals were enrolled during these 12 seasons and contributed 24,399 visits (Supplementary Figure 1.1). Among them, 12,057 individuals over 9 seasons (the 2008-2009 season, the 2010-2011 through 2018-2019 seasons) were included in the main analyses that study the impact of clinical infection (Supplementary Table 1.2). These individuals contributed 15,362 visits. Among the 7,262 individuals who had received one dose of the current seasonal influenza vaccine ≥14 days prior to illness onset, 83.0% (n=6,024) had also been vaccinated in the previous season, and 92.2% (n=6,693) had been vaccinated in at least one of the three seasons immediately before the enrollment season. Among those who presented with acute respiratory symptoms and were eligible for enrollment in the prior season, 68.4% (754/1,102) were enrolled.

4.1 Impact of waning vaccine protection

On average, repeat vaccinees of similar age, sex, and comorbidities were vaccinated 1.1 (95%CI: 1.0-1.2) weeks earlier than non-repeat vaccinees (Figure 1A). Also, consistent with previous work [20] we estimated waning of vaccine protection with time since vaccination in the current season (Figure 1C). Thus, when estimating the impact of repeat vs. non-repeat vaccination on odds of clinical infection in the current season, we adjusted for the timing of vaccination in the current season to account for waning vaccine protection within a season (see subsection 6.1 for detailed methods). After adjusting for the timing of vaccination in the current season, we found that repeat vaccinees had 1.03 (95%CI: 0.89, 1.18) times the odds of testing positive for type B than non-repeat vaccinees, similar to the OR of 1.06 (95%CI: 0.92-1.22) before adjustment (Figure 1C). Likewise, the adjustment did not strongly alter the odds of clinical infection with A/H1N1pdm09 among repeat vaccinees compared with non-repeat vaccinees (pre-adjustment OR=1.08 (95%CI: 0.94-1.24) to post-adjustment OR=1.03 (95%CI: 0.90-1.19)). The adjustment did not change the observation that repeat vaccinees had higher odds of clinical infection with A/H3N2 than non-repeat vaccinees (post-adjustment OR=1.11 (95%CI: 1.02, 1.21) vs. pre-adjustment OR=1.13 (95%CI: 1.04,1.23)). The effect of repeated vaccination against A/H3N2 was particularly strong among the 10-19-year-olds (post-adjustment OR=1.48, 95%CI: 1.18, 1.87; Supplementary Figure 2.2). In summary, adjusting for the timing of vaccination in the current season did not notably change the findings of a marked repeat vaccination effect for A/H3N2 and had little to no effect for A/H1N1pdm09 and type B (see Supplementary Figure 2.3 for variation in estimates by season and site).

In models accounting for the timing of vaccination as well as for whether an individual was vaccinated in the previous season, we observed that odds of infection against all three (sub)types increased within a season (Figure 1C). Compared with individuals not vaccinated in the current season (with the highest risk of testing positive), individuals vaccinated 2-9 weeks before testing had lower OR (0.29 [95%CI 0.23, 0.35]) than those vaccinated 18-21 weeks before testing (OR 0.66; 95%CI 0.56, 0.78) for A/H1N1pdm09-associated illness. Similarly, compared with individuals not vaccinated in the

current season, the odds of testing positive for infection with type B increased from 0.37 (95%CI 0.30, 0.45) among individuals vaccinated 2-9 weeks before testing to 0.54 (95%CI: 0.46, 0.64) 18-21 weeks before testing. Compared with individuals not vaccinated in the current season, the odds of testing positive for infection with A/H3N2 increased from 0.68 (95%CI: 0.61, 0.76) among individuals vaccinated 2-9 weeks before testing to 0.84 (95%CI: 0.75, 0.94) 18-21 weeks before testing. In the 2014-2015 season, when there was a mismatch between the A/H3N2 component and the circulating strains, the odds of infection decreased with time from vaccination in Wisconsin (Supplementary Figure 2.1). Excluding the 2,850 individuals (5.1%) who vaccinated after the start of an influenza season (i.e., after the first case was detected at each site) did not qualitatively change the estimates (Supplementary Figure 2.4).

