Cross-reactive and Vaccine-Induced Antibody to an Emerging Swine-Origin Variant of Influenza A Virus Subtype H3N2 (H3N2v)

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Background. Cases of infection due to a novel swine-origin variant of influenza A virus subtype H3N2 (H3N2v) have recently been identified in the United States, primarily among children. We estimate levels of cross-reactive antibody to H3N2v by age and assess whether seasonal trivalent inactivated influenza vaccine (TIV), with or without adjuvant, may increase seroprotection.

Methods. Antibody to H3N2v was assessed by hemagglutination inhibition (HI) assay and, for a subset, also by microneutralization assay. Seroprevalence and seroprotection were defined as an HI titer of ≥40, and levels were compared with those for ancestral and contemporary human strains. The analysis included 1116 sera collected during fall 2010, corresponding to approximately 100 sera per decade of life. Vaccine-induced antibody levels were also assessed in sera from 136 children aged <10 years and 65 adults aged 20–59 years before and after receipt of 2010–2011 split TIV and in sera from 182 elderly individuals aged ≥65 years before and after receipt of 2011–2012 split TIV (for 31 individuals), MF59-adjuvanted TIV (for 72), or unadjuvanted subunit TIV (for 79).

Results. The overall prevalence of HI titers of ≥40 against A(H3N2)v was 25%. No children aged <5 years and <20% of individuals aged ≤14 years or ≥40 years had an HI titer of ≥40. Conversely, among individuals aged 15–39 years, half of teens and adults showed H3N2v seroprotection. Following TIV receipt, <15% of individuals in any vaccine group developed a 4-fold increase in antibody level.

Conclusions. A substantial proportion of adolescents and young adults have cross-reactive antibody against emerging H3N2v, whereas children and older adults show broad susceptibility. Recent formulations of TIV do not substantially increase seroprotection. A specific vaccine would be needed if H3N2v establishes epidemic spread.

Clinical Trials Registration. NCT01140009 and NCT01368796.

Human influenza viruses evolve rapidly to escape immunity induced by previous infections, with antibodies to surface proteins, particularly hemagglutinin (HA), driving antigenic variation over successive years. The short life span of swine, however, precludes such immune selection pressure [1]. Pigs are generally slaughtered at a young age, which leads to the herd remaining mostly influenza naive. Consequently, epizootic swine influenza viruses retain close resemblance to their ancestral strains over many decades, whereas over a similar period human influenza viruses accrue significant genetic diversity. Ultimately, the increasing antigenic separation between swine and human viruses has important implications for susceptibility and zoonotic risk among humans.

Influenza in pigs was first recognized during the 1918–1919 influenza A virus subtype H1N1 pandemic, during which an association was observed between illness in swine herds and farm families [2, 3]. Accordingly, classic swine and human H1N1 viruses share a common ancestor in the 1918–1919 pandemic strain [4, 5]. In 2009, the World Health Organization (WHO) declared a pandemic

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due also to a swine-origin H1N1 subtype virus [6]. Phylogenetic analysis showed that the HA sequence of the 2009 pandemic influenza A virus subtype H1N1 (A[H1N1]pdm09) strain most closely resembled that of the 1918 strain and was antigenically distinct from recent human H1N1 viruses and vaccine components [7, 8]. Serosurveys also showed that most humans lacked preexisting protection against A(H1N1)pdm09, with the notable exception of very old individuals, who were likely exposed to 1918-like H1N1 strains during childhood [7, 9, 10].

Influenza A virus subtype H3N2 (A[H3N2]) emerged in humans during the 1968 pandemic [11]. These viruses were subsequently detected in pigs between 1968 and 1997 but occurred infrequently relative to the classic H1N1 lineage [12, 13]. In 1998, however, swine-adapted A(H3N2) viruses derived from a contemporary human H3N2 virus spread rapidly through the US swine population [14]. The first documented human infection due to swine H3N2 (swH3N2) in North America occurred in 2005 in an Ontario farm worker [14], with sporadic human cases observed thereafter.

During the latter half of 2011, 12 cases of human infection with a swH3N2 variant, H3N2v [15], were identified across 5 US states, primarily among children [16, 17], with an additional pediatric case reported in another US state in April 2012 [18]. Three of the 13 individuals were hospitalized. Six had no swine exposure, raising concern about human-to-human transmission, a potential event recently confirmed in ferret studies [19]. Although select zoonotic swH3N2 viruses from 2009 and 2010 show some cross-reactivity with seasonal human strains from the early 1990s [20], phylogenetic analysis indicates that the HA of 2011 H3N2v and other recent swH3N2 viruses descended from and bears closest resemblance to the HA of a common human H3N2 ancestor strain that circulated in the mid-1990s, notably, the A/Wuhan/359/1995(H3N2) vaccine reference strain (hereafter, "A/Wuhan") [21, 22]. A/Wuhan is estimated to share 90% similarity with H3N2v in its HA1 surface protein [22] but has neither circulated in humans nor been a component of the trivalent inactivated influenza vaccine (TIV) since 1997–1998, when it was replaced by A/Sydney/5/1997(H3N2) (hereafter, "A/Sydney") [23, 24], itself bearing 87.8% similarity to H3N2v [22]. Additionally, certain swH3N2 viruses detected in North America [25, 26] and elsewhere [27] since 2009, including H3N2v [15–17], acquired the A(H1N1)pdm09 matrix gene, distinguishing them from prior swH3N2 viruses and possibly contributing to greater transmission potential [21]. In that context, advance knowledge of age-related serosusceptibility to H3N2v in the population and of possible TIV-induced improvement could inform the likelihood of epidemic growth and the development and targeting of interventions and other risk assessment and response activities.

