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

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# Abstract

Across decades of simultaneous seasonal circulation in humans, influenza A subtypes H1N1 and H3N2 have consistently caused 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 has a greater impact in young adults. These differences in age distribution may in fact be driven by birth year-specific differences in childhood immune imprinting. Individuals gain particularly strong, lifelong immune memory against the influenza viruses encountered in childhood, but it is not clear whether narrow immune memory from imprinting to a particular antigenic subtype, or broader immune memory from imprinting to a particular phylogenetic group (with conserved antigenic properties) governs cohort-specific differences in seasonal influenza risk. Another hypothesis is that subtype-specific differences in evolutionary rate, rather than imprinting effects, explain observed differences in age distribution.

Using two large, seasonal influenza data sets, we performed likelihood-based model fitting and model selection. Results showed that narrow, 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 against seasonal influenza. Separate analysis of 2009 pandemic data showed some support for broadly protective imprinting during the first pandemic wave, but not during the second pandemic wave. Altogether, these population-level patterns mirror within-host immunological outcomes, where only exposure to novel strains, not familiar seasonal strains, can elicit broadly protective antibody responses. These findings confirm that narrow, homologous immunity drives protection against seasonal influenza, also highlight the difficulty of inducing broadly protective immune responses against familiar, seasonal strains using vaccines. Furthermore, these results imply that H1N1’s mortality burden (currently low) may increase in the future as cohorts with different childhood imprinting eventually age in to the high-risk elderly group.

# Introduction

Childhood exposures to influenza leave an immunological imprint, which has reverberating, lifelong impacts on the quality and specificity of immunity against influenza viruses encountered later in life. Foundational work on this phenomenon, also known as original antigenic sin (1) or antigenic seniority (2), showed that individuals of all ages maintain the highest serological titers against influenza strains encountered in childhood, and not necessarily against contemporary strains of the same subtype. Although foundational studies by Francis (1), and later Lessler et al. (2), argued that immune imprinting from childhood would not interfere with effective, *de-novo* antibody responses later in life, their findings ignited decades of scientific interest in the potential negative impacts of antigenic sin (3,4).

A new wave of studies has instead focused on potential benefits from childhood immune imprinting, which is thought to have shaped cohort-specific immunity against every pandemic in the modern epidemiological record (5–11). Furthermore we now know that immune imprinting can provide broad, heterologous (cross-subtype) protection against novel, emerging avian influenza viruses (12). As avian and pandemic influenza viruses were historically considered too novel to face any pre-existing immunity as they emerged into humans, the existence of any protection from imprinting is a welcome benefit.

Recent studies have also highlighted imprinting’s ability to shape multiple layers of influenza immune memory, both broad and narrow. Influenza’s immunodominant epitopes, the primary targets of most antibody responses, show considerable structural diversity and drift antigenically over time. As a result, most seasonal influenza immunity provides only narrow, ephemeral, protection. Until recently, broader, cross-subtype (heterologous) responses were considered rare or anomalous, and so research on immune imprinting focused primarily on narrow, within-subtype (homologous) responses.

More recently, the 2009 H1N1 pandemic, and subsequent efforts to develop a universal influenza vaccine drew attention to antibody responses that can indeed provide broad, heterologous protection (13–15). Broadly protective antibodies that target conserved epitopes on the HA stalk have been particularly well studied, and are common in existing, human antibody repertoires (13–16). These broadly protective antibodies typically provide cross protection at the HA group-level, i.e. across all subtypes in genetic group 1, or group 2 of the HA tree (14). Recently, we showed that childhood imprinting strongly shapes the population-level impacts of these broadly protective, HA group-level responses against avian influenza. Birth cohorts show strong, lifelong protection against novel, avian influenza viruses from the same HA group as the seasonal strains encountered in childhood (12).

Similar imprinting effects may also shape how protection against specific seasonal influenza subtypes is distributed across birth years. Since 1977, two distinct subtypes of influenza A, H1N1 and H3N2, have circulated seasonally in humans, and these subtypes show consistent differences in age distribution (17–20). H3N2 causes the vast majority of cases in older adults, while H1N1 causes a greater proportion of cases in younger cohorts. These differences in age distribution are qualitatively consistent with childhood imprinting patterns, in that older cohorts were almost certainly exposed to historical variants of H1N1 in childhood, and may now be preferentially protected against modern, seasonal H1N1 (18–20). Likewise, younger cohorts have the highest probabilities of childhood imprinting to H3N2, which is consistent with greater incidence of the opposite seasonal subtype, H1N1.

