Birth year, immune history and differences in risk from seasonal influenza H1N1 and H3N2

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# Abstract

Across decades of co-circulation in humans, seasonal influenza A subtypes H1N1 and H3N2 have been characterized by different age distributions of infection and mortality. H3N2 typically causes the majority of cases in high-risk elderly cohorts, and the majority of overall deaths, whereas H1N1 causes fewer deaths overall and has an impact shifted more towards young and middle-aged adults compared to H3N2. We re-examine possible drivers of these patterns, motivated by the recent discovery of broadly-protective immunity arising from influenza viruses encountered in childhood. Using two large, epidemiological data sets, we tested the possibility that immune imprinting shapes seasonal flu epidemiology via narrow immune memory to a particular subtype, or via broader immune memory that acts across subtypes. We also explore a separate hypothesis about evolutionary rate. Likelihood-based model comparison showed that within-subtype imprinting is the strongest driver of cohort-specific seasonal influenza risk. The data did not support a strong effect of evolutionary rate, or of broadly protective imprinting. Our findings emphasize that childhood exposures can imprint a lifelong immunological bias toward particular influenza subtypes, and that these cohort-specific biases shape epidemic age distributions. These results illuminate the epidemiological impacts of antigenic seniority, indicating that less “senior” antibody responses acquired later in life do not provide the same strength of protection as responses imprinted in childhood. Finally, these results imply that H1N1’s mortality burden (currently low) may increase in the coming decades, as cohorts that lack H1N1-specific imprinting eventually become elderly.

# Introduction

Childhood exposures to influenza leave an immunological imprint, which has reverberating, lifelong impacts on immune memory. Foundational work on original antigenic sin (1) and antigenic seniority (2) showed, using serological data, that individuals maintain the highest titers against influenza strains encountered in childhood. But how these serological patterns map to functional immune protection, and shape birth year-specific risk during outbreaks, remains an active research frontier. One key open question is whether immunological benefits from childhood imprinting are always accompanied by immunological costs. Another key uncertainty is what breadth of cross-protection we should expect childhood immune imprinting to provide against influenza, and how the expected breadth of cross-protection shifts with epidemic context.

Numerous epidemiological examples highlight childhood imprinting’s potential to provide immunological benefits. During every pandemic in the modern epidemiological record, certain birth cohorts have shown pre-existing immunity, attributed to cross-protective responses primed in childhood (3–9). Childhood imprinting can also protect against novel, emerging avian influenza viruses (8,10). As avian and pandemic influenza viruses were historically considered too novel to encounter substantial population immunity as they emerged into humans, the existence of any protection from imprinting is a welcome discovery.

But whether preferential immune memory of strains encountered in childhood interferes with effective *de novo* responses, and immune protection, against drifted or shifted strains encountered later in life has been debated for years [CITE]. Adults certainly maintain the ability to mount new antibody titers against drifted influenza strains, but these responses are accompanied by back-boosting of existing cross-reactive memory [CITE]. In theory, repeated back-boosting of responses primed earliest in life could give rise to serological antigenic seniority, even in the absence of any antigenic interference between existing immune memory and *de novo* responses. On the other hand, immunological studies have demonstrated the potential for antigenic interference, where upon infection by a drifted influenza strain, cross-reactive memory B cells can exclude *de novo* clones from the naïve B cell pool, leading immune memory to focus on the few epitopes held in common between current and past strains [CITE]. Such antigenic interference may contribute to decreased vaccine efficacy after repeated immunization [CITE], but so far, the epidemiological impacts of antigenic interference remain poorly understood.

Recent studies have also highlighted the ability of imprinting to shape multiple layers of influenza immune memory, both broad and narrow. Seasonal influenza immunity usually provides narrow, ephemeral, cross-protection, only against closely related variants of the same hemagglutinin (HA) or neuraminidase (NA) subtype. This sort of narrow, within-subtype immunity famously drives antigenic drift [CITE] and vaccine escape (11). Within the host, narrow, within-subtype immunity is driven by B cell memory of immunodominant epitopes on the head of HA, which are genetically variable and structurally diverse, but provide easy targets for antibodies [CITE].

Until recently, broader cross-subtype (heterosubtypic) protection was considered rare or anomalous, and so research on immune imprinting focused primarily on narrow within-subtype (homosubtypic) patterns. But the 2009 H1N1 pandemic, and subsequent efforts to develop a universal influenza vaccine, have drawn attention to more broadly protective antibody responses, which target conserved HA epitopes, and can provide broad protection across all HA subtypes in the same phylogenetic group (12–14). (HA group 1 contains seasonal subtypes H1 and H2, while group 2 contains seasonal H3[CITE].) Broadly protective B cell memory is common in existing human antibody repertoires, but is rarely deployed except against antigenically novel HA subtypes, whose conserved epitopes may provide the only recognizable immune targets (12–15). Recently, we showed that broadly protective childhood imprinting to a particular HA group strongly shapes birth year-specific risk from emerging, avian influenza viruses (8,10). Thus, imprinting can act both at the narrow, within-subtype level or at the broad, HA group-level.

