PREVALENCE OF ANTIBODIES AGAINST SEASONAL INFLUENZA A AND B VIRUSES IN CHILDREN IN THE NETHERLANDS

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Running title: Seroprevalence of antibodies to influenza in children

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To gain insight in the age at which children become infected with influenza viruses for the first time, we analyzed the seroprevalence of antibodies against influenza viruses in children 0-7 years of age in The Netherlands. Serum samples were collected during a cross-sectional population-based study in 2006 and 2007 and were tested for the presence of antibodies against influenza A/H1N1, A/H3N2 and B viruses representative for previous influenza seasons using the hemagglutination inhibition assay. The seroprevalence of antibodies to influenza was higher in children 1-6 months of age than that of children 7-12 months of age, which likely reflects the presence of maternally derived antibodies. The proportion of study subjects >1 year of age with detectable antibodies against influenza viruses gradually increased with age until they reached the age of six when they all had antibodies to at least one influenza A virus. These findings may have implications for the development of vaccination strategies aiming at the protection of young children against seasonal and/or pandemic influenza virus infection.
Infection with influenza viruses is an important cause of illness in children with estimated annual attack rates in this age group ranging between 20 and 30% during epidemics (9, 11). Especially young children with underlying disease are at risk for severe disease after infection with an influenza virus, but it has been demonstrated that also the hospitalization rates attributable to influenza virus infection of young children without underlying disease are similar to those observed among older adults (18, 23). Furthermore, the importance of influenza as a cause of severe disease was demonstrated during the 2003-2004 influenza season when a newly emerged drift variant caused an unusual high number of severe fatal cases of influenza amongst children (19). In addition, the pandemic caused by the influenza A/H1N1(2009) virus has highlighted the importance of influenza viruses as a cause of morbidity and mortality in infants (2, 12).

Furthermore, since children have a high number of contacts relative to other age groups, and have a tendency to make contacts within their own age group, they may have the highest incidence of infection after the introduction of a newly emerging virus (22). In addition, they also may shed virus for a prolonged period of time and have higher virus loads in the nasopharynx (10, 14). Therefore, children most probably play an important role in the transmission of virus and are considered efficient vectors for spreading the disease.

To prevent morbidity and mortality of children due to infection with influenza viruses, a number of countries, including the USA, have recommended vaccinating all healthy children 6-59 months of age against influenza (8, 15). In various studies, it has been demonstrated that annual vaccination against seasonal influenza is beneficial for children and reduces the transmission of virus (21, 27, 33, 35, 37, 43). However, the impact of vaccination will be influenced by the immune status of the vaccinated individuals. Since they will be more at risk to become infected and develop disease, naïve subjects most likely will benefit from vaccination more than children that already have experienced an infection with one or more influenza viruses. In addition, it can be anticipated that with increasing age the chance of having experienced an influenza virus infection also increases. However, at present it is not fully clear at which age children become infected for the first time and develop influenza virus specific immunity and detailed sero-epidemiological studies of this age group are largely lacking (36, 42). Here we report the seroprevalence of antibodies against influenza A/H1N1, A/H3N2 and B viruses in children from one month to seven years of age in The Netherlands. To this end, serum samples were used that were collected during a cross-sectional population-based study designed to represent the population of The Netherlands (40). These serum samples were tested for the presence of antibodies against representative influenza A/H1N1, A/H3N2 and B viruses from multiple influenza seasons using the hemagglutination inhibition (HI) assay, which is the gold standard for the demonstration of antibodies against influenza viruses (3). In addition, we were able to discriminate between antibodies against various antigenically distinct influenza A/H1N1 and influenza A/H3N2 viruses and antibodies to influenza B viruses from B/Victoria/2/87 and B/Yamagata/16/88 lineages. In children >1 year of age, there was a gradual, age-related increase in the seroprevalence of antibodies against all influenza viruses until in all children >6 years of age antibodies against at least one influenza virus were detected. Results obtained in this study give more insight in the rate of infection of children with influenza viruses during non-pandemic seasons and may aid policy making regarding the implementation of vaccination strategies in this vulnerable age group.
MATERIALS AND METHODS

Collection of serum samples
Serum samples were collected during a nationwide cross-sectional population-based study which was performed in The Netherlands from February 2006 to June 2007 (PIENTER 2 Study) to evaluate the Dutch national immunization program (40). For this purpose, serum samples were collected from in total 6386 individuals (aged 0-79 years, men and women). For our study, 720 serum samples obtained from children 0-7 years of age were used. Fifty-six samples were obtained from children 1-6 months of age and 98 serum samples were obtained from children 7-12 months of age. The number of samples obtained from children that were one, two, three, four, five, six and seven years of age was 57, 80, 93, 91, 72, 75 and 97, respectively.