It remains unclear whether these ostensible cohort effects arise due to immune memory imprinted at the narrow, within-subtype level, or at the broader group level. The well-established impacts of narrow, within-subtype seasonal influenza immunity intuitively suggest narrow, subtype-level imprinting may have the strongest impacts. If HA subtype-level imprinting is the key driver, then childhood imprinting to H1, or to H3 might provide preferential lifelong protection against seasonal variants of the same HA subtype. 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 antibodies involved in group-level protection usually play a minimal role in immunity against familiar, seasonal influenza viruses, these antibodies can rise in frequency and play a strong role in immunity if the host lacks immune memory of more variable, immunodominant epitopes (13,14). 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 antibodies are unable to recognize their drifted, variable targets. If HA group-level imprinting strongly shapes cohort effects, 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. 1). Furthermore, because broadly protective influenza immunity is most often deployed against antigenically novel strains, whose conserved epitopes may provide the only recognizable immune targets, we predict that broad, group-level imprinting will show stronger impacts during the 2009 H1N1 pandemic than during normal, seasonal H1N1 circulation.

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 H1N1 drifts more slowly than H3N2, 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 (21).

Using two large data sets on seasonal influenza incidence, which together represent 22,041 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. We compared age distributions of infection caused by H1N1 and H3N2, and confirmed that subtype-specific differences in risk are consistent with previously observed patterns, and with histories of childhood imprinting. 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 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 influenza incidence.

# The Data

We analyzed two large epidemiological data sets. The first was provided by the Arizona Dept. of Health Services (AZDHS), and contained 18,812 H1N1 and H3N2 cases, confirmed using a combination of rapid tests, serology and PCR, per established department protocols (22). Cases were observed across 22 years of influenza surveillance, from the 1993-1994 influenza season through the 2014-2015 season, although observed case counts increased after the 2009 pandemic precipitated increases in sampling efficiency (Table 1). Per epidemiological standards, the Arizona influenza season was defined as beginning in epidemiological week 40 (around early October) and ending in week 39 of the following year.

A second data set provided by the INSIGHT outpatient study (http://insight.ccbr.umn.edu/) contained 3,612 PCR-confirmed H1N1 and H3N2 cases, observed across 16 countries between 2009 and 2016. The INSIGHT data 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. Note that we use the non-specific term “recent influenza vaccination” because some study sites recorded whether patients had been vaccinated in the past 6 months, and others recorded whether patients had been vaccinated in the past 12 months. To facilitate comparison between data sets, we defined INSIGHT cases enrolled from Oct-May as part of the Northern Hemisphere (NH) influenza season, and cases enrolled from June-Sept as part of the Southern Hemisphere (SH) season. These definitions were used to facilitate comparison between data sets. October 1 roughly aligns with the week 40 NH season start date used in the Arizona data set.

### Differences between datasets

The INSIGHT dataset was smaller, and sampled across a shorter period of time than the AZDHS data. The INSIGHT data also excluded children under age 18, and enrolled relatively few elderly individuals and young adults (Fig. S7). However, the INSIGHT data sampled a greater geographic range, contained denominator data on the age distribution of all tested cases, and contained details on patient medical history that were not available in the AZDHS data.

# The Model

## Reconstructed imprinting patterns

Reconstructed, birth year-specific probabilities of imprinting mirror the timeline of influenza circulation in humans (Fig. 1A). Older cohorts born between pandemics in 1918 and 1957 imprinted to H1N1, and middle-aged cohorts born between pandemics in 1957 and 1968 imprinted to H2N2. Ever since its emergence in 1968, H3N2 has dominated seasonal circulation in humans, and caused the majority of imprinting in younger cohorts. However, H1N1 has also caused some seasonal circulation since 1977, and thus a fraction of post-1977 cohorts are imprinted to H1N1.