In a recent study to assess vulnerability to emerging swH3N2 infections, we measured the levels of cross-reactive antibody before and after vaccination with 2010–2011 TIV in Canadian children aged <10 years and adults aged 20–59 years [22]. That study, however, was limited by a small sample size and narrow age categories, and the swH3N2 virus against which antibody was measured was not from the H3N2v lineage that recently caused US zoonotic infections [22]. Here, we more precisely delineate age-specific levels of cross-reactive antibody to H3N2v in an expanded set of >1000 sera collected from across the life span, with approximately 100 sera obtained per decade from <1 to 100 years of age. By using H3N2v, we also assess whether TIV receipt increases cross-reactive antibody levels, including those in elderly participants who received various formulations with and without adjuvant.

METHODS

Age-Based Cross-reactive Antibody

We used a convenience sample of anonymized residual sera previously collected at community-based centers from the urban Lower Mainland area of British Columbia, Canada, between September and October 2010. These sera were assembled as part of 1-year postpandemic evaluation activities previously described for other time points during the 2009 pandemic [7, 28]. Approximately 100 sera per decade of life were assembled, with age groups for young children further subdivided into <5 years and 5–9 years and slight oversampling for children aged <2 years. If a seroprevalence of 10% is assumed, 100 sera would provide a precision of ±6% with a 95% confidence interval (CI); if seroprevalence were instead 50%, the corresponding precision would be ±10%. The University of British Columbia research ethics board approved this study.

Vaccine-Induced Cross-reactive Antibody

Sera from 3 TIV immunogenicity trials were used to assess vaccine-induced cross-reactive antibody to H3N2v: one involving children aged <10 years, a second involving adults aged 20–59 years, and a third involving elderly individuals aged ≥65 years.

Children and adults in Quebec had received 2010–2011 TIV (Fluviral; GSK, Laval, Quebec) during August–November 2010; study protocols have been described previously [22, 29, 30]. Elderly participants in British Columbia and Quebec had been randomly assigned by age and sex to receive one of 3 formulations of 2011–2012 TIV during September–October 2011: Vaxigrip (split; Sanofi Pasteur, Lyon, France), FLUAD (MF59-adjuvanted subunit; Novartis Pharmaceuticals, Dorval, Quebec), or Agriflu (unadjuvanted subunit; Novartis Pharmaceuticals, Dorval, Quebec). A smaller sample size for the Vaxigrip group was originally planned with randomization respected on that basis. Eligibility criteria required all elderly individuals to have received at least 1 TIV dose in the prior 2

years. All pediatric and young adult participants and >80% of elderly participants in the current analysis had previously received the 2009 AS03-adjuvanted monovalent A(H1N1) pdm09 vaccine (Arepanrix; GSK, Quebec).

Both 2010–2011 and 2011–2012 TIVs shared the same WHO-recommended vaccine components: A/Perth/16/2009 (H3N2)-like, A/California/07/2009(H1N1)-like, and B/Brisbane/60/2008-like antigens.

All sera were collected before vaccination and 21–28 days after receipt of the last age-appropriate vaccine dose. Sera from adults who received 2010–2011 TIV and from elderly individuals who received 2011–2012 TIV were collected as part of clinical trials (NCT01140009 and NCT01368796, respectively). Ethics boards of the Centre Hospitalier Universitaire de Québec and the University of British Columbia approved these studies.

Antibody Testing
Viruses
A summary of study viruses is provided in Appendix A (Supplementary Materials). Testing for hemagglutination inhibition (HI) antibody titers to A/Indiana/10/2011 (representative H3N2v was kindly provided by the Centers for Disease Control and Prevention [CDC; Atlanta, GA]; hereafter, "A/Indiana"), passaged once in embryonated chicken eggs, was performed at the National Microbiology Laboratory (Winnipeg, Canada). Ferret antiserum against A/Indiana, also provided by the CDC, was used as a control. The microneutralization (MN) assay used the same A/Indiana virus provided by the CDC, after 3 passages in Madin-Darby canine kidney (MDCK) cells.

For comparison, testing for HI antibody titers to ancestral (A/Sydney) and contemporary (A/Brisbane/10/2007; hereafter, "A/Brisbane") human influenza H3N2 reference viruses was performed at the British Columbia Centre for Disease Control (Vancouver, Canada) after 2 and 3 passages, respectively, of viruses in MDCK cells. A/Sydney was present in 1998–1999 and 1999–2000 TIVs for the northern hemisphere and was the most readily available and closely related human ancestor strain of H3N2v [21, 22, 24]. A/Brisbane was present in 2008–2009 and 2009–2010 TIVs from the northern hemisphere and represents the most recently circulating H3N2 strain in the years before fall 2010 [24]. HA1 nucleotide sequence analysis confirmed the antigenic integrity of passaged test viruses.