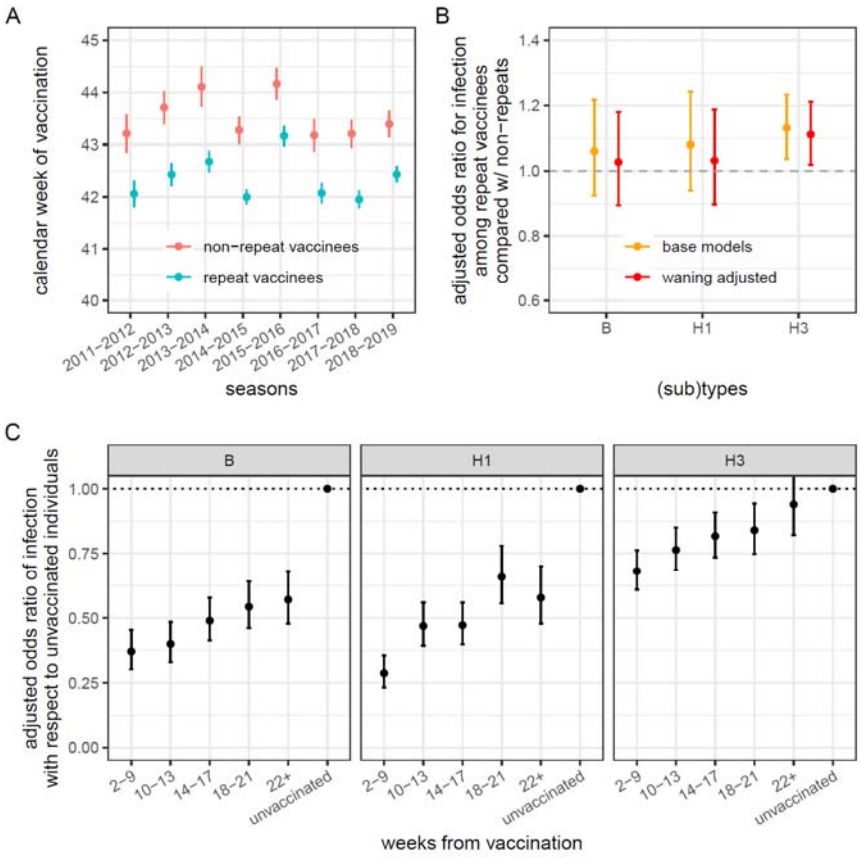

Figure 1: Impact of waning influenza vaccine effectiveness within a season. A) Average calendar week of vaccination among repeat and non-repeat vaccinees over the study enrollment seasons. Repeat vaccinees consistently get vaccinated earlier than non-repeat vaccinees. B) Adjusted odds ratio for clinical infection among individuals vaccinated this season stratified based on whether the individuals were also vaccinated in the prior season (repeat vaccinees) or not (non-repeat vaccinees) before (yellow) and after (red) adjusting for the timing of vaccination within a season. Site- and season-specific data were shown in Supplementary Figure 2.3). C) Adjusted odds ratio of clinical infection comparing individuals vaccinated 2-9, 10-13, 14-17, 18-21, and 22+ weeks before testing positive with respect to those not vaccinated in the current season. Site- and season- specific data were shown in Supplementary Figure 2.1. See Supplementary Section 2 for detailed definitions of the quantities reported here.

4.2 Impact of clinical infection history

We used data from only the MCHS to assess the impact of clinical infection history since it was the only site in the US Flu VE Network that had linked previous enrollment and testing history on enrolled individuals. We found that prior clinical infections of the homologous (sub)type protected against clinical infections of type B or A/H3N2, with more recent infections conferring stronger protection (Figure 2A); those infected with type B more than 6 seasons ago had 3.44 (95%CI: 1.04-11.4) times the odds of testing positive for type B in the current season than those who were clinically infected in the previous 1-2 seasons (Figure 2A). A similar trend was observed for clinical infections against A/H3N2 (OR=33.6, 95%CI: 4.52-250, Figure 2A). We did not find clinical infections of a heterologous (sub)type to be protective (Supplementary Figure 3.2). Due to the limited number of A/H1N1pdm09 infections during our enrollment period, we were not able to assess the impact of homologous infection with A/H1N1pdm09.

We also found that having confirmed influenza virus infections in previous seasons can influence people's decision to vaccinate in the current season. Unvaccinated individuals were more likely to vaccinate in the current season (OR=1.23, 95%CI: 1.11-1.35) if they were clinically infected in the previous season than if they were not infected. However, individuals who became infected after being vaccinated in a previous season were as likely to be unvaccinated in the current season as those not infected (OR=0.97, 95%CI: 0.85-1.10), with the exception of the oldest age group: adults over the age of 65 years who experienced a confirmed infection in the previous season were less likely to be unvaccinated in the current season (OR=0.62, 95%CI: 0.42-0.91) compared with those in the same age group who did not experience a confirmed infection (Figure 2B).

Estimating the effect of repeated vaccination requires estimating the joint effect of vaccination in the previous and current season on infection status in the current season. Because clinical infection in the previous season is both affected by vaccination in the previous season and a confounder for the effect of vaccination in the current season on infection in the current season, we adjusted for it using inverse-probability weighting. We found that the adjustment had little influence on the estimated effect of repeated vaccination against A/H1N1pdm09 (Figure 2C). After we adjusted for clinical infection in the previous season, repeat vaccinees enrolled during the 2008-2009 season and between the 2010-2011 and the 2018-2019 seasons had 1.30 (95%CI: 0.94-1.68) times the odds of testing positive for A/H1N1dpm09 than those who were only vaccinated in the current season. Accounting for clinical infection history did not significantly change the estimates for the effect of repeated vaccination against A/H3N2 (from 1.01, 95% CI: 0.83-1.22 to 1.16, 95%CI: 0.96-1.40 post adjustment) or against type B (from 1.25, 95%CI: 0.94-1.68 to 1.12, 95%CI: 0.87-1.45 post adjustment; Figure 2C). Excluding the 108 individuals who presented with acute respiratory illness but refused enrollment in the previous season did not significantly change the results (Supplementary Figure 3.3). The distributions of the estimated stabilized weights were mean=1.00, range=0.70–1.32, IQR=1.00–1.00 for infection against A/H3N2, A/H1N1, and type B. Not adjusting for waning vaccine protection in the weighted outcome model yielded similar results (Supplementary Figure 3.4, see methods in Supplementary Section 3).