We reconstructed birth year-specific probabilities of imprinting to H1N1, H2N2 and H3N2 using methods described previously (12). 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 some probability of remaining naïve to influenza, and not yet having imprinted. Code to perform reconstructions is available at in the Supplementary Materials.

## Expected age distributions under alternate imprinting models

All tested models assumed childhood imprinting to H1N1 would protect against modern, seasonal H1N1, and that childhood imprinting to H3N2 would protect against modern, seasonal H3N2. 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. However, differences in the shape of predicted risk in middle-aged, H2N2 imprinted cohorts provided leverage to differentiate between imprinting at the HA subtype, HA group or NA subtype level (Fig. 1B).

To tease apart age-specific risk factors from birth year-specific imprinting effects, we noted that age-specific risk factors 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. But 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 (i.e. as a function of imprinting status). 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 age 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, we fitted a suite of models to each data set using a multinomial likelihood, and then performed model selection using AIC. 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). 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. 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 (17–20). 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 observed, 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 cocirculated (≥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 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). 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. 1), predictions from the two best models were highly collinear, except in their risk predictions among middle-aged, H2N2-imprinted cohorts (birth years 1957-1968). 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 scores (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 smaller sample sizes, increased noise from geographic variation, and smaller apparent differences in subtype-specific impact in the INSIGHT data (Table 2, Fig. 3). 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 can be interpreted as the proportional support for a given model, out of all models tested. 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. Imprinting protection was consistently associated with moderate reductions in risk in protected birth years (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), which is consistent with the fact that the INSIGHT data contained fewer confirmed cases and showed weaker age-specific or subtype-specific differences in risk. 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).

## Strength and specificity of imprinting protection against 2009 pandemic H1N1

The AZDHS data contained large numbers of H1N1 cases confirmed during the 2009 pandemic (Table 1). We repeated model selection on these pandemic H1N1 cases to assess the strength and breadth of imprinting protection against the pandemic strain during the first pandemic wave (2008-2009 influenza season), and during the second pandemic wave (2009-2010 season). Minimal H3N2 circulation during the pandemic prevented us from re-fitting the age-specific risk curve to pandemic data. Instead, we input the age-specific risk curve fitted to seasonal AZDHS data, and then re-fit models assuming HA group, HA subtype and NA subtype-level imprinting to H1N1 data from the first and second pandemic wave.

Results showed that, under any imprinting model, the estimated strength of imprinting protection was strongest during the first pandemic wave, intermediate during the second wave, and weakest during seasonal influenza circulation (Fig. 6A). Model selection on first-wave pandemic data (2008-2009 influenza season) showed the strongest support for HA group-level imprinting (Fig. 5B). However, model selection on data from the second pandemic wave was consistent results from seasonal influenza data, where narrower, NA and HA subtype-level imprinting received the most statistical support (ΔAIC = 0, 20.5, respectively).

## Effect of evolutionary rate

To test the impact of antigenic advance on epidemic age distribution, we used publicly available data from *Nextstrain* (23–25) to calculate the annual antigenic advance of H1N1 and H3N2, relative to the previous influenza season (Methods). 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 the epidemic strain’s antigenic advance since the previous season. In other words, strains that have not changed antigenically since the previous season should be more restricted to causing cases in immunologically naïve children. Furthermore, if the rate of antigenic advance is the dominant driver of age-specific influenza risk, then in seasons where H1N1 and H3N2 show similar rates of advance, epidemics caused by either subtype should converge in age distribution.

In the AZDHS data, annual antigenic advance was somewhat negatively associated with the fraction of H3N2 cases observed in children, but the Pearson correlation was not strong enough to reach significance in any age group (Fig. 6A). 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.

Differences in H1N1 and H3N2’s age-specific impacts persisted, even after controlling for antigenic advance. 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 (Fig. 6A). Smoothed density plots showed no clear relationship between annual antigenic advance and age distribution (Fig. 6B). Overall, the data showed no evidence that H1N1 and H3N2’s age-specific impacts converge when rates of antigenic advance are similar, and although weak effects cannot be ruled out, these analyses did not reveal a strong relationship between antigenic advance and epidemic age distribution.

# Discussion

Altogether, results showed that childhood imprinting to a particular NA or HA subtype is the most likely driver of observed differences in the age-specific impacts of seasonal H1N1 and H3N2. The data did not support strong effects from broader, HA group-level imprinting, or from differences in each subtype’s rates of antigenic advance. 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.

