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

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# Abstract

For the past four decades, two seasonal subtypes of influenza A, H1N1 and H3N2, have circulated in humans, and have caused different age distributions of infection. H3N2 typically causes the majority of cases, and the majority of deaths in high-risk elderly cohorts, whereas H1N1 has a greater impact in young adults. These ostensible differences in age-specific risk may in fact be driven by birth year-specific differences in childhood immunological 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 group of subtypes with conserved antigenic properties governs cohort-specific differences in seasonal influenza risk. We analyzed two large, seasonal influenza data sets, which together included 22,041 confirmed influenza A cases across 18 years and five continents. We performed likelihood-based model fitting and model selection to determine whether broad or narrow imprinting protection best explained cohort-specific seasonal influenza risk. We also considered the potential impact of differences in H1N1 and H3N2’s rates of antigenic evolution on subtype-specific differences in age distribution. Results show narrow, within-subtype childhood imprinting is the strongest driver of cohort-specific seasonal influenza risk, whereas broad, cross-subtype protection has little detectable impact. Differences in H1N1 and H3N2’s rates of antigenic evolution were not the dominant driver of subtype-specific differences in age distribution, but may have weaker impacts. Altogether, these findings suggest the subtype-specific burden of seasonal influenza may shift in the near future. Elderly cohorts at the highest risk of mortality are currently imprinted to, and protected against subtype H1N1, but in the future H1N1 may become more deadly as cohorts imprinted to the mismatched H2N2 subtype become elderly. With implications for universal influenza vaccine development, these findings also highlight the difficulty of inducing broadly protective immune responses against seasonal influenza.

# 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 Francis, and later Lessler, originally 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.

These 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 [CITE]. Broadly protective antibodies that target conserved epitopes on the HA stalk have been particularly well studied, and are common in existing, human antibody repertoires [CITE]. Stalk-specific 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 [XITE]. Recently, we showed that childhood imprinting strongly shapes the population-level impacts of these broadly protective 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 (13–16). 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 (14–16). 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 strong impacts from narrow, subtype-level imprinting. 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 [CITE], these antibodies can rise in frequency and play a strong role in immunity if the host lacks immune memory of more variable, immunodominant epitopes [CITE]. 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 seasonal influenza 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).

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 (17).

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 across time, and space. 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 could be a dominant driver of observed subtype-specific differences in age distribution, we analyzed the relationships between the annual magnitude of antigenic advance, and the shape of age-specific risk.

# Methods

## Data

We analyzed two large epidemiological data sets. The 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 (18). 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 northern hemisphere (NH) influenza season was defined as beginning in epidemiological week 40 (around early October) and ending in week 39 of the following year. For most AZDHS cases, date of birth and birth year was extracted directly from patient medical records. We estimated patient age at the time of testing by subtracting their birth year from the year of case observation.

A second data set provided by the INSIGHT outpatient study [REF] contained 4,006 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 relevant medical history for each patient: antiviral treatment used (Y/N), presence of any underlying conditions (Y/N), and recent influenza vaccination (Y/N). 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 in Oct-May as part of the Northern Hemisphere (NH) influenza season, and cases enrolled in June-Sept as part of the Southern Hemisphere (SH) season. October 1 roughly aligns with the week 40 NH season start date used in the Arizona data set. Table ## shows confirmed case counts from each country and season.

We excluded 7 subjects whose ages did not fall in the focal age range (18-90). We excluded 94 subjects for whom season, age, vaccination status, antiviral treatment use or presence of underlying conditions was unknown. We also excluded 3 cases of co-infection with multiple subtypes. After excluding 104 cases in total, we analyzed a data set of 11,575 tested cases, including 1,998 confirmed H1N1 cases and 2,080 confirmed H3N2 cases.

The INSIGHT data contained patient age, and the exact date of case observation, but did not record patient birth year. Birth year can be approximated as (observation year)-(age), but this approximation is slightly biased, and biased in different ways for cases observed during the NH and SH influenza seasons. Cases observed earlier in the year (e.g. in January) are more likely to have a birthday that has not yet passed in the current calendar year, whereas cases observed later in the year (e.g. in December) are most likely to have already had a birthday pass in the current calendar year. Using logic laid out in Fig S9, we used the following three formulas to obtain 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 (Fig S9).

# Results

## Expected impacts from imprinting

### 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.

### 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).

### 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, S1-S8). These patterns are qualitatively consistent with previously reported differences in subtype-specific impact (13–16), and consistent with the predicted effects of cohort-specific imprinting (Fig 1). 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 adults.

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), and only included cases observed in adults, age 18+ (although the AZDHS data included children, the INSIGHT study did not). Figures S1-S7 show similar plots for all countries and seasons represented in the data, plots of AZDHS data that include children, and plots showing alternate smoothing parameters.

## Imprinting model selection

To test quantitatively whether observed, subtype-specific differences in age distribution were most consistent with narrow imprinting to specific HA or NA subtypes, or with broad, HA group-level imprinting, we fitted a suite of models to each data set using maximum likelihood, and then performed model selection using AIC. Technical details and a link to all relevant code are provided in the Methods.

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. 4-5, Table 3). The AZDHS data showed a preference for NA subtype-level imprinting over HA subtype-level imprinting (ΔAIC=23.4), and effectively no statistical support for broad, HA group-level imprinting (ΔAIC=245), and no support for models that did not contain imprinting effects (ΔAIC=380). Visual assessment of model fits (Fig. 4##-##) confirmed that models containing imprinting effects at the narrow, NA or HA subtype levels provided the best fits to data. As expected (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 than model selection on INSIGHT data, possibly due to lower sample sizes and smaller apparent differences in subtype-specific impact within the INSIGHT data. Although no single model fit to INSIGHT data was definitively preferred (six had ΔAIC<4, and differences between fits were minor (Fig. 6, 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, 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. 5E).

