Effect of Priming With Seasonal Influenza A(H3N2) Virus on the Prevalence of Cross-Reactive Hemagglutination-Inhibition Antibodies to Swine-Origin A(H3N2) Variants

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Abstract

Background—Recent outbreaks of swine-origin influenza A(H3N2) variant (H3N2v) viruses have raised public health concerns. Previous studies indicated that older children and young adults had the highest levels of hemagglutination-inhibition (HI) antibodies to 2010–2011 H3N2v viruses. However, newly emerging 2013 H3N2v have acquired antigenic mutations in the hemagglutinin at amino acid position 145 (N145K/R). We estimated the levels of serologic cross-reactivity among humans primed with seasonal influenza A(H3N2) (sH3N2), using postinfection ferret antisera. We also explored age-related HI antibody responses to 2012–2013 H3N2v viruses.

Methods—Human and ferret antisera were tested in HI assays against 1 representative 2012 H3N2v (145N) and 2 2013 H3N2v (145K/R) viruses, together with 9 sH3N2 viruses circulating since 1968.

Results—Low levels of cross-reactivity between the H3N2v and sH3N2 viruses from the 1970s–1990s were observed using postinfection ferret antisera. The overall seroprevalence among the sH3N2-primed population against 2012–2013 H3N2v viruses was >50%, and age-related seroprevalence was observed. Seroprevalence was significantly higher to 2013 H3N2v than to 2012 H3N2v viruses among some children likely to have been primed with A/Sydney/5/97-like (145K) or A/Wuhan/359/95-like viruses (145K).

Conclusions—A single substitution (N145K/R) was sufficient to affect seropositivity to H3N2v viruses in some individuals. Insight into age-related antibody responses to newly emerging H3N2v viruses is critical for risk assessment and pandemic preparedness.

Keywords

Triple-reassortant swine influenza A(H3N2) viruses (tr-H3N2 SIVs), which contain genes from human, classic swine, and avian influenza A viruses, have been enzootic among swine herds in the United States since 1998 [1, 2]. The genes encoding the principal surface glycoprotein of the tr-H3N2 SIVs, hemagglutinin (HA), are genetically similar to the 1995, 1997, or 2010–2011 human seasonal influenza A(H3N2) viruses (sH3N2) [1–3]. The first human case in the United States was identified in 2009 [4], and 2009–2010, 7 additional tr-H3N2–infected cases were reported in the United States, primarily among children [5].

In 2010, a novel reassortant H3N2 SIV containing 7 genes from the tr-H3N2 and the matrix gene from influenza A(H1N1)pdm09 virus was identified in US pigs [6, 7]. From August 2011 to October 2016, a total of 364 human infections with the novel reassortant H3N2 variant (H3N2v) viruses were reported from 13 states [8, 9] (http://www.cdc.gov/flu/swineflu). Twenty-one patients were hospitalized, and 1 fatal case was identified. The number of H3N2v-infected cases was probably underestimated, and limited person-to-person transmission was likely [10]. However, no sustained or community transmission of this virus had been identified [10]. The majority of patients with H3N2v virus were children aged <12 years and had reported swine exposure at agricultural fairs [8, 9].

Antibodies against HA are a primary determinant of protection to influenza infection [11]. Serum hemagglutination-inhibition (HI) antibodies that bind or block the receptor-binding site of HA are widely accepted immune correlates of protection [12]. An HI titer of 40 is generally considered a 50% protective titer for sH3N2 viruses in adult populations [12]. Previous seroprevalence studies conducted by 6 countries indicated that approximately 25%–50% of the human populations were seropositive (with HI titers 40) to 2010–2011 H3N2v viruses [13–18]. Age-related seroprevalence, based on the percentage of HI titers 40, was noted. The older children and young adults had the highest levels of seropositivity to H3N2v viruses, but most young children (aged <10 years) and older adults (aged 40–60 years) had few or no cross-reactive HI antibodies to these viruses. The reasons for this apparent age-related seroprevalence were not discussed in detail [13–18]. Immunization with the 2010–2011 or 2011–2012 trivalent inactivated seasonal influenza vaccine did not result in HI antibody response to H3N2v viruses in children aged <3 years [19], but it slightly boosted the levels of cross-reactive HI antibodies among some older children, adults, and the elderly [15, 19]. On the other hand, vaccination with inactivated A/Beijing/32/1992 vaccine or 2011–2012 trivalent inactivated seasonal influenza vaccine failed to induce HI antibody response to 2011 H3N2v virus and did not provide any significant cross-protection against the H3N2v virus infection in ferrets [20].

The sH3N2 HA genes introduced into the US SIV gene pool in the 1990s followed an evolutionary path in the swine population separate from their human counterparts [5, 21]. This resulted in 2009–2012 H3N2 SIVs, including H3N2v virus, being antigenically distinct from 1990s tr-H3N2 SIVs and sH3N2 viruses circulating since 1979, observed with HI tests

using postinfection ferret antisera [15, 22]. Contemporary H3N2 SIVs demonstrated substantial antigenic diversity [21], and continuous genetic and antigenic evolutions of H3N2 SIVs could generate new SIVs with greater antigenic distance from historical and contemporary sH3N2 viruses. Of particular note are the 2013 H3N2v strains, which possess substitutions at position HA-145 (N145K/R). These H3N2v viruses caused 18 human infections in 2013, mainly among children 10 years old (http://www.cdc.gov/flu/swineflu).