Based on widely used rules of thumb for the interpretation of ΔAIC values, HA subtype-level imprinting effects (AZDHS ΔAIC=23.42), would normally be ruled inferior to NA subtype-level imprinting. However, predictions from models containing HA and NA-subtype level imprinting were very similar, differing only slightly in the predicted protection status of H2N2 imprinted cohorts. Because the dataset was large, these small differences in model fit produced substantial differences in likelihood and in AIC. It is possible that some combination of effects from both HA and NA subtype-level imprinting shapes seasonal influenza risk, but given the extensive collinearities between predictions of the two best models, we could neither directly test, nor definitively rule out this hypothesis.

The low diversity of influenza circulation in the past century, and unbalanced sampling across time, space and age groups limited the scope of inference supported by this study, or any study relying exclusively on cross-sectional population data. Deeper insights into the relative impacts of HA-specific or NA-specific childhood imprinting will most likely need to come from immunological data. The recent NIH initiative to fund large cohort studies [CITE], is a promising development, but these studies may not yield results for over a decade. Development of an assay to diagnose imprinting status in cross-sectional data from individuals, or from banked blood and serum samples would also be a major step forward.

Evidence that narrow, within-subtype imprinting has much stronger impacts than broader, HA group-level imprinting is consistent with decades of research on seasonal influenza immunity, where narrow, homologous immune memory is known to drive well-documented epidemiological and phylodynamic patterns. Still, given that narrow, within-subtype immunity is known to decay rapidly in the face of antigenic drift, it is somewhat surprising that signatures of homosubtypic imprinting protection persist across an entire human lifetime, remaining evident even in the oldest cohorts. On average, H1N1 and H3N2 viruses drift by 0.62 and 1.01 antigenic units per year, respectively [cite Bedford eLife paper], which roughly corresponds to a two-fold drop in titer for every 1.61, or 0.99 years of antigenic evolution between strains. Strains that circulated more than 15 years apart usually show no measurable serological cross-protection [Bedford, elife]. Thus, it is somewhat puzzling that narrow, homologous influenza immunity primed in childhood provides any meaningful protection after adolescence, let alone decades later in old age.

One possible explanation for the evident longevity of homologous childhood imprinting protection is that imprinting to a particular HA or NA subtype builds strong memory of epitopes conserved among homologous variants of the same subtype, but not across subtypes. Another possible explanation is that the memory B cell clones developed during the first childhood influenza exposure later adapt via somatic hypermutation to “follow” homologous 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 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 (born in the 1920s and 1930s). Only H1N1 circulated in humans from 1918 to 1957, so the oldest subjects in the data (born shortly after the 1918 pandemic) would not have encountered an influenza virus of any other subtype until 20 or 30 years or age. Decades of early-life exposures to diverse H1N1 variants may have reinforced and expanded the breadth of H1N1-specific immune memory in these cohorts. Furthermore, H1N1 lineages that emerged in both 1977 and 2009 shared some antigenic properties with strains that circulated earlier in the 20th century, during older cohorts’ childhoods [CITE]. It is unclear whether younger cohorts born since 1977, who have been exposed to a mixture of H1N1 and H3N2 strains in their first decades of life, will show such strong, subtype-specific biases in imprinting protection by the time they become elderly.

Although the data did not support effects from broad, HA group-level imprinting against seasonal influenza, or during the second wave of the 2009 H1N1 pandemic, broad, HA group-level imprinting was supported during the first pandemic wave. In other words, population-level impacts of broadly protective immunity appear to decay rapidly as novel influenza strains establish in humans and become familiar to our immune systems. These population-level results mirror recent within-host immunological data. Individuals who lacked pre-existing titers against the 2009 pandemic strain initially deployed broadly protective antibodies specific to conserved epitopes on the HA stalk. However, on second exposure to the 2009 strain, all subjects had developed immune memory of more variable, immunodominant epitopes, and reverted to antibody responses that provided only narrow, homologous cross-protection. Following on these observations, the conceptual basis for HA stalk-based universal influenza vaccines is that exposure to an influenza virus whose variable, immunodominant epitopes are novel [CITE], or inaccessible [CITE] reliably stimulates broadly protective antibody responses, although exposure to a familiar, seasonal strain does not [CITE].