Since 1977, two distinct subtypes of influenza A, H1N1 and H3N2, have circulated seasonally in humans, and differences in their age-specific impacts are widely believed to be driven by cohort-specific differences in childhood imprinting (8,16–19). Childhood imprinting effects are widely believed to driver these differences in age distribution, but it remains unclear whether imprinting protection against seasonal influenza acts at the narrow, within-subtype level [CITE], or at the broader HA group level [CITE]. H3N2 causes the vast majority of cases in older adults, while H1N1 causes a greater proportion of cases in younger adults. These differences in age distribution are qualitatively consistent with childhood imprinting patterns, in that older cohorts (i.e. those born before 1957 when H2N2 replaced H1N1) were almost certainly exposed to historical variants of H1N1 in childhood, and may now be preferentially protected against modern, seasonal H1N1 (17–19). Likewise, younger adults have the highest probabilities of childhood imprinting to H3N2, which is consistent with greater incidence of the opposite seasonal subtype, H1N1.

Here, we aim to test whether birth year-specific risk from seasonal influenza is primarily driven by broad imprinting protection at the HA phylogenetic group-level, or by narrower imprinting protection specific to a single HA or NA subtype. Childhood imprinting specific to a particular hemagglutinin subtype might provide exceptionally strong, lifelong protection specific to H1 or to H3. Similarly, childhood imprinting might act strongly at the NA subtype level, providing lifelong protection specific to N1 or to N2 (Fig. ***1***). Alternatively, broad HA group-level imprinting might drive seasonal influenza cohort effects. Although the B cell clones involved in group-level protection usually play a minimal role in immunity against familiar, seasonal influenza viruses, these broadly protective B cells can rise in frequency and play a strong role in immunity if the host lacks immune memory of more variable, immunodominant epitopes (12–14,20,21). Thus, in theory, HA group-level immune memory may serve as a second line of defense against drifted seasonal strains, called in as backup to target conserved epitopes when narrow, first-line memory B cells are unable to recognize their drifted, variable targets. If HA group-level imprinting strongly shapes seasonal influenza risk, then cohorts imprinted to H1 or H2 (both group 1) should be protected against modern, seasonal H1N1, while only cohorts imprinted to H3 (group 2) would be protected against modern, seasonal H3N2 (Fig. 1A-B).

In addition to cohort effects from childhood imprinting, differences in H1N1 and H3N2’s rates of antigenic drift may also contribute to differences in subtype-specific age distribution. Subtype H3N2 drifts slightly faster than H1N1, and as a result, H3N2 may be more able to cause infections in older, immunologically experienced cohorts, whereas H1N1 may be relatively restricted to incidence in immunologically naïve children (22).

Using two large data sets on seasonal influenza incidence, which together represent 13,063 confirmed influenza A cases across 18 years and 15 countries, we tested whether observed differences in age distribution of H1N1 and H3N2 cases are primarily driven by cohort effects from childhood imprinting, or by other factors. To test whether HA group-level imprinting, HA subtype-level imprinting, NA subtype-level imprinting or no effect of imprinting was most consistent with observed patterns, we developed a suite of models, fitted models to data using maximum likelihood, and performed model selection using the Akaike Information Criterion (AIC). Additionally, to test whether differences in H1N1 and H3N2’s rates of antigenic advance were a dominant driver of observed subtype-specific differences in age distribution, we analyzed the relationships between the annual magnitude of antigenic advance, and season-specific age distributions of infection.

# The Data

We analyzed two large epidemiological data sets. The first was provided by the Arizona Department of Health Services (AZDHS), and contained 9,451 seasonal H1N1 and H3N2 cases, reported through a state-wide passive surveillance system. Cases of all ages were confirmed at clinical laboratories throughout the state (independent and hospital-associated lab facilities), and then reported back to AZDHS. Laboratories confirmed cases using a combination of rapid tests, serology, viral isolation and PCR, following AZDHS case definitions (23). Cases were observed and reported across 22 years of influenza surveillance, from the 1993-1994 influenza season through the 2014-2015 season, but the number of cases reported each year has increased over time. All clinical laboratories were mandated to report influenza cases starting in 2004, and later, the 2009 pandemic marked the beginning of a dramatic uptick in per-season sample sizes (Table 1). The Arizona influenza season was defined as beginning in epidemiological week 40 (around early October) and ending in week 39 of the following year. We excluded cases observed in the 2008-2009 and the 2009-2010 seasons from our analysis, as these fell during the first or second waves of the 2009 H1N1 pandemic (24), during which epidemic age distributions and underlying drivers of immune memory differed markedly from normal, seasonal influenza circulation (12,14,16).

A second data set provided by the INSIGHT influenza outpatient study (http://insight.ccbr.umn.edu/) contained 3,612 PCR-confirmed H1N1 and H3N2 cases, observed across 16 countries between 2010 and 2016 (Table 2). The study enrolled adults ages 18 and over who sought health care for influenza-like illness at a participating outpatient clinic. The INSIGHT data sampled a greater geographical range, and contained information not available in the AZDHS data, including the age distribution of cases that tested negative for influenza, and three binary medical history variables: antiviral treatment used, presence of any underlying conditions, and recent influenza vaccination. However, the range of age groups sampled was more restricted in the INSIGHT dataset. The INSIGHT data excluded children under age 18, and enrolled relatively few elderly and young adult subjects, whereas the AZDHS data contained cases large numbers of cases at the extremes of age, including in children (Fig. S1). To facilitate comparison between data sets, we defined INSIGHT cases enrolled from Oct-May as part of the Northern Hemisphere influenza season, and cases enrolled from June-Sept as part of the Southern Hemisphere season. October 1 roughly aligns with the week 40 Northern Hemisphere season start date used in the Arizona data set.