Amino acid position 145, located in antigenic site A of H3 HA near the tip of the globular head and the receptor-binding site [23], has been under continual antibody-driven selection since the 1968 H3N2 pandemic [24, 25]. The amino acid substitutions at residue 145 of sH3N2 viruses (S145N, N145K, K145N, and N145S) have been identified in multiple antigenic drift events in the past [26–29]. A single N145K substitution was shown to have a large antigenic effect responsible for the A/Sichuan/1987 to A/Beijing/1989 and A/Beijing/1992 to A/ Wuhan/1995 cluster transitions detected by ferret antisera [26, 27]. Convalescent serum samples collected in 1991 from children (aged 12–14 years) infected with sH3N2 viruses containing 145K either lost or reduced their binding capacity to a mutant virus as well as to the 1993–1994 field strains containing 145N [30]. Moreover, the S145N/R or N145K substitution was associated with an emergence of a new antigenic cluster or outliers in European and the US pigs [21, 31].

The human infections with H3N2v viruses possessing the HA N145K/R mutations in 2013 are of public health concern. In the current study, we evaluated the levels of HI antibodies to 1 2012 H3N2v (145N) virus, 2 2013 H3N2v (145K/R) viruses, and 9 1968–2007 sH3N2 viruses in a large set of human serum samples (from subjects aged 6 to 80 years) and postinfection ferret antisera. We also explored whether previous exposures to different sH3N2 antigenic variants is associated with age-related cross-reactivity in US children. Analysis of the antigenic relationships among H3N2v and sH3N2 viruses and continuous monitoring of age-based seroprevalence to newly emerging H3N2v drift strains are important for risk assessment and development of medical countermeasures.

MATERIAL AND METHODS

Viruses

Influenza viruses were propagated either in the allantoic cavity of 10-day-old embryonated eggs or in Madin-Darby Canine Kidney cells, following procedures published elsewhere [32] under BSL2-enhanced conditions for H3N2v or BSL2 conditions for sH3N2 viruses.

Serum Samples

Human serum samples collected in 2010 (N = 1007; subjects aged 6 to 80 years) were randomly selected from a large set of serum samples provided by the National Health and Nutrition Examination Survey. The protocol for testing of these serum samples at the Centers for Disease Control and Prevention (CDC) was reviewed and approved by the Institutional Review Board of the CDC's National Center for Immunization and Respiratory Diseases and the Research Ethics Review Board of CDC's National Center for Health

Statistics. Postinfection ferret antisera were prepared by intranasal inoculation with a single dose of wild-type virus.

HI Assay

Serum samples were treated with receptor destroying enzyme (Denke-Seiken) to remove nonspecific inhibitors and adsorbed with packed turkey red blood cells to remove nonspecific agglutinins before testing with 4 HA units of virus and 0.5% turkey red blood cells by means of HI assays [33].

Statistical Analyses

Fisher exact tests and *t* tests were used to analyze seroprevalence and geometric mean titers (GMTs), respectively. Differences were considered significant at *P* < .05. SAS software (version 9.3; SAS Institute) was used for statistical analyses.

RESULTS

Antigenic Analysis

The antigenic relationship among 3 2012–2013 H3N2v and 9 sH3N2 viruses associated with the majority of severe epidemics since 1968 was determined by HI assays using primary-infection ferret antisera. Three H3N2v viruses possessing 145N (A/Ohio/13/2012 [OH/13/12]), 145K (A/Indiana/06/2013 [IN/6/13]), or 145R (A/Indiana/17/2013 [IN/17/13]) were tested with HI assays, together with sH3N2 viruses representative of 9 antigenic clusters since 1968. Nine sH3N2 viruses, their prevalent eras, prominent seasons, and vaccine-use eras in the United States are summarized in Table 1, based on previous publications [34, 35] and the annual US influenza surveillance data.

As shown in Table 2, the 3 H3N2v viruses were well inhibited by ferret antisera raised against these viruses, although somewhat reduced cross-reactivity (2–4 fold) was noticed between 2012 H3N2v (145N) and 2013 H3N2v (145K/R) viruses. However, these viruses were not inhibited or were poorly inhibited by antisera raised against all 9 sH3N2 viruses. The 3 antisera raised by H3N2v viruses also demonstrated low levels of cross-reactivity to the sH3N2 viruses from the1970s–1990s, and 2 antisera raised to WH/95 or BJ/92 showed low levels of cross-reactivity with the 2012 and/or 2013 H3N2v viruses. These data indicate that the 2012–2013 H3N2v viruses were antigenically distinct from the 1968–2007 sH3N2 viruses but antigenically related to the sH3N2 viruses from the1970s–1990s.

Age-Related Seroprevalence

To determine the seroprevalence of HI antibodies to OH/13/12, IN/6/13, and IN/17/13, a large set of human serum samples (N = 1007) collected from consecutive ages (6 to 80 years) in 2010 were examined in HI assays. Most samples yielded similar HI titers against IN/6/13 and IN/17/13 (data not shown). Therefore, seroprevalence, determined on the basis of an HI titer 40, and HI GMTs were evaluated for OH/13/12 and IN/6/13 across 9 separate age group categories (Table 3). We also evaluated seroprevalence and HI GMT differences between OH/13/12 (145N) and IN/6/13 (145K).

The overall seroprevalence was similar against OH/13/12 (54%) and IN/6/13 (55%), although the 2 viruses showed distinctive age-related patterns. Low seropositive rates for both H3N2v viruses were observed among children 6–9 years old. The proportion of seropositivity increased with age, peaked among those aged 20–29 years, then dropped to approximately one-third among those aged 40–49 or 50–59 years, and remained between 39% and 52% among those aged 60–69, 70–79, or 80 years. The GMTs had the same age-related trend as the seroprevalence. However, significant differences ($P < .01$) in seroprevalence and HI GMTs between IN/6/13 and OH/13/12 were observed in those aged 10–19 or 30–39 years (Table 3).