Our population-level findings, together with recent immunological evidence, highlight that narrow immunity is not an intrinsic property of the influenza virus, but an emergent property of within-host competition between broadly and narrowly neutralizing antibody clones. Due to structural properties of the hemagglutinin antigen, whose variable epitopes are exposed and highly immunogenic, narrowly neutralizing antibodies can usually exclude their less-fit, broadly neutralizing competitors [CITE]. But exposure to novel avian or pandemic strains (whose conserved epitopes may be the only recognizable immune targets) can facilitate competitive release, and broad immune protection).

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 was collected across five continents, whereas all the AZDHS data all came from a single US state. 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, one previous study also observed greater differences between the age distribution of H1N1 and H3N2 within data collected in the US than within data collected in Europe (17). It is possible that the US’s climatic or demographic characteristics, or high rates of influenza vaccination in the US [CITE], somehow magnify subtype-specific differences in age distribution.

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 differences in sampling between the INSIGHT and AZDHS data almost certainly also contributed to differences in the apparent strength of signal in each data set. The INSIGHT study did not enroll children, and enrolled relatively few young and few elderly adults, cohorts where subtype-specific differences in imprinting were particularly distinct (Fig. S7). This dearth of cases at the extremes of age may have dampened the signal of subtype-specific differences in risk in the INSIGHT data. The age distribution of tested cases was not known in the AZDHS data, but given the large numbers of confirmed cases in children, teens and the elderly (Fig S7), it is obvious that the extremes of age were comparatively well-sampled. To illustrate the impact of uneven sampling across age groups, we down-sampled the AZDHS data to match the sample size and age distribution of all confirmed 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. S7).

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 our ability to test more complex hypotheses in this study. 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 surveillance data are already shared publicly by WHO [CITE], and by the US CDC [CITE], data on patient ages is not currently reported, or is obscured by aggregation into broad age categories.

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 season-specific attack rates. Thanks to ambitious and well-funded open science initiatives like the GISAID genetic database, and the *Nextstrain* project, 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. The expense and difficulty of maintaining large, public databases should not be taken for granted, and those responsible for collecting and curating high-quality data deserve more professional credit for their work. But more systematic sharing of influenza surveillance data, standardization of sampling effort, and reporting of age-specific denominators could represent a turning point in the scientific community’s ability to link influenza's genetic and antigenic properties with epidemiological outcomes.

Epidemiologically, these results imply that the incidence and mortality impacts of H1N1 may increase in elderly cohorts over time. Currently, H3N2 causes many times the number of influenza-related deaths as H1N1. These patterns may reflect intrinsic differences in virulence, but we speculate that population-level imprinting patterns may also shape H3N2’s greater mortality impact. The vast majority of influenza-related deaths occur in adults over age 65, cohorts whose imprinting protection currently limits the incidence of clinically-attended H1N1 infection. In the future, H2N2 imprinted cohorts (born c. 1950-1968) will eventually become elderly, and will instead have imprinting protection against H3N2 (NA subtype-level), or may lack imprinting protection against either seasonal subtype (HA subtype-level). In either scenario, the mortality burden of H1N1 is predicted to increase, while the mortality burden of H3N2 may decrease.

On one hand, this study’s failure to detect any signal of broadly protective imprinting in seasonal influenza surveillance data is unsurprising. Narrow, homologous immunity is a hallmark of seasonal influenza’s evolutionary and epidemic dynamics, which drives selection for immune escape, permits repeated infections, and demands frequent vaccine updates. But on the other hand, these results raise new questions about the longevity of apparent protection from narrow, homologous immune responses primed in childhood, and about the underlying within-host dynamics of B cell repertoire development. With implications for universal influenza vaccine development, these results also highlight the difficulty of inducing broadly protective antibody responses against familiar, seasonal influenza strains.

# Methods

## Data inclusion criteria

We analyzed all reported cases in the AZDHS data, except one case whose year of birth was recorded in error and had not yet occurred. In the INSIGHT data, we excluded ## cases with missing information in any of five key categories: age, date of enrollment, vaccination, underlying conditions and antiviral treatment. We excluded an additional ## recorded cases who were too young to meet the study’s inclusion criteria, and 3 cases whose infecting subtype could not be unambiguously identified due to possible coinfection.