## Strength 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, which may have differed from protection against seasonal H1N1. Because H3N2 circulation during the pandemic was minimal, we were unable to fit a pandemic-specific age-specific risk curve, and instead input the age-specific risk curve fitted to seasonal influenza data.

Results showed that, under any imprinting model, the estimated strength of protection was stronger against pandemic H1N1 than against seasonal H1N1 (Fig. 6A), during both the first and second pandemic waves (2008-2009 and 2009-2010 influenza seasons, respectively). Model selection on data from the second pandemic wave was consistent with model selection on seasonal influenza data, in that models containing NA and HA subtype-level imprinting received the most support (ΔAIC = 0, 20.5, respectively). However, model selection on first-wave pandemic data (2008-2009 influenza season) showed the strongest support for HA group-level imprinting (Fig. 6B).

## Effect of evolutionary rate

In addition to cohort effects, differences in H1N1 and H3N2’s rates of antigenic advance may contribute to differences in subtype-specific age distribution. H3N2’s faster average rate of antigenic evolution may allow it to more rapidly escape immune memory in older adults, whereas H1N1 may be more restricted to infecting young, immunologically naïve children (17).

To test the impact of antigenic advance on epidemic age distribution, we used publicly available data from Nextstrain to calculate ΔA, which we defined as the annual antigenic advanced of circulating H1N1or H3N2 strains, relative to the previous influenza season. 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 ΔA, and the fraction of cases in immunologically experienced adults should be positively related to ΔA. Furthermore, if the rate of antigenic drift is the dominant driver of age-specific influenza risk, and has a stronger impact than cohort effects, then seasonal epidemics caused by H1N1 or H3N2 strains with similar ΔA should converge in age distribution.

In the AZDHS data, ΔA 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 fact, ΔA was not significantly associated with the fraction of H3N2 cases occurring in any age group (Fig. 7). 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. However, plots show that H1N1 data points tended to cluster separately from H3N2, and that H1N1 consistently caused fewer cases in the youngest children (0-10), and in the oldest adults (71-85), than H3N2 strains with similar ΔA. Similarly, H1N1 consistently caused more cases in teens and young adults (11-25), than H3N2 strains with similar ΔA. Altogether, these data show that differences in cohort-specific impacts from H1N1 and H3N2 persist, even after controlling for the strain-specific rate of antigenic advance.

### Models fit to AZDHS Data

We fitted 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, additional free parameters estimated the relative risk of confirmed H1N1 or H3N2 infection given imprinting protection.

Age-specific risk curves fitted to AZDHS data consistently showed similar patterns, with the risk of severe infection highest in young children, and then decreasing over time (Fig. ##). The estimated relative risk of infection, given protective imprinting, was always less than one, indicating some level of protection (Table ##). The estimated impact of imprinting on H1N1 risk was consistently stronger than on H3N2 risk (TABLE ##).

### Models fit to INSIGHT data

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 respectively characterized the relative risk of any influenza infection, given antiviral treatment or given the presence of underlying conditions. We assumed vaccination might have slightly different effects on H1N1 risk and H3N2 risk, so factor V added two free parameters which characterized the relative risk of H1N1 or of H3N2 infection, given vaccination against influenza. We tested all possible combinations of V, T, and U, in addition to age-specific risk (A) and each of the three imprinting hypotheses (S, N and G), for a total of ## tested INSIGHT models.

Additionally, the INSIGHT study collected denominator data on the age distribution of cases that tested negative for influenza. Thus, in all models fitted to INSIGHT data, we input the age distribution of all tested cases from each country and influenza season as the null, expected age distribution. Because these denominator data were not available in the AZDHS dataset, the age-specific risk curves fit to the INSIGHT and AZDHS data have different interpretations. The age-specific risk curve fitted to AZDHS data represented all facets of the infection and case observation process, including the age-specific risk of developing influenza-like illness and seeking medical treatment, and the age-specific rate of testing positive for influenza. On the other hand, INSIGHT denominator data allowed us to characterize the age-specific risk of developing influenza and seeking treatment empirically. As a result, the fitted age-specific risk curve only represents residual, age-specific differences in the rate of testing positive for influenza, and thus, the age-specific risk curve fitted to INSIGHT data shows much less variation between age groups than the curve fitted to AZDHS data (compare Fig. 4A to Fig. 5A).

In models fitted to INSIGHT data, relative risk estimates indicated a positive association between antiviral treatment and confirmed infection, and a negative association between vaccination in the previous year and confirmed infection. Effects from underlying conditions were small and rarely included in preferred models (Table ##). Imprinting protection was consistently associated with small reductions in risk, and



**Figure 1. Expected age distributions of infection under different imprinting models. (A)** Reconstructed, birth year-specific probabilities of imprinting. **(B**) Expected imprinting protection against H1N1 or H3N2 under the three tested models. (**C**) Cartoon of age-specific risk curve. The shape of this curve is purely hypothetical, but each tested model fit a similar step function to data. (**D**-**F)** Fraction of each birth year unprotected by their childhood imprinting (as in A) determines the shape of birth year-specific risk. (**G-I)** A linear combination of age-specific risk (C), and birth year-specific risk (D-F) gave the expected age distribution of H1N1 or H3N2 cases under each model. Note: In all panels except B and C, reconstructed imprinting probabilities are specific to Peru, 2016, but provide a representative example of reconstructions from other countries and years.



**Figure 2. Observed age distributions, Arizona, 1993-2015**. Points show fraction of confirmed H1N1 or H3N2 cases observed in each single year of age (18-90). Lines show a smoothing spline fit to observed distributions. **(A)** All confirmed cases. (**B-G**) Season-specific age distributions.

