Potent Protection against H5N1 and H7N9 Influenza via Childhood Hemagglutinin Imprinting*

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Abstract

Two zoonotic influenza A viruses (IAV) of global concern, H5N1 and H7N9, exhibit unexplained differences in age distribution of human cases. Using data from all known human cases of these viruses, we show that an individual's first IAV infection confers lifelong protection against severe disease from novel hemagglutinin (HA) subtypes in the same phylogenetic group. Statistical modeling shows protective HA imprinting is the crucial explanatory factor, providing 75% protection against severe infection and 80% protection against death for both H5N1 and H7N9. Our results enable us to predict age distributions of severe disease for future pandemics and demonstrate that a novel strain's pandemic potential increases yearly when a group-mismatched HA subtype dominates seasonal influenza circulation. These findings open new frontiers for rational pandemic risk assessment.

The spillover of novel influenza A viruses (IAV) is a persistent threat to global health. H5N1 and H7N9 are particularly concerning avian-origin IAVs, each having caused hundreds of severe or fatal human cases (1). Despite commonalities in their reservoir hosts and epidemiology, these viruses show puzzling differences in age distribution of observed human cases (1,2). Existing explanations, including possible protection against H5N1 among older birth-year cohorts exposed to the neuraminidase of H1N1 as children (3,4) or age biases in exposure to infected poultry (5–7), cannot fully explain these opposing patterns of severe disease and mortality. Another idea is that severity of H5N1 and H7N9 differs by age, leading to case ascertainment biases (1), but no explanatory mechanism has been proposed.

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The key antigenic determinants for IAV susceptibility are the virus's two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), where different numbered subtypes canonically indicate no cross-immunity. However, recent experiments have revealed that broadly-protective immune responses can provide cross-immunity between different HA subtypes, particularly subtypes in the same phylogenetic group (8–14). (HA group 1 contains (human seasonal) subtypes H1, H2 and avian H5, while group 2 contains seasonal H3 and avian H7; Fig. 1A, Fig. S1). Combining these insights into heterosubtypic immunity with the concept of 'original antigenic sin' (15) or 'antigenic seniority' (16), we hypothesized that individuals imprint on the HA group of their first IAV exposure and thereby experience a reduced risk of severe disease from novel IAVs within that same phylogenetic group. This hypothesis predicts that the 1968 pandemic, which marked the transition from an era of group 1 HA circulation (1918–1968) to a group 2-dominated one (1968-present) (Fig. 1B), caused a major shift in population susceptibility that would explain why H5N1 cases are generally detected in younger people than H7N9 (2,17–19). Our analysis of human cases of H5N1 and H7N9 revealed strong evidence that childhood HA imprinting indeed provides profound, lifelong protection against severe infection and death from these viruses. These findings allowed us to develop new approaches for IAV pandemic risk assessment, preparedness and response, but also raise possible challenges for future vaccination strategies.

Reconstructing IAV exposure history by birth year

To investigate whether an individual's initial childhood exposure to IAV influences later susceptibility to H5 and H7 viruses, we estimated the fraction of each birth-year cohort from 1918 to 2015 with first exposure to H1, H2, or H3 – or the fraction still naïve – for each country in our study (China, Egypt, Cambodia, Indonesia, Thailand, Vietnam). We estimated the annual probability of IAV infection in children using published age-seroprevalence data (20,21) and then rescaled this baseline attack rate to account for year-to-year variability in IAV circulation intensity (Supplementary Text).

One resulting country-specific reconstruction of HA history is depicted in Fig. 1C. While H3N2 has dominated since 1968, a non-negligible fraction of many birth-year cohorts from the 1970s onwards was exposed first to H1N1 viruses, with notable peaks near the re-emergence of H1N1 in 1977 and the 2009 pandemic.