Interestingly, 8% of individuals (83 of 1007; median age, 17 years) showed the pattern of higher seroprevalence to IN/6/13 than to OH/13/12 (HI titers 40 to IN/6/13 and 4-fold higher for IN/6/13 than for OH/13/12). In contrast, 4% (38 of 1007; median age. 47 years) showed the pattern of higher seroprevalence to OH/13/12 than to IN/6/13 (HI titers 40 to OH/13/12 and 4-fold higher for OH/13/12 than for IN/6/13). These 2 patterns were also age related: 61% with higher seroprevalence to IN/6/13 were aged 8–20 years, and 95% with higher seroprevalence to OH/13/12 were aged 21 years.

Likely Association Between Seroprevalence in Older Children and First Exposure to sH3N2 Viruses in the 1990s

To determine whether the HI antibodies cross-reactive to 2012–2013 H3N2v viruses were associated with exposures to historic sH3N2 viruses, all serum samples from children (n = 211; aged 6–18 years) from a set of 1007 samples were tested against 2 H3N2v and 8 sH3N2 viruses. These serum samples showed unmeasurable HI antibody response (titer, <10) against AI/68, VIC/75, and BK/79 (data not shown). The age distributions for HI antibodies to the 2 H3N2v and 5 1987–2007 sH3N2 viruses are presented in Figure 1.

The majority of children (79%) aged 6–9 years (born 2001–2004) showed seropositivity to BR/07, but 17% showed seropositivity to the 2 H3N2v viruses. Increased seroprevalence to IN/6/13 (46%) and OH/13/12 (36%) were observed among 10–11-year-olds (born 1999–2000), along with increasing seroprevalence (86%) to SY/97, which circulated between 1997 and 2000. Further increased seroprevalence to IN/6/13 (80%) was observed in the 12–13- and 14–15-year age groups (born 1995–1998), accompanied by increased seroprevalence (77%) to WH/95, which circulated between 1995 and 1998. Among 16–18-year-olds (born 1992–1994), seroprevalence (~90%) to IN/6/13 and OH/13/12 was the highest, with increasing seroprevalence (88%) to BJ/92, which circulated between 1992 and 1996. Analysis using GMTs demonstrated the same age-related trend as seroprevalence. These data suggested that HI antibodies cross-reactive to 2012 and/or 2013 H3N2v viruses among children (aged 10–18 years) were probably associated with their first exposure to different sH3N2 viruses in the 1990s.

To further clarify the age-related seroprevalence in the older children, the HI data from 141 children (aged 12–18 years) were selected and further analyzed, and 124 children (88%) were seropositive to either IN/6/13 or OH/13/12, or both. Finally, we selected the 118 H3N2v-seropositive children and grouped them into 3 groups based on the 3 unique HI antibody patterns against the 4 1987–1997 sH3N2 viruses (Figure 2 and Supplementary

Tables 1–3). Group 1 (median age, 13 years in 2010) included children showing the highest HI titers to SY/97, which were significantly higher (4-fold) than titers to WH/95, BJ/92, and SH/87 (Figure 2A and Supplementary Table 1). Group 2 (median age, 15 years in 2010) included children exhibiting similar levels (within 2-fold) of HI antibody titers against SY/97 and WH/95, with titers to WH/95 virus significantly higher than those to BJ/92 and SH/87 (Figure 2B and Supplementary Table 2). Group 3 (median age, 17 years in 2010) included children with similar levels of HI titers to SY/97, WH/95, and BJ/92, with titers to BJ/92 significantly higher than those to SH/87 (Figure 2C and Supplementary Table 3).

Consistent with recent publications [36–38], our data also showed varied levels of cross-reactive HI antibodies to SH/87, which circulated before the birth of these children (Table 1 and Supplementary Tables 1–3). Considering that the first sH3N2 virus infection can occur among children aged 2 months to 7 years old [39, 40], the age distribution (Figure 3) correlated well with what is known as the "period of prevalence" of the SY/97, WH/95, and BJ/92 antigenic clusters (Supplementary Table 4). Thus, it is reasonable to suggest that most children, if not all, in groups 1, 2, and 3 were highly likely to be primed with the SY/97, WH/95, and BJ/92 clusters, respectively. It is noteworthy that 6 H3N2v-seropositive children were excluded from the analysis (data not shown), including 1 child with the highest HI titers to sH3N2 viruses circulated after 2000, 1 with low HI titers to the sH3N2 viruses tested, and 4 with HI titers to WH/95 2-fold higher than those to BJ/92 but titers to IN/6/13 4-fold higher than those to OH/13/12. The antibody population of these 4 serum samples was verified by antibody adsorption assays; WH/95-specific antibody, but not BJ/92-specific antibody, was detected, suggesting that these children were probably primed with WH/95-like viruses (data not shown). Overall, the 118 samples analyzed in Figure 2 represented 95% of H3N2v-seropositive children available in this age group.

All children in group 1, likely to have been primed with SY/97-like virus, showed seropositivity to IN/6/13 and SY/97; 62% also showed seropositivity to OH/13/12 (Figure 2D). Although only a small portion of the children (21%) showed significantly higher HI titers (4 fold) against IN/6/13 than against OH/13/12, the data suggested that a certain portion of antibodies in these children could recognize an epitope containing 145K on IN/6/13 HA. All children in group 2, likely to have been primed with WH/95-like virus, showed seropositivity to IN/6/13, WH/95, and SY/97, but only 43% showed seropositivity to OH/13/12 (Figure 2E). Most children (91%) in group 2 had significantly higher HI titers ((4 fold) against IN/6/13 than against OH/13/12, thus suggesting that dominant HI antibodies cross-reacting with IN/6/13 in most WH/95-primed children bind to an epitope containing 145K. Most children (98%) in group 3, likely to have been primed with BJ/92-like virus, showed seropositivity and similar levels of HI antibody responses to the 2 H3N2v and the 3 sH3N2 viruses (Figure 2F). Taken together, these data suggested that primary infections with the SY/97, WH/95, or BJ/92 cluster were highly likely to be associated with the age-related seroprevalence to 2012–2013 H3N2v viruses in older children.