# The Model

## Reconstructed imprinting patterns

Reconstructed, birth year-specific probabilities of imprinting mirror the timeline of influenza circulation in humans (Fig. 1A). Most individuals born between pandemics in 1918 and 1957 experienced a first influenza A virus (IAV) infection by H1N1, and middle-aged cohorts born between pandemics in 1957 and 1968 almost all were first infected by H2N2 (note that because the first influenza exposure may occur after the first year of life, individuals born in the years leading up to a pandemic have some probability of first infection by the new pandemic subtype). Ever since its emergence in 1968, H3N2 has dominated seasonal circulation in humans, and caused the majority of first infections in younger cohorts. However, H1N1 has also caused some seasonal circulation since 1977, and thus a fraction of those born a few years before 1977 to present will have first seen H1N1.

We reconstructed birth year-specific probabilities of first infection by H1N1, H2N2 and H3N2 using methods described previously (10). We repeated reconstructions for every country and year of case observation in the data. Country-specific reconstructions differed only in the virological surveillance data used to estimate the fraction of seasonal influenza cases caused by H1N1 or H3N2 in recent decades, and year-specific reconstructions differed only in the birth years that remained young enough (ages 0-12) to have a non-negligible probability of remaining naïve to IAV. Code to perform reconstructions is available at %%DOI HERE%%.

## Expected age distributions under alternate imprinting models

All models that included imprinting effects assumed a first childhood exposure to H1N1 would later protect against modern, seasonal H1N1, and that a first exposure to H3N2 would later protect against modern, seasonal H3N2 (Fig. 1B). Collinearities between the predictions of different imprinting models (Fig. 1G-I) were inevitable, given the limited diversity of influenza circulation in humans over the past century. Note that middle-aged cohorts, which were first infected by H2N2, are crucial, because they provide the only leverage to differentiate between imprinting at the HA subtype, NA subtype or HA group-level level (Fig. 1).

To tease apart age-specific risk factors from birth year-specific imprinting effects, we noted that age-specific risk factors for influenza infection are largely subtype-independent. Specifically, age-specific risk, or age-specific probabilities of case ascertainment could be influenced by medical factors like age-specific vaccine coverage, age-specific risk of severe disease, and immunosenescence, or by behavioral factors like age-assorted social mixing, and age-specific healthcare seeking behavior. All these factors should have similar impacts on any influenza subtype.

Thus, we fit a single step function to characterize the shape of age-specific risk of any confirmed influenza infection. Then, we modeled residual, subtype-specific differences in risk as a function of birth year, which enabled us to focus on the possible role childhood imprinting status. Note that for a given birth cohort, age-specific risk changed over time, and depended specifically on the individual’s age in the year of case observation, whereas birth year-specific risk was fixed for all years of case observation. Finally, each tested model used a linear combination of age-specific risk (Fig. 1C) and birth year-specific risk (Fig. 1D-F) to generate an expected distribution of H1N1 or H3N2 incidence (Fig. 1G-I).

To test quantitatively whether observed, subtype-specific differences in age distribution were most consistent with imprinting at the HA subtype, NA subtype or HA group level, or with no contribution of imprinting, we fitted a suite of models to each data set using a multinomial likelihood and then performed model selection using the Akaike information criterion (AIC). AIC is a measure of statistical support, which favors simple models that fit the data well (i.e. models with high likelihood and relatively few parameters) (25,26). ΔAIC is used to compare a focal model’s performance to the best tested model, and as a rule of thumb, ΔAIC>2 indicates at least some statistical preference for the better model (26). Technical details and a link to all relevant code are provided in the Methods.

### AZDHS Models

We fit a set of four models to the AZDHS data set. The simplest model contained only age-specific risk (A), and more complex models added effects from imprinting at the HA subtype level (S), at the HA group level (G), or at the NA subtype level (N): AS, AG, and AN, respectively. The age-specific risk curve took the form of a step function, in which relative risk was fixed to 1 in age bin 0-4, and one free parameter was fit to relative risk in each of the following 12 age bins: {5-10, 11-17, 18-24, 25-31, 32-38, 39-45, 46-52, 53-59, 60-66, 67-73, 74-80, 81+}. Within models that contained imprinting effects, two additional free parameters estimated the relative risk of confirmed H1N1 or H3N2 infection given imprinting protection.

### INSIGHT Models

When fitting to the INSIGHT data, which contained additional medical details, the suite of tested models included three additional risk factors: vaccination (V), antiviral treatment (T), and presence of underlying conditions (U). Factors T and U each added one free parameter, which characterized the relative risk of any influenza infection, given antiviral treatment or given the presence of underlying conditions. Factor V added two free parameters which characterized the relative risk of H1N1 or of H3N2 infection, given recent influenza vaccination. Differences in vaccine effectiveness across study years would not have been identifiable, and so we did not include them in the model. We tested all possible combinations of V, T, and U, in and each of the three imprinting hypotheses (S, N and G), for a total of 32 tested INSIGHT models. All tested models contained age-specific risk (A).

### Interpretation of the age-specific risk curve

The INSIGHT study collected denominator data on the age distribution of all tested cases (including cases that tested negative for influenza), whereas the AZDHS data contained only information on confirmed cases, with no age-specific denominators. When fitting to INSIGHT data, we input the age distribution of all tested cases as the null, expected age distribution in each country and season. Because these denominator data were not available in the AZDHS dataset, the age-specific risk curves fit to each data set must be interpreted differently. Age-specific risk curves fitted to INSIGHT data only represented age-specific differences in the rate of testing positive for influenza, residual to observed denominators. On the other hand, age-specific risk curves fitted to the AZDHS data captured all aspects of the infection and case observation process. Thus, curves fitted to INSIGHT data showed much less variation between age groups than curves fitted to AZDHS data.