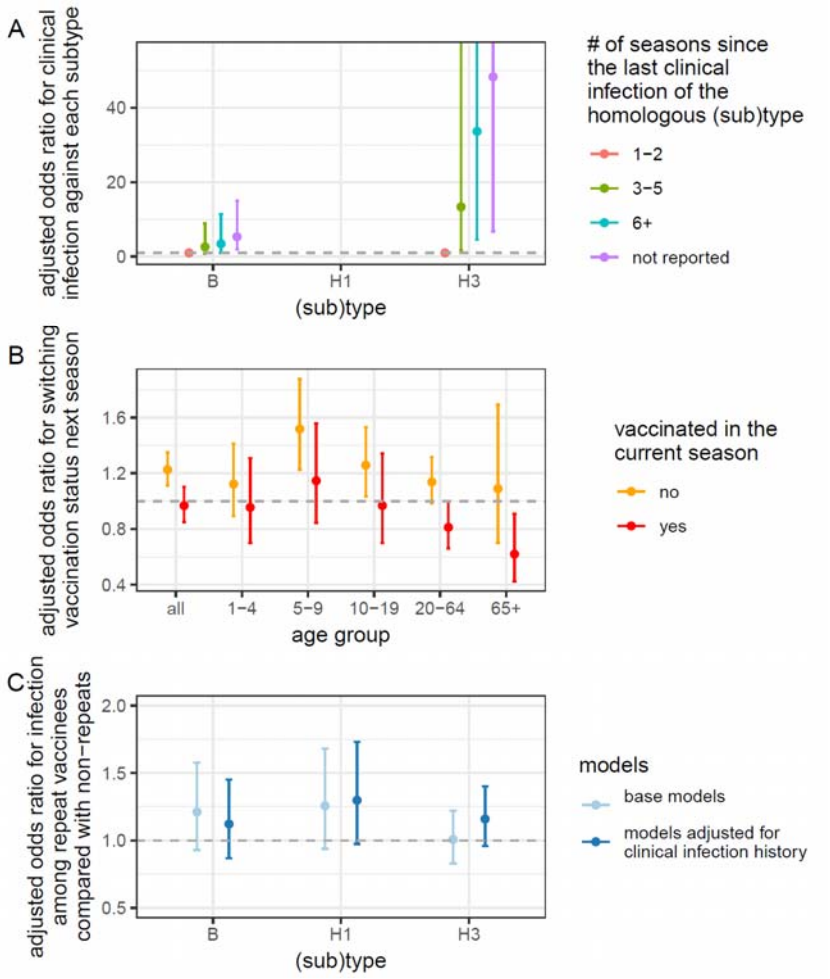

Figure 2: Impact of clinical infections on the estimated effect of repeated vaccination among participants from the Marshfield Clinic Health System. A) Association between recent clinical infections and odds of current-season clinical infection. More distant clinical infections of the homologous subtype are associated with a higher odds of current-season clinical infection. B) Tendency to switch vaccination status in the current season after clinical infection in the previous season. Compared with individuals without confirmed infections, unvaccinated individuals who were clinically infected in the previous season were more likely to vaccinate in the current season. C) Estimated effect of repeat vaccination after adjusting for recent clinical infections. Adjusted odds ratio for clinical infection comparing repeat vaccinees with non-repeat vaccinees before (light blue) and after adjusting for clinical infection status in the previous season (dark blue) using inverse-probability weighting. Adjustment did not significantly impact the estimates. In all panels, error bars indicate 95% confidence intervals.

4.3 Impact of clinical and subclinical infection history

4.3.1 Infection block hypothesis

In the previous section we estimated that repeat vaccinees had a 10% increase (OR~1.1) in the odds of current seasonal infection, an effect that could be partially mediated by clinical infection in the prior season, a version of the infection block hypothesis. In this section, we further explore using a theoretical model, the degree to which subclinical infection – which would not be observed in any of

the data sets we consider – could fully explain the observed repeat vaccination effect. To do so, we evaluated the proportion of repeat and non-repeat vaccinees who would have had to have been subclinically infected in previous seasons in order to reproduce the elevated odds of clinical infection among repeat vaccinees observed in the US Flu VE Network, assuming that the subclinical infection-block hypothesis was the only explanation for the observed elevated risk.

Under the infection block hypothesis [6,35–37], if we assumed a 2% clinical attack rate among those not vaccinated in a season based on estimates from the US [47], a 50% VE against clinical infection would lead to a clinical attack rate of 1% among vaccinees. We also assumed that the current-season clinical attack rate among the subset of current-season vaccinees who were not infected in the previous season is 1.5%. (This rate is higher than the expected 1% clinical attack rate for vaccinees overall because it includes only vaccinees without additional protection from recent infection.) In Figure 3, we show that for example, if the protection against future clinical infections after a subclinical infection is 30% (the first panel) and 20% of repeat vaccinees were subclinically infected in the prior season (the x-axis), then 45% of non-repeat vaccinees would have to have been subclinically infected in the prior season (the y-axis) to observe the estimated effect of repeated vaccination in the US Flu VE Network (i.e., odds ratio for clinical infection comparing repeat vaccinees with non-repeat vaccinees against A/H3N2 or type B of 1.1).

We found that for a range of hypothetical protections after subclinical infection (30, 50, and 70% protection against future clinical infection) and given our assumptions about clinical attack rates, to produce the observed OR in the US Flu VE Network, non-repeat vaccinees would have to be subclinically infected in the prior season at a substantially higher rate than repeat vaccinees (Figure 3). Larger differences in subclinical attack rates between the two groups or stronger protection against clinical infection after subclinical infection would each lead to a greater excess of clinical infections in the current season among repeat vaccinees compared with non-repeat vaccinees (Figure 3), leading to increased odds of clinical infection among repeat vaccinees, consistent with the infection block hypothesis.

When repeat and non-repeat vaccinees experienced subclinical infections in the previous season at the same rate, a scenario represented by the diagonal lines in Figure 3, the higher rate of clinical infection among non-repeat vaccinees compared with repeat vaccinees (1% vs. 2% based on our assumption) could elevate OR to only about 1.01 (vs. the observed ORs of about 1.1 in the CDC Flu VE Network). For thoroughness, we explored the possibility that prior-season clinical infection fully mediates the relationship between prior-season vaccination and the odds of current-season clinical infection using a theoretical model. Under a range of parameters and assumptions, the protection conferred by clinical infection in the prior season led to little variation in the estimated effect of repeated vaccination in the theoretical model, suggesting that prior-season clinical infection is unlikely to be an important mediator in this relationship (See Supplementary Section 5).

The annual incidence of clinical influenza virus infection has been estimated to be as high as 6% in some settings and higher among the younger age group [47]. We therefore considered an alternative assumption of 6% incidence of clinical infection among unvaccinated individuals and 3% among vaccinated individuals. Compared with estimates from a low incidence setting, we would then expect a greater excess of clinical infections in the current season among repeat vaccinees compared with non-repeat vaccinees when non-repeat vaccinees experience a higher rate of subclinical infections in the prior season than repeat vaccinees (Supplementary Figure 4.1).

4.3.2 Enhanced vaccine immunogenicity hypothesis

Studies have shown that recent infection can lead to better vaccine immunogenicity and thus potentially better vaccine protection among recently infected vaccinees [38,40]. We show that this hypothesis can be incorporated into the same framework given a different interpretation of the same parameters (see Supplementary Section 4). If recent infection improves vaccine immunogenicity and thus vaccine-induced protection, a smaller difference in rates of subclinical infection between repeat and non-repeat vaccinees would generate the same estimated effect of repeated vaccination against infection in the current season (Supplementary Figure 4.2).

For example, in the scenario we described in the second paragraph of section 4.3.1, we would observe the expected effect of repeated vaccination (OR=1.1) when the difference in the rate of subclinical infection between repeat and non-repeat vaccinees is 25% (rates among the two groups

are 20% and 45% respectively). If recent infection boosts VE from 50% to 76% while other assumptions stay the same, only a difference of 10% in the rate of subclinical infection between the two groups (20% and 30% respectively) is needed to produce the same expected effect of repeated vaccination (Supplementary Figure 4.2).

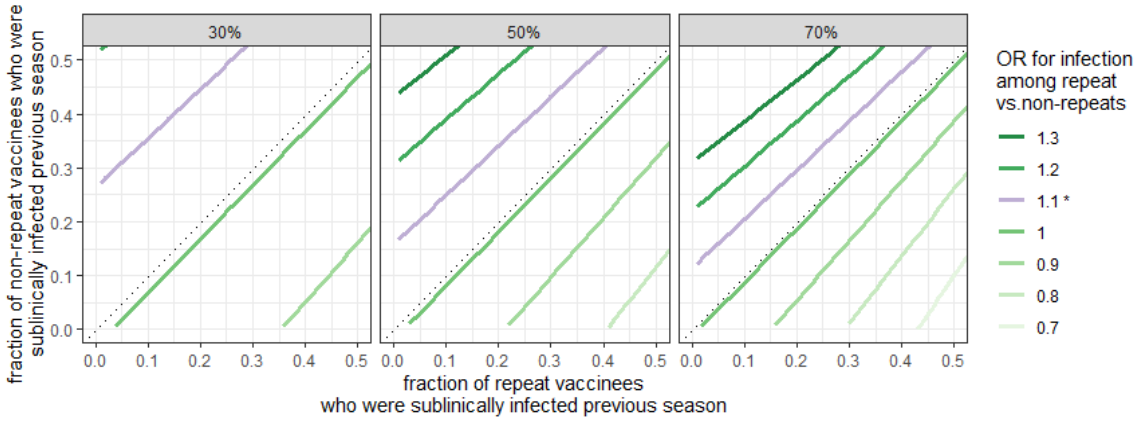

Figure 3: The fraction of repeat and non-repeat vaccinees who would need to have been subclinically infected in the previous season to reproduce the estimated effect of repeated vaccination in the US Flu VE Network. See Supplementary Section 4 for detailed methods. The estimated effect of repeated vaccination (OR=1.1) in the US Flu VE network is colored in purple. The results shown here are generated assuming vaccine effectiveness against clinical infection is 50%; clinical attack rate among vaccinees in a season is 1%; current-season clinical attack rate among the subset of current-season vaccinees not infected in the previous season is 1.5%; and clinical infection in the previous season perfectly protects against clinical infection in the following season. Each facet represents a predetermined protection against clinical infection after subclinical infection (i.e., 30%, 50%, 70%). The legend represents the estimated effect of repeated vaccination given the difference in subclinical attack rate among repeat vaccinees (x-axis) and non-repeat vaccinees (y-axis) and assumptions stated above. Only the plausible range of subclinical attack rate among repeat vaccinees (x-axis) and non-repeat vaccinees (y-axis; 0-50%) are shown in the figure.

5 Discussion

Observational studies [9–15,17,18,43], mostly using the test-negative design [10–15,17,18,43], have provided critical information on influenza VE. These studies can have biases and uncontrolled confounding that affect inference, including inference of the effectiveness of the vaccine in different subpopulations [49–53]. Reduced VE in repeat vaccinees has been a troubling, intermittent, and largely unexplained phenomenon [10,11]. We studied a component phenomenon, which is that the absolute risk (or odds) of infection among vaccinees in the current season is less if they were unvaccinated last season than if they were vaccinated last season. We observed waning of vaccine protection and repeat vaccinees' tendency to vaccinate earlier within a season compared with non-repeat vaccinees. We showed that clinical infection impacts individuals' decisions to vaccinate in the next season, and these infections in the past 1-2 seasons could confer strong protection against reinfection. However, these potentially biasing factors – prior-season infection and timing of vaccination – were insufficient to fully explain the higher risk of infection in the repeat vaccinees vs. non-repeat vaccinees in our study population. We showed that the residual repeat vaccination effect might be explained by different rates of subclinical infection between repeat and non-repeat vaccinees. These subclinical infections could result in a higher estimated odds of clinical infection among repeat vaccinees via two proposed mechanisms, the infection block hypothesis [6,35–37] and the hypothesis of enhanced vaccine immunogenicity and protection post-infection [38,54].

Our refined estimates of the effect of repeated vaccination are overall consistent with published studies. Similar to results from published meta-analyses [10,11] and a study that spans an eight-year period in Wisconsin and includes a subset of our data [12], repeat vaccination reduced VE for clinical

infection with A/H3N2. During our 8 recruitment seasons that include 6 seasons with strong A/H3N2 circulation, we observed mildly elevated odds of clinical infection in repeat vaccinees in all but the 2018-2019 season, some of which have previously been reported using the US Flu VE Network data [9,17]. In the 2014-2015 season, although a higher odds of infection among repeat vaccinees was observed for A/H3N2 in Canada and Europe [14,15], we observed similar and statistically significant results in only one of five US Flu VE Network sites. For A/H1N1pdm09, we found slightly increased odds of clinical infection among repeat vaccinees in the 2013-2014 season, which has also previously been reported using the same data [53]. However, we did not observe increased odds of clinical infection with A/H1N1pdm09 among repeat vaccinees when data were pooled across all 8 seasons, including 3 seasons with strong A/H1N1pdm09 circulation, consistent with results from published meta-analyses that cover broader geographic areas and different but overlapping seasons [10,11]. For type B, we found increased odds of clinical infection after repeated vaccination in the 2014-2015, 2015-2016, and 2016-2017 seasons, as well as when data were pooled across the enrollment seasons, although there is no statistical significance [11]. These results for the three (sub)types were upheld after accounting for waning of vaccine protection.

Mostly due to lack of data, clinical infection history has typically not been accounted for when estimating influenza VE. We found differences in clinical infection history between repeat and non-repeat vaccinees. Recent clinical infections seem on average to motivate unvaccinated individuals to vaccinate in the next season, making non-repeat vaccinees more likely to have experienced recent, potentially immunizing infections at the start of a season than the repeat vaccinees. The strength of this association varied by age. However, accounting for clinical infection history did not substantially change the estimated effect of repeat vaccination, indicating that confounding by prior-season clinical infection through its effect on current-season vaccination behavior may not fully explain the elevated odds of infection among repeat vaccinees. Aside from its potential role as a confounder, we also explored the possibility that prior-season clinical infection acts as a mediator of the relationship between prior-season vaccination and the odds of current-season clinical infection using a theoretical model (see Supplementary Section 5), and we found it unlikely to act as an important mediator. Verifying the finding in surveillance data requires methods that can tease apart the direct and indirect effect of vaccination after taking into account the interaction of vaccination and infection over a multi-year period.

Although we did not measure the rate of subclinical infections through serological studies, we found in theoretical analyses that protection would be reduced in repeat vaccinees to the extent estimated in the US Flu VE Network if they experience a lower rate of partially protective subclinical infections in the prior season compared to non-repeat vaccinees. We observed that, when the protection against future clinical infection after subclinical infections is 70%, the rate of subclinical infections would have to be lower in repeat vaccines than in non-repeat vaccines by about 10 percentage points to create the observed increase in clinical infection risk among repeat vaccinees. This absolute difference would increase to about 25 percentage points if the protection against future clinical infection is about 30%. This finding provides an explanation for increased risk after repeated vaccination after adjusting for clinical infection history alone. We demonstrated mathematically that smaller differences in subclinical infection rates could generate observed differences in VE if subclinical infection also substantially enhances the immunogenicity of vaccination in the next season. Understanding the difference in rate of subclinical infection between the two groups and how it varies by season might be crucial to explaining the variation in the effect of repeated vaccination.

The sensitivity of the estimated effect of repeated vaccination to differences in attack rates and infection-associated protection suggests a possible explanation for the observed variability in the estimated effect of repeat vaccination and other VE measures across locations and time. Using data from the CDC FluView database, we observed substantial variability in the relative sizes of influenza epidemics across the regions encompassing the five sites of the US Flu VE Network, which might be interpreted as a rough proxy for incidence. There is also well known variability in the sizes of influenza epidemics over time, and spatiotemporal variability in circulating clades that could affect the strength of protection conferred by infection. These possible explanations for VE variability suggest a need to try to account for past infections, so that VE estimates can be compared across populations stratified by similar infection history. Longitudinal cohort studies that involve blood collection, active surveillance, and sequencing can be useful for identifying subclinical infections, and coupling these observations with healthcare-seeking behavior and PCR testing can help test support for the infection block and

enhanced immunogenicity hypotheses [55]. Eventually, stratification on infection history may be possible through surrogate immune markers and feasible under other study designs, e.g., by measuring antibodies to nucleoprotein, which should be boosted only on infection, and hemagglutinin, which should be boosted after either exposure type.

The study has several limitations. Throughout our analysis, we assumed that influenza vaccination with any type of influenza vaccine confers complete protection in a subset of vaccinees. We did not consider the "leaky" vaccine effects, where vaccines are partially protective in all recipients, and which can lead to observed decline in VE without the need for waning vaccine protection [56]. Although the test-negative study, by selecting only patients who seek medical care, is designed to reduce the difference in health-seeking behavior between cases and non-cases, it does not eliminate it [50]. We were not able to test the representativeness of the enrolled patients across US Flu VE Network sites, although estimates excluding participants who refused enrollment in the previous season in MCHS were consistent with our main results, providing some confidence the enrolled population is generalizable to the population from which they were sampled. We did not explore birth cohort effects or the effects of antigenic distance on protection [57–60]. The timing of vaccination each season and vaccination status in the previous season were determined based on electronic medical records. We found that 7.5% (2,267/30,338) of individuals from the 5 sites who self-reported to have been vaccinated in the current season lacked an electronic record of vaccination. However, excluding these individuals did not qualitatively change the results. We attempted to adjust for clinical infection in the previous season using data from MCHS as a proxy for longitudinal follow-up data. Two-thirds of enrolled individuals in a season consented to enrollment in the previous season if eligible and invited to participate. However, the proportion of patients with ARI who were screened for enrollment varied by season and may have been somewhat affected by the volume of patients each day and the staffing capacity. As a result, we were not able to capture all patients who presented with ARI in the catchment area. Since we assumed individuals without an enrollment record in the previous season were not clinically infected, some of them may be misclassified.

The practical benefits of annual vaccination programs should not be extrapolated from this analysis of the relative risk of infection in repeat vaccinees compared to non-repeat vaccinees. The choice between an annual and a non-annual vaccination program should be based on assessments of the infection risk among all repeat and non-repeat vaccinees as well as the unvaccinated. Our analysis does not compare the risk of infection between repeat vaccinees and those vaccinated in the prior season only, who would be part of a hypothetical non-annual vaccination program.

Our study provides evidence that two potential factors, timing of vaccination and clinical infection history, are insufficient to fully explain increased risk in repeat vaccinees compared with non-repeat vaccinees. Clinical infection history is further unlikely to act as a strong mediator to explain the repeated vaccination effect. We find that under reasonable assumptions, the infection block hypothesis involving subclinical infection in the previous season may explain the observation of higher incidence of clinical influenza among those vaccinated in both the previous and current seasons than among those vaccinated only in the current season, thus acting as a potential mediator. Estimation of VE requires careful consideration of both time since vaccination and infection history of different subpopulations in a geographic setting.

Acknowledgements

QB, BAD, ML, SC conceived of the study. HQM, ETM, MG, KJW, BG, BF collected the data. QB performed the analyses and generated all figures. QB, BAD, ML, SC wrote the manuscript. All authors revised the manuscript. This study was partially supported by the Collaborative Influenza Vaccine Innovation Centers (CIVICs) contract 75N93019C00051 (S.C. and Q.B.). ML acknowledges support from US NIH grant 1R01GM139926 to University of Michigan. We thank Benjamin A. Cowling for comments. Authors report no conflict of interest

US Flu VE Network Investigators:

Baylor Scott & White Health – Kempapura Murthy, Chandni Raiyani, Kayan Dunnigan, Muffadal Mamawala
CDC – Jessie R. Chung, Manish Patel
Henry Ford Medical Center – Lois Lamerato
Kaiser Permanente Washington – Michael L. Jackson, C. Hallie Phillips, Erika Kiniry
Marshfield Clinic Research Institute – Edward A. Belongia, Jennifer P. King
University of Michigan – Arnold S. Monto
University of Pittsburgh – Richard K. Zimmerman, Mary Patricia Nowalk, Krissy Moehling Geffel

References

1. Vaccines against influenza WHO position paper – November 2012. Wkly Epidemiol Rec **2012**; 87:461–476.

2. Principi N, Camilloni B, Esposito S, ESCMID Vaccine Study Group (EVASG). Influenza immunization policies: Which could be the main reasons for differences among countries? Hum Vaccin Immunother **2018**; 14:684–692.

3. CDC. Weekly Flu Vaccination Dashboard. 2022. Available at: https://www.cdc.gov/flu/fluvaxview/dashboard/vaccination-dashboard.html. Accessed 23 December 2022.

4. [No title]. Available at: https://www.ecdc.europa.eu/sites/default/files/documents/seasonal-influenza-antiviral-use-2018.pdf. Accessed 23 December 2022.

5. Hoskins TW, Davies J, Smith AJ, Allchin A, Miller C, Pollock TM. INFLUENZA AT CHRIST'S HOSPITAL: MARCH, 1974. The Lancet. 1976; 307:105–108. Available at: http://dx.doi.org/10.1016/s0140-6736(76)93151-2.

6. Hoskins TW, Davies JR, Smith AJ, Miller CL, Allchin A. Assessment of inactivated influenza-A vaccine after three outbreaks of influenza A at Christ's Hospital. Lancet **1979**; 1:33–35.

7. Skowronski DM, Janjua NZ, De Serres G, et al. A Sentinel Platform to Evaluate Influenza Vaccine Effectiveness and New Variant Circulation, Canada 2010–2011 Season. Clinical Infectious Diseases. 2012; 55:332–342. Available at: http://dx.doi.org/10.1093/cid/cis431.

8. Ng S, Fang VJ, Ip DKM, et al. Humoral antibody response after receipt of inactivated seasonal influenza vaccinations one year apart in children. Pediatr Infect Dis J **2012**; 31:964–969.

9. Ohmit SE, Petrie JG, Malosh RE, et al. Influenza Vaccine Effectiveness in the Community and the Household. Clinical Infectious Diseases. 2013; 56:1363–1369. Available at: http://dx.doi.org/10.1093/cid/cit060.

10. Belongia EA, Skowronski DM, McLean HQ, Chambers C, Sundaram ME, De Serres G. Repeated annual influenza vaccination and vaccine effectiveness: review of evidence. Expert Rev Vaccines **2017**; 16:1–14.

11. Ramsay LC, Buchan SA, Stirling RG, et al. The impact of repeated vaccination on influenza vaccine effectiveness: a systematic review and meta-analysis. BMC Med **2019**; 17:9.

12. McLean HQ, Thompson MG, Sundaram ME, et al. Impact of Repeated Vaccination on Vaccine Effectiveness Against Influenza A(H3N2) and B During 8 Seasons. Clinical Infectious Diseases. 2014; 59:1375–1385. Available at: http://dx.doi.org/10.1093/cid/ciu680.

13. Ohmit SE, Thompson MG, Petrie JG, et al. Influenza Vaccine Effectiveness in the 2011–2012 Season: Protection Against Each Circulating Virus and the Effect of Prior Vaccination on Estimates. Clinical Infectious Diseases. 2014; 58:319–327. Available at:

http://dx.doi.org/10.1093/cid/cit736.

14. Skowronski DM, Chambers C, Sabaiduc S, et al. A Perfect Storm: Impact of Genomic Variation and Serial Vaccination on Low Influenza Vaccine Effectiveness During the 2014-2015 Season. Clin Infect Dis **2016**; 63:21–32.

15. Valenciano M, Kissling E, Reuss A, et al. Vaccine effectiveness in preventing laboratory-confirmed influenza in primary care patients in a season of co-circulation of influenza A(H1N1)pdm09, B and drifted A(H3N2), I-MOVE Multicentre Case-Control Study, Europe 2014/15. Euro Surveill **2016**; 21:ii=30139.