Comparison of Amino Acid Sequence in Antigenic Site A Among H3N2 Viruses

To better understand the molecular basis of the different seroprevalence patterns to OH/13/12 and IN/6/13 in children, we compared HA1 amino acid sequences of the H3N2v and

the sH3N2 viruses. Because there was only a single amino acid difference at position 145 (antigenic site A) in the entire HA1 domain between the OH/13/12 and IN/6/13, but the 2 viruses demonstrated a moderate antigenic variation (2–4-fold HI titer differences; Table 2), our analysis focused on antigenic Cross-Reactive Antibody to H3N2v Viruses • JID 2017:216 (Suppl 4) • S545 site A. An amino acid difference in antigenic site A between the sH3N2 and the H3N2v viruses is presented in Table 4. The IN/6/13 and WH/95 shared several amino acids in antigenic site A, particularly the 145K, which might provide an explanation of the high seroprevalence to IN/6/13 among possible WH/95-primed children [30]. The data also suggested that the majority of HI antibodies cross-reactive to IN/6/13 among WH/95-primed children bound to an epitope possessing 145K. A single N145K substitution in H3N2v virus HA is sufficient to change an individual's seropositivity and significantly influence seroprevalence in some age groups.

DISCUSSION

Using human and postinfection ferret antisera, we evaluated the levels of HI antibodies against 2012–2013 H3N2v viruses, together with 9 epidemiologically important sH3N2 viruses circulating since 1968. Children born after 2000 had lowest level of cross-reactive HI antibodies to 2012–2013 H3N2v viruses tested. This is consistent with results of previous population serosurveys with 2010–2011 H3N2v viruses [13–18], suggesting that young children are at high risk of infection with the H3N2 SIVs. In agreement with the serological findings, the majority of confirmed H3N2v virus infections in 2011–2015 occurred in children 10 years old [9] (http://www.cdc.gov/flu/swineflu). However, more than half of individuals born before 2000 showed seropositivity to 2012–2013 H3N2v viruses.

Low degrees of serologic cross-reactivity between 1975–1997 sH3N2 and the 2012–2013 H3N2v viruses were observed in ferrets after primary infection. However, a high degree of cross-reactivity was observed in some individuals after multiple exposures. Moreover, age-related seroprevalence was observed in humans, and most older children and young adults showed seropositivity to the H3N2v viruses. A 2016 study indicated that HI antibody patterns in ferrets and young children after primary infection with sH3N2 viruses is generally similar [38]. An earlier study found that primary infection with sH3N2 virus could occur in 2-month-old infants, and the second infection in those 12 months old [40].

Our cross-HI data demonstrated that most individuals tested in this study had experienced secondary or even >2 exposures to sH3N2 viruses. Secondary exposures with antigenic drifted strains could shape HI antibody patterns by producing antibodies targeting different subdominant epitopes on the HA [41–43], which may be responsible for the difference observed between the ferrets and humans in this study. It would be interesting to study how the age-related HI antibodies change as an individual experiences first, second, or multiple exposures to sH3N2 antigenic variants. Consistent with previous findings [36, 37, 44], our data indicated that primary exposure with sH3N2 viruses influenced HI antibody response thereafter. Because HI antibodies target the HA globular head and neutralizing antibodies target both globular head and stalk domain [45], it would also be interesting to investigate to what extent such primary exposure with sH3N2 virus affects the neutralizing antibody response.

A single substitution (N145K) was sufficient to affect individuals' seropositivity to 2012–2013 H3N2v viruses. Significantly higher seropositivity (80%) to IN/6/13 (145K) compared with OH/13/12 (145N; 44%) was observed in children born between 1995 and 1998. The seropositivity to IN/6/13 among older children was also remarkably higher than that to 2011 H3N2v viruses possessing 145N [13, 14, 16]. BJ/92, WH/95, and SY/97 are the representative sH3N2 viruses circulated between 1992 and 2000. To better understand the age-related antibody patterns, we have, for the first time, determined the priming sH3N2 virus in older children with unknown exposure histories. The priming sH3N2 viruses, determined based on each child's HI antibody pattern, were consistent with surveillance data reflecting the period during which the 3 sH3N2 antigenic cluster circulated in the US population. Grouping the children into 3 sH3N2 primed groups allowed us to understand how sH3N2 priming histories influence cross-reactivity for 2012–2013 H3N2v viruses. For example, most children who were highly likely to have been primed with WH/95-like viruses showed similar levels of HI antibody responses to the WH/95, SY/97, and IN/6/13 viruses, all 3 containing 145K, but their HI antibody levels against OH/13/12 (145N) and BJ/92 (145N) were significantly lower or even undetectable.

Previous studies showed that convalescent serum samples from children infected with BJ/89-like viruses (145K) and antisera from ferrets infected with WH/95-like virus (145K) had significantly decreased HI activity to H3N2 viruses possessing 145N [30, 46]. The addition of glycans at Asn133 on SY/97 HA should not mask the 140 loop [47]. Moreover, a human 145K-specific monoclonal antibody could cross-react with SY/97-like viruses [48]. The HI pattern in the WH/95-primed children, together with previous findings [30, 38, 46–48] suggests that reinfection with SY/97-like viruses probably occurred in these children. The B memory cells targeting an epitope possessing 145K might be restimulated by reinfection with SY/97-like viruses, which might resulted in dominance of the antibodies directed to the epitope possessing 145K in WH/95-primed children [41]. These antibodies are likely to recognize WH/95 (145K), SY/97 (145K), and IN/6/13 (145K) but not OH/13/12 (145N).