# Results

### Subtype-specific differences in age distribution

In both AZDHS and INSIGHT data, H3N2 consistently caused more cases in older cohorts, while H1N1 caused more cases in younger cohorts (Figs. 2-3, S2-S7). These patterns are qualitatively consistent with the predicted effects of cohort-specific imprinting (Fig 1), and with previously reported differences in age distribution (16–19). Overall, differences between H1N1 and H3N2’s age distributions were more pronounced in the Arizona data than in the INSIGHT data. But despite some variation between countries, seasons and data sets, observed patterns never contradicted the expected effects of imprinting. Whenever subtype-specific differences in age distribution were apparent, H3N2 always caused greater impacts in the oldest cohorts, while H1N1 caused greater impacts in young and middle-aged adults (Figs. 2-3).

To facilitate comparison between subtypes, and across data sets, Figs. 2-3 only included data from countries and seasons in which H1N1 and H3N2 both circulated (≥50 confirmed cases of each subtype). Figures S2-S7 show similar plots for all countries and seasons represented in the data, and plots showing alternate smoothing parameters.

## Imprinting model selection

Model selection on seasonal influenza data supported effects from narrow, within-subtype imprinting to NA or HA. Whether we fit to INSIGHT or to AZDHS data, models containing NA subtype-level imprinting received the most statistical support, and models containing HA subtype-level imprinting were the second most preferred in terms of AIC (Fig. 4, Table 3). The AZDHS data showed a preference for NA subtype-level imprinting (ΔAIC=0) over HA subtype-level imprinting (ΔAIC=23.42), and effectively no statistical support for broad, HA group-level imprinting (ΔAIC=245.18), or for an absence of imprinting effects (ΔAIC=380.47). This latter point is crucial since it shows that first infection leaves a lifelong imprint of susceptibility to seasonal influenza, just as it does avian-origin influenza (10, 12), and that this imprint is not erased even after decades of exposure to seasonal influenza strains mismatched to the first exposure in childhood. Visual assessment of model fits (Fig. 4F,G) confirmed that models containing imprinting effects at the narrow, NA or HA subtype levels provided the best fits to data. As expected (see Fig. 1G-I), predictions from the two best models were highly collinear, except in their risk predictions among middle-aged, H2N2-imprinted cohorts (birth years 1957-1968), and some other minor differences arising from normalization across birth-years. Predictions of two best models (HA and NA subtype-level imprinting) were much more similar to each other than to predictions from models with much worse AIC (HA group-level imprinting and no imprinting).

Model selection on AZDHS data showed much greater differences in AIC, and much stronger effects of all tested factors than model selection on INSIGHT data (Fig. 4, Table 3). This was unsurprising due to smaller sample sizes, increased noise from geographic variation, and smaller apparent differences in subtype-specific impact in the INSIGHT data. Although no single model fit to INSIGHT data was definitively preferred (six had ΔAIC<4, and differences between fits were negligible (Fig. 4, Table 3)), results of model selection on INSIGHT data aligned qualitatively with model selection on AZDHS data, in that none of the six best models contained HA group-level imprinting effects (Table 3), and overall, NA subtype-level imprinting or HA subtype-level imprinting received the most statistical support. Akaike weights are derived from AIC, and can be interpreted as the fraction of statistical support allocated to a given model, out of all models tested (25). The total Akaike weight for INSIGHT models including NA subtype level imprinting was 0.38, and for models including HA subtype-level imprinting was 0.34. Models including HA group-level imprinting or no imprinting received much less support, with Akaike weights of 0.04 and 0.23 respectively (Fig. 4E).

## Fitted risk patterns

When fitted to AZDHS data, age-specific risk curves took similar forms in all models, with risk decreasing rapidly from birth through adolescence, and then decreasing much more slowly until the end of life (Fig. 4A). When fitted to INSIGHT data, age-specific risk effects always took values close to 1, indicating that the age distribution of confirmed influenza cases was roughly proportional to denominator data on the age distribution of all tested cases (Fig. 4C, Fig. S1).

Tables S1-S3 show parameter estimates and 95% profile confidence intervals from all models fitted to AZDHS And INSIGHT data. Fits to AZDHS data estimated moderate reductions in risk due to imprinting protection, and fits to INSIGHT data estimated weak reductions in risk (Fig. 4B,D, Table S2-S3). Overall, risk parameters fitted to the INSIGHT data took values closer to 1 and had wider confidence intervals than risk parameters fitted to the Arizona data (Fig. 4, Tables S1-S3). As fitted to INSIGHT data, the estimated relative risk of infection given antiviral treatment was usually greater than one, which may reflect that antiviral treatment is often prescribed in response to a positive influenza test. Vaccination was consistently associated with small reductions in risk, although confidence intervals often overlapped the null value of one. The presence of underlying conditions did not strongly impact relative risk and was not included in preferred models (Table 3, Tables S1-S3).

## Effect of evolutionary rate

To test the impact of antigenic evolutionary rate on epidemic age distribution, we used publicly available data from *Nextstrain* (27,28), and from one previously published study (29), to calculate annual antigenic advance, which we defined as the antigenic distance between strains of a given lineage (pre-2009 H1N1, post-2009 H1N1 or H3N2) that circulated in consecutive seasons (Methods). The “antigenic distance” between two strains is used as a proxy for immune cross-protection and can be obtained using a variety of methods that map serological or genetic data into Euclidian space (29,30), or onto phylogenetic trees (28).