Selection of representative influenza viruses
Representative influenza A/H3, A/H1 and B viruses were selected that circulated in The Netherlands in seasons 1999/2000 to 2006/2007 based on data collected by the National Influenza Center for the World Health Organization (WHO) in the Netherlands (4-7, 29-32). For most seasons, vaccine strains were used, but when epidemiological strains could be identified that gave higher antibody titers with reference ferret serum these were included as well (Table 1). Furthermore, influenza B viruses of both B/Victoria/2/87-like and B/Yamagata/16/88-like lineages (Victoria- and Yamagata-lineages) were used for each year, although in some seasons only influenza B viruses belonging to one lineage were detected in clinical specimens in The Netherlands. In addition, data collected by the Dutch National surveillance program was used to assess the severity of the influenza seasons and to evaluate relative dominance of each of the influenza virus types and subtypes.

Before use in the HI assay, vaccine strains were inoculated in the allantoic cavity of 11-days old embryonated chicken eggs while epidemiological strains were propagated in confluent Madin-Darby Canine Kidney (MDCK) cells. Allantoic fluid was harvested after two days and culture supernatant was harvested after cytopathologic changes were complete and both were cleared by low speed centrifugation. Sera of children between one month and 12 months of age were tested for the presence of antibodies against all influenza viruses representative for the six preceding influenza seasons to analyze the presence of maternal antibodies, while serum samples collected from children older than one year were tested for the presence of antibodies against all influenza viruses of seasons that they might have been exposed to according to their age (Table 1).

Serological testing
Serum samples were tested for the presence of antibodies against the hemagglutinin of the respective influenza viruses by HI assay as described previously (25). In brief, serum samples were treated with cholera filtrate and heat inactivated at 56°C for one hour. Duplicate two-fold serial dilutions of pre-treated serum samples were subsequently incubated with 4 HA units of an influenza virus or phosphate buffered saline (PBS) for 30 minutes at 37°C and subsequently 1% turkey erythrocytes was added. Hemagglutination patterns were read after incubation for one hour at 4°C. The highest dilution of serum that still gave complete inhibition of the hemagglutination was recorded as titer and when duplo results were different, geometric mean titers were calculated. Serum samples were considered negative when they failed completely to inhibit agglutination of erythrocytes (antibody titer < 10) by any of the selected viruses. Serum samples of ferrets collected before and after infection with each of the respective influenza viruses were used as negative and positive control in the HI assay.

Statistical analysis
Pearson’s correlation coefficient was used to calculate correlations between antibody titers detected against multiple variants of influenza A/H3N2, A/H1N1 and B viruses. Furthermore, assuming binominal distribution, the
two-sided exact 95% confidence interval (CI) was calculated for seroprevalences of antibodies against influenza A/H3N2, A/H1N1 and B viruses using Stata/SE software version 11.0. Statistical analysis of differences between children one to six months of age and six to 12 months was performed using the chi-square test. The Cochrane-Armitage Trend test was performed to evaluate the presence of an age-related trend in the presence of antibodies against influenza viruses using SAS software version 9.2.
RESULTS

Influenza epidemics from 1999 to 2007 in The Netherlands

Using epidemiological and virological data, we were able to assess the relative severity of the influenza epidemics in the Netherlands from 1999 to 2007 and the causative viruses. During most seasons, influenza viruses caused moderate epidemics, except for the 2004/2005 season that was relatively severe and caused by influenza A/H3N2 viruses predominantly and the 2000/2001 season that was relatively mild and caused by influenza A/H1N1 viruses. Furthermore, most seasons were dominated by influenza A/H3N2 viruses, while during the 2002/2003 and 2005/2006 influenza seasons, both influenza A/H3N2 and B viruses were co-dominant. During most seasons, the majority of isolated influenza B viruses belonged to the Yamagata-lineage. However, during the 2002/2003 influenza seasons, in which influenza B viruses were co-dominant, only viruses from the Victoria-lineage were isolated in The Netherlands. During most epidemics from 1999 to 2007, influenza A/H1N1 viruses caused only low influenza activity, except during the 2000/2001 season (Table 1).