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**Figure 3. Observed age distributions, INSIGHT outpatient data, 2009-2017**. Panels follow the same format as in Fig. 1. **(A)** All confirmed cases. (**B-F**) Season-specific age distributions. (**G-L**) Country-specific age distributions.



**Figure 4**. **Results of model selection on AZDHS data**. Fitted effects from AN, the best model, of (**A)** age and **(B)** imprinting, and **(C)** maximum likelihood parameter estimates with 95% profile confidence intervals. In A-C, Black indicates a subtype-independent risk effect, blue indicates an effect specific to H1N1 risk, and red specific to H3N2 risk. Dashed lines show relative risk of 1; values below 1 indicate reduced risk, while values above 1 indicate elevated risk, relative to baseline.**(D)**, Model comparison results, with shaded rectangles indicating which factors were included in each model. **(E-F)** model fits to observed data.

**Figure 5. Results of model selection on INSIGHT data**. Formatting is as described in Fig. 4. Fitted effects from the best model (ATVN) of **(A)** age, **(B)** antiviral **(C)** vaccination, and **(D)** imprinting. **(E)** Total Akaike weight attributed to models containing specific forms of imprinting. **(F)** Maximum likelihood parameter estimates, and profile confidence intervals. **(G)** Model comparison results. Colors of the best four models correspond to predictions in **H-I**. Fits to H1N1 (**H)** and H3N2 **(I)** data from the four best models, and from the null model (age effects only).



**Figure 6. Model selection on H1N1 pandemic data.** (A) Estimated reductions in risk from any kind of protective imprinting were stronger during the first and second pandemic waves than against normal seasonal influenza. (B) Fits to data from the 2008-2009 influenza season (first pandemic wave) showed the strongest support for HA group-level protection. (C) During the 2009-2010 season (second pandemic wave), NA subtype-level and HA subtype-level imprinting again received the strongest support, as for seasonal influenza.



**Figure 6. Relationship between subtype, annual rate of antigenic advance and fraction of cases in each age group.** Each data point represents a single influenza season in which at least 100 confirmed cases of a given subtype were observed. Blue label shows Pearson correlation between the fraction of cases observed in each age group and the annual rate of antigenic advance of H3N2 (the only lineage with enough data points to support a meaningful statistic). Dashed blue line shows linear trend line fitted using lm() in R.

|  |  |  |
| --- | --- | --- |
| **Season** | **Confirmed H1N1** | **Confirmed H3N2** |
| 1993-94 | 0 | 101 |
| 1994-95 | 12 | 38 |
| 2002-03 | 71 | 8 |
| 2003-04 | 0 | 71 |
| 2004-05 | 0 | 131 |
| 2005-06 | 1 | 321 |
| 2006-07 | 212 | 28 |
| 2007-08 | 196 | 244 |
| 2008-09\* | 2717 | 382 |
| 2009-10\* | 6261 | 1 |
| 2010-11 | 472 | 1204 |
| 2011-12 | 595 | 348 |
| 2012-13 | 80 | 1578 |
| 2013-14 | 1475 | 151 |
| 2014-15 | 5 | 2109 |
| **Total** | **12097** | **6715** |

**Table 1. Surveillance data, Arizona, USA.** Data representing the first and second waves of the 2009 H1N1 pandemic (\*) and were analyzed separately. Following standard definitions, northern hemisphere seasons spanned epidemiological week 40-39, or roughly Oct.-Sept of the following year.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Season** | **Confirmed H1N1** | **Confirmed H3N2** | **Country** | **Total confirmed** |
| NH 2009-10\* | 317 | 0 | Argentina | 1552 |
| SH 2010\* | 122 | 27 | Australia | 43 |
| NH 2010-11 | 93 | 13 | Austria | 9 |
| SH 2011 | 25 | 243 | Belgium | 527 |
| NH 2011-12 | 1 | 123 | Chile | 22 |
| SH 2012 | 24 | 49 | Denmark | 67 |
| NH 2012-13 | 115 | 105 | Estonia | 98 |
| SH 2013 | 248 | 95 | Germany | 78 |
| NH 2013-14 | 218 | 140 | Greece | 163 |
| SH 2014 | 36 | 349 | Japan | 12 |
| NH 2014-15 | 47 | 320 | Peru | 131 |
| SH 2015 | 36 | 386 | Poland | 123 |
| NH 2015-16 | 271 | 80 | Spain | 49 |
| SH 2016 | 432 | 90 | Thailand | 858 |
| NH 2016-17 | 13 | 60 | UK | 14 |
| **Total** | **1998** | **2080** | USA | 332 |

**Table 2. INSIGHT study data.** NH signifies the northern hemisphere season, Oct-May, and SH signifies the southern hemisphere season, June-Sept. Data collected during the 2009 pandemic (\*) were excluded from analysis of seasonal epidemiology.

|  |  |
| --- | --- |
| **Model** | **ΔAIC** |
| **AZDHS models** | |
| AN | 0.00 |
| AS | 23.42 |
| AG | 245.18 |
| A | 380.47 |
| **INSIGHT models** | |
| ATVN | 0.00 |
| ATVS | 0.25 |
| ATV | 0.98 |
| ATUVN | 2.00 |
| ATUVS | 2.25 |
| ATUV | 2.96 |
| ATVG | 4.26 |
| ATN | 6.16 |
| ATUVG | 6.25 |
| ATS | 6.40 |
| ATUN | 8.10 |
| ATUS | 8.33 |
| AT | 8.67 |
| AVN | 8.79 |
| AVS | 8.95 |
| AV | 9.54 |
| ATU | 10.56 |
| AUVN | 10.79 |
| AUVS | 10.95 |
| ATG | 11.35 |
| AUV | 11.54 |
| AVG | 12.99 |
| ATUG | 13.23 |
| AN | 14.02 |
| AS | 14.18 |
| AUVG | 14.99 |
| AUN | 16.00 |
| AUS | 16.16 |
| A | 16.18 |
| AU | 18.14 |
| AG | 19.17 |
| AUG | 21.12 |

**Table 3.** Model comparison. Model name abbreviations specific which factors were included. A = age-specific risk, N = NA subtype-level imprinting, S = HA subtype-level imprinting, G = HA group-level imprinting, V = vaccination, U = underlying conditions, T = antiviral treatment. Only INSIGHT data contained sufficient details to test factors V, U and T.