If the rate of antigenic drift is a strong driver of age-specific influenza risk, then the fraction of influenza cases observed in children should be negatively related to annual antigenic advance (22). In other words, strains that have not changed much antigenically since the previous season should be unable to escape pre-existing immunity in immunologically experienced adults, and more restricted to causing cases in immunologically naïve children; strains that have changed substantially (i.e. those exhibiting higher antigenic advance) will be less restricted to children. Consistent with this expectation, the AZDHS data showed a slight negative association between annual advance and the fraction of H3N2 cases observed in children, but the Pearson correlation was not strong enough to reach significance in any age group (Fig. 5A). The data contained too few influenza seasons with >100 confirmed H1N1 cases to support meaningful Pearson correlation coefficients specific to pre-2009 or post-2009 H1N1 lineages.

Furthermore, if evolutionary rate is the dominant driver of epidemic age distribution, then outbreaks caused by H1N1 and H3N2 should converge in age distribution when annual antigenic advance is similar.However, the data showed that differences in H1N1 and H3N2’s age-specific impacts persisted, even when lineages showed similar annual advance (Fig. 5A). When comparing the fraction of cases observed in specific age classes, H1N1 data consistently clustered separately from H3N2, with H1N1 consistently causing fewer cases in children (0-10), and elderly adults (71-85), and more cases in adults than H3N2 strains with similar rates of antigenic advance. Smoothed density plots showed no clear relationship between annual antigenic advance and age distribution (Fig. 5B). Overall, the data did not show a strong signal that epidemic age distribution varies with the magnitude of antigenic drift.

# Discussion

Our analyses of two large datasets of influenza cases confirmed a difference in age-specific impacts of seasonal H1 and H3, which was consistent across multiple countries and influenza seasons. We analyzed several possible drivers of these differences, and found greatest support for the hypothesis that immunological imprinting leads to lasting protection against the NA or HA subtype of the first influenza strain encountered in childhood. The data did not support strong effects from broader HA group-level imprinting, as recently detected for novel zoonotic subtypes (10), or from differences in each subtype’s rates of antigenic evolution (22).

Model comparison on both data sets independently provided the strongest support for effects from childhood imprinting to NA. Although NA is not as intensively studied as HA, these results emphasize the importance of both antigens as drivers of protection against seasonal influenza (31,32). The models containing NA and HA subtype-level imprinting produced very similar fits to data, and independently emerged as the top two models in terms of AIC, whether we fit to INSGITH or AZDHS data. We interpret the results as providing equivocal support for HA or NA subtype-level imprinting. This interpretation deviates from widely used rules of thumb for the interpretation of ΔAIC values, where the model based on HA subtype-level imprinting effects (ΔAIC=23.42) would normally be ruled definitively inferior to NA subtype-level imprinting after fitting to AZDHS data. But because the AZDHS dataset was very large, minute differences between the fit of models containing NA and HA subtype-level imprinting produced substantial differences in likelihood and hence in AIC.

Realistically, some combination of effects from both HA and NA subtype-level imprinting probably shape seasonal influenza risk. Unfortunately, given extensive collinearities between predictions of the simple, single-antigen models considered here, we could neither directly test, nor definitively rule out more complicated models of combined effects from imprinting to HA and NA, or to other antigens such as internal proteins. Collinearities in model predictions emerged inevitably from influenza’s limited history of circulation in humans across the past century, and will limit the scope of inference supported by any study relying solely on population-level data. Deeper insights into the respective roles of HA, NA and other influenza antigens as drivers of cohort effects will most likely need to come from focused immunological cohort studies in which individual histories of influenza infection are known, such as those recently funded by the National Institutes of Health (33). Alternatively, the development of immunological biomarkers for diagnosis of imprinting status in individual patients could substantially increase the power of epidemiological inference, which currently relies instead on probabilistic reconstructions of imprinting histories according to birth year (10).

Our finding that narrow, within-subtype imprinting has much stronger impacts than broader, HA group-level imprinting on seasonal influenza is consistent with decades of research on seasonal influenza immunity, where narrow, homosubtypic immune memory is known to drive well-documented epidemiological and phylodynamic patterns, such as antigenic drift, waning immunity and the need for frequent vaccine updates (22,34). Still, given that narrow, within-subtype immunity is known to decay rapidly in the face of antigenic drift, it is striking that signatures of narrow, within-subtype imprinting protection persist across an entire human lifetime, and remain evident even in the oldest cohorts in the data. On average, H1N1 and H3N2 viruses drift by 0.62 and 1.01 antigenic units per year, respectively (29), which roughly corresponds to a two-fold drop in HI titer for every 1.61, or 0.99 years of antigenic evolution between strains. Strains that circulated more than 14 years apart do not show measurable cross-protective titers (29). In this context, it is not obvious that narrow, homosubtypic influenza immunity primed in childhood should provide any meaningful protection after adolescence, let alone decades later in old age. However, we note that the serological assays used to map antigenic cross-reactivity, (hemagglutination inhibition and microneutralization) measure only serum antibodies. The HI assay only measures antibodies specific to sites near the receptor binding domain, and neither assay captures effects from cellular immunity and other mechanisms of protection (13,14). Both assays are imperfect correlates of in-vivo protection in humans or animal models (13,35–37).