Age-dependent seroprevalence of antibodies against individual influenza virus strains

First, the prevalence of antibodies directed against individual influenza virus strains was assessed using serum samples of children one to seven years of age. Strains were used against which based on their age at the time point of sampling, the study subjects potentially could have developed an antibody response. As shown in figure 1A, an age-dependent increase in the proportion of subjects with antibodies to selected A/H3N2 strains was observed. The highest prevalence of antibodies to a single strain was observed against influenza viruses A/NL/118/01 and A/Wyoming/3/03 (100%) in subjects of seven years old.

A similar pattern was observed for the prevalence of antibodies to individual influenza A viruses of the H1N1 subtype, although the overall seroprevalence was lower (Figure 1B). The highest seroprevalence to individual strains was observed to influenza viruses A/NL/128/04 (77%) in subjects seven years of age, which was similar to that against most other H1N1 strains.

The seroprevalence of antibodies to individual influenza B virus strains displayed a different pattern and was largely depending on the lineage of the influenza B virus that was used. In general, higher seroprevalences of antibodies against influenza B viruses of the Yamagata-lineage were detected than those to viruses of the B/Victoria lineage (B/Malaysia/2506/04 and B/Shangdon/7/97) (Figure 1C).

Seroprevalence during first year of life

Serum samples of children one to twelve months of age were not only tested for the presence of antibodies to influenza viruses from the 2006/07 season, but also for those specific for older strains since it was anticipated that these sera also might contain maternally derived antibodies.

In 15% (CI 6-27%) of the children between one and six months of age, antibodies were detected against at least one of the influenza A/H1N1 viruses tested, while only 4% (CI 1-10%) of children between 7 and 12 months of age had antibodies against A/H1N1 viruses (Figure 2A and 2C). In 43% (CI 30-57%) and 36% (CI 23-50%) of the children one to six months of age, antibodies were detected against at least one influenza A/H3N2 or B virus respectively. In the serum samples obtained from children 7-12 months of age the proportion of subjects with antibodies to these viruses was 19% (CI 12-28%) and 5% (CI 2-12%), respectively (Figure 2A). The significant differences in the prevalence of antibodies to A/H3N2 and B viruses between the two age groups could be largely attributed to a difference in the proportion of serum samples containing antibodies to strains from previous influenza seasons like A/Wyoming/3/03, A/Panama/07/99, A/Sydney/5/97 (all A/H3N2) and B/Yamanashi/429/99 (Figures 2B and 2D). This indicates that the relatively high seroprevalence of antibodies in children 1-6 months of age indeed can be attributed to maternally derived antibodies.
Age-dependent seroprevalence of antibodies to any influenza A or B virus

The seroprevalence to individual influenza virus strains was used to calculate the proportion of subjects with antibodies to at least one influenza A or B virus. Within the influenza A viruses the relative contribution of antibodies to influenza A/H3N2 and A/H1N1 viruses was discriminated and within the influenza B viruses, those to the Yamagata and Victoria-lineage.

As shown in figure 3, the seroprevalence of antibodies to influenza A viruses declined after 6 months of age. Thereafter, with increasing age the proportion of subjects with antibodies to influenza A viruses increased steadily. At the age of six virtually all subjects (99%; CI 93-100) had developed antibodies to an influenza A virus. For subjects >2 years of age, the proportion with antibodies to influenza A/H3N2 viruses was significantly higher than those with antibodies to A/H1N1 viruses.

A similar pattern was observed for the development of antibodies to influenza B viruses. After six months the proportion of subjects with antibodies to an influenza B virus dropped to 5% (CI 2-12%). With increasing age a gradual incline was observed of the proportion of children with antibodies to influenza B virus. At age seven, 72% (CI 61-80%) of the subjects had developed antibodies to at least one influenza B virus. The seroprevalence of antibodies to influenza B viruses of the Yamagata lineage was higher that of the Victoria lineage. Using the Cochrane-Armitage trend test, the presence of a significant age-related trend in the increase of seroprevalence of antibodies to influenza A/H1N1, A/H3N2 and B viruses was demonstrated (p<0.01).