# Discussion

We analyzed two large, epidemiological data sets on influenza A to assess whether suspected impacts from childhood imprinting consistently shape birth year specific risk from H1N1 and H3N2 influenza. First, we reconstructed birth year-specific imprinting histories for all birth years from 1918 to the present, and then developed three antigen-specific hypotheses to describe expected patterns if childhood imprinting to conserved HA epitopes, variable HA epitopes, or to NA was the dominant driver of birth year-specific risk (Fig. \ref{fig1}). Then, we compared differences in the age distribution of risk from H1N1 and H3N2 across influenza seasons from 2007 to 2016, and found that H3N2 consistently caused more cases than H1N1 in older cohorts, while H1N1 consistently had the highest impacts in younger cohorts. In general, these differences between subtype-specific age distributions were qualitatively consistent with population-level imprinting protection (Fig. \ref{fig1}), although the magnitude and shape of differences between H1N1 and H3N2 distributions varied somewhat across time and space (\ref{fig2}-\ref{fig3}). Finally, we fit a suite of models to data using maximum likelihood, and performed model comparison to test which candidate imprinting hypothesis, if any, was most consistent with observed differences in risk from H1N1 or H3N2. Model comparison on both INSIGHT and Arizona data sets showed the greatest support for imprinting effects at the HA subtype level, or NA imprinting.

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 four 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 US data than within European data (13). On the other hand, differences between datasets may also be due, at least partially, to differences in case ascertainment. The INSIGHT study did not enroll children, and its enrolled adult populations contained relatively few subjects under the age of 25, or over the age of 70 (Fig. S##), cohorts in which differences in imprinting status are most pronounced. This dearth of cases at the extremes of age may have dampened the signal of subtype-specific differences in risk. 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 \ref{figS8}), it is obvious that the extremes of age were comparatively well-sampled. To illustrate this sampling effect, we down-sampled the AZDHS data to match the age distribution of all tested cases from the INSIGHT study, and found that apparent differences in age-specific risk from H1N1 and H3N2 became weaker as a result of differences in age-specific sampling. (Fig. S##).

The potential for age-specific sampling biases to erode or magnify the signal of imprinting effects highlights some limitations of existing epidemiological data. The largest, long-term epidemiological data sets on influenza come from massive, global surveillance efforts. But 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 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 and vaccine effectiveness. 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 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 if influenza surveillance data were more shared more systematically, and if single year of age information and sampling denominators were included, it could represent a turning point in the scientific community’s ability to link influenza's genetic and virologic characteristics with population-level disease outcomes.

%% Interpretation of INSIGHT fits

cmt{I NEED TO WORK ON TRANSITION}

Comparison of models fit to INSIGHT data showed nearly equal statistical support for five tested models that all had $\Delta AIC < 4$. The spread of $\Delta AIC$ values among all tested models was also much narrower among models fit to INSIGHT data than among models fit to Arizona data. This is unsurprising given that the signal of imprinting effects was weaker overall in the INSIGHT data than in the Arizona data (Fig. \ref{fig2}-\ref{fig3}). The simplest model fit to INSIGHT data (A), whose fit was based solely on the observed age distribution of all tested cases, and on the fitted age-specific risk curve, provided a fairly good fit to observed data, and additional effects from patient medical history or imprinting improved on this simplest fit only marginally (Fig. \ref{fig5} G-H). Because the fitted data set was large (4,078 confirmed influenza A cases), these small improvements in fit translated to statistically meaningful differences in model likelihood, and in AIC.

%% Interpretation of AZ fits

Among models fit to the Arizona data, the spread of $\Delta AIC$ values was much wider, and visually, competing models showed much more dramatic differences in goodness of fit (Fig. \ref{fig4}). The best model fit to Arizona data, AN, would normally be interpreted as having definitive statistical support. Based on widely used rules of thumb for the interpretation of $\Delta AIC$ values, the second best model (AS, $\Delta AIC=21.65$), would normally be ruled out as inferior, and we should conclude that childhood imprinting to NA is a much better predictor of subtype-specific seasonal influenza risk than HA subtype-level imprinting. However, the only qualitative difference between the predictions of model AN (best) and model AS (second best), is the protection status of H2N2 imprinted cohorts against seasonal H3N2 (fits in Fig. \ref{fig4} E-F, consistent with differences between hypotheses in Fig. \ref{fig1} B). Visually, neither model AN nor AS is a precise fit to the observed data in the H2N2-imprinted region where their predictions differ (Fig. \ref{fig4} E-F), and so we hesitate to assert that NA-specific childhood imprinting is a much better predictor of seasonal influenza risk than HA subtype-specific imprinting. Rather, fits to the AZ data set show that models including either NA-level or HA subtype-level childhood imprinting provide better fits to data than models assuming HA group-level imprinting (AG), or no imprinting effects. However, none of the tested models provided a perfect fit to the Arizona data in all birth cohorts, indicating that models considering only the impacts of childhood imprinting to one antigen, HA or NA, are probably insufficient to precisely capture observed epidemiological patterns. Some combination of effects from HA imprinting, NA imprinting, and from imprinting to internal proteins most likely drives birth year-specific risk.

%% Complexity/colinnearity issues

We considered testing more complex models that would have incorporated a mixture of imprinting effects, but ultimately decided that, given extensive collinearities between the predictions of even the simple models tested here, the data were unlikely to support a more complex analysis. Deeper insights into the relative contributions of HA and NA to seasonal imprinting patterns are needed, but given the limited diversity of influenza circulation in humans over the past century, epidemilogical data can support a limited scope of inference. Deeper insights will most likely need to come from immunological data. The recent NIH initiative to fund large cohort studies [CITE], and technological advances in the sequencing of B and T cell repertoires [CITE].

%% HA and NA subtype-level protection is best

Model comparison on both data sets independently provided the strongest support for effects from childhood imprinting to NA, or subtype-level protection from imprinting to HA. These results are consistent with decades of research on seasonal influenza immunity, in which titers against HA and NA have always been considered the strongest correlates of protection, and in which narrow, homosubtypic immunity has been considered the norm against seasonal influenza.

%% Potential immunological explanation

Still, it is somewhat surprising given the known influence of antigenic drift, that signatures of homosubtypic imprinting protection persist across an entire human lifetime. On average, antigenic clusters persist for only 3.3 years [SMITH 2004]. Circulating H1N1 and H3N2 viruses drift by 0.62 and 1.01 antigenic units per year, respectively [cite Bedford eLife paper], which roughly correponds to a two-fold drop in titer for every 1.61, or 0.99 years of antigenic evolution between viruses. Cross-protective titers between viruses sampled 15 or more years apart are consistently too low to measure [Bedford, elife], indicating that cross-protection should be weak or absent. Thus, it is somewhat puzzling that influenza immunity primed in childhood provides any meaningful protection after adolescence, let alone throughout adulthood and into old age.

One possible explanation for the evident longevity of protection from childhood imprinting is that imprinting to a particular HA or NA subtype builds strong memory to epitopes that are relatively conserved at the subtype level, and drift more slowly than the epitopes that react in hemagglutination inhibition assays.

Another possible explanation is that the exact B cell clones developed during the first childhood influenza exposure are not necessarily identical to the clones deployed against strains encountered later in life. Memory B cells maintain some phenotypic plasticity, and through somatic hypermutation, have some capacity to tailor their binding affinity to match newly encountered antigens. 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 raw prototypes that can be rapidly and effectively tailored to recognize drifted influenza strains of the same subtype, but less effectively tailored to recognize more distantly related, heterosubtypic strains. A third possibility is that childhood imprinting depends strongly on a combination of B and T cell memory. The protective effects of T cells is not measured in the HI or neutralization assays used to build antigenic maps and characterize antigenic drift, and so these studies may systematically underestimate the functional strength of cross-immunity between drifted strains of the same subtype.

%% Group-level

Neither the INSIGHT nor the Arizona data set supported a strong role of HA group-level imprinting. Previously, we showed that childhood imprinting at the HA group level (presumably driven by memory of conserved HA epitopes) is a surprisingly strong predictor of birth year-specific risk from two emerging, avian influenza A subtypes, H5N1 and H7N9. Conserved HA epitopes are known to play a particularly strong role in the immune responses against novel influenza subtypes like avian H5 and H7 [CITE]. On the other hand, more variable HA epitopes are known to be immunodominant upon exposure to a more familiar, seasonal HA subtype [CITE]. Thus, it is not surprising that HA group-level imprinting appears to play a strong role against novel, emerging influenza viruses, whereas subtype-level imprinting plays a dominant role against established, seasonal variants.

%% Epidemiological interpretation

The vast majority of influenza-related deaths occur in adults over age ##, and H3N2 causes an estimated ## times as many deaths in these cohorts than H1N1. This study suggests that the low number of H1N1-related deaths in elderly cohorts is at least partially explained by the fact that childhood imprinting appears to reduce the incidence of clinically attended H1N1 disease in high-risk elderly age groups. H2N2 imprinted cohorts (born c. 1950-1968) will eventually replace the H1N1-imprinted cohorts currently at the highest risk of influenza-related mortality. Results from this study suggest that in the future, elderly H2N2 imprinted cohorts will not be preferentially protected against H1N1 from their childhood imprinting, and so epidemiologists may expect the mortality burden of H1N1 to increase in the future. H2N2-imprinted cohorts may instead enjoy some protection against H3N2 given childhood imprinting to the same NA subtype, or based on HA subtype-level imprinting, may not be protected against either circulating seasonal subtype. In the latter case, the overall mortality impact of influenza A may increase in the future.

NEED SOME SORT OF PUNCHY CONCLUSION...TO WRITE AFTER WE DISCUSS AND SETTLE ON A CORE MESSAGE.

# Methods

The Arizona data set contained the variables birth year, subtype and season for 18,813 cases confirmed in the state of Arizona. AZDHS staff removed identifying details from the data, and only variables of interest to our study were shared. Birth year was extracted from each patient's reported date of birth, except in the 1993-1994, and 1994-1995 seasons, where birth year was approximated as (year of observation)-(age at time of observation). Birth year was necessary to estimate probabilities of imprinting protection for each patient. We calculated patient age as (year observed - birth year), where year observed was set to the second year of the influenza season associated with any case (e.g. 2014 for a case observed in the 2013-2014 season). We excluded one case whose birth year was not available, and ultimately analyzed 18,812 cases.

AZDHS staff classified cases into seasons using the standard definition, with the Northern Hemisphere (NH) season beginning in week 40, and ending in week 39 of the following calendar year (roughly Oct-Sept). Note that in the Arizona data, most H1N1 cases from the 2009 H1N1 pandemic were confirmed and reported during the 2009-2010 season, although some cases from early pandemic waves were reported during the 2008-2009 season. The relatively low number of pandemic H1N1 cases reported during the 2008-2009 season was due to lower case incidence early in the pandemic, and to limited testing capacity during the first pandemic wave. %% See pdf in raw\_data folder

\subsubsection\*{INSIGHT Data}

The INSIGHT outpatient data contained 11,679 total cases, and the variables: age, date of enrollment, antiviral treatment used (Y/N), presence of any underlying conditions (Y/N), influenza subtype (H1N1, H3N2, B or negative), and country. The data also contained variables for influenza vaccination in the past 6 months, or influenza vaccination in the past 12 months. We combined these data into a single binary variable, which classified subjects as vaccinated if they reported recent influenza vaccination at either timescale.

We used patient age and date of study enrollment to impute year of birth. Birth year can be approximated as (observation year)-(age), but this approximation is slightly biased, and biased in different ways for cases observed during the NH and SH influenza seasons. Cases observed earlier in the year (e.g. in January) are more likely to have a birthday that has not yet passed in the current calendar year, whereas cases observed later in the year (e.g. in December) are most likely to have already had a birthday pass in the current calendar year. Thus, the time of year at which a case is observed impacts how birth year should be estimated. Using logic laid out in Fig \ref{figS9}, we used the following three formulas to obtain 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 (Fig \ref{figS9}). Table \ref{tabS1} shows

We excluded 7 subjects whose ages did not fall in the focal age range (18-90). We excluded 94 subjects for whom season, age, vaccination status, antiviral treatment use or presence of underlying conditions was unknown. We also excluded 3 cases of co-infection with multiple subtypes. After excluding 104 cases in total, we ultimately analyzed a data set of 11,575 tested cases, including 1,998 confirmed H1N1 cases and 2,080 confirmed H3N2 cases. Cases were confirmed in 16 countries (Argentina, Australia, Austria, Belgium, Chile, Denmark, Estonia, Germany, Greece, Japan, Peru, Poland, Spain, Thailand, UK, and USA), and our study analyzed cases observed during 16 influenza seasons, starting with the 2009-2010 Northern Hemisphere season (which included the second wave of the 2009 H1N1 pandemic), and ending with the 2016-2017 Northern Hemisphere season. To facilitate comparison between data sets, we defined INSIGHT cases enrolled in Oct-May as part of the Northern Hemisphere (NH) influenza season, and cases enrolled in June-Sept as part of the Southern Hemisphere (SH) season. October 1 roughly aligns with the week 40 NH season start date used in the Arizona data set. Table \ref{tabS2} shows confirmed case counts from each country and season.

\subsection\*{Splines}

In Figures \ref{fig2}-\ref{fig3}, smoothing splines were fit to aid visual interpretation of noisy data. We fit splines using the command \textit{smooth.spline(x = AGE, y = FRACTIONS, spar = 0.8)} in R version 3.5.0. Variables \textit{AGE}, and \textit{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. \ref{figS3}, \ref{figS6} \& \ref{figS7}. Observed differences in H1N1 and H3N2 age distribution were insensitive to our choice of smoothing parameter; although the overall shape of fitted splines changed with different smoothing parameter choices, differences between the splines fit to H1N1 data and the splines fit to H3N2 data usually remained qualitatively consistent within a given season or country.

\subsection\*{Reconstruction of imprinting patterns}

Using methods described previously [CITE], we estimated the probability that an individual born in a given year would have first been exposed in childhood to an H1N1, H2N2 or H3N2 influenza A virus. For birth years of ages 0-12 in the year of case observation, reconstructions included the probability that an individual remained naive to influenza A, and had not yet imprinted. We repeated reconstructions for all birth years, and for all countries and years of case observation relevant to the data. Code to perform reconstructions is available at in the Supplementary Materials.

We calculated probabilities of birth year-specific protection for each of the three tested imprinting hypotheses using the rules defined in Table \ref{tab1}, where $p\_{HxNx}$ gives the estimated probability of imprinting to subtype \textit{HxNx} for a given birth year.

\begin{table}[!ht]

\begin{adjustwidth}{0in}{0in} % comment out/remove adjustwidth environment if table fits in text column.

\centering

\caption{{\bf Estimated probabilities of imprinting protection}, where $p\_{HxNy}$ gives the probability of having imprinted to subtype $HxNy$ in childhood.}

\begin{tabular}{l|l|l}

\hline

\textbf{Imprinting hypothesis} & \textbf{Challenge} & \textbf{p(protected)} \\

\hline

HA subtype-level protection & H1N1 & $p\_{H1N1}$ \\

& H3N2 & $p\_{H3N2}$ \\

\hline

NA subtype-level protection & H1N1 & $p\_{H1N1}$ \\

& H3N2 & $p\_{H3N2}+p\_{H2N2}$$ \\

\hline

HA group-level protection & H1N1 & $p\_{H1N1}+p\_{H2N2}$ \\

& H3N2 & $p\_{H3N2}$ \\

\end{tabular}

\label{tab1}

\end{adjustwidth}

\end{table}

\subsection\*{Model formulation}

Information on individual case medical histories was available in the INSIGHT data set, and so we were able to model age-specific impacts from vaccination prior to the influenza season of interest (V), of underlying symptoms (U) and of antiviral treatment (T) explicitly when fitting to these data. However, when fitting to the Arizona dataset, these patient details were not reported, and so tested models only included age-specific risk (A), and birth year-specific risk from imprinting, which could provide protection at the HA subtype (S), HA group (G), or NA subtype (N) level.

We assumed underlying symptoms (U) and antiviral treatment (T) would have similar impacts on H1N1 and H3N2’s age distributions and therefore fit a single curve to describe these impacts on both H1N1 and H3N2, however, we fit distinct impacts of vaccination (V), and of imprinting protection (I) for each subtype. Below, the abbreviation I will be used to represent any of the three possible imprinting hypotheses, G, S or N.