Aside from the limitations of common serological assays, one potential biological explanation for the evident longevity of homosubtypic childhood imprinting protection is that imprinting to a particular HA or NA subtype builds strong memory of epitopes conserved among homosubtypic variants of the same subtype, but not across subtypes. One recent study suggests that B cell memory shifts over time to focus on conserved influenza epitopes, as sequential exposures to drifted H1N1 or H3N2 variants repeatedly back-boost cross-reactive memory of epitopes that have not changed over time (38). Another explanation supported by recent immunological data (21), is that the memory B cell clones developed during the first childhood influenza exposure later adapt via somatic hypermutation to “follow” homosubtypic antigenic targets as they drift over time. Thus, childhood imprinting may provide preferential, lifelong protection against a particular HA or NA subtype by filling a child's memory B cell repertoire with clones that will serve in the future, not as final products but as prototypes that can be rapidly and effectively tailored to recognize drifted influenza strains of the same subtype.

A third possibility is that signals of imprinting protection are anomalously strong in the current cohort of elderly adults. For nearly four decades, from 1918-1957, H1N1 persisted as the only strain circulating in humans. The oldest subjects in our data were born slightly after its emergence in 1918, and would not have encountered an influenza virus of any subtype but H1N1 until after age 30. Decades of early-life exposures to H1N1 variants may have reinforced and expanded the breadth of H1N1-specific immune memory in these oldest cohorts. But this strong protection against H1N1 seems to come at a cost; even after decades of seasonal H3N2 exposure later in adulthood, older cohorts have evidently failed to develop equally strong protection against H3N2. Another factor that may have amplified the signal of subtype-specific bias in today’s oldest cohort is antigenic similarity between H1N1 strains that circulated earlier in the 20th century, (which caused imprinting in older cohorts) and modern H1N1 lineages that emerged in 1977 and in 2009 (3,4,39,40). Given that younger cohorts, especially those born before 1977, have had much more varied early life exposures to both H1N1 and H3N2, it is unclear whether equally strong, subtype-specific biases in imprinting protection will persist when the next generation becomes elderly.

One additional consideration in this context is that group 1 antigens appear to induce narrower immune responses than structurally distinct group 2 antigens, which may be better able to induce cross-group responses (Zost, Wu, Hensley, Wilson JID 2019). This may be because most group 1 stem-binding antibodies utilize VH1-69 germline, whereas group 2 stem-binding antibodies utilize a wider array of germlines including VH1-2, VH1-3, VH1-18 and VH3-53 (Zost 2019). Perhaps imprinting with group 1 antigens tends to trap individuals in narrower responses that offer good protection against relatively similar strains to that of first exposure but relatively poor adaptability to other subtypes.

Our failure to detect a strong signal of impact from evolutionary rate on age distributions of H1N1 and H3N2 cases was surprising, but is consistent with growing recognition that existing methods to map antigenic distance between strains do not always capture realized patterns of cross-reactivity in the human population. Humans, who have complex histories of influenza exposure, may be primed to target different influenza epitopes than lab ferrets exposed to a single influenza strain, yet antigenic distance between strains is estimated using such ferret data. As a result, antigenic changes or glycosylation events that cause antigenic escape in large subsets of the human population may be perceived as neutral in existing antigenic maps (41,42). One epidemiologically impactful example of this sort of incognito (unmapped) antigenic escape was observed during the 2013-2014 H1N1 epidemic (41). On the other hand, mapped changes in antigenic position are usually assumed to cause antigenic escape in the entire human population, whereas in reality, these changes may only cause antigenic escape in a subset of the population with unlucky immune histories. We speculate that a clearer relationship between epidemic age distribution and antigenic drift would emerge if antigenic distance measures were modified to incorporate cohort-specific variation in immune history.

Overall, differences between age distributions of infection caused by H1N1 or H3N2 were much more pronounced in the AZDHS data than in the INSIGHT data. Differences between the datasets may arise due to geographic variation in influenza’s epidemiology; the INSIGHT data were collected across five continents, whereas all the AZDHS data came from a single US state. Climatic or demographic characteristics, or high rates of influenza vaccination (44,45), may magnify subtype-specific differences in age distribution within the United States. It is noteworthy that within the INSIGHT data, the subset of cases observed in the United States showed more dramatic differences in age distribution than data collected in many other countries (Fig. 3). Similarly, apparent differences between H1N1 and H3N2’s age distributions were greater in the United States than in Europe in one previous study (18).

On the other hand, the United States was not the only country in the INSIGHT data to show relatively strong differences in age distribution (Fig. 3), and study-specific differences in sampling almost certainly were another contributing factor to apparent differences between the datasets. The INSIGHT study did not enroll children, and a dearth of enrolled cases in the youngest and oldest included age groups may have dampened the signal of subtype-specific differences in risk (Fig. S1). To illustrate the impact of uneven sampling across age groups, we subsampled the AZDHS data to match the sample size and age distribution of all confirmed influenza A cases from the INSIGHT study. Filtering the AZDHS data in this way made differences in age-specific risk from H1N1 and H3N2 appear much smaller (Fig. S1D).

The potential for age-specific sampling biases to erode or magnify the signal of imprinting effects highlights some limitations of existing epidemiological surveillance data, which in turn limited this study’s scope of inference. The largest, long-term epidemiological data sets on influenza come from massive, global surveillance efforts. But due to practical and economic constraints, these data are often collected opportunistically, meaning that sampling effort is uneven over time, and across age groups, and denominator data are rarely documented or shared. Furthermore, while some aspects of surveillance data are already shared publicly by WHO (46), and by the US CDC (47), data on patient ages is not currently reported, or is obscured by aggregation into broad age categories. Focused clinical studies like INSIGHT can yield more extensive information on individual medical histories and on sampling denominators, but such clinical data sets may contain orders of magnitude fewer cases than state or country-wide surveillance data sets, and may not sample all age groups. Arguably, only epidemiological surveillance contains enough confirmed cases to characterize epidemic age distributions with precision across multiple countries and influenza seasons. Several hundred confirmed cases of each subtype are necessary in each sampled country and influenza season to populate epidemic distributions across the population (roughly 100 single years of age, or single years of birth).