Estimated attack rates

The differences in seroprevalence of antibodies to the respective influenza viruses at various ages were used to estimate attack rates. The proportion of children with antibodies to influenza A/H3N2 viruses, only increased 1.5% between children 7-12 months of age and one year of age. The highest increase in the seroprevalence of antibodies against influenza A/H3N2 viruses was observed at age two and three. At these ages, the proportion of subjects with antibodies increased with 25% each year. The highest increase in seroprevalence of antibodies to influenza A/H1N1 viruses were observed at age three (18%) and at age six (26%). During the first year of life, only a minority of the subjects acquired antibodies to influenza B viruses (5%). The highest increase in the seroprevalence in antibodies against influenza B viruses was observed at age three (20%) and five (19%). These increases could be attributed largely to the development of antibodies directed to influenza B viruses of the Yamagata lineage. In general, the increase in the seroprevalence of antibodies against viruses from the Victoria-lineage was modest, with the exception of a 14% increase observed in subjects five years of age.

Correlation between antibody titers against multiple influenza virus strains

As serum samples were tested for antibodies against various influenza viruses, we determined the correlation between antibody titers against different strains within a (sub)type (Figure 4). In general, antibody titers to various influenza A/H1N1 viruses correlated well (R=0.8), and also those against strains of each of the lineages of influenza B viruses (R>0.8). In contrast, antibody titers against viruses from the two different influenza B lineages correlated poorly (R<0.1), although in some samples antibodies against viruses from both lineages were detected. The correlation of antibody titers against different influenza A/H3N2 viruses was dependent on the year of isolation and most likely on the antigenic match between the two strains that were studied. For example a good correlation was observed between titers against A/New York/55/04 and A/Hiroshima/52/05 and between A/Panama/2007/00 and A/Wyoming/3/03, whereas titers between A/Wyoming/3/03 or A/Panama/2007/99 and A/Hiroshima/52/05 correlated poorly. Figure 4 shows an example of the correlations between antibody titers that were observed with the serum samples obtained from children four years of age.
DISCUSSION

In the present study, the seroprevalence of antibodies against influenza viruses in The Netherlands was investigated in children. Sera were collected from February 2006 to June 2007 in a cross-sectional population-based study and were tested for the presence of antibodies against influenza virus strains representative for viruses that circulated during the life span of the children tested. Since the persistence of maternally derived antibodies is short-lived and probably less than six months (17), sera of children <12 months of age were also tested for antibodies against older influenza viruses that may have infected their mothers.

Indeed the seroprevalence of antibodies to influenza viruses was relatively high in children between one and six months of age which could be attributed to the presence of maternally derived antibodies to older influenza virus strains. The seroprevalence was lower in children between six months and one year of age, but showed an age-dependent increase until the age of seven, when all of the children had developed antibodies to at least one influenza A virus and 72% antibodies to at least one influenza B virus. The increase in the seroprevalence was not caused by differences in the geometric mean titer (GMT) against influenza viruses, since GMTs against the respective strains were independent of age. Also high antibody titers were observed in serum samples collected from some children 7-12 months of age, reflecting recent infections with the corresponding viruses.

In children of all ages, the seroprevalence of antibodies to influenza A/H3N2 viruses was higher than the seroprevalence of antibodies against influenza A/H1N1 or B viruses. This is in accordance with epidemiological data from the Netherlands collected between 1999 and 2007. During influenza seasons in this period, influenza A/H3N2 viruses were detected predominantly in clinical specimens compared to influenza A/H1N1 and influenza B viruses. In addition, we observed a relative strong increase in the seroprevalence of antibodies against influenza A/H1N1 viruses in children six years of age compared to children of other ages, which could be attributed to the dominant circulation of influenza A viruses of this subtype during the 2000/2001 influenza season. Furthermore, the presence of antibodies to influenza B viruses of the B/Yamagata lineage and the B/Victoria lineage could be discriminated. These two lineages are antigenically distinct and cross-react poorly (20, 34). In addition in young children that most likely had been infected with only one influenza B virus only antibodies were detected against influenza B viruses of a single lineage. In older children antibodies were detected against influenza B viruses of both lineages, with is in accordance with the possibility that these children have been infected subsequently with both viruses during their life. Overall, the sero-prevalence of antibodies to influenza B viruses of the B/Yamagata-lineage is higher than those specific for viruses of B/Victoria-lineage. This correlates with epidemiological data which indicate that in five out of eight seasons under investigation only viruses from the B/Yamagata-lineage were isolated and in two other seasons viruses of both lineages were co-dominant.