## Classification of pandemic cases

The first wave of the 2009 H1N1 pandemic occurred during the summer of 2009 [CITE], and fell at the end of the 2008-2009 AZDHS influenza season. In the northern hemisphere, the second wave of the 2009 H1N1 pandemic occurred during the fall of 2009 and winter of 2010, falling within the 2009-2010 AZDHS season. Granted, the data provided no ability to separate pandemic cases from pre-pandemic H1N1 circulation occurring early in the 2008-2009 season, and so a small number of non-pandemic H1N1 cases may have been mis-classified. But due to the dramatic increase in the number of recorded H1N1 cases during the first pandemic wave (Table 2), and due to the H1N1’s distinct age distribution of incidence 2008-2009 season (Fig. ##), we are confident that the majority of H1N1 cases observed during the 2008-2009 season were indeed part of the first pandemic wave.

The INSIGHT study began collecting data in response to the 2009 pandemic, and did not capture the first pandemic wave. Few H1N1 cases were recorded during the second pandemic wave in the Northern and Southern Hemispheres (Table 3, 2009-2010 NH season, 2010 SH season), and signals in the INSIGHT data were inherently weak, so we did not analyze these pandemic data.

## Estimation of age from birth year from age 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. 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 insure that the minimum estimated age was 0, where the second year in the corresponding influenza season 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 the precise temporal resolution of INSIGHT dates of enrollment. 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. Using logic laid out in Fig. S9, we used the following three formulas to estimate three possible birth years for cases observed in the NH influenza season: (current year)-(age)-1, (current year)-(age)-, and (current year)-(age)-+1. We then took a weighted average of the three relevant, birth year-specific imprinting protection probabilities, using weights 0.0625, 0.875 and 0.0625, respectively. Meanwhile, for cases observed in a SH season, only two birth years were possible. Here, we used the formulas: (current year)-(age)-1, and (current year)-(age) to calculate both possible birth years, with each receiving a probabilistic weight of 0.5.

## 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. S##-##. Although the exact shape of fitted splines changed was sensitive to our choice of smoothing parameter, qualitative differences between H1N1 and H3N2’s age distributions 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:

1

Note ***1H1N1*** is an indicator function that takes value 1 if *p* describes the expected age distribution of H1N1 cases, and 0 otherwise, thus including subtype-specific risk factors only in relevant subtype-specific predictions. Similarly, ***1H3N2*** takes value 1 if *p* describes the expected age distribution of H3N2 cases. Subtype-independent risk factors always modulate *p,* regardless of the focal subtype.

### 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 the. Model.

### 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 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 risk distribution given by equation 2 so that predicted risk across all age groups summed to 1.

**2**

### Antiviral treatment (T)

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* is defined as:

### Underlying conditions (U)

The underlying conditions risk factor takes the same form as factor *T*:

**3**

### Vaccination (V) and Imprinting (I)

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 *fI* be vectors describing the fraction of cases of each age that were vaccinated against influenza, or that were protected against strain *HxNy* by their childhood imprinting.

4

5

## Model fitting and model comparison

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.

## Antigenic advance

To estimate annual antigenic advance in specific influenza seasons, as shown in Fig. 6, we calculated the average antigenic position per-lineage, per-season, and then found the distance between the average antigenic position in consecutive seasons. We obtained antigenic position estimates for H3N2 strains (starting in 2002), and for post-pandemic H1N1 strains using publicly available *Nextstrain* data. Antigenic position values were calculated using genetic data extracted from GISAID [CITE], and using the CTiter method described by ## et al., [CITE]. Pre-2009 H1N1 antigenic position estimates, were not reported on *Nextstrain*, and instead were extracted from a study published by Bedford et al. [CITE]. Antigenic distance estimates reported by Bedford et al., are proportional to those reported on *Nextstrain*, but greater in absolute magnitude [CITE]. We scaled pre-2009 H1N1 distance estimates appropriately to match *Nextstrain* estimates.

## 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 an INSIGHT data file with scrambled column entries.