Children who were likely to have been primed with SY/97-like virus showed significantly higher levels of HI antibodies to SY/97, compared with 2012–2013 H3N2v viruses, indicating that the majority of these antibodies could not cross-react with 2012–2013 H3N2v viruses. Some SY/97-primed children showed the pattern of higher seroprevalence to IN/6/13 than to OH/13/12, suggesting that some antibodies binding to an epitope possessing 145K have, in part, contributed to the cross-reactivity to IN/6/13 virus. Children primed with BJ/92-like virus showed equally high levels of HI antibody activity to 2012–2013 H3N2v and 1992–1997 sH3N2 viruses, regardless of 145N or 145K, suggesting that there are alternate common epitope(s) between the sH3N2 and 2012–2013 H3N2v viruses. The pattern of higher HI antibody activity to OH/13/12 than to IN/6/13 was observed mainly in individuals born before 1989. It is unknown whether this pattern was associated with exposures to sH3N2 viruses possessing 145N, which circulated in 1970s and 1980s [26]. Further studies are warranted to clarify the influence of priming histories in the cross-reactivity to H3N2v viruses among adults and elderly. The nature of age-related cross-reactive antibodies in different age populations should also be further addressed.

H3N2v viruses can efficiently replicate and transmit in mammals [49]. The continued evolution of H3N2 SIVs among pigs may generate a novel virus, to which the majority of humans, particularly older children and younger adults, may have little or no preexisting immunity. Improved surveillance in pigs for newly emerging H3N2 SIVs remains important. Insight into age-related cross-reactive antibody patterns against circulating SIVs among different age groups is critical in identifying the risks of viruses in causing human infection and interspecies transmission with these viruses, and their pandemic potentials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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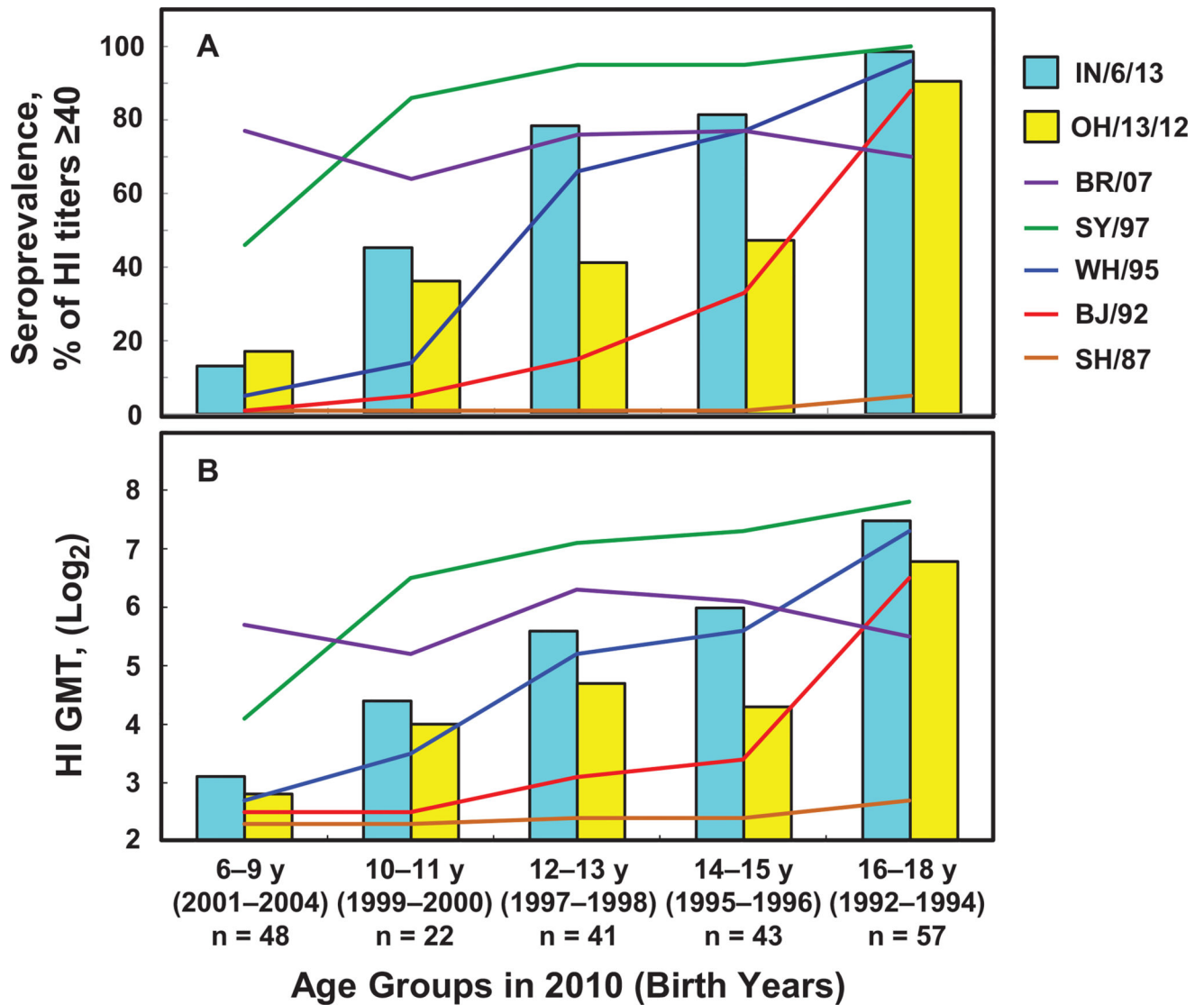