Assuming that children that were infected with influenza viruses also developed antibodies against the corresponding virus, we calculated the estimated attack rates based on the sero-conversion rates at the respective ages. Influenza A/H3N2 viruses had the highest attack rates in children that were between two and four years old. However, it can not be excluded that the attack rate of older children was underestimated, since subsequent infection with viruses of the same subtype may have remained undetected due to the presence of antibodies induced by previous infections. The estimated attack rates based on the sero-conversion rates are comparable with the attack rates during inter-pandemic influenza seasons reported by others (9, 11). Strikingly, in children <2 years of age, the attack rates were relatively low compared to older children. Since the length and severity of the influenza seasons between 2004 and 2006 was not different from most other seasons and antibody titers in seropositive subjects was not age dependent, differences in exposure to influenza viruses may explain the observed differences in attack rates. To account for potential confounding differences in the length and severity of flu seasons experienced between each age group, birth and sample collection dates were used to calculate the duration of flu season time each subject would have experienced. Further, these values were...
weighted using influenza-like illness data (24) as a measure of epidemic severity during each weekly period.

When these values were used to control for differences in circulating flu conditions throughout the lives of the subjects forming each year group, a similar pattern of increases in seroprevalence was still encountered (Figure 5). Of note, when instead of HI titer $\geq 10$ a threshold of HI $\geq 40$ was used for seropositivity, essentially the same results were obtained since infection-induced antibody titers were generally higher than 40.

In addition, vaccination against seasonal influenza is currently only recommended in The Netherlands for children that are at high risk for developing complications after infection with influenza due to underlying disease, and therefore is considered a minor confounding factor in the present study.

Our results regarding the relatively high seroprevalence in infants <7 months of age coincide with those reported for newborns (13, 41) and it is likely that transplacentally acquired maternal antibodies can protect young infants to a certain extent (26, 28). The high seroprevalence in children <7 months of age is explained by the presence of antibodies to older influenza viruses to which their mothers may have been exposed. In addition, since vaccination against influenza is not recommended for (pregnant) mothers in The Netherlands, the proportion of vaccinated mothers is most likely very low. Indeed the titers to these older strains decline rapidly and are not detectable in children 7-12 months of age. The presence and duration of maternal antibodies against influenza has been demonstrated previously (38-39, 41). It is unlikely that the children <7 months of age had experienced an infection with influenza viruses since the day of birth and day sample collection were in between two influenza seasons for 20 of these children, including 14 with antibodies to various older influenza virus strains. In addition, two children >7 months were sero-negative and may not have been exposed to influenza viruses for the same reason. The presence of maternal antibodies against various influenza A and B viruses in infants <7 months of age seems in paradox with the high hospitalization rate in this age group (23). However, in a substantial proportion of these infants (30%) antibodies against any influenza virus were not detectable which may constitute the subjects highly susceptible to infection with influenza virus.

As expected, the antibody titers against antigenically related influenza A and B viruses correlated well. In contrast, antibodies to influenza B viruses of the B/Yamagata and B/Victoria lineages did not cross-react. Furthermore, a poor correlation was observed when antibody titers against antigenically distinct A/H3N2 viruses were compared. Apparently, there is heterogeneity in the antibody repertoire of various subjects, which dictates the level of cross reactivity with different influenza viruses.

Collectively, in this study we determined the seroprevalence of antibodies against various influenza viruses in children from 0-7 years of age during non-pandemic influenza seasons. We demonstrated that at seven years of age, all children developed antibodies against at least one of the influenza viruses tested. Furthermore, the highest attack rates calculated based on the seroprevalence of antibodies to influenza A viruses was observed in children two and three years of age. These data provide information on the age at which children experience their first infections with influenza viruses and develop immunity to these viruses. This type of information may aid decision making for the implementation of vaccination strategies that aim at achieving optimal protective immunity against seasonal and pandemic influenza. Ideally, in infants vaccines are used that not only induce antibodies to seasonal influenza viruses but also immunity to influenza A viruses of other subtypes (1, 16).