As we enter the era of big data, one of the next great challenges for influenza epidemiology will be to understand how measurable genetic and antigenic properties of the circulating viruses impact population-level outcomes, like age-specific risk, birth year-specific risk, vaccine effectiveness and cohort-specific attack rates. Thanks to ambitious and well-funded open science initiatives like the Influenza Research Database (48), the GISAID genetic database(49), and the *Nextstrain* project (28,50), the genetic and antigenic history of influenza circulation in humans is already well-documented and freely available to scientists. The difficulty of accessing corresponding antigenic and epidemiological data remains a key stumbling block, one that could be addressed in part by better recognizing the expense and difficulty of maintaining large public databases. We emphatically echo earlier calls (51) for more systematic sharing of single year-of-age in influenza surveillance data, standardization of sampling effort, and reporting of age-specific denominators, which could represent a turning point in the scientific community’s ability to link influenza's genetic and antigenic properties with epidemiological outcomes.

Our results imply mortality from H1N1 may increase in the future as the imprinting status of elderly cohorts shifts. The vast majority of influenza-related deaths occur in adults over age 65, and H3N2 currently causes many times the number of fatalities as H1N1 (20,52).

These patterns may reflect intrinsic differences in virulence, but we speculate that imprinting protection, which currently limits the incidence of clinically-attended H1N1 infection, may also dramatically reduce H1N1-related mortality. In the future, H2N2 imprinted cohorts (born c. 1950-1968) will become elderly, and their imprinting protection will act instead against seasonal H3N2 (via NA subtype-level imprinting), or against no seasonal strains (via HA subtype-level imprinting). If these cohorts show strong, subtype specific biases from childhood imprinting when they become elderly, the overall mortality burden of H1N1, or of both seasonal subtypes may increase if H1N1 and H3N2 remain endemic in humans.

Altogether, this analysis confirms previous speculation (17) that observed differences in the birth year-specific impacts of H1N1 and H3N2 are indeed driven by cohort-specific differences in childhood imprinting. The finding that such imprinting patterns act at the narrow, HA or NA subtype-level against seasonal influenza enables prediction of the future epidemiological impact of specific seasonal subtypes in high-risk elderly cohorts. Furthermore, the data’s lack of support for broader, HA group-level imprinting effects highlights the difficulty of inducing broadly protective B cell responses against familiar, seasonal strains. Overall, these findings further our understanding of how serological antigenic seniority translates to functional immune protection, and shapes cohort-specific risk during epidemics. The fact that elderly cohorts show relatively weak immune protection against H3N2, even after living through decades of seasonal H3N2 circulation, suggests that antibody responses acquired in adulthood do not provide the same strength of immune protection as responses primed in childhood. These findings support the hypothesis that serological antigenic seniority is not just an emergent property of repeated back-boosting of the titers primed earliest in life, but instead reflect (at least in this case) active interference between memory B cells and *de novo* responses (11). It remains to be seen whether the strong, lifelong immunological biases observed in elderly cohorts within this study are an intrinsic feature of childhood imprinting, or whether these biases have become usually entrenched in the elderly cohorts studied here, due to decades of exclusive H1N1 circulation across the first half of the 20th century.

# Methods

## Data inclusion criteria

From the AZDHS data, we excluded 58 cases with birth years before 1918 (whose imprinting status could not be inferred unambiguously), and one case whose year of birth was recorded in error and had not yet occurred. In the INSIGHT data, we excluded 94 cases with missing information in any of five key categories: age, date of enrollment, vaccination, underlying conditions and antiviral treatment. We excluded an additional 7 cases that fell outside the focal age range of 18-90, and 3 cases whose infecting subtype could not be unambiguously identified due to coinfection.

## Estimation of age from birth year in AZDHS data

The AZDHS data contained three variables, influenza season, birth year and confirmed subtype. For most cases, birth year was extracted directly from the reported date of birth in patient medical records, but age was not known. To fit age-specific risk curves to the AZDHS data, we estimated patient age at the time case observation using the formula [year of observation]-[birth year]. To ensure that the minimum estimated age was 0, the second year in the influenza season of case observation was considered the calendar year of observation (e.g. 2013 for the 2012-2013 season).

## Estimation of birth year from age in the INSIGHT data

The INSIGHT data contained patient age, and the exact date of case enrollment, but not birth year. We estimated birth year using a method that took advantage of precise dates of case enrollment available in the INSIGHT data. The simplest approximation of birth year would have been (observation year)-(age), but this approximation is slightly biased, as cases observed earlier in the year (e.g. in January) are less likely to have passed a birthday in the current calendar year. As shown in Fig. S8, we determined the possible birth dates of an individual observed during each month of the year. We then determined that on average, cases observed during months in the Northern Hemisphere season (Oct.-May) had the following probabilities of birth in each of three possible years, relative to the year of case observation:

**1**

Cases observed during months of the Southern Hemisphere had different probabilities:

**2**

Using these probabilities, we took a weighted average of birth year-specific imprinting probabilities for cases observed in the Northern Hemisphere or Southern Hemisphere influenza seasons.