Antibody titers were measured in duplicate by the HI assay, according to standard WHO protocol [31, 32]. The HI assay used 4 HA units of reference virus and 0.5% turkey erythrocytes for A/Indiana and A/Brisbane and 0.7% guinea pig erythrocytes for A/Sydney [33]. Serial 2-fold dilutions began at 1:10. The HI titer was the inverse of the highest serum dilution to inhibit hemagglutination. HI titers of <10 were assigned a value of 5. The MN assay was performed according

to protocols previously described [7, 28]. The MN titer was defined as the inverse of the serum dilution immediately preceding wells with cytopathic effects. The geometric mean titer individual (GMT) of duplicate results was used in assessing antibody end points.

Statistical Analysis
Main antibody end points were group GMTs and prevalence of an HI titer of ≥40, by convention the 50% seroprotective threshold for evaluating vaccine antigens [32, 34]. The proportion with HI titers of ≥20, 80, and 160 was also explored. To account for differences in sampling approach and source population age structure, overall seroprevalence was directly age-standardized to the 2010 population of the Lower Mainland [35]. For the vaccine studies, the ratio of the GMT after vaccination to the GMT before vaccination (ie, the GMTR) and the proportion of individuals who seroconverted (defined as a 4-fold increase from prevaccination to postvaccination titers or as an increase from a titer of <10 before vaccination to a titer of ≥40 after vaccination) were also derived.

We explored the distribution of natural log–transformed titers by age, using scatter plots with overlaying penalize B-splines and their 95% CIs. Smoothening parameters for the penalize B-spline were automatically data determined. We also explored natural log–transformed mean titers by 1-year age intervals. Pearson correlation coefficients of natural log–transformed titers were computed across virus strains.

RESULTS

Age-Based Cross-Reactive Antibody
The age-based design included 1116 sera. The median age of sampled individuals was 43 years and ranged from <1 to 100 years (Table 1). The seroprevalence, determined on the basis of an HI titer of ≥40, is described below by age for each virus, followed by comparison across viruses (Figure 1). The GMTs had the same trend as the prevalence of an HI titer of ≥40. Similar age-related trends were also found using HI titers of ≥20, 80, or 160 (Appendix B1; Supplementary Materials).

Swine-Origin A/Indiana Strain
The overall age-standardized prevalence of an HI titer of ≥40 for A/Indiana was 25% (Table 1). No child <5 years of age and <15% 5–9 years of age had a titer of ≥40 (Table 1 and Figure 1). This proportion increased significantly to include nearly half of children 10–19 years old. On further subdivision, the seroprevalence among 27 children aged 10–14 years was significantly lower (26%; 95% CI, 9%–43%), than that among 72 teens aged 15–19 years (57%; 95% CI, 46%–68%). The proportion of individuals with an HI titer of ≥40 peaked at nearly 60% among adults aged 20–29 years, dropping to

Table 1. Geometric Mean Titers and Proportion With Hemagglutination Inhibition (HI) Antibody Titers ≥40, by Age and H3N2 Influenza Virus Strain, Among Participants in a 2010 Age-Based Cross-reactive Antibody Study

Age	Participants (No.)	Median Age (Years)	Female Sex (%)	GMT (95% CI), by Strain[a]			Titer ≥40 (%) (95% CI), by Strain[a]		
				A/Indiana	A/Sydney	A/Brisbane	A/Indiana	A/Sydney	A/Brisbane
<2 y	45	1	33	5.2 (4.9–5.5)	5.4 (4.9–5.9)	6.5 (5.2–8.1)	0	0	9 (1–17)
2–4 y	84	3	51	5.4 (5.2–5.6)	6.2 (5.5–6.9)	20.5 (14.4–29.2)	0	1 (0–4)	37 (27–47)
5–9 y	95	7	48	10.1 (8.4–12.1)	26.9 (19.6–36.8)	**80.9 (59.4–110.2)**	12 (5–18)	43 (33–53)	**78 (70–86)**
10–19 y	99	17	59	30.4 (24.1–38.4)	**247.8 (199.7–307.6)**	36.8 (28.7–47.1)	48 (39–58)	**96 (92–100)**	58 (48–67)
20–29 y	98	25	68	**42.8 (33.9–54)**	99.3 (80.6–122.3)	16.4 (12.3–21.7)	**59 (49–69)**	91 (85–97)	28 (19–36)
30–39 y	100	35	62	21.6 (17.6–26.4)	53.3 (43.7–65.0)	14.5 (11.4–18.4)	35 (26–44)	80 (72–88)	31 (22–40)
40–49 y	100	45	54	8.8 (7.5–10.3)	46.3 (36.1–59.3)	11.7 (9.2–14.8)	7 (2–12)	61 (51–71)	25 (17–34)
50–59 y	100	55	49	9.0 (7.8–10.4)	42.7 (33.7–54.2)	13.0 (9.8–17.2)	8 (3–13)	63 (54–73)	24 (16–32)
60–69 y	99	65	49	8.9 (7.6–10.4)	46.3 (36.1–59.3)	15.6 (11.8–20.6)	9 (3–15)	62 (52–71)	28 (19–37)
70–79 y	99	74	54	12.7 (10.5–15.4)	49.5 (37.2–66.0)	22.9 (16.7–31.2)	21 (13–29)	65 (55–74)	41 (32–51)
80–89 y	100	84	56	15.7 (12.8–19.3)	47.4 (35.7–63.0)	25.3 (18.7–34.3)	20 (12–28)	60 (50–70)	46 (36–56)
90–100 y	97	92	57	12.9 (10.5–16)	48.0 (36.6–62.9)	25.3 (18.6–34.5)	21 (13–29)	65 (55–75)	46 (36–56)
Overall	1116	43	54	13.2 (12.4–14)	43.9 (40.2–48.0)	20.6 (18.8–22.5)	25 (23–28)[b]	61 (58–64)[b]	34 (31–38)[b]

Sera were collected in September and October 2010. Bold indicates ages with the highest virus-related titers and proportions with an HI titer of ≥40, compared with adjacent age groups.

Abbreviation: CI, confidence interval.

[a] A/Indiana denotes A/Indiana/10/2011(H3N2), a representative virus of the swine origin H3N2v. A/Sydney denotes A/Sydney/5/1997(H3N2), an ancestral human influenza A(H3N2) virus and component of the northern hemisphere influenza vaccines for 1998–1999 and 1999–2000. A/Brisbane denotes A/Brisbane/10/2007 (H3N2), a contemporary human influenza A(H3N2) virus and component of the northern hemisphere influenza vaccines for 2008–2009 and 2009–2010.

[b] Proportion is directly age standardized to the 2010 Lower Mainland British Columbia population.
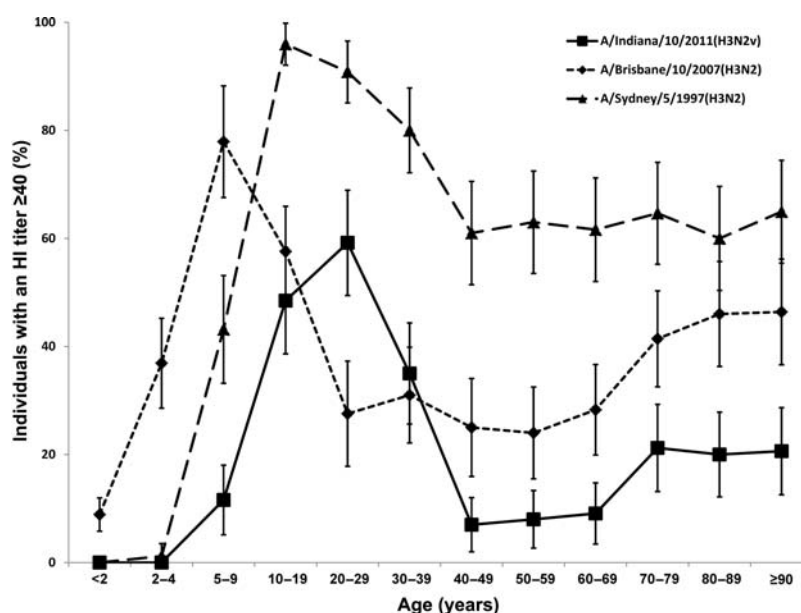

Figure 1. Hemagglutination inhibition (HI) antibody titers of ≥40, by age and H3N2 influenza virus strain, among participants in a 2010 age-based cross-reactive antibody study. Sera were collected in September and October 2010. A/Indiana/10/2011(H3N2) denotes a representative virus of the swine origin H3N2v. A/Sydney/5/1997(H3N2) denotes an ancestral human influenza A(H3N2) virus and component of the northern hemisphere influenza vaccines for 1998–1999 and 1999–2000. A/Brisbane/10/2007(H3N2) denotes a contemporary human influenza A(H3N2) virus and component of the northern hemisphere influenza vaccines for 2008–2009 and 2009–2010. Whiskers denote 95% confidence intervals.

about one-third for those aged 30–39 years, to <10% for those aged 40–69 years, and to 20% for those ≥70 years.

MN antibody titers and the proportion of individuals who were seropositive on the basis of a corresponding threshold titer are shown for select pediatric and adult age groups in Appendix B2 (Supplementary Materials). The proportion who were seroprotected, as defined by an HI titer of ≥40, most closely corresponded with the proportion of individuals with an MN titer of ≥80.

Ancestral A/Sydney Strain

The overall age-standardized prevalence of an HI titer of ≥40 for A/Sydney was 61% (Table 1). Fewer than 1% of children aged <5 years but nearly half of those aged 5–9 years had an HI titer of ≥40 (Table 1 and Figure 1). Virtually all children aged 10–19 years and young adults aged 20–29 years (≥90%) had a titer of ≥40, compared with 80% of adults aged 30–39 years. Among individuals aged ≥40 years, the proportion exceeded 60% for all age-specific categories.

The proportion of individuals with a MN titer to A/Sydney of ≥40 was similar to the proportion with an HI titer of ≥40 in a subset of 50 sera randomly selected from children 5–9 years old (46% [95% CI, 32%–60%] and 42% [95% CI, 28%–56%], respectively) and children 10–19 years old (96% [95% CI, 90%–100%] and 94% [95% CI, 87%–100%]).

Contemporary A/Brisbane Strain

The overall age-standardized prevalence of an HI titer of ≥40 for A/Brisbane was 34% (Table 1). Fewer than 10% of infants and toddlers <2 years of age had a titer of ≥40. The prevalence increased to about one-third of preschool children aged 2–4 years and peaked at nearly 80% among children aged 5–9 years (Table 1 and Figure 1). This proportion dropped to about 60% of children aged 10–19 years and was significantly lower among adults (20%–30%) and elderly individuals (40%–50%).

Comparison Across Viruses

Age-related curves of seroprevalence, determined on the basis of an HI titer of ≥40, were similar for A/Indiana, A/Sydney, and A/Brisbane, with increases associated with increasing age among children but decreases associated with increasing age among younger adults and plateau across middle-age (Figure 1). Among adults, the age of peak antibody levels for A/Indiana overlapped or was slightly greater than that for A/Sydney but was about 2 decades greater than that for the recent seasonal A/Brisbane strain. The latter strain showed the expected pattern of highest seroprevalence among young school-aged children (ie, those aged 5–9 years). This is illustrated by scatter plots in Appendix C (Supplementary Materials) and in Figure 2, which display mean titers by 1-year age intervals in relation to the emergence of A/Wuhan and

A/Sydney ancestral human strains and historical data on the circulation of other influenza A virus subtypes. Antibody titers plateaued across middle-aged and elderly adults for all viruses, with an increase in A/Indiana titers suggested among very old individuals, although this was not apparent for A/Sydney or A/Brisbane (Figure 1 and Appendix C [Supplementary Materials]).

The correlation was 0.61 between A/Indiana and A/Sydney titers ($P < .0001$), 0.31 between A/Indiana and A/Brisbane titers ($P < .0001$), and 0.41 between A/Sydney and A/Brisbane titers ($P < .001$).

Vaccine-Induced Cross-reactive Antibody

There were 136 children and 65 young adults included in 2010–2011 immunogenicity assessment. The 2011–2012 study involving elderly individuals included 31 participants vaccinated with split TIV, 72 vaccinated with the MF59-adjuvanted subunit formulation, and 79 vaccinated with the unadjuvanted subunit formulation (Table 2).

Antibody responses among pediatric (2010–2011) [29], adult (2010–2011) [30], and elderly (2011–2012) participants included here showed that each of the respective seasonal A/H3N2 TIV components met all 3 Committee for Proprietary Medicinal Products criteria for annual vaccine evaluation [32], with the sole exception of the unadjuvanted subunit formulation, which did not reach the 30% seroconversion criterion specified for elderly individuals (authors' unpublished data).

Prevaccination antibody findings for A/Indiana were consistent with findings from the age-based seroprevalence study (Table 2). Children <10 years old showed virtually no cross-reactive antibody, whereas a higher proportion of adults (20%–25%) showed an HI titer of ≥40. That proportion was significantly higher among young adults aged 20–39 years (45%; 95% CI, 28%–63%), compared with those aged 40–59 years (6%; 95% CI, 0%–15%) or 65–85 years (20%; 95% CI, 14%–26%).

Vaccination with seasonal TIV did little to increase the level of cross-reactive antibody to A/Indiana: seroconversion rates were <15% in all age and vaccine groups. With further stratification, those 20–39 years and 40–59 years had seroconversion rates of 12% (95% CI, 1%–24%) and 9% (95% CI, 0%–20%), respectively. Among elderly individuals, the MF59-adjuvanted subunit formulation did not yield an enhanced response to A/Indiana, compared with the split or unadjuvanted subunit products.

DISCUSSION

Here we estimate that up to a quarter of the population may be protected against the newly emerging swine-origin H3N2v, on the basis of the conventional seroprotective threshold titer of 40, but with significant variation in cross-reactive antibody
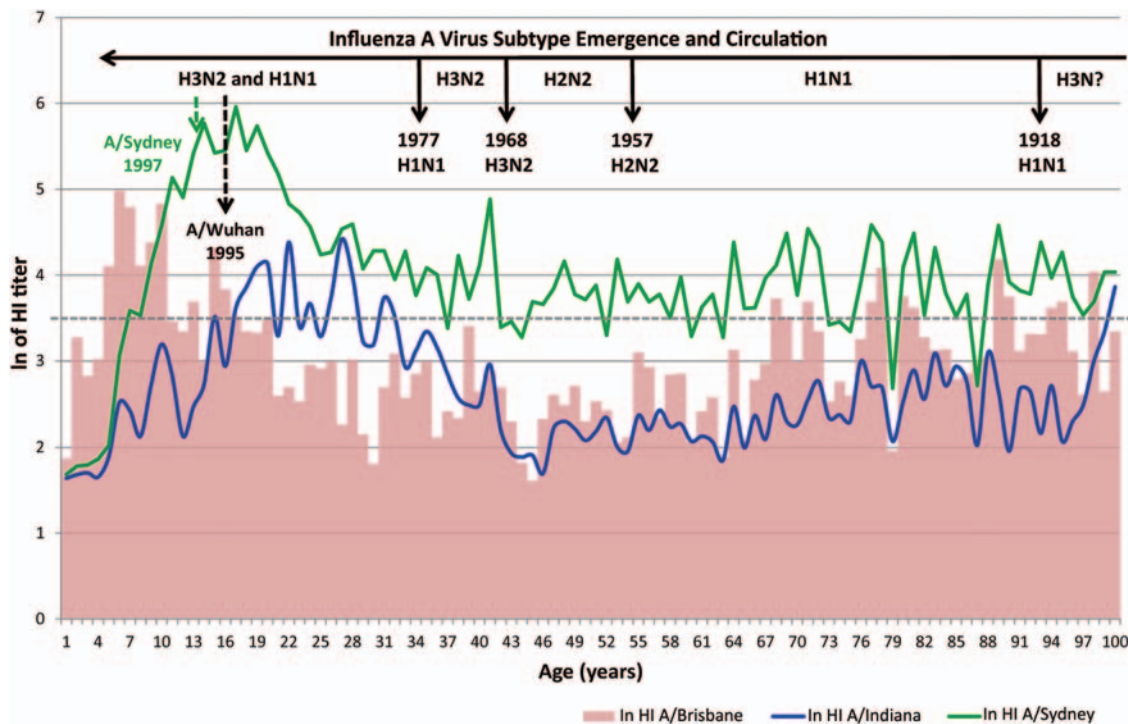
Figure 2. Natural logarithm (ln) of hemagglutination inhibition (HI) antibody titers, by age, among participants in a 2010 age-based cross-reactive antibody study, with superimposed timing of influenza A virus subtype and strain emergence. Sera were collected in September and October 2010. The solid black horizontal line depicts circulation of influenza A virus subtypes, specified in black bold font (eg, H3N2, H1N1, and H2N2) to be read from right to left, beginning with the solid black vertical arrows indicating the year of first emergence of those subtypes and corresponding to the 2010 ages shown in the x-axis. For example, the H2N2 subtype emerged in 1957 (corresponding with age 53 years in 2010) and circulated until it was replaced by the H3N2 subtype in 1968 (corresponding with age 42 years in 2010). Dotted vertical lines indicate the year of first emergence of ancestral human A/Sydney/5/1997 (green) and A/Wuhan/359/1995 (black) ancestral strains corresponding to the 2010 ages shown in the x-axis (13 years and 15 years, respectively). The dotted horizontal line corresponds to a seroprotective threshold, defined as an HI titer of ≥40. A/Indiana denotes A/Indiana/10/2011, a representative virus of the swine origin H3N2v. A/Wuhan denotes A/Wuhan/359/1995, an ancestral human influenza A(H3N2) virus and component of the northern hemisphere influenza vaccines for 1996–1997 and 1997–1998. A/Sydney denotes A/Sydney/5/1997, an ancestral human influenza A (H3N2) virus and component of the northern hemisphere influenza vaccines for 1998–1999 and 1999–2000. A/Brisbane denotes A/Brisbane/10/2007, a contemporary human influenza virus and component of the northern hemisphere influenza vaccines for 2008–2009 and 2009–2010.

levels by age. We show that vaccination with recent seasonal TIV does not meaningfully improve seroprotection and that an MF59-adjuvanted formulation cannot be relied on to stimulate better cross-reactive antibody responses against that novel virus in elderly individuals. These findings have both practical and scientific implications for further risk assessment and response.

During the latter half of 2011 and first half of 2012, 12 cases of H3N2v infection were reported among children <10 years old, with 1 case reported in a middle-aged adult [16–18]. Given the nondistinctive clinical presentation, the presence of cases without swine exposure, and findings from recent ferret-transmission studies [19], it is anticipated that other unrecognized cases and human-to-human spread may have occurred. Surveillance reports likely represent a fraction of the true infection rate, although the age distribution of reported cases likely reflects the main distribution of vulnerability by age. Consistent with these early surveillance findings, we identified that all children <5 years old and >80% up to 14 years old lack seroprotection. Among adults aged ≥40 years, we also estimate that >80% lack protective levels of antibody. Identification of a single affected adult to date among surveillance reports may reflect limitations in surveillance sensitivity and/or differences in exposure opportunities, hygienic practices, and other protective measures between adults and children. Conversely, between the ages of 14 and 40 years, about half of teens and young adults had an HI titer of ≥40, which is suggestive of immunity. This profile is markedly different from prepandemic serosurvey estimates for the A(H1N1)pdm09 virus, to which we and others identified broad susceptibility across all age groups except very old individuals [7, 9, 10]. In comparison, our findings for the emerging H3N2v may thus be interpreted as partially reassuring but also partially cautionary.

Table 2. Hemagglutination Inhibition (HI) Antibody Titers for A/Indiana/10/2011 Before and After Receipt of 2010–2011 or 2011–2012 Trivalent Inactivated Influenza Vaccine (TIV), by Age, Among Participants in a Study of Vaccine-Induced Cross-reactive Antibody

Age (Mean/Median), Vaccine (No. of Participants)	GMT (95% CI)	HI Titer ≥40 (%) (95% CI)	GMTR[a]	Seroconversion (%)[b] (95% CI)
Children 17–120 mo (63/63 mo), split 2010–2011 TIV[c] (Fluviral) (n = 136)				
Before vaccination	5.4 (5.1–5.7)	1 (0–2)	NA	NA
After vaccination	8.4 (7.2–9.8)	10 (5–15)	1.56	10 (5–15)
Adults 20–59 y (40/39 y), split 2010–2011 TIV (Fluviral) (n = 65)				
Before vaccination	13.7 (10.4–18.1)	26 (15–37)	NA	NA
After vaccination	21.7 (16.3–28.7)	38 (26–50)	1.58	11 (3–19)
Elderly 65–85 y (73/73 y), all 2011–2012 TIV vaccines (n = 182)				
Before vaccination	13.2 (11.6–15.1)	20 (14–26)	NA	NA
After vaccination	16.6 (14.4–19.1)	27 (20–33)	1.26	3 (0–5)
Elderly 65–84 y (74/74 y), split 2011–2012 TIV (Vaxigrip) (n = 31)				
Before vaccination	12.1 (8.8–16.6)	13 (0–25)	NA	NA
After vaccination	18.3 (12.5–26.7)	32 (15–50)	1.51	7 (0–21)
Elderly 65–85 y (74/74 y), adjuvanted subunit 2011–2012 TIV (FLUAD) (n = 72)				
Before vaccination	12.7 (10.3–15.5)	21 (11–30)	NA	NA
After vaccination	16.4 (13.4–20.2)	25 (15–35)	1.29	3 (0–7)
Elderly 65–83 y (73/72 y), unadjuvanted subunit 2011–2012 TIV (Agriflu) (n = 79)				
Before vaccination	14.2 (11.5–17.6)	23 (13–32)	NA	NA
After vaccination	16.1 (12.8–20.2)	27 (17–37)	1.13	0

Abbreviations: CI, confidence interval; GMT, geometric mean titer; NA, not applicable.

[a] The ratio of GMTs is calculated as the GMT after vaccination divided by the GMT before vaccination.

[b] Seroconversion is defined as a 4-fold increase from prevaccination to postvaccination titers or as an increase from a titer of <10 before vaccination to a titer of ≥40 after vaccination.

[c] Results after vaccination are for age-appropriate doses and were measured 3–4 weeks following receipt of 1 dose, for those who had previously been vaccinated, or following receipt of 2 doses, for those who had never been vaccinated.

The high proportion of young adults with an HI titer of ≥40 against H3N2v indicates that the population is not entirely immunologically naive. On either side of that reassurance, however, important subgroups of the population appear to be susceptible. Young children are thought to amplify respiratory virus transmission through their extensive social networks [36] and greater virus shedding [37], and in that regard their susceptibility may be a more relevant concern than protection in adults. In addition, H3N2 subtype viruses tend to cause more severe illness [37–39], and elderly individuals in particular show higher rates of hospitalization and death during seasons in which H3N2 subtypes are circulating [38, 39]. Amplification among susceptible children and a higher risk of severe complications among vulnerable elderly individuals could together yield a substantial disease burden. Ongoing surveillance for and epidemiologic monitoring of H3N2v infections thus remain important.

To understand age-related findings, we compared the pattern of cross-reactive antibody levels to newly emerging swine-origin A/Indiana to the pattern for previously circulating A/Sydney and A/Brisbane human strains. While A/Wuhan is phylogenetically most closely related to A/Indiana [21, 22], it caused only moderate H3N2 activity during a single winter

(1996–1997) in Canada [40]. Conversely, during the subsequent 1997–1998 season, the notorious antigenic drift strain, A/Sydney, triggered the most severe influenza epidemic in Canada in 20 years, dwarfing the activity of A/Wuhan and the activity of past H3N2 strains several-fold; over the successive 2 seasons, A/Sydney caused comparable or worse epidemic peaks that have seldom been surpassed since [23, 41]. The impact of A/Sydney may be evident in the substantial antibody levels we measured more than a decade later in all but the very young age group. The age-associated pattern of A/Indiana antibody levels does not precisely mirror that of A/Sydney antibody levels, with a correlation coefficient of about 60%. A/Sydney several years subsequent to, and is 96.4% similar in its HA1 peptide to A/Indiana's closest ancestor, A/Wuhan [22]; interpretation of age-related trends should take these antigenic and time differences into account. The substantial proportion (>40%) of children 5–9 years of age in 2010 with HI titers to A/Sydney of ≥40 may be surprising given that cohort's birth subsequent to A/Sydney circulation. This, however, may be explained by cross-reactivity to other closely related A/Sydney descendant strains.

Antibody levels peaked at 5–9 years of age for the contemporary A/Brisbane strain, at 10–19 years for the ancestral

A/Sydney strain, and at 15–29 years of age for the newly emerging swine-origin A/Indiana strain. Taken together, these age-related findings suggest cohort effects to explain cross-reactive antibody to H3N2v. These cohort effects may be predicated on childhood priming to related ancestral strains, with robust memory responses to the priming virus carried forward with age and reinforced through subsequent boosting exposures [42, 43]. Subsequent declines in cross-reacting antibody levels through middle age may reflect differences in particular priming exposure in childhood and/or more limited opportunities for subsequent boost through adulthood. The suggestion of increased levels of antibody to A/Indiana among very old individuals (ie, those approaching 100 years of age) is based on a small sample size and is thus inconclusive. It is intriguing to consider that individuals 98–100 years old in 2010 (ie, people who were born during 1910–1912) would have been 5–7 years of age when an H3 subtype virus last dominated (during 1889–1917), prior to its replacement by the 1918 H1N1 pandemic strain (Figure 2) [42]. However, relatedness of pre-1918 strains to the emerging H3N2v cannot be known in the absence of recovered virus from that period. An H3N2 virus next emerged during the 1968 pandemic [11], becoming a probable priming subtype for those who were ≤42 years of age in 2010 (Figure 2). Other childhood non-H3 subtype priming exposures (H1N1 and H2N2) in the intervening period may be relevant in interpreting the lower levels of cross-reactive antibody to H3N2v among individuals >42 years of age, despite their greater cumulative exposure to influenza across the lifespan (Figure 2). These reflections, however, are speculative and would require specific evaluation.

The limitations of this study also warrant careful consideration. Interlaboratory and intralaboratory variability in HI assay results is widely recognized; comparison across test viruses assessed in different laboratories requires particular caution. We emphasize the interpretation of major age-related trends by virus within the same laboratory. Titers measured by HI, unlike those measured by MN, do not necessarily represent functional antibodies, and the threshold of 40 indicates a seroprotective level of 50% (rather than 100%) that may not apply to zoonotic infections or to all age groups [44, 45]. Levels of cross-reactive antibody to A/Indiana that were considered cross-protective were frequently close to threshold titers; as such, the proportion considered protected may be unstable and should be interpreted cautiously. In general, HI has been thought to overestimate cross-reactive responses to heterologous strains [45, 46], but among tested subsets, we found comparable or higher titers to A/Indiana by the MN assay. A seroprotective threshold based on MN analysis has not been defined. The proportion of individuals considered seroprotected against A/Indiana on the basis of an HI titer of ≥40 was most closely aligned in our study with the proportion

with an MN threshold titer ≥80, a finding also highlighted by others [47]. Levels of antibodies to other virus domains (eg, HA stalk and neuraminidase) and/or cell-mediated immunity may also contribute to protection and vary by age but were not assessed. We assessed levels of cross-reactive antibody to a representative H3N2v strain, but this may not reflect findings for other swine-origin H3N2 variants. It is reassuring, however, that, using the same pediatric and adult vaccine sera but with a different swine-origin H3N2 lineage and at a different laboratory, we found similar age-related and vaccine-induced trends [22]. Our results are also consistent with H3N2v serologic findings from smaller serosurveys recently reported from the United States [47] and Norway [48]. Sera included in our study were sampled from the eastern province of Quebec and from the westernmost province of British Columbia—areas located >4000 km apart—and yielded similar results, suggesting broad geographic relevance of these findings in the northern hemisphere. However, sera were not randomly selected, and full epidemiologic details were not available to guide interpretation or generalization to other areas. In British Columbia, seasonal influenza vaccine coverage is estimated at approximately 30% overall, with the highest coverage among elderly individuals (approximately 65%); uptake of the 2009 adjuvanted monovalent A(H1N1)pdm09 vaccine was approximately 45% overall, with coverage highest among children and elderly individuals (55%–60%) [28, 49]. We do not know the vaccination history of individuals whose sera were included in the age-based serosurvey, but our vaccine study group suggests prior seasonal or pandemic vaccinations are unlikely to have substantially increased cross-reactive H3N2v antibody levels. Nevertheless, population differences in prior infection, vaccination history, and other characteristics may be relevant to consider in extrapolating our findings to other settings. These findings should be confirmed elsewhere.

In conclusion, our serologic findings suggest substantial protection against H3N2v in late adolescence and young adulthood but broad susceptibility in children and older adults. The current seasonal TIVs, including available adjuvanted formulations for elderly individuals, do not substantially increase cross-protection, indicating that a specific vaccine would be needed in the event of epidemic spread. Ongoing surveillance and evaluation are needed to confirm these findings and to inform further risk analysis and response. Although only a single human case of H3N2v infection has so far been detected during the first half of 2012 [16–18], swine influenza viruses do not show typical winter seasonality, and further resurgence remains possible [3]. In that regard, knowledge of population immunity and variation in seroprotection by age are critical in guiding clinician diagnostic suspicion, informing surveillance efforts at the local and national levels, modeling epidemic likelihood, and, ultimately, supporting policy makers in their planning and preparedness activities.

Supplementary Data

Notes

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