Figure 1.
Prevalence of hemagglutination-inhibition (HI) antibodies cross-reactive to A(H3N2) variant (H3N2v) swine influenza viruses in US children is likely to be associated with exposures to 1990s human seasonal influenza A(H3N2) (sH3N2) viruses. Serum samples from children aged 6 to 18 years (n = 211) were collected in 2010. Seroprevalence (*A*) and HI geometric mean titers (GMTs) (*B*) for 2 H3N2v viruses are depicted in bars for A/Indiana/06/2013 (IN/6/13) and A/Ohio/13/2012 (OH/13/12). The seroprevalence and GMTs for the 5 human sH3N2 viruses isolated between 1987 and 2007 are shown as lines. Age groups at the time of serum sample collection (2010) and years of birth are shown on the x-axis. The seroprevalence and HI GMTs against IN/6/13 compared with OH/13/12 were significantly higher in 12–13 and 14–15 age groups.
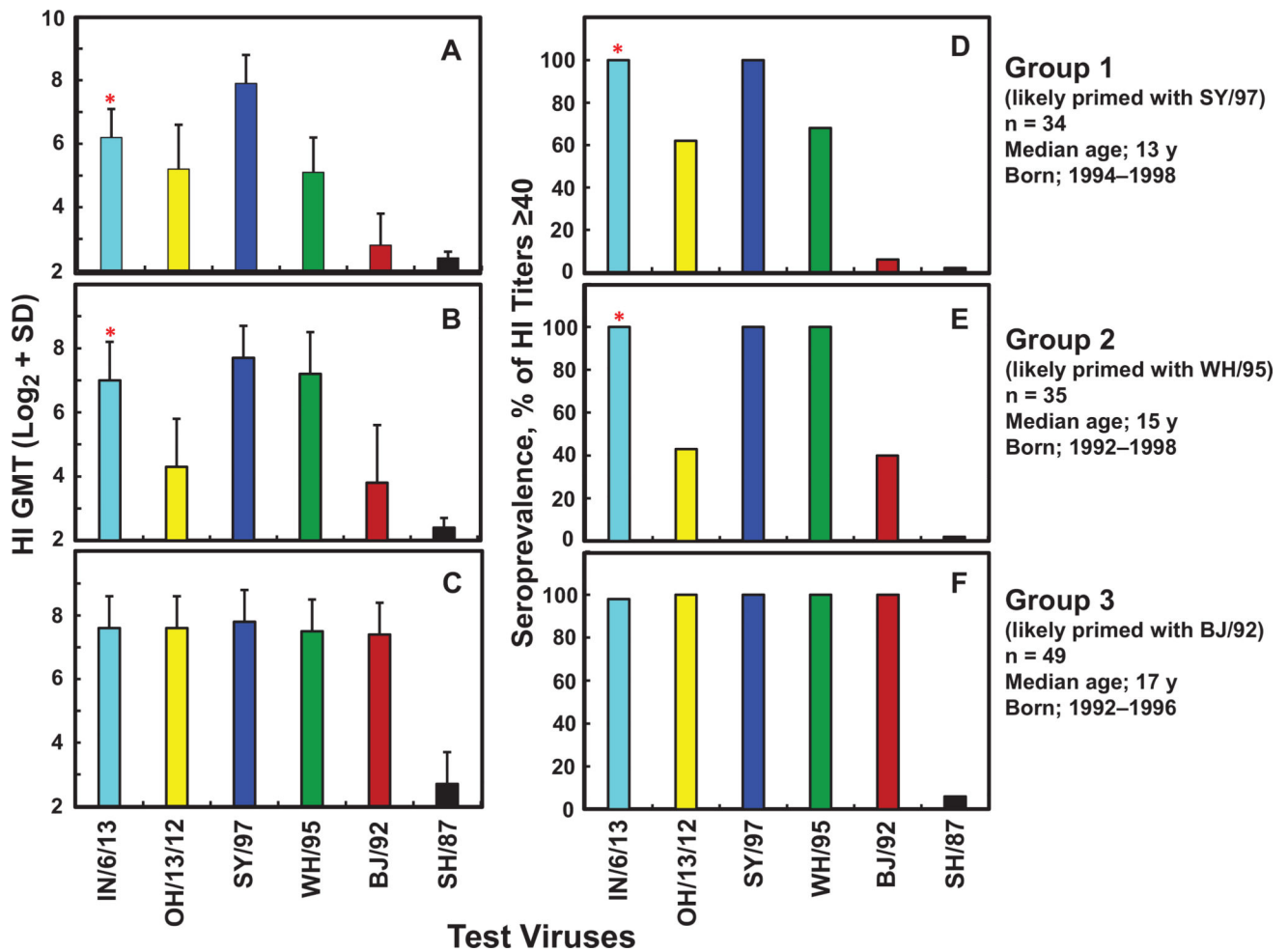
Figure 2.
Priming with different 1990s human seasonal influenza A(H3N2) (sH3N2) viruses in US children influences seroprevalence of hemagglutination-inhibition (HI) antibodies to 2012–2013 influenza A(H3N2) variant (H3N2v) virus. Serum samples from children aged 12–18 years (n = 118) were collected in 2010; median ages are for this year. HI geometric mean titers (GMTs) and seroprevalence against the 2 H3N2v and 4 sH3N2 viruses are shown for 3 groups of children likely to have been primed with SY/97 (*A, D*), WH/95 (*B, E*), or BJ/92 cluster (*C, F*). *Significantly higher seroprevalence and HI GMTs against A/Indiana/06/2013 (IN/6/13) compared with A/Ohio/13/2012 (OH/13/12) in groups 1 and 2 (*P* < .01). Abbreviation: SD, standard deviation.

Figure 3.
Age distribution in 3 groups of children (aged 12–18 years, n = 118) likely to have been primed with SY/97, WH/95, or BJ/92 cluster. Median ages shown are for 2010.

Table 1

Periods of Prevalence for sH3N2 Viruses Used in Current Study

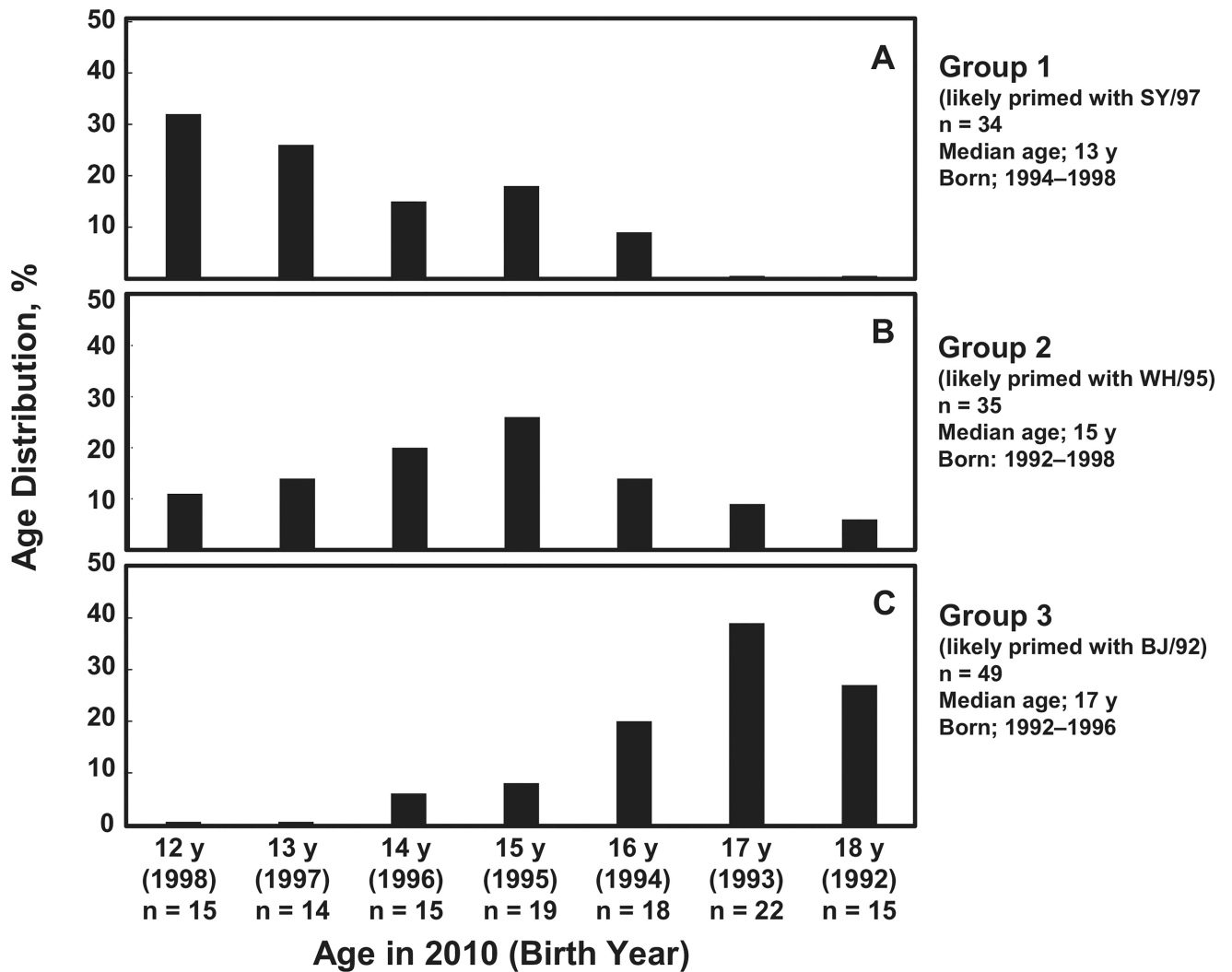| Reference Strain (Abbreviation)	Eras of Prevalence[a]	Prominent Seasons[b]	Vaccine-Use Eras[a]
A/Aichi/2/1968 (AI/68)	1968–1972	1968–1969; 1969–1970; 1971–1972	1968–1969; 1969–1970; 1970–1971; 1971–1972; 1972–1973
A/Victoria/3/1975(VIC/75)	1975–1977	1975–1976	1976–1977; 1977–1978
A/Bangkok/1/1979(BK/79)	1979–1983	1980–1981; 1982–1983	1980–1981; 1981–1982; 1982–1983;
A/Shanghai/11/1987(SH/87)	1987–1990	1987–1988; 1989–1990	1989–1990
A/Beijing/32/1992(BJ/92)	1992–1996	1992–1993; 1993–1994	1993–1994
A/Wuhan/359/1995(WH/95)	1995–1998	1996–1997	1996–1997; 1997–1998
A/Sydney/5/1997(SY/97)	1997–2000	1997–1998; 1998–1999; 1999–2000	1998–1999; 1999–2000
A/Fujian/411/2002(FJ/02)	2002–2005	2003–2004	2004–2005
A/Brisbane/10/2007(BR/07)	2007–2009	2007–2008	2008–2009; 2009–2010

Abbreviation: sH3N2, human seasonal influenza A(H3N2).

[a] Data from previous publications [34, 35] and annual US influenza virus surveillance data.

[b] Prominent influenza seasons were defined as those in which 20% of isolates typed and subtyped in that season were the sH3N2 virus [35].