16. Kissling E, Pozo F, Buda S, et al. Low 2018/19 vaccine effectiveness against influenza A(H3N2) among 15-64-year-olds in Europe: exploration by birth cohort. Euro Surveill **2019**; 24. Available at: http://dx.doi.org/10.2807/1560-7917.ES.2019.24.48.1900604.

17. McLean HQ, Thompson MG, Sundaram ME, et al. Influenza vaccine effectiveness in the United States during 2012-2013: variable protection by age and virus type. J Infect Dis **2015**; 211:1529–1540.

18. Kissling E, Pozo F, Buda S, et al. Low 2018/19 vaccine effectiveness against influenza A(H3N2) among 15–64-year-olds in Europe: exploration by birth cohort. Eurosurveillance. 2019; 24. Available at: http://dx.doi.org/10.2807/1560-7917.es.2019.24.48.1900604.

19. Ferdinands JM, Shay DK. Magnitude of potential biases in a simulated case-control study of the effectiveness of influenza vaccination. Clin Infect Dis **2012**; 54:25–32.

20. Ray GT, Lewis N, Klein NP, et al. Intraseason Waning of Influenza Vaccine Effectiveness. Clin Infect Dis **2019**; 68:1623–1630.

21. Ferdinands JM, Gaglani M, Martin ET, et al. Waning Vaccine Effectiveness Against Influenza-Associated Hospitalizations Among Adults, 2015–2016 to 2018–2019, United States Hospitalized Adult Influenza Vaccine Effectiveness Network. Clinical Infectious Diseases. 2021; 73:726–729. Available at: http://dx.doi.org/10.1093/cid/ciab045.

22. Young B, Sadarangani S, Jiang L, Wilder-Smith A, Chen MI-C. Duration of Influenza Vaccine Effectiveness: A Systematic Review, Meta-analysis, and Meta-regression of Test-Negative Design Case-Control Studies. J Infect Dis **2018**; 217:731–741.

23. Pebody RG, Andrews N, McMenamin J, et al. Vaccine effectiveness of 2011/12 trivalent seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: evidence of waning intra-seasonal protection. Eurosurveillance. 2013; 18. Available at: http://dx.doi.org/10.2807/ese.18.05.20389-en.

24. Kissling E, Valenciano M, Larrauri A, et al. Low and decreasing vaccine effectiveness against influenza A(H3) in 2011/12 among vaccination target groups in Europe: results from the I-MOVE multicentre case–control study. Eurosurveillance. 2013; 18. Available at: http://dx.doi.org/10.2807/ese.18.05.20390-en.

25. Jiménez-Jorge S, de Mateo S, Delgado-Sanz C, et al. Effectiveness of influenza vaccine against laboratory-confirmed influenza, in the late 2011-2012 season in Spain, among population targeted for vaccination. BMC Infect Dis **2013**; 13:441.

26. Andrews N, McMenamin J, Durnall H, et al. Effectiveness of trivalent seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: 2012/13 end of season results. Euro Surveill **2014**; 19:5–13.

27. Ferdinands JM, Fry AM, Reynolds S, et al. Intraseason waning of influenza vaccine protection: Evidence from the US Influenza Vaccine Effectiveness Network, 2011-12 through 2014-15. Clin Infect Dis **2017**; 64:544–550.

28. Kissling E, Nunes B, Robertson C, et al. I-MOVE multicentre case–control study 2010/11 to

2014/15: Is there within-season waning of influenza type/subtype vaccine effectiveness with increasing time since vaccination? Eurosurveillance. 2016; 21. Available at: http://dx.doi.org/10.2807/1560-7917.es.2016.21.16.30201.

29. Belongia EA, Sundaram ME, McClure DL, Meece JK, Ferdinands J, VanWormer JJ. Waning vaccine protection against influenza A (H3N2) illness in children and older adults during a single season. Vaccine **2015**; 33:246–251.

30. Puig-Barberà J, Mira-Iglesias A, Tortajada-Girbés M, et al. Waning protection of influenza vaccination during four influenza seasons, 2011/2012 to 2014/2015. Vaccine **2017**; 35:5799–5807.

31. Sullivan SG, Komadina N, Grant K, Jelley L, Papadakis G, Kelly H. Influenza vaccine effectiveness during the 2012 influenza season in Victoria, Australia: influences of waning immunity and vaccine match. J Med Virol **2014**; 86:1017–1025.

32. Petrie JG, Ohmit SE, Truscon R, et al. Modest Waning of Influenza Vaccine Efficacy and Antibody Titers During the 2007–2008 Influenza Season. Journal of Infectious Diseases. 2016; 214:1142–1149. Available at: http://dx.doi.org/10.1093/infdis/jiw105.

33. Radin JM, Hawksworth AW, Myers CA, Ricketts MN, Hansen EA, Brice GT. Influenza vaccine effectiveness: Maintained protection throughout the duration of influenza seasons 2010-2011 through 2013-2014. Vaccine **2016**; 34:3907–3912.

34. Gherasim A, Pozo F, de Mateo S, et al. Waning protection of influenza vaccine against mild laboratory confirmed influenza A(H3N2) and B in Spain, season 2014–15. Vaccine. 2016; 34:2371–2377. Available at: http://dx.doi.org/10.1016/j.vaccine.2016.03.035.

35. Skowronski DM, Janjua NZ, Hottes TS, De Serres G. Mechanism for Seasonal Vaccine Effect on Pandemic H1N1 Risk Remains Uncertain. Clin. Infect. Dis. 2011; 52:831–2; author reply 832–3.

36. Skowronski DM, De Serres G, Crowcroft NS, et al. Association between the 2008-09 seasonal influenza vaccine and pandemic H1N1 illness during Spring-Summer 2009: four observational studies from Canada. PLoS Med **2010**; 7:e1000258.