For each unique country and season in which cases were observed, define $p$ as a vector whose entries, $p\_a$ represent the expected case age distribution for a given subtype, i.e., the expected probability that a randomly drawn H1N1 or H3N2 case was observed in an individual of age a. Then, use a linear model to define $p$, as follows:

\begin{equation}

p = null\*A\*T\*U\*\mathbbm{1}\_{H1N1}(V\_{H1N1}\*I\_{H1N1})\*\mathbbm{1}\_{H3N2}(V\_{H3N2}\*I\_{H3N2})

\end{equation}

Note $\mathbbm{1}\_{H1N1}$ is an indicator function that takes value 1 if $p$ describes the expected age distribution of H1N1 cases, and 0 otherwise. %Thus, $\mathbbm{1}\_{H1N1}$ and $\mathbbm{1}\_{H3N2}$ ensure that subtype-specific risk factors are only included in relevant subtype-specific predictions, whereas subtype-independent factors are always included, regardless of the focal subtype.

\subsubsection\*{Null}

When fitting to INSIGHT data, the null expected age distribution was proportional to the total number of subjects enrolled in the study, regardless of their influenza test outcome. Crucially, this null prediction ensured that the predicted number of cases was only greater than 0 in countries, seasons and ages where one or more subjects was enrolled. This null distribution was particularly important when fitting to the INSIGHT data, where the number of confirmed cases was often low, and where the age distribution of cases in a given country and season was not always fully populated (Table \ref{tabS2}).

AZDHS only collects surveillance data on confirmed influenza cases, and not on the age distribution of ILI cases that tested negative for influenza. Thus, we were unable to include a null distribution in models fitted to data from Arizona. Crucially, this technical difference in model formulation leads to a different interpretation of free parameters fitted to the Arizona and INSIGHT data sets, as noted above.

%When fitting to Arizona data, where no denominator data was available, the fitted age-specific risk curve describes a combination of the age-specific probability of developing ILI and seeking medical treatment, and of testing positive (i.e. the age distribution of all positive tests). On the other hand, when fitting to the INSIGHT data, the null distribution controls for the age-specific probability of developing ILI and seeking medical treatment, while and all other factors describe only the relative risk that an administered test is positive (i.e. the age-specific test positive rate). The age-specific rate of positive tests (INSIGHT fits) is much less variable across age groups than the age distribution of all positive tests (AZ fits), which is why the age-specific risk curve fitted to the AZ data (Fig. \ref{fig4} A), shows a much wider range of relative risk estimates than the age-specific risk curve fitted to the INSIGHT data (Fig. \ref{fig5} A).

\subsubsection\*{Age-specific risk}

Define age-specific risk, ($A$), as a step function, with each step representing the expected fraction of cases of a given subtype observed in each of $Z$ age bins. Fit $Z-1$ free parameters, $r\_z$ to describe relative risk in each age bin, except one arbitrarily chosen reference bin, in which relative risk is fixed to 1. Below, $\mathbbm{1}\_z$ are indicator functions that take value 1 if a given vector entry belongs in age bin \textit{z}, and 0 otherwise. 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.

\begin{equation}

A = norm(\mathbbm{1}\_{1}+\mathbbm{1}\_{2}r\_2...\mathbbm{1}\_{z-1}r\_{z-1}+\mathbbm{1}\_{z}r\_z)

\end{equation}

\subsubsection\*{Antiviral treatment}

Within a given country and season, $f\_T$ is a vector whose entries describe the fraction of tested cases of a given age that received antiviral treatment. Free parameter $r\_T$ defines the relative risk of a positive test, given antiviral treatment. Then, define risk factor \textit{T} as:

\begin{equation}

T = f\_Tr\_T+(1-f\_T)

\end{equation}

\subsubsection\*{Underlying conditions}

The underlying conditions risk factor takes the same form as factor \textit{T}:

\begin{equation}

U = f\_Ur\_U+(1-f\_U)

\end{equation}

\subsubsection\*{Vaccination and Imprinting}

Factors describing risk from vaccination (\textit{V}) and imprinting (\textit{I}) took forms similar to risk factors \textit{T} and \textit{U} (equations 3 and 4), but with subtype-specific impacts. An indicator function defined whether a given prediction vector described risk of confirmed H1N1 or H3N2 infection:

\begin{equation}

V\_{HxNy} = \mathbbm{1}\_{HxNy}(f\_V\*r\_V+(1-f\_V))

\end{equation}

Below, $f\_I$ describes the probability that an individual of a given age, and observed during a particular influenza season, is protected from the subtype of interest by their childhood imprinting.

\begin{equation}

I\_{HxNy} = \mathbbm{1}\_{HxNy}(f\_I\*r\_I+(1-f\_I))

\end{equation}

\subsection\*{Model fitting and model comparison}

We simultaneously estimated all free parameter values using maximum likelihood estimation (MLE). We calculated likelihood profiles for each fitted free parameter, and defined profile confidence intervals based on the range of fixed parameter values for which the profile likelihood exceeded a minimum threshold. The threshold was defined using the method of likelihood ratios, in which Wilks's Theorem states that $-2\ln(\frac{L\_{profile}}{L\_{full}}) \sim \chi ^2$. Then, the threshold is given by the value of $L\_{profile}$ for which the left-hand side of the above inequality is equal to the 95th quartile of a $\chi ^2$ distribution with one degree of freedom. All models fit to a given data set were compared using AIC.

\subsection\*{Code and data availability}

Code to perform all analyses and construct all plots is available \#\#HERE\#\#. Data from AZ AVAILABILITY? Data from the INSIGHT study are available by application, pending approval from the study's scientific review committee (LINK). Because we are not free to share the INSIGHT data, the posted code contains an INSIGHT data file with scrambled column entries.

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%% Supporting information

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\section\*{Supporting Information}

%These commands reset the figure counter and add "S" to the figure caption (e.g. "Figure S1"). This is in case you want to add actual figures and not just captions.