Table 2

HI Reactions of H3N2v and Human sH3N2 Viruses Using Ferret Antisera

| Viruses | HI Antibody Titers With Primary-Infection Ferret Antisera[a] | | | | | | | | | | | | |
| | H3N2v Viruses | | | Human sH3N2 | | | | | | | | | Passage |
	OH/13/12	IN/5/13[b]	IN/17/13	HK/68	VIC/75	BK/79	SH/87	BJ/92	WH/95	SY/97	FJ/02	BR/07	
OH/13/12	1280[c]	160	320	<	10	<	<	160	<	<	<	<	C3
IN/6/13[b]	320	640[c]	640	<	10	<	10	40	20	<	<	<	C2
IN/17/13	640	320	1280[c]	<	<	<	<	80	20	<	<	<	C2
AI/68	<	<	<	1280[c]	20	<	40	<	<	<	<	<	E6
VIC/75	40	<	10	<	640[c]	<	10	<	<	<	<	<	E6
BK/79	40	<	<	<	80	320[c]	20	<	<	<	<	<	E9
SH/87	20	<	<	<	10	<	640[c]	40	<	<	<	<	EX/E6
BJ/92	40	<	<	<	<	<	10	1280[c]	<	<	<	<	E2/E4
WH/95	<	40	40	<	<	<	<	80	320[c]	40	<	<	E2/E5
SY/97	<	20	20	<	10	<	<	10	40	1280[c]	80	20	C2/E3
FJ/02	<	<	<	<	<	<	<	<	<	80	2560[c]	160	CX/C5
BR/07	<	<	<	<	<	<	<	<	<	<	160	1280[c]	E2/E3

Abbreviations: H3N2v, influenza A(H3N2) variant; HI, hemagglutination-inhibition; IN/5/13, A/Indiana/5/2013; IN/6/13, A/Indiana/06/2013; IN/17/13, A/Indiana/17/2013; OH/13/12, A/Ohio/13/2012; sH3N2, human seasonal influenza A(H3N2).

[a] Serum samples from ferrets infected with a single dose of H3N2 wild-type virus. Less than symbols (<) denote HI titers <10.

[b] IN/6/13 and A/Indiana/5/2013 (IN/5/13) have identical hemagglutinin sequences.

[c] Reactions to the homologous virus or antigenically closely related virus.

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Table 3

Cross-Reactive HI Antibodies to H3N2v Viruses, by Age Group, in US Human Serum Samples Collected in 2010

Age Group in 2010, y	Birth Years	Subjects, No.	HI Titer 40 (95% CI), %		HI GMT (95% CI)	
			OH/13/12	IN/6/13	OH/13/12	IN/6/13
6–9	2001–2004	48	17 (6–27)	13 (3–22)	9 (7–11)[a]	10 (8–13)[a]
10–19	1991–2000	188	62 (55–69)[b]	82 (77–88)[b]	51 (41–64)[b]	90 (76–108)[b]
20–29	1981–1990	121	88 (83–94)	90 (85–95)	105 (84–131)	97 (81–117)
30–39	1971–1980	121	74 (66–81)[b]	55 (47–64)[b]	49 (41–58)[b]	32 (27–39)[b]
40–49	1961–1970	125	34 (25–42)	30 (22–38)	18 (15–22)	18 (15–22)
50–59	1951–1960	99	35 (26–45)	35 (26–45)	19 (15–24)	20 (15–25)
60–69	1941–1950	118	44 (35–53)	48 (39–57)	25 (20–32)	27 (21–35)
70–79	1931–1940	108	52 (42–61)	50 (41–59)	32 (25–41)	28 (22–36)
80	1908–1930	79	46 (35–57)	39 (28–50)	23 (17–31)	20 (15–27)
All ages	1908–2004	1007	54 (51–57)	55 (52–58)	33 (31–36)	35 (32–38)

Abbreviations: CI, confidence interval; GMT, geometric mean titer; H3N2v, influenza A(H3N2) variant; HI, hemagglutination-inhibition; IN/6/13, A/Indiana/06/2013; OH/13/12; OH/13/12, A/Ohio/13/2012.

[a] *P* = .04 for difference in HI GMT between OH/13/12 and IN/6/13.

[b] *P* < .01 for difference in seroprevalence or HI GMT between OH/13/12 and IN/6/13.

Table 4

Antigenic Site A Variation Among Human sH3N2 Viruses and 2012–2013 H3N2v Viruses

Strain	Amino Acid Residues in Antigenic Site A[a]														Glycosylation Sites, No.[b]	Accession No.[c]
	122	124	126	131	133	135	137	138	140	142	143	144	145	146		
sH3N2 viruses																
SH/87	N	D	N[d]	T	S	G	Y	A	K	G	S	V	N	S	1	AF008886
BJ/92	…	…	…	A	D	…	…	…	…	…	…	…	…	…	1	CY113677
WH/95	…	G	…	A	D	T	…	…	…	…	…	…	K	…	1	EU501120
SY/97	N[d]	S	…	A	N[d]	T	…	…	…	S	…	I	K	…	3	CY112885
BR/07	N[d]	S	…	…	N[d]	T	S	…	I	R	…	N[d]	…	…	4	EU199366
H3N2v viruses																
OH/13/12	Q	S	…	A	D	Y	…	…	R	…	…	…	…	…	1	EPI381857
IN/6/13	Q	S	…	A	D	Y	…	…	R	…	…	…	K	…	1	EPI461874
IN/17/13	Q	S	…	A	D	Y	…	…	R	…	…	…	R	…	1	EPI462212

Abbreviations: H3N2v, influenza A(H3N2) variant; IN/6/13, A/Indiana/06/2013; IN/17/13, A/Indiana/17/2013; OH/13/12, A/Ohio/13/2012; sH3N2, human seasonal influenza A(H3N2).

[a]The aa sequence in antigenic site A; ellipses denote sequence identical to that for SH/87 virus.

[b]Numbers of potential glycosylation sites in antigenic site A.

[c]Hemagglutinin sequences were accessed from the National Center for Biotechnology Information or the GISAID database.

[d]Potential glycosylation sites (N-X-S/T).