H5N1 and H7N9 cases track HA imprinting patterns

Next, we compiled data on all known human cases of H5N1 and H7N9 with reported patient age (Fig. 2A,B). These data encompass mostly clinically severe and fatal cases; total incidence remains unknown. Thus, our analysis focused on the determinants of severe cases. The possible existence of many undetected mild cases, as hypothesized for H7N9 (1), is consistent with HA imprinting since broadly-protective immune responses are expected to provide partial protection (8,14), i.e., reduce severity without preventing infection altogether (4,12,22–25).

The preponderance of observed H7N9 cases among older cohorts, and H5N1 cases among younger cohorts, is clear (Fig. 2A,B). These patterns reflect birth year, not age: H5N1 cases occurred over 19 years from 1997–2015, yet cases from all years exhibit similar dependence on birth year. Analysis of 361 H5N1 cases in Egypt, the one country with many cases across the last decade, shows no trend in case birth years through time, while case age increased steadily (p=0.0003, Spearman's correlation; Fig. S2). So, on average, the same birth cohorts remained at high risk of severe infection, even as members grew ten years older.

Fig. 2C and D depict the case data normalized to demographic age distributions in affected countries. (If all birth cohorts had equal risk of severe infection, case incidence would be proportional to age distribution.) Bars above the midline thus represent birth years showing excess risk, while bars below indicate a shortfall. This normalization highlights two points: first, excess incidence and mortality data for H5N1 and H7N9 are near-mirror images of each other. Second, the group 1 to group 2 HA transition in 1968 is the key inflection point, with those born before the emergence of H3N2 showing protection against severe cases of H5N1 but not H7N9, and those born after 1968 showing the opposite pattern. For H7N9 severe case incidence also spikes in birth years around 1977 and 2009, when resurgent H1N1 circulation would have caused considerable mismatched imprinting. One-sided binomial exact tests showed excess H5N1 incidence had a lower probability of occurring in cohorts born before 1968 ($p<1e^{-10}$), while excess H7N9 incidence was more probable in these same cohorts ($p<1e^{-9}$). The same pattern held for excess mortality (Supplementary Text). These patterns suggest that the immune system imprints on conserved HA epitopes from the first-ever exposure to IAV, resulting in heterosubtypic (but within-group) protection against severe infection.

Even more striking is the tight correspondence of observed H5N1 and H7N9 incidence and mortality with *a priori* predictions based on HA imprinting patterns and demographic age distributions (Fig. 2). We emphasize that the black lines in Fig. 2 are not fitted to the case data, but are independent predictions (Fig. 1C). Differences between the predictions and data are remarkably small—some noise arises from generalization across time and countries (e.g. attack rates for the reconstruction came from German data, but focal populations are largely Asian), and from small case numbers. Incorporating additional epidemiological factors and estimating the protective efficacy of imprinting further improved correspondence between predictions and data (Fig. S3). In contrast, NA imprinting patterns (which fully capture patterns of childhood exposure to N1) are a poor fit to H5N1 and H7N9 data from 1957–1968 cohorts (Fig. S4), and NA-mediated protection is not supported by statistical modeling.

HA imprinting explains age distributions

To formally assess the HA imprinting hypothesis alongside previous explanations (1,3–7) for observed H5N1 and H7N9 age distributions, we developed a set of multinomial models. These models related the probability that a case occurred in a given birth cohort to country- and year-specific demography, and risk factors including age-based risk of exposure to poultry, age-based risk of severe disease or case ascertainment, and reconstructed patterns of first exposure (and hence potential immunological imprinting) to HA or NA subtypes (Table S1). Model comparison showed HA imprinting was the dominant explanatory factor for

observed incidence and mortality patterns for both H5N1 and H7N9. It was the only tested factor included in all plausible models for both viruses (i.e. all models with Akaike weights greater than $4e^{-5}$).

The best models also included age-based risk of severe disease, echoing patterns known from seasonal influenza epidemiology. Age-based poultry exposure risk (estimated based on contact data from China (6, 7)) was included for H7N9 but not H5N1, perhaps reflecting that age-specific poultry exposures vary across the multiple countries affected by H5N1 or that humans interact differently with ill (H5N1-infected) versus asymptomatic (H7N9-infected) poultry. In models including HA imprinting, NA imprinting never showed any significant effect (Table S2). Remarkably, despite differences between the viruses and age cohorts involved, the estimated protective effects of HA imprinting were nearly identical for H7N9 and H5N1. In all models, protective HA imprinting reduced the risk of severe infection with H5N1 or H7N9 by ~75%, and the risk of death by ~80% (Table 1, Figs. S5–S7, Table S2).

Antigenic seniority across influenza subtypes

Most individuals born before the emergence of H3N2 in 1968, and exposed first to group 1 HA antigens (Fig. 1), have also been exposed to H3N2 after 1968—probably multiple times. Yet these seasonal group 2 exposures later in life evidently fail to override group 1 HA imprinting from childhood (Fig. 2). The birth-year specific protection seen for human H5N1 and H7N9 thus clearly indicates that clinically relevant antigenic seniority— preferential recall of immunological reactivities to antigens encountered earlier in life upon later exposure to cross-reactive antigens (16)—can act across HA subtypes of the same HA group, not just within subtypes as often assumed.

While the precise mechanism underlying antigenic seniority in this context remains to be determined, antibodies directed against conserved HA epitopes provide a plausible explanation for protection at the level of HA groups. For example, research following the 2009 H1N1 pandemic drew attention to the fact that primary exposure to a novel IAV can preferentially boost broadly-protective antibodies that bind conserved HA head or stem epitopes shared by different HA subtypes (8–14), even though immune memory against more variable epitopes on the novel HA head may be absent. This absence may in fact enable robust expression of otherwise subdominant, broadly-protective responses to conserved epitopes such as those on the HA stem (8). In particular, primary exposure to H5N1 or H7N9 can activate HA stem-specific reactivities induced by previous infection by H1 or H3, respectively (12,13,26). Indeed, others have suggested that heterosubtypic antibodies might attenuate disease from other IAV strains and may be imprinted to some degree by childhood exposure, though their serological assays provided no ability to detect or predict actual patterns of protection relevant to H5N1 and H7N9 in human populations (27).

Given the immunodominant nature of HA head reactivities (13,14,26,28), conserved HA head epitopes shared within, but not between, HA groups (29) may play a role in these patterns of protection. Cross-reactive HA-specific CD4+ or CD8+ T cell responses should also be investigated, since they too are likely to be disproportionately shared within HA

groups (given the sequence similarities within each clade) and might be especially capable of facilitating the sort of long-term immunity indicated by the data.

Nevertheless, current data, including the high degree of sequence conservation of stem domains within each HA group (Fig. 1A, Fig. S1), seem most consistent with a stem-directed mechanism for the antigenic seniority acting at the HA-group level (13). Divergence in HA stem amino acid sequences within each phylogenetic *group* is comparable to divergence in globular head sequences within a single HA *subtype* (i.e. the scale at which antigenic seniority is already known to act (16); Fig. S1), but stem divergences between the two HA groups are markedly higher. Notably, H3 and H7 are as divergent as any pair of group 2 HAs; since H3 childhood exposure provides protection against H7 it may thus protect as well or better against the other group 2 HAs (H4, H10, H14, H15), but perhaps not at all against more divergent group 1 HAs (Fig. S1C). Similarly, the joint consideration of protein sequence conservation patterns (Fig. 1A, Fig. S1) along with immunological and epidemiological data suggests that H1 or H2 childhood exposure may protect generally against zoonotic group 1—but not group 2—HAs.

The putative generality in HA imprinting protection patterns for novel HA subtypes other than H5N1 or H7N9 is tentatively supported by the preponderance of HA group-mismatched childhood exposures among the small number of clinically significant human cases detected to date: pooling data from 28 human cases of H5N6, H6N1, H7N7, H9N2, H10N7 and H10N8, age patterns are consistent with HA imprinting (p=0.019; see Supplementary Text), but case numbers are insufficient to investigate particular subtypes. Immunological experiments (e.g. using chimeric HA proteins (12)) are needed to systematically map HA cross-protection patterns across all HA subtypes, both within and between HA groups.

Rational projections of future pandemic risk

For any new pandemic IAV strain capable of efficient human-to-human transmission, HA imprinting patterns would combine with age-based mixing patterns (30–32) to determine the epidemiological impacts of the first pandemic wave. We created projections for a putative pandemic-capable strain of subtype H5 or H7—such as a gain-of-function strain or a natural variant with mutations increasing human-to-human transmissibility. The data on observed H7N9 and H5N1 cases enabled us to quantify how matched HA imprinting reduces the probability of developing a severe infection, but not how matched imprinting affects an individual's probability of acquiring a milder infection or the infectivity of such mild infections. People who become infected despite prior immunity likely transmit influenza at reduced rates owing to diminished viral titers and viral shedding, as observed in humans and in animal models (4,12,22–25). We thus assumed, conservatively, that imprinting does not change the probability of acquiring infection upon exposure, but can reduce severity and infectivity in individuals with protective HA imprinting.

Fig. 3A illustrates the projected age-structured attack rate of severe cases for hypothetical pandemics of H5 or H7 IAV occurring in 2015 in the United Kingdom. The projected risk profiles for severe infection are shaped strongly by HA imprinting, including the prediction that individuals above 50 years of age (i.e. born well before 1968 and first exposed to a

group 1 HA) would experience much lower morbidity than younger age groups in an H5 pandemic. Similar projections for China and Vietnam reveal the influence of demographic differences between countries (Fig. S8). The qualitative patterns in projected age impacts are robust to a wide range of assumptions about how seasonal influenza vaccination might affect imprinting (Fig. 3A), and to the assumed infectivity of mild cases arising in individuals with protective HA imprinting (Fig. S8A).

Projections for pandemics occurring a decade from now highlight predictable shifts in severe disease risk patterns as the imprinted population ages, with the key pivot point around birth years near 1968 shifted to older ages (Fig. S8). Impacts in the youngest age groups would depend on patterns of IAV circulation in the coming decade. All pandemic projections that account for HA imprinting exhibit markedly lower severe attack rates than projections assuming no imprinting protection (Fig. 3A, Fig. S8). Total attack rates (including mild and subclinical cases) would be higher and more evenly distributed across age groups than the severe attack rates shown here.

Over any prolonged period when IAV circulation is dominated by one HA group, imprinting generates growing herd immunity against zoonotic IAV strains from that group. Conversely, zoonotic strains from the mismatched HA group benefit from the rising proportion of humans without protection. So long as mild cases arising in people with group-matched imprinting contribute any less to transmission than unprotected cases, or if some fraction of infection events is prevented by imprinting-derived immunity, imprinting will alter the transmissibility of zoonotic IAV strains in the human population. This is summarized by the effective reproductive number, R_{eff}, which quantifies transmission in a partially immune population (Fig. 3B). Crucially, a zoonotic strain that is initially subcritical (i.e. with $R_{eff} < 1$ and therefore unable to spread sustainably) could—due solely to susceptibility changes in the human population— emerge as supercritical, and hence as a pandemic threat, if the mismatched HA group dominates IAV circulation for a sufficient period (Fig. 3B).

Our work implies that we have never seen a true 'virgin soil' influenza pandemic, and that all prior estimates of R_0 for pandemic IAVs are systematic underestimates since they do not account for protection induced by HA imprinting. Conversely, we see that imprinting raises the threshold R_0 necessary for a novel subtype to invade. Interestingly, the co-circulation of group 1 and 2 HAs since 1977 has balanced herd immunity in a way that increases the inherent transmissibility needed for a novel subtype from either HA group to invade. As a generality, R_{eff} for zoonotic influenza strains will change through time depending on seasonal influenza patterns and demographic background, and the magnitude of change will depend on the infectivity of imprinting-protected cases (Fig. S9).

Discussion

Our findings show that major patterns in zoonotic IAV epidemiology, previously attributed to patient age, are in fact driven by birth year. IAV strains circulating during an individual's childhood confer long-term protection against novel HA subtypes from the same phylogenetic group. Hence, antigenic seniority extends across IAV subtypes, introducing previously unrecognized generational structure to influenza epidemiology. These immune

imprinting effects have implications for public health and highlight that influenza virulence represents a joint phenotype between virus and host—even for strains not yet adapted to the human population.

These findings support the hypothesis that the unusually high mortality in young adults during the 1918 H1N1 (group 1) pandemic may have arisen primarily from mismatched H3 (group 2) imprinting in the cohort born between ~1880 and 1900 (19). This same cohort was strongly affected during the (group 1) 1957 pandemic (33); yet they suffered no excess mortality when they were even older, during the (group 2) 1968 pandemic (34). The possibility that mismatched HA imprinting currently contributes to the greater health impacts of seasonal H3N2 (relative to H1N1) in today's older age classes is worth investigating. And a diagnostic assay to determine whether an individual was imprinted on a group 1 or group 2 HA may be useful for individualized clinical care and vaccine design strategies, both for pandemic and seasonal IAVs.

Our findings raise questions about whether seasonal influenza vaccination might boost broadly-protective anti-HA responses (27) or alter imprinting from natural infection in IAV-naïve children. By exposing IAV-naïve children simultaneously to group 1 (H1N1) and group 2 (H3N2) antigens, vaccination might confer dual imprinting to both HA groups, or prevent strong imprinting against either HA group—or it could have no effect beyond delaying the age of imprinting via the first natural infection. Our sensitivity analyses demonstrated that, given the low IAV vaccination coverage in H5N1- and H7N9-affected countries, none of these effects would change our study's conclusions (Fig. S7). However, to properly inform early childhood vaccine policy, future research must determine which, if any, of these effects occur.

HA group imprinting also might complicate 'universal' vaccination approaches targeting conserved HA epitopes. Our findings indicate potent, long-lasting cross-protection between subtypes, putatively based on such responses. However, universal vaccination may have to outperform natural infection in its ability to induce broad immunity in the face of previous imprinting. The persistence of group 1 imprinting in older adults, despite decades of natural exposure to H3N2 after 1968 (Fig. 2), and the relative weakness of group 2 anti-HA stem reactivities in these older cohorts (11), suggest HA exposures later in life do not readily alter broadly-protective responses in individuals already imprinted to a particular HA group. To be effective, would bivalent (group 1 and group 2 HA stem) universal vaccines need to be delivered to infants prior to natural IAV infection? Or, might universal vaccines even impair natural, long-term protection of the sort we have detected against H5N1/H7N9 if received prior to an individual's first natural IAV infection?

Our findings are consistent with the known potential for repeated infection by seasonal IAV subtypes. Group-matched imprinting, like other broadly-protective IAV immune responses, is expected to protect against severe disease but not necessarily against infection (8,12,14). This parallels the reduced severity observed for repeat infections with seasonal strains (22,23,25). Furthermore, re-exposure to a seasonal subtype typically elicits memory responses against the immunodominant HA head, which mask subdominant broadly-protective responses involved in group-level imprinting (26).

For any country with suitable contact and demographic data, the methods shown here can provide rolling estimates of which age groups would be at highest risk for severe disease, should particular novel HA subtypes emerge. Such projections could guide cohort- or region-specific prevention, preparation, or control. Quantitative projections of changes in R_{eff}, and hence pandemic risk, will require further research into the protection arising from matched imprinting: is some fraction of cases prevented entirely, and by what factor is infectivity reduced in mild cases arising in protected individuals?

Our findings show that emergence risk cannot be considered in isolation, even for 'novel' pathogens that have not circulated in humans before. These pathogens are commonly assumed to have a blank slate of immunologically naïve humans to infect, but cross-protection from related pathogens can generate substantial population immunity. When this community of related pathogens undergoes major shifts, as during influenza pandemics, the landscape of population immunity changes accordingly. Thus emergence of novel pathogens can be governed by bottom-up control, with population immunity acting in an important and predictable manner to modulate the widely-recognized effects of virological and ecological risk factors. This perspective opens new frontiers for quantitative and mechanistic analysis of emergence risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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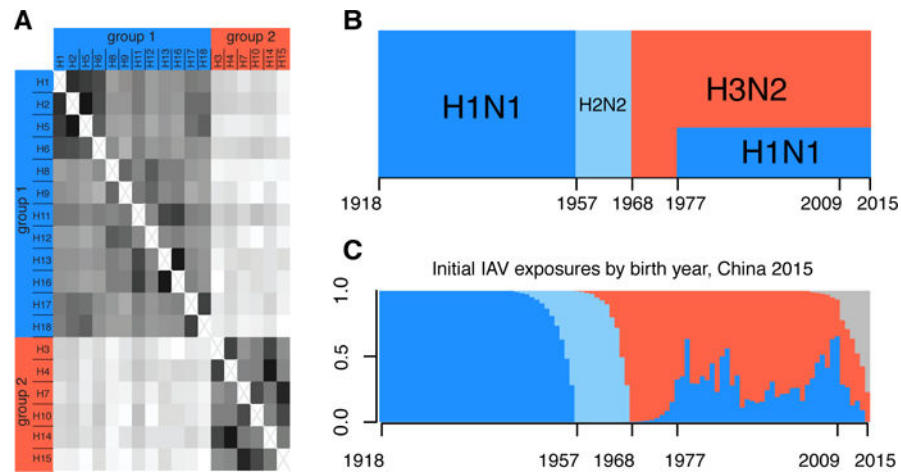
Fig. 1.

HA groups and reconstruction of 20th century HA imprinting. (**A**) HA groups 1 and 2, and pairwise amino acid similarities in the HA stem region. Darker colored cells indicate higher similarity (see Fig. S1). Each within-group subtype pair is more similar (83.2%–97.8%) than any between-group pair (75.9%–81.7%). (**B**) History of seasonal IAV circulation, and (**C**) estimated fraction of each birth cohort in China with initial exposure to each subtype. Estimated patterns in other countries (not shown) are identical up to 1977, and very similar thereafter. Pandemic years are marked on the horizontal axis. Blue represents group 1 HA viruses, red represents group 2, and grey represents naïve children who have not yet experienced an IAV infection.
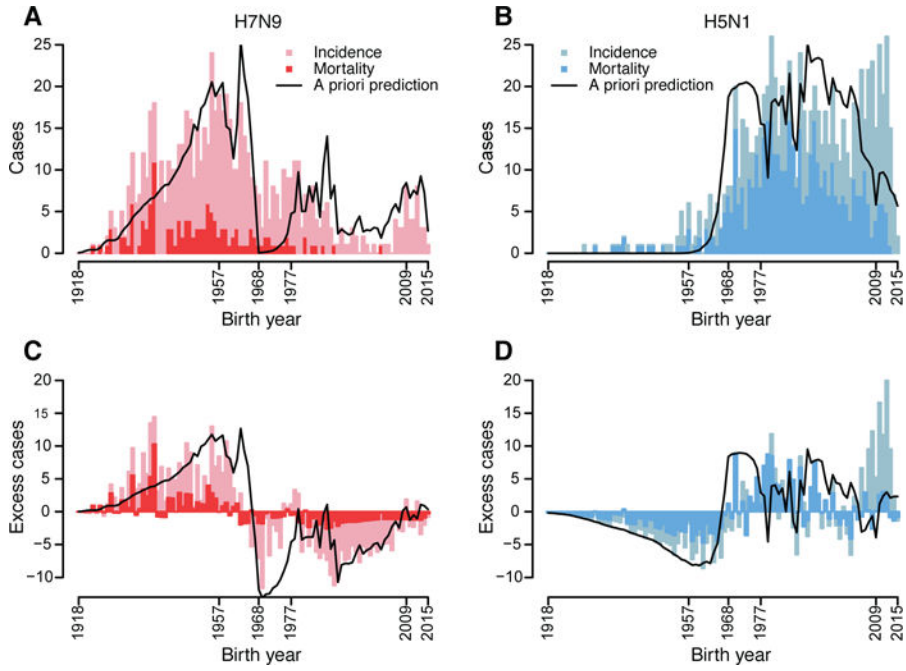
Fig. 2.
H7N9 and H5N1 observed cases and deaths by birth year (bars). Black lines show *a priori* prediction based on demographic age distribution and reconstructed patterns of HA imprinting. (**A**) 680 H7N9 cases, from China, 2013–2015. (**B**) 835 H5N1 cases, from Cambodia, China, Egypt, Indonesia, Thailand and Vietnam, 1997–2015. (**C**, **D**) Case data normalized to demographic age distribution from appropriate countries and case observation years.
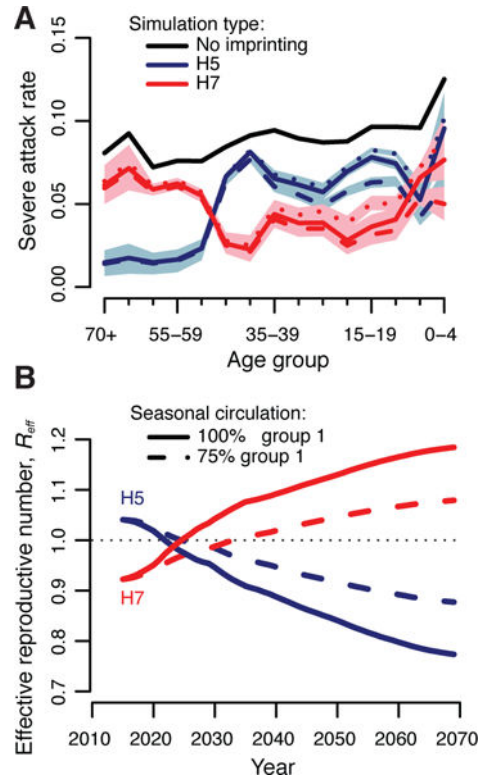
Fig. 3.

Projected effects of HA imprinting on future pandemics. (**A**) Attack rate of severe cases, by age group, for hypothetical H5 (blue) and H7 (red) IAV pandemics in 2015 (R_0=2.5, relative infectiousness of imprinting-protected individuals (a)=0.5), assuming UK demography and age-structured mixing (Supplementary Text). Lines show the average of 100 simulated outcomes, and shaded regions show the central 95%. Three vaccination scenarios explored: vaccination of IAV-naïve children could cause dual imprinting to both HA groups (dashed lines), prevent imprinting to both groups (dotted lines), or have no effect on imprinting (solid lines). (**B**) Projected change in R_{eff}, for hypothetical H5 (blue) or H7 (red) IAV with R_0=1.2 and a=0.5, if group 1 IAVs make up 100% or 75% of seasonal circulation after 2015.

Table 1

Estimated protection from HA imprinting.

Factors in model	HA imprinting protection (95% CI)	AIC	Akaike weight
H5N1			
DAH	0.75 (0.65–0.82)	0.00	0.9994
DEAH	0.83 (0.76–0.88)	15.35	4.65E-4
DEANH	0.83 (0.73–0.88)	17.32	1.74E-4
DH	0.80 (0.71–0.85)	69.18	9.50E-16
DEH	0.87 (0.80–0.90)	103.31	3.69E-23
DENH	0.86 (0.78–0.90)	105.29	1.37E-23
H7N9			
DEAH	0.76 (0.67–0.82)	0.00	1.00
DAH	0.81 (0.74–0.87)	42.87	4.09E-10
DEH	0.84 (0.78–0.88)	61.59	4.23E-14
DENH	0.83 (0.75–0.88)	62.26	3.02E-14
DH	0.88 (0.84–0.92)	138.40	8.83E-31

D=Demography, E=Exposure to poultry, A=High-risk age groups, H=HA imprinting, N=NA imprinting (see Methods, Table S1).