\setcounter{figure}{0}

\setcounter{table}{0}

\renewcommand{\thefigure}{S\arabic{figure}}

\renewcommand{\thetable}{S\arabic{table}}

\subsection\*{Figures}

%% -----------------------

%% Figure S1

\begin{figure}[ht]

\includegraphics[width=\textwidth]{age-dists-by-season-ARIZONA-ages0-90.pdf}

\caption{\color{Gray} \textbf{AZ age distributions, including children ages 0-17.} Alternate version of Fig. \ref{fig2} showing observed age distributions with children ages 0-17 included. Children ages 0-17 were not enrolled in the INSIGHT study, and so children ages 0-17 were excluded from main text Fig. \ref{fig2}, in order to facilitate comparison between the Arizona and INSIGHT data sets.}

\label{figS1} % \label works only AFTER \caption within figure environment

\end{figure}

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%% Figure S2

\begin{figure}[ht]

\includegraphics[width=\textwidth]{age-dists-ARIZONA-all-seasons.pdf}

\caption{\color{Gray} \textbf{AZ age distributions, all seasons.} Alternate versions of Fig. \ref{fig2} showing observed age distributions from all influenza seasons. Observed case fractions (points) were only plotted if 10 or more cases of a given subtype were confirmed, to avoid extreme stretching of the y axis. Smoothing splines were only plotted if 50 or more cases of a given subtype were observed, as fits to fewer data points would not have been meaningful.}

\label{figS2} % \label works only AFTER \caption within figure environment

\end{figure}

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%% Figure S3

\begin{figure}[ht]

\includegraphics[width=\textwidth]{age-dists-by-season-ARIZONA-hilowsmoothpar.pdf}

\caption{\color{Gray} \textbf{Alternate smoothing parameters, AZ age distributions.} Alternate versions of Fig. \ref{fig2} B-G, with smoothing parameters chosen to fit smoothing splines that are less (\textbf{A-F}), or more (\textbf{G-L}) smooth than the splines shown in the main text.}

\label{figS3} % \label works only AFTER \caption within figure environment

\end{figure}

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%% Figure S4

\begin{figure}[ht]

\includegraphics[width=\textwidth]{age-dists-INSIGHT-all-countries.pdf}

\caption{\color{Gray} \textbf{INSIGHT age distributions, all countries.} Observed age distributions from all countries in which one or more confirmed cases of either subtype was observed, (as opposed to Fig. \ref{fig3}, in which only countries reporting $>=$ 50 cases of both subtypes were shown). Smoothing splines were only plotted if 50 or more cases of a give subtype were observed, as fits to fewer data points would not have been meaningful.}

\label{figS4} % \label works only AFTER \caption within figure environment

\end{figure}

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%% Figure S5

\begin{figure}[ht]

\includegraphics[width=\textwidth]{age-dists-INSIGHT-all-seasons.pdf}

\caption{\color{Gray} \textbf{INSIGHT age distributions, all seasons.} Alternate versions of Fig. \ref{fig3} showing observed age distributions from all seasons in which one or more confirmed cases were observed. Smoothing splines were only plotted if 50 or more cases of a give subtype were observed.}

\label{figS5} % \label works only AFTER \caption within figure environment

\end{figure}

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%% Figure S6

\begin{figure}[ht]

\includegraphics[width=\textwidth]{age-dists-INSIGHT-hiSmoothPar.pdf}

\caption{\color{Gray} \textbf{INSIGHT age distributions, increased smoothness.} Alternate versions of Fig. \ref{fig3}, except with a higher smoothing parameter value (greater smoothness) used to fit smoothing splines.}

\label{figS6} % \label works only AFTER \caption within figure environment

\end{figure}

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%% Figure S7

\begin{figure}[ht]

\includegraphics[width=\textwidth]{age-dists-INSIGHT-lowSmoothPar.pdf}

\caption{\color{Gray} \textbf{INSIGHT age distributions, increased smoothness.} Alternate versions of Fig. \ref{fig3}, except with a lower smoothing parameter value (less smoothness) used to fit smoothing splines.}

\label{figS7} % \label works only AFTER \caption within figure environment

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%% Figure S8

\begin{figure}[ht]

\includegraphics[width=\textwidth]{all-tested-cases.pdf}

\caption{\color{Gray} \textbf{Age distributions of all tested and confirmed cases.} \textbf{A}, Counts and \textbf{B}, frequencies from the Arizona and INSIGHT data. Note that data on the age distribution of all tested cases was not available in the Arizona data. The Arizona data contains comparatively more confirmed cases at the extremes of age.}

\label{figS8} % \label works only AFTER \caption within figure environment

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%% Figure S9

\begin{figure}[ht]

\includegraphics[width=\textwidth]{FigS8-birth-yr-est-table.pdf}

\caption{\color{Gray} \textbf{Rationale for birth year estimation, INSIGHT data.} \textbf{A}, This worked example illustrates that the month of case observation determines potential birth dates, and cases observed earlier in the year have a higher probability of a birthday falling in the previous year. Because NH season cases are observed late in year y0, or early in year y1, an individual of age 0, observed during the NH 2000-2001 season would most likely have been born in 2000, with low probabilities of birth in 1999 or in 2001. However, an individual of age 0 observed in the SH season has an equal probability of birth in 2000 or 2001. \textbf{B}, the patterns shown in \textbf{A}, were generalized to develop a scheme to calculate the weighted average birth year for individuals of any age, whose cases were observed in a NH season spanning years y0-y1, or in a SH season during year y1.}

\label{figS9} % \label works only AFTER \caption within figure environment

\end{figure}

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We reconstructed birth year-specific childhood imprinting patterns, as described previously (12). Figure 1A shows one representative reconstruction for Peru during the 2016 Southern Hemisphere (SH) influenza season. Reconstructions for other countries differ only in the underlying data used to inform the fraction of cases caused by H1N1 or H3N2 in recent decades.