37. Cowling BJ, Ng S, Ma ESK, et al. Protective Efficacy of Seasonal Influenza Vaccination against Seasonal and Pandemic Influenza Virus Infection during 2009 in Hong Kong. Clinical Infectious Diseases. 2010; 51:1370–1379. Available at: http://dx.doi.org/10.1086/657311.

38. Auladell M, Phuong HVM, Mai LTQ, et al. Influenza virus infection history shapes antibody responses to influenza vaccination. Nat Med **2022**; 28:363–372.

39. Krammer F. The human antibody response to influenza A virus infection and vaccination. Nat Rev Immunol **2019**; 19:383–397.

40. Hall V, Foulkes S, Insalata F, et al. Protection against SARS-CoV-2 after Covid-19 Vaccination and Previous Infection. N Engl J Med **2022**; 386:1207–1220.

41. VanderWeele TJ. Explanation in causal inference: developments in mediation and interaction. International Journal of Epidemiology. 2016; :dyw277. Available at: http://dx.doi.org/10.1093/ije/dyw277.

42. Hernan MA, Robins JM. Causal Inference. CRC Press, 2019.

43. Kim SS, Flannery B, Foppa IM, et al. Effects of Prior Season Vaccination on Current Season Vaccine Effectiveness in the United States Flu Vaccine Effectiveness Network, 2012–2013 Through 2017–2018. Clinical Infectious Diseases. 2021; 73:497–505. Available at: http://dx.doi.org/10.1093/cid/ciaa706.

44. Kieke AL, Kieke BA Jr, Kopitzke SL, et al. Validation of Health Event Capture in the Marshfield Epidemiologic Study Area. Clin Med Res **2015**; 13:103–111.

45. Li KQ, Shi X, Miao W, Tchetgen ET. Double Negative Control Inference in Test-Negative Design Studies of Vaccine Effectiveness. ArXiv **2022**; Available at: https://www.ncbi.nlm.nih.gov/pubmed/35350548.

46. Robins JM, Hernán MÁ, Brumback B. Marginal Structural Models and Causal Inference in Epidemiology. Epidemiology. 2000; 11:550–560. Available at: http://dx.doi.org/10.1097/00001648-200009000-00011.

47. Jackson ML, Jackson LA, Kieke B, et al. Incidence of medically attended influenza infection and cases averted by vaccination, 2011/2012 and 2012/2013 influenza seasons. Vaccine. 2015; 33:5181–5187. Available at: http://dx.doi.org/10.1016/j.vaccine.2015.07.098.

48. Cohen C, Kleynhans J, Moyes J, et al. Asymptomatic transmission and high community burden of seasonal influenza in an urban and a rural community in South Africa, 2017–18 (PHIRST): a population cohort study. The Lancet Global Health. 2021; 9:e863–e874. Available at: http://dx.doi.org/10.1016/s2214-109x(21)00141-8.

49. Lipsitch M, Tchetgen Tchetgen E, Cohen T. Negative controls: a tool for detecting confounding and bias in observational studies. Epidemiology **2010**; 21:383–388.

50. Sullivan SG, Tchetgen Tchetgen EJ, Cowling BJ. Theoretical Basis of the Test-Negative Study Design for Assessment of Influenza Vaccine Effectiveness. Am J Epidemiol **2016**; 184:345–353.

51. Chua H, Feng S, Lewnard JA, et al. The Use of Test-negative Controls to Monitor Vaccine Effectiveness: A Systematic Review of Methodology. Epidemiology **2020**; 31:43–64.

52. Jackson ML, Nelson JC. The test-negative design for estimating influenza vaccine effectiveness. Vaccine. 2013; 31:2165–2168. Available at: http://dx.doi.org/10.1016/j.vaccine.2013.02.053.

53. Gaglani M, Pruszynski J, Murthy K, et al. Influenza Vaccine Effectiveness Against 2009 Pandemic Influenza A(H1N1) Virus Differed by Vaccine Type During 2013–2014 in the United States. Journal of Infectious Diseases. 2016; 213:1546–1556. Available at: http://dx.doi.org/10.1093/infdis/jiv577.

54. Altarawneh HN, Chemaitelly H, Ayoub HH, et al. Effects of Previous Infection and Vaccination on Symptomatic Omicron Infections. N Engl J Med **2022**; 387:21–34.

55. Monto AS, DeJonge PM, Callear AP, et al. Coronavirus Occurrence and Transmission Over 8 Years in the HIVE Cohort of Households in Michigan. J Infect Dis **2020**; 222:9–16.

56. Tokars JI, Patel MM, Foppa IM, Reed C, Fry AM, Ferdinands JM. Waning of Measured Influenza Vaccine Effectiveness Over Time: The Potential Contribution of Leaky Vaccine Effect. Clin Infect Dis **2020**; 71:e633–e641.

57. Smith DJ, Forrest S, Ackley DH, Perelson AS. Variable efficacy of repeated annual influenza vaccination. Proc Natl Acad Sci U S A **1999**; 96:14001–14006.

58. Arevalo P, McLean HQ, Belongia EA, Cobey S. Earliest infections predict the age distribution of seasonal influenza A cases. Elife **2020**; 9. Available at: http://dx.doi.org/10.7554/eLife.50060.

59. Skowronski DM, Chambers C, De Serres G, et al. Serial Vaccination and the Antigenic Distance Hypothesis: Effects on Influenza Vaccine Effectiveness During A(H3N2) Epidemics in Canada, 2010-2011 to 2014-2015. J Infect Dis **2017**; 215:1059–1099.

60. Flannery B, Smith C, Garten RJ, et al. Influence of Birth Cohort on Effectiveness of 2015-2016 Influenza Vaccine Against Medically Attended Illness Due to 2009 Pandemic Influenza A(H1N1) Virus in the United States. J Infect Dis **2018**; 218:189–196.