## Splines

In Figures 2-3, smoothing splines were fit to aid visual interpretation of noisy data. We fit splines using the command *smooth.spline(x = AGE, y = FRACTIONS, spar = 0.8)* in R version 3.5.0. Variables *AGE* and *FRACTIONS* were vectors whose entries represented single years of age, and the fraction of cases observed in the corresponding age group. The smoothing parameter 0.8 was chosen to provide a visually smooth fit. Alternative smoothing parameter choices (0.6 & 1.0) are shown in Figs. S3, S6-S7. Although the choice of smoothing parameter changed the shape of each fitted spline, qualitative differences between splines fitted to H1N1 or H3N2 were robust.

## Model formulation

For each unique country and season in which cases were observed, define *p* as a vector whose entriesrepresent the expected probability that a randomly drawn H1N1 or a randomly drawn H3N2 case was observed in an individual of age *a*. Each model defined, *p* as a linear combination of age-specific risk, birth year-specific risk (i.e. imprinting effects), and other medical history variables, and *p* took slightly different shapes for expected H1N1 and H3N2 case age distributions. All tested models were nested within the equation:

**3**

To include risk factors that only modulated risk from one subtype, we included indicator functions ***1H1N1*** and ***1H3N2***, which took value 1 if *p* described the expected age distribution of H1N1 or H3N2 cases, respectively, and 0 otherwise.

### Denominator data (D)

When fitting to INSIGHT data, *D* was a vector whose entries were proportional to the age distribution of all tested cases within a given country and year. As noted above, corresponding denominator data were not available in the AZDHS dataset, and so factor D was not included in models fit to AZDHS data.

### Age-specific risk (A)

Age-specific risk was defined as a step function, in which relative risk was fixed to value 1 in an arbitrarily chosen age bin, and then *z*-1 free parameters, denoted *r*2 to *rz*, were fit to describe relative risk in all other age bins. Below, ***1i*** are indicator functions specifying whether each vector entry is a member of age bin *i.* To obtain the predicted fraction of cases observed in each single year of age, we normalized the risk distribution so that predicted risk across all age groups summed to 1.

**4**

### Antiviral treatment (T) and underlying conditions (U)

Within each country and season, *fT* defined a vector whose entries describe the fraction of tested cases of a given age that had received antiviral treatment. Free parameter *rT* defines the relative risk of any confirmed influenza infection, given antiviral treatment. Then, risk factor *T* was defined as:

**5**

And risk factor *U* was described similarly:

**6**

### Imprinting (I) and vaccination (V)

Factors describing risk from vaccination and imprinting took forms similar to risk factors *T* and *U*, but with subtype-specific impacts. An indicator function defined whether a given prediction vector described risk of confirmed H1N1 or H3N2 infection. Let *fV* and *fIHxNy* be vectors describing the fraction of cases of each age that were recently vaccinated against influenza, or that were protected against strain *HxNy* by their childhood imprinting. Note that we used the general definition “recent influenza vaccination” because some INSIGHT study sites recorded whether patients had been vaccinated in the last 6 months, while other recorded vaccination in the last 12 months. We defined *rvHxNy* and *rIHxNy* as free parameters describing the risk of confirmed *HxNy* infection, given vaccination, or given imprinting protection. Finally, risk factors describing the effect of vaccination (V) and imprinting (I) were defined as:

**7**

**8**

## Likelihood

We used equations 3-8 to generate predicted case age distributions (*p*) for each influenza season (s) and country (c) in which cases were observed in the data. Then, the likelihood was obtained as a product of multinomial densities across all countries and seasons observed in the data. If *ncs* represents the total number of cases observed in a given country and season, *x0cs,…xmcs*each represent the number of cases observed in each single year of age/single year of birth, and if *p0cs…pmcs* each represent entries in the model’s predicted age/birth year-distribution of cases, then the likelihood is given by:

## Model fitting and model comparison

We fit models containing all possible combinations of the above factors to influenza data from each unique country and season in the AZDHS data, or in the INSIGHT data. We simultaneously estimated all free parameter values using the optim() function in R. We calculated likelihood profiles and 95% profile confidence intervals for each free parameter. Confidence intervals were defined using the method of likelihood ratios (27).

## Antigenic advance

We obtained antigenic distance estimates from *Nextstrain (nextstrain.org)*(28,50)*,* and from source data associated with Figure 3 in Bedford et al. (30). *Nextstrain* calculates antigenic distance using genetic data from GISAID (49), and using methods described by Neher et al. (29). We analyzed “CTiter” estimates from *Nextstrain*, which correspond to Neher et al.’s “tree model” method. Datasets from *Nextstrain* and Bedford et al. both contained redundant antigenic distance estimates for the H3N2 lineage, but only Bedford et al. analyzed the pre-2009 H1N1 lineage, and only *Nextstrain* data analyzed the post-2009 H1N1 lineage. The antigenic distance estimates reported by Bedford et al. were roughly proportional to those reported on *Nextstrain*, but greater in absolute magnitude (29). To enable direct comparison among all three lineages on the same plot axes, we rescaled pre-2009 H1N1 estimates from Bedford et al. using the formula *dNextstrain* = 0.47*dBedford*. The scaling factor was chosen so that directly-comparable H3N2 distance estimates obtained using each method were well-aligned (Fig. S9). The *Nextstrain* data files used in this analysis are archived at ###//CODE\_LINK//####.

## Code and data availability

Code to perform all reported analyses and construct all plots is available \#\#HERE\#\#. AZDHS data is available as a supplementary data file. Data from the INSIGHT study are available by application, pending approval from the study's scientific review committee (<http://insight.ccbr.umn.edu/index.php>). Because we are not free to share the INSIGHT data, the posted code contains a dummy INSIGHT data file with scrambled column entries. In other words, the data files are formatted properly, and the code will run, but the actual data entries have no biological meaning.

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