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Table 1. Influenza virus epidemics in the Netherlands during influenza seasons from 1999-2007

<table>
<thead>
<tr>
<th>Season</th>
<th>Selected influenza viruses</th>
<th>Dominance of (sub)type(a)</th>
<th>Dominance B-lineage</th>
<th>Age at which may be exposed</th>
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<tr>
<td></td>
<td>A/H3N2</td>
<td>A/H1N1</td>
<td>B</td>
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<td>LA</td>
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<td>0-8</td>
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<td>LA</td>
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<tr>
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\(a\) D = dominant, CD= co-dominant, LA = Low activity, NI = no viruses isolated of this subtype.
LEGENDS

Fig 1. Seroprevalence of antibodies against individual influenza viruses. Serum samples of children 0-7 years of age were tested for the presence of antibodies against representative influenza A/H3N2 (A), A/H1N1 (B) and B (C) virus strains. For each age group, representative influenza viruses were selected to which they may have been exposed according to their age. Indicated are the strains that have been used to evaluate the presence of antibodies in the serum samples and the percentage of serum samples in which antibodies were detected against each influenza virus antigen. Shaded area: not tested.

Fig 2. Seroprevalence of antibodies against influenza viruses in children 1-12 months of age. Seroprevalences of antibodies against influenza A/H3N2, A/H1N1 and B viruses of the 2000-2007 influenza season in children one to six months of age (grey bars) and seven to 12 months of age (white bars) (A). Serum samples were tested for the presence of antibodies against multiple antigens as is indicated for influenza A/H3N2 (B), influenza A/H1N1 (C) and B (D) viruses. Bars indicate the percentage of the serum samples in which antibodies were detected and error bars indicate the 95% confidence intervals.

Fig 3. Seroprevalence of antibodies against influenza A and B viruses depends on age. Percentages of serum samples of children in which antibodies were detected against at least one of the representative influenza viruses were calculated for influenza A/H1N1 (light grey bars), influenza A/H3N2 (white bars) and all influenza A viruses (dark grey bars) (A). The same procedures was used to calculate the seroprevalence of antibodies against at least one of the influenza B viruses from the Victoria-lineage (light grey bars), the Yamagata-lineage (white bars) and all influenza B viruses (dark grey bars (B). Bars indicate the percentage of the serum samples in which antibodies were detected and error bars indicate the 95% confidence intervals.

Fig 4. Correlation of antibody titers against individual influenza A virus strains in four year old children. Correlation between the antibody titers against multiple representative influenza A/H3N2 viruses, influenza A/H1N1 viruses and influenza B viruses. Dots indicate individual serum samples and Pearson correlation coefficient was calculated for all datapoints for which antibodies against at least one influenza virus was detected. For influenza B viruses, the letter behind the name of each strain indicates the lineage to which the virus belongs (V= Victoria-lineage, Y= Yamagata-lineage).

Fig 5. The difference in proportion of seropositive individuals for each age group compared to the previous age group. Unadjusted (light grey) and adjusted (dark grey) proportions controlled for estimated differences in the severity of flu incidence throughout the life of individuals in each group. For the adjustment, firstly the mean total weighted season time experienced by the individuals of each age group was calculated using information about date of birth, date of sample collection and relevant influenza-like illness data. Next, the differences in this mean for each age group compared to the previous age group were calculated, alongside an overall mean difference between age groups. Finally, the adjustments were made by scaling the value for each age group by the factor by which it differed from the overall mean for the dataset, to account for age groups that had lived through a time of abnormally high or low flu incidence. For age 0, only individuals greater than 220 days old were included to reduce the chance of detecting potential maternal immunity rather than genuine exposure and values for age 0 were plotted assuming a previous seroprevalence of 0%. Error bars indicate the 95% confidence intervals.
REFERENCES


Graph A and B illustrate seroprevalence for H1N1, H3N2, HxN1, and HxN2 subtypes across different strains:

- **A**: Comparison of seroprevalence for H1N1 and H3N2 across A/HongKong/1/68, A/New York/55/68, A/Japan/305/57, and A/Norway/1/09 strains.
- **B**: Comparison of seroprevalence for H1N1, H3N2, HxN1, and HxN2 across A/New York/55/68, A/Wyoming/2103, A/Panama/10/99, and A/Sydney/597/97 strains.

Graph C and D show seroprevalence for A/NewCaledonia/2009, A/Scotland/Ireland/2006, B/Malaysia/266/04, B/Norway/1007, B/Leamington/1007, B/Guangdong/120/06, and B/Yamagata/4/09 strains:
