Risk factors and attack rates of seasonal influenza infection: results of the SHIVERS seroepidemiologic cohort study

A brief, summary of this article's main point:

New Zealand's sero-epidemiological cohort study found Neuraminidase-inhibition assay identified more influenza virus infections than hemagglutination-inhibition assay. This result highlights the importance to measure serologically defined infections against not just haemagglutinin but also neuraminidase antigens in future sero-epidemiologic cohort studies.

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Abstract

Background: Understanding the attack rate of influenza infection and the proportion who become ill by risk group is key to implementing prevention measures. While population-based studies of anti-haemagglutinin antibody responses have been described previously, studies examining both anti-haemagglutinin and anti-neuraminidase antibodies are lacking.

Methods: In 2015, we conducted a sero-epidemiologic cohort study of individuals randomly selected from a population in New Zealand. We tested paired sera for haemagglutinin-inhibition (HAI) or neuraminidase-inhibition (NAI) titres for seroconversion . We followed participants weekly and performed influenza PCR for those reporting influenza-like illness (ILI).

Results: Influenza infection (either HAI or NAI seroconversion) was found in 321 (35%; 95%CI:32-38%) of 911 unvaccinated participants, of which 100 (31%) seroconverted to NAI alone. Young children and Pacific peoples experienced the highest influenza infection attack rates, but overall only a quarter of all infected reported influenza-PCR-confirmed ILI and one-quarter of these sought medical attention. Seroconversion to NAI alone was higher among children aged <5 years vs. those aged ≥5 years (14% vs 4%; p<0.001) and among those with influenza B vs A(H3N2) virus infections (7% vs 0.3%; p<0.001).

Conclusions: Measurement of anti-neuraminidase antibodies in addition to anti- hemagglutinin antibodies may be important in capturing the true influenza infection rates.

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Keywords: influenza, infection attack rate, influenza-like illness, sero-epidemiologic cohort, haemagglutinin-inhibition antibody, neuraminidase-inhibition antibody

Introduction:

Influenza infection results in various outcomes from asymptomatic infection to severe respiratory disease, cardiovascular complications, and death [1]. Few data exist on the seasonal influenza infection attack rates across age and other risk groups and the proportion of infections that progress to mild or severe illness. This lack of understanding hampers the ability to assess severity and burden in different target groups which guide our prevention and control measures such as vaccination, antiviral prophylaxis and non-pharmaceutical interventions. It also poses challenges to assess the relative severity of novel influenza strains and predict their behaviours with modelling.

Sero-epidemiologic studies can estimate the true age-specific incidence of influenza infection because asymptomatic infections are detected. Cross-sectional population serosurveys use differences in seropositivity proportions of antibodies before and after the influenza season to determine the attack rate [2, 3]. This approach may lead to misclassification of some infections due to inability to link pre- and post-season antibody titres to a specific individual, and low specificity resulting from cross-reactive antibodies, especially in seasonal influenza infections [4]. Cohort studies provide more reliable and precise estimates of incidence rates and risk profiles of infection by using paired pre- and post-season serum samples from the same individual. Household sero-epidemiologic cohort studies for seasonal influenza [5-8] are useful for estimating the secondary attack rates and effects of intervention within households including children, but do not fully represent the entire community [9, 10].

The gold standard for serologic detection of recent influenza infections is to demonstrate seroconversion, a fourfold or greater increase in antibody titre relative to a baseline sample, to the circulating influenza suspected of causing the infection [4]. Antibody against haemagglutinin, which generally neutralizes viral infectivity, has been widely used in sero-epidemiologic studies to define influenza infection through seroconversion. However, some influenza infected individuals may not seroconvert to haemagglutinin, leading to underestimation of true infection rates [11-13]. Antibody against neuraminidase, while inducing 'infection permissive' immunity by attenuating infection, limits viral spread within the host and ameliorates the clinical course of infection and disease [14, 15]. Although anti-neuraminidase seroconversion has been used in human challenge studies [13, 16], vaccine efficacy trials [17, 18] and investigations of naturally acquired infections [19, 20], it has not been applied to measure infection attack rates in population-based sero-epidemiologic cohort studies. Estimation of influenza infection through anti-haemagglutinin and anti-neuraminidase seroconversion may capture additional infections missed when measuring anti-haemagglutinin seroconversion alone. Accurate estimate of the number of influenza infections is critically important in the calculation of case fatality or case hospitalization rates. This can result in more precise estimation of the burden and severity of seasonal influenza and help prediction of pandemic influenza transmission and optimize influenza countermeasures. Comparative patterns of anti-haemagglutinin and anti-neuraminidase responses are essential to improve understanding of host immune responses, correlates of protection, and to optimize serologic diagnoses.

We describe here a sero-epidemiologic cohort study conducted in a Southern Hemisphere country, New Zealand, through the SHIVERS (Southern Hemisphere Influenza and Vaccine Effectiveness Research and Surveillance) project. It aimed to estimate attack rates (a measure of cumulative incidence of infection over the season) of both influenza infection (symptomatic or not) and influenza-PCR-confirmed ILI across risk groups in our unvaccinated cohort and the proportion of those that sought medical care.

Methods

Full methods are described in supplementary materials. The study was approved by the Northern A Health and Disability Ethics Committee (NTX/11/11/102 AM13). We obtained written informed consent from all participants (or guardians of children).

Sampling frame and sample size

We included all 88,011 persons enrolled in 14 selected Auckland general practices (GPs) as our source population [21]. We stratified the population by age (0-4, 5-19, 20-64, ≥65 years) and ethnicity (Maori, Pacific, Asian, European and others). Within each stratum, we performed simple random sampling to select a sample to represent different ages and ethnicities but purposely over-sampled young children and Maori and Pacific ethnicities to get more robust rates in these hard-to-reach groups. We estimated a minimum sample size of 800 for an assumed 26% risk of infection and two-sided 95% confidence intervals of +/- 12%, 30% risk of ILI (+/- 11%), 7% risk of influenza-PCR-confirmed ILI (+/- 29%).

Data collection

During February-April 2015, we contacted the selected individuals by letter with follow-up phone calls. If individuals declined or could not be reached after 6 attempts, they were replaced by another randomly selected person from the same stratum. We administered phone questionnaires to participants before and after the study period for information on influenza vaccination, occurrence of respiratory illness during study period and risk factors. We collected paired sera from all participants: pre-season during March to mid-June and post-season during mid-October to December.

During May-September 2015, we conducted surveillance for cases of ILI (defined as *'an acute respiratory illness with a history of fever or measured temperature of ≥38°C, and cough, and onset within the past 7 days'*). We texted/emailed participants weekly whether or not they had ILI. Participants could also report ILI by phones. Subsequently, nurses contacted those with reported ILI to verify their ILI status and arranged to collect a nasopharyngeal or throat swab within 14 days of reported onset.

Study data were captured using REDCap electronic data capture tools [22].

Laboratory methods

We tested respiratory samples for influenza RNA by real-time reverse transcriptase (rRT) PCR assay [23]. We used standard haemagglutination inhibition (HAI) and neuraminidase inhibition (NAI) assays [2, 24] to assess anti-haemagglutinin and anti-neuraminidase antibody titres (Supplement detailed influenza strains used in the assays).

Outcomes

We defined 'Seroconversion' as a fourfold or greater rise in HAI or NAI antibody titres in paired sera with the second HAI titre ≥40. Titres of 10 were assigned a value of 5 for computational purposes.

Influenza infection was defined as HAI or NAI seroconversion or influenza-PCR-confirmed ILI.

Illnesses were classified as 'ILI' when nurses confirmed participant responses of "Yes" to the weekly question "*have you had cough and fever in the previous 7 days.*" Illnesses among those who

responded “Yes” but the nurses upon examination determined them as not meeting the above criteria were classified as ‘mild illness not-ILI’. Those who responded “No” to the weekly question were classified as ‘not ILI’. ‘Mild illness not-ILI’ and ‘not-ILI’ were combined as ‘no ILI’.

Statistical analysis

Analyses were performed in Stata 14.1 (StataCorp LLC). Participants who had received the 2015 southern hemisphere influenza vaccine were excluded from the analysis because serology assays cannot distinguish whether antibody titre rises are due to vaccinations or naturally acquired infections.

The observed attack rates of ILI and influenza-PCR-confirmed ILI were corrected each week to account for: a) missed weekly reports by applying the percent of reports that were classified as ILI to those non-responders (Corrected number of ILI events = Total possible number of responses x Actual number of ILI/Actual number of responses); b) missed swabs from ILI cases by applying the influenza positivity rate of those tested to those non-tested (Corrected number of influenza-PCR-confirmed ILI events = Corrected number of ILI x Actual number of influenza-PCR-confirmed ILI/Actual number of ILI swabs). The above adjustments for the missed weekly reports and missed ILI swabs were applied on a week-by-week basis for each stratum (age and ethnicity) to account for differences over the season . Then we aggregated the relevant events for the season (22 weeks). To estimate the number of persons who experienced at least one ILI or influenza-PCR-confirmed ILI event during the season, we divided the number of the relevant events by the average number of the events per person: Corrected number of persons with ILI or influenza-PCR-confirmed ILI = Corrected ILI or influenza-PCR-confirmed ILI events ÷ (Actual number of ILI or influenza-PCR-confirmed ILI events/Actual number of persons with ILI or influenza-PCR-confirmed ILI events).

Two independent methods were employed to calculate the percentage of infections as measured by seroconversion that led to influenza disease: 1) dividing the corrected number of persons with influenza-PCR-confirmed ILI by the total number of seroconverters; and 2) subtracting ILI rates in non-seroconverters from those in seroconverters in order to account for the fact that some ILI would be attributable to other non-influenza pathogens or factors unrelated to infections or some influenza-associated ILI cases would be negative by PCR due to late swabbing, sample type and sampling techniques.

The risks of influenza infection and of developing ILI once infected were examined for five potential risk factors (age, sex, ethnicity, social deprivation, underlying medical conditions). Univariate and multivariate analyses were performed using rate ratios from Poisson regression generalised linear models.

Finally, we weighted the attack rates in our study population by age and ethnicity strata to arrive at a total infection rate for the whole Auckland region; 95% confidence intervals for proportions were calculated using the binomial distribution, applying weighting where appropriate.

Results

In 2015, New Zealand's influenza surveillance data showed influenza A(H3N2) and B (mainly B/Victoria lineage) predominance [25].

Of 14,825 randomly selected persons, 1514 (10%) agreed to participate, supplied paired sera, and completed the study questionnaires (supplement Figure S1). Of these participants, 603 (40%) reported receipt of the 2015 influenza vaccine. This report focuses on the remaining 911 unvaccinated individuals with full information and paired sera. When the unvaccinated study

population was compared to the central and south-eastern Auckland population, children aged <5 years were over-represented and older adults (≥65 years) were under-represented (supplement Table S1).

Overall attack rate of influenza infection

Of all 911 unvaccinated participants, 321 (35%) seroconverted to either HAI or NAI (Figure 1). Of these, 175 (55%) seroconverted to both HAI and NAI, 46 (14%) to HAI only, and 100 (31%) to NAI only. Of 590 non-seroconverters, no influenza-PCR positives were detected.

The risk of influenza infection was significantly higher in children aged 0-19 years than other age groups (46% vs 26%; p<0.001); among Pacific peoples compared to other ethnicities (46% vs 34%; p=0.014); and among A(H3N2) infected persons compared to influenza B infected (21% vs 18%; p=0.046) (Table 1).

The overall infection attack rate, when weighted to the age and ethnicity structure of the Auckland population, was 32% (95%CI:29-35) when influenza infection was defined as HAI or NAI seroconversion or influenza RNA detection; 24% (95%CI:21-27) when only influenza RNA detection or HAI seroconversion were included.

Influenza-like illness (ILI) and influenza-PCR-confirmed ILI

From 27-April to 27-September, participants responded to 17,121 of 20,042 (85%) potential person-weeks ILI reminders. Responses included 347 (2%, 347/17121) ILI events reported from 287 persons for an observed ILI attack rate of 32% (287/911). The corrected ILI attack rate during the surveillance period was 36% (95%CI:33-39). Children aged <5 years and Pacific peoples had

significantly higher ILI attack rates than other age groups (67% vs 30%, p<0.01) or other ethnicities (53% vs 34%, p<0.01) respectively (Table 1).

Of the 287 persons with ILI, swabs were collected from 209 (73%) with a median of 8.2 days after onset (range 2-15 days). Of these 209 persons, 50 (24%) were positive for influenza virus by PCR.

Influenza viruses were detected continuously from 27-June to 27-September with two distinct circulation patterns: A(H3N2) virus predominated during weeks 26-33, whereas influenza B virus (mainly B/Victoria lineage) predominated during weeks 34-39. No influenza A(H1N1)pdm09 virus was detected (Figure 2). The corrected number of influenza-PCR-confirmed ILI (adjusting for non-swabbing and non-reporting) was 76 for a corrected influenza-PCR-confirmed ILI attack rate of 8% (95%CI:7-10) among the entire unvaccinated cohort. The highest attack rate of influenza-PCR-confirmed ILI was in children aged 0-4 years (14%). Influenza-PCR-confirmed ILI attack rates decreased with increasing age (Table 1).

Of all 321 who seroconverted to HAI or NAI, an estimated 24% (76/321) experienced influenza-PCR-confirmed ILI. Using the alternative approach to estimate proportion of serological infections leading to ILI (subtracting rates of ILI in non-seroconverters from those in seroconverters), we found a corrected ILI proportion attributable to influenza of 30% [56% (180/321) in seroconverters minus 26% (151/590) in non-seroconverters]. Infections of A(H3N2) and influenza B virus had similar proportions of ILI, 19% (37/193) and 25% (39/159) respectively (Table 1).

We were concerned that we may have missed substantial numbers of symptomatic influenza illness from those who reported ILI but were subsequently not confirmed to meet the case definition

(termed ‘mild illness not-ILI’) because the fever may have abated by the time the nurse examined the case of ILI. However, we found proportion of seroconverters with ‘mild illness not-ILI’ was similar to those with ‘no ILI’ reported (28% vs 23% respectively; p=0.125), but much lower than those with ILI (28% vs 48% respectively; p<0.001) (Supplement Table S2).

Of the 50 influenza-PCR-confirmed ILI, 13 (26%) consulted GPs. The corrected number of influenza-PCR-confirmed ILI seeking consultation (adjusting for non-swabbing and non-reporting) was 15 for a rate of 1.6% (95%CI:0.9-2.7) among the entire unvaccinated cohort. None of influenza-PCR-confirmed ILI or ILI infections were hospitalized (Supplement Table S3).

HAI versus NAI seroconversion

Different patterns of HAI and NAI seroconversion rates were observed across risk groups. NAI only seroconversion was significantly higher in young children aged <5 years than in other age groups (14% vs 4%; p<0.001) and also in individuals infected with influenza B compared to those with A(H3N2) virus (7% vs 0.3%; p<0.001) (Figure 3 and supplement Table S4).

Risk factors

Multivariate analysis showed that age was an independent risk factor for influenza infection. Children aged <5 and 5-19 years had significantly higher risk of infection compared to adults aged 20-64 years (Table 2). Although the risk of infection was similar among children <5 and 5-19 years, the risk of influenza-PCR-confirmed ILI was significantly higher in children aged <5 years compared to adults aged 20-64 years. Pacific peoples also had significantly higher risk of influenza infections compared with Maori and European and other ethnic groups. The risk of influenza infection was 1.6 (95%CI:1.1-2.3) times higher in persons with at least one child aged 5-19 years in the household compared to persons living in households without children (data not shown).

Other variables, sex, social deprivation, underlying conditions, smoking, poor housing conditions and household size did not show any independent effect on infection and influenza-PCR-confirmed ILI.

Discussion

The SHIVERS sero-epidemiologic cohort study, to our knowledge, is the first report to quantify attack rates of influenza infection and disease using assays to measure seroconversion against both haemagglutinin and neuraminidase antigens in a sero-epidemiologic cohort for all age groups. A third of unvaccinated individuals in our cohort were infected with influenza of which one-quarter developed influenza-PCR-confirmed ILI and only a quarter of these sought medical attention. Seroconversion with anti-neuraminidase antibody alone constituted one-third of all seroconverted individuals, and was particularly frequent among children less than 5 years and influenza B virus infected individuals.

When influenza infection was weighted to the Auckland population assuming unvaccinated, we estimate that 32% of the Auckland population were infected with influenza virus during the study. When NAI antibody titres were measured, we identified an additional 31% of influenza infections associated solely with anti-neuraminidase antibody. Our study highlights the importance of measuring serologically defined infections against not just haemagglutinin but also neuraminidase antigens to understand the true epidemiology and immunology of influenza and better guide prevention and control measures. Anti-neuraminidase antibody's broad cross protection and long duration of immunity against the 1968 Hong Kong A(H3N2) pandemic was demonstrated in US-Tecumseh's sero-epidemiologic cohort study. Infections with the newly emerged influenza A/Hong

Kong/68 (H3N2) virus (against which the population had no specific H3 anti-haemagglutinin antibody) were significantly more frequent in subjects without anti-neuraminidase antibody than in those with antibody acquired by prior infection with the preceding influenza A(H2N2) viruses [20]. The importance of pre-existing NAI titres as predictors of immunity against the 2009 pandemic virus has also been demonstrated [19, 26] and such data are valuable for better modelling influenza transmission.

Using the criteria of HAI seroconversion or influenza RNA detection, we found an attack rate of 24%, similar to studies in the UK (19%)[9], Vietnam (17-26%) [10], and US-Tecumseh (25-34%) [27]. This consistency, despite varying study designs, laboratory methods, data availability and circulating viruses, suggests that annually 15-35% of the unvaccinated population is infected with seasonal influenza. Additionally, we found that seasonal influenza infections resulted in the highest infection attack rates in children and the risk decreased with increasing age. This pattern, also observed elsewhere from historical North America [7], contemporary Europe [9] and South East Asia [10] studies, may represent a generalizable finding globally. This probably reflects relative immunologic naivety of children and long-lived and cross-protective immunity against seasonal influenza virus [28]. Furthermore, we found that Pacific peoples had significantly higher infection attack rate than other ethnicities, similar to our 2009 pandemic serosurvey [2]. This may be due to higher transmission of respiratory viruses [29] (possibly among communities with household crowding), higher levels of comorbidities, delays in seeking treatment, lack of knowledge of preventing spread or genetic susceptibility. The proportion of Maori/Pacific populations may have lower herd immunity because of lower vaccination rates [30, 31].

Most influenza infections do not lead to illness. We found 24% of HAI seroconverters developed PCR-confirmed influenza disease, similar to the UK study (25%) [9]. Additionally, we found 19% of A(H3N2) and 25% of influenza B virus infections led to influenza disease, different from studies in

US-Tecumseh [27] with 15-25% of A(H3N2) and 19-34% of influenza B infections, and Viet Nam [10] with 11% of A(H3N2) and 15% of influenza B infections leading to clinical illness. These varying proportions between studies may reflect different case definitions, study designs, climate settings, degrees of antigenic drift, circulating strains, and propensity to report illness.

Most individuals with symptomatic influenza did not consult healthcare providers. Our finding that 26% of the influenza-PCR-confirmed ILI cases sought medical care was lower than studies in US-Michigan (32%) [32] and US-Tecumseh (38%) [8], but higher than the UK study (17%) [9]. These differences may reflect primary health care accessibility, health seeking behaviour and cultural practices within these countries. We found a higher rate (1.6%) of GP consultations in participants with influenza-PCR-confirmed ILI compared with that (0.6%) of the GPs registered population with influenza-PCR-confirmed ILI [25]. This difference may be due to propensity to consult GPs if ill in our study cohort.

The serologically confirmed symptomatic and asymptomatic influenza infections provide an accurate denominator of the total burden of influenza for assessing severity by calculating case fatality and case hospitalization ratios. Previous studies showed that asymptomatic fractions among total influenza infections ranged from 65-85% in sero-epidemiologic studies adjusted for background illnesses, a pooled mean of 16% (95%CI:13-19) in outbreak investigations, and 33% in volunteer challenge studies respectively [33] [34] [35]. For sero-epidemiologic studies, varying case definitions of asymptomatic infection have been used, including completely asymptomatic [27], or absence of acute respiratory illness [9], or absence of influenza-like illness (fever and cough) [10]. We showed that 70-76% of seroconverted individuals did not develop ILI; however, some of these individuals would have developed milder afebrile illness.

Limitations

Our study had several limitations: First, we only conducted the cohort study in 2015 rather than multiple influenza seasons. We were not able to study A(H1N1)pdm09 attack rates due to its low circulation. Second, we did not estimate true proportions of clinical disease among those infected since we did not elicit clinical information or test those not meeting ILI case definition. Some influenza cases with ILI may have been missed because of delays in reaching the case, but proportions of ILI due to influenza by subtracting rates of ILI in non-seroconverters from those in seroconverters produced similar results. Third, we had high weekly ILI responding rates (~85%). We assumed that ILI rates in not-always-responders is likely to be similar to that in every-week-responders. There did not appear to be any systematic bias by age or ethnicity when compared these among non-respondents and respondents. Even if there was any bias, it would not contribute significantly due to small proportions (15%) of non-respondents. Fourth, fourfold or greater increase of HAI or NAI titres was used to define seroconversion. While this is relevant for diagnosis of an individual case to account for inherent measurement errors, we may miss those who were truly infected but did not show fourfold rises [36]. Fifth, our sample had small numbers of participants aged <1 and ≥65 years, resulting in less precise estimates. Last, we only studied unvaccinated persons who may be different from vaccinated persons. Therefore, our extrapolation to all of Auckland only represents rates among unvaccinated people. The vaccination rate in Auckland is not recorded in registries so calculating attack rates among the total population is not possible.

Conclusions

Our study provides more precise measurement for influenza infection and illness attack rates across risk groups. A substantial fraction of seroconverted individuals only to neuraminidase antigen in an age- and virus-specific manner highlights the importance of measuring serologically defined infections against not just haemagglutinin but also neuraminidase antigens in future sero-epidemiologic cohort studies. Accurate estimates of true infection rates are critically important for

modelling transmission and impact of influenza and guiding countermeasure strategies such as antivirals, vaccines, hospital use and behavioural interventions. The comparative patterns of the antibody responses to the two most abundant and immunogenic antigens of influenza virus will improve understanding of immune correlates of protection and optimize pandemic and seasonal vaccine design and vaccination policies.

References

1. Cox NJ, Subbarao K. Influenza. Lancet **1999**; 354:1277-82.

2. Bandaranayake D, Huang QS, Bissielo A, et al. Risk factors and immunity in a nationally representative population following the 2009 influenza A(H1N1) pandemic. PLoS One **2010**; 5:e13211.

3. Van Kerkhove MD, Hirve S, Koukounari A, Mounts AW, group HNpsw. Estimating age-specific cumulative incidence for the 2009 influenza pandemic: a meta-analysis of A(H1N1)pdm09 serological studies from 19 countries. Influenza Other Respir Viruses **2013**; 7:872-86.

4. Katz JM, Hancock K, Xu X. Serologic assays for influenza surveillance, diagnosis and vaccine evaluation. Expert Rev Anti Infect Ther **2011**; 9:669-83.

5. Dingle JH, Badger GF, Feller AE, Hodges RG, Jordan WS, Jr., Rammelkamp CH, Jr. A study of illness in a group of Cleveland families. I. Plan of study and certain general observations. Am J Hyg **1953**; 58:16-30.

6. Elveback LR, Fox JP, Ketler A, Brandt CD, Wassermann FE, Hall CE. The Virus Watch program: a continuing surveillance of viral infections in metropolitan New York families. 3. Preliminary report on association of infections with disease. Am J Epidemiol **1966**; 83:436-54.

7. Monto AS. Studies of the community and family: acute respiratory illness and infection. Epidemiol Rev **1994**; 16:351-73.

8. Monto AS, Kioumehr F. The Tecumseh Study of Respiratory Illness. IX. Occurence of influenza in the community, 1966--1971. Am J Epidemiol **1975**; 102:553-63.

9. Hayward AC, Fragaszy EB, Bermingham A, et al. Comparative community burden and severity of seasonal and pandemic influenza: results of the Flu Watch cohort study. Lancet Respir Med **2014**; 2:445-54.

10. Horby P, Mai le Q, Fox A, et al. The epidemiology of interpandemic and pandemic influenza in Vietnam, 2007-2010: the Ha Nam household cohort study I. Am J Epidemiol **2012**; 175:1062-74.

11. Hung IF, To KK, Lee CK, et al. Effect of clinical and virological parameters on the level of neutralizing antibody against pandemic influenza A virus H1N1 2009. Clin Infect Dis **2010**; 51:274-9.

12. Miller E, Hoschler K, Hardelid P, Stanford E, Andrews N, Zambon M. Incidence of 2009 pandemic influenza A H1N1 infection in England: a cross-sectional serological study. Lancet **2010**; 375:1100-8.

13. Memoli MJ, Shaw PA, Han A, et al. Evaluation of Antihemagglutinin and Antineuraminidase Antibodies as Correlates of Protection in an Influenza A/H1N1 Virus Healthy Human Challenge Model. MBio **2016**; 7:e00417-16.

14. Eichelberger MC, Wan H. Influenza neuraminidase as a vaccine antigen. Curr Top Microbiol Immunol **2015**; 386:275-99.

15. Johansson BE, Cox MM. Influenza viral neuraminidase: the forgotten antigen. Expert Rev Vaccines **2011**; 10:1683-95.

16. Murphy BR, Kasel JA, Chanock RM. Association of serum anti-neuraminidase antibody with resistance to influenza in man. N Engl J Med **1972**; 286:1329-32.

17. Kendal AP, Noble GR, Dowdle WR. Neuraminidase content of influenza vaccines and neuraminidase antibody responses after vaccination of immunologically primed and unprimed populations. J Infect Dis **1977**; 136 Suppl:S415-24.

18. Ogra PL, Chow T, Beutner KR, et al. Clinical and immunologic evaluation of neuraminidase-specific influenza A virus vaccine in humans. J Infect Dis **1977**; 135:499-506.

19. Couch RB, Atmar RL, Franco LM, et al. Antibody correlates and predictors of immunity to naturally occurring influenza in humans and the importance of antibody to the neuraminidase. J Infect Dis **2013**; 207:974-81.

20. Monto AS, Kendal AP. Effect of neuraminidase antibody on Hong Kong influenza. Lancet **1973**; 1:623-5.

21. Huang QS, Turner N, Baker MG, et al. Southern Hemisphere Influenza and Vaccine Effectiveness Research and Surveillance. Influenza Other Respir Viruses **2015**; 9:179-90.

22. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform **2009**; 42:377-81.

23. Shu B, Wu KH, Emery S, et al. Design and performance of the CDC real-time reverse transcriptase PCR swine flu panel for detection of 2009 A (H1N1) pandemic influenza virus. Journal of clinical microbiology **2011**; 49:2614-9.

24. Sandbulte MR, Gao J, Straight TM, Eichelberger MC. A miniaturized assay for influenza neuraminidase-inhibiting antibodies utilizing reverse genetics-derived antigens. Influenza Other Respir Viruses **2009**; 3:233-40.

25. Lopez L, Wood T, Prasad N, Huang QS. Influenza surveillance in New Zealand, 2015. Available at: https://surv.esr.cri.nz/PDF_surveillance/Virology/FluAnnRpt/InfluenzaAnn2015.pdf.

26. Marcelin G, Bland HM, Negovetich NJ, et al. Inactivated seasonal influenza vaccines increase serum antibodies to the neuraminidase of pandemic influenza A(H1N1) 2009 virus in an age-dependent manner. J Infect Dis **2010**; 202:1634-8.

27. Monto AS, Koopman JS, Longini IM, Jr. Tecumseh study of illness. XIII. Influenza infection and disease, 1976-1981. Am J Epidemiol **1985**; 121:811-22.

28. Duvvuri VR, Moghadas SM, Guo H, et al. Highly conserved cross-reactive CD4+ T-cell HA-epitopes of seasonal and the 2009 pandemic influenza viruses. Influenza Other Respir Viruses **2010**; 4:249-58.

29. Khieu TQ, Pierse N, Telfar-Barnard LF, Huang QS, Baker MG. Estimating the contribution of influenza to hospitalisations in New Zealand from 1994 to 2008. Vaccine **2015**; 33:4087-92.

30. Everitt AR, Clare S, Pertel T, et al. IFITM3 restricts the morbidity and mortality associated with influenza. Nature **2012**; 484:519-23.

31. Zhang YH, Zhao Y, Li N, et al. Interferon-induced transmembrane protein-3 genetic variant rs12252-C is associated with severe influenza in Chinese individuals. Nat Commun **2013**; 4:1418.

32. Ohmit SE, Petrie JG, Malosh RE, et al. Influenza vaccine effectiveness in the community and the household. Clin Infect Dis **2013**; 56:1363-9.

33. Leung NH, Xu C, Ip DK, Cowling BJ. Review Article: The Fraction of Influenza Virus Infections That Are Asymptomatic: A Systematic Review and Meta-analysis. Epidemiology **2015**; 26:862-72.

34. Wang TE, Lin CY, King CC, Lee WC. Estimating pathogen-specific asymptomatic ratios. Epidemiology **2010**; 21:726-8.

35. Carrat F, Vergu E, Ferguson NM, et al. Time lines of infection and disease in human influenza: a review of volunteer challenge studies. Am J Epidemiol **2008**; 167:775-85.

36. Cauchemez S, Horby P, Fox A, et al. Influenza infection rates, measurement errors and the interpretation of paired serology. PLoS Pathog **2012**; 8:e1003061.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

QSH, DB, AB, RS, AM, TM, JR, AT, SL, NT, BM, JC, EGR, MGB, RW, GM, PT, CCG, CM, SR, AT, CW, ST, JD, DG, MGT, MAW designed and operationalized the cohort study; QSH, RS, AB, TW, JR, AT, WG, JB, SSW, NP, TM, SL, EC, AR, JH, GM, BM, JC, EGR provided the testing and the meta data collection used in this analysis; QSH, MAW, TW, DB, ECN, SSW, RW, MGT actively contributed to the design of the analysis; TW, BW, ECN did the data analysis; QSH wrote the first draft of the report and QSH and MAW signed off the final draft for clearance; All authors contributed to the interpretation of the results, revision of the manuscript critically for intellectual content and have given final approval of the version to be published.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention, US Department of Health and Human Services, the Institute of Environmental Science and Research (ESR) and other collaborating organizations.

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Tables

Table 1. Influenza seroconversion, influenza-like illness, influenza-confirmed ILI, and proportion of seroconversions leading to influenza-PCR-confirmed ILI among the cohort

Column name		A	B	C	D	E	F	G	H	I	J	K	L
Characteristics		Cohort		Seroconverted		Observed			Corrected				
		No.	Col (%)	No.	Infection attack rate (C/A%:CI)	No. person with ILI (E/A%)	No. ILI with swabs (F/E%)	No. swabs with PCR-confirmed Influenza (G/F%)	No. person with ILI	ILI attack rate (H/A%:CI)	No. ILI with PCR-confirmed influenza	Influenza-PCR-confirmed ILI attack rate (J/A%:CI)	% influenza-PCR-confirmed ILI among serocoonverted (J/C%:CI)
Overall		911	100	321	35 (32, 38)	287 (32)	209 (73)	50 (24)	329	36 (33, 39)	76	8 (7, 10)	24 (19, 29)
Age (years)	0-4	160	18	77	48 (40, 56)	89 (56)	73 (82)	16 (22)	107	67 (59, 74)	22	14 (9, 20)	29 (19, 40)
	5-19	264	29	117	44 (38, 51)	70 (27)	34 (49)	15 (44)	81	31 (25, 37)	27	10 (7, 15)	23 (16, 32)
	20-64	444	49	116	26 (22, 31)	119 (27)	94 (79)	18 (19)	133	30 (26, 35)	25	6 (4, 8)	22 (15, 30)
	65+	43	5	11	26 (14, 41)	9 (21)	8 (89)	1 (13)	10	23 (12, 39)	1	2 (0, 12)	9 (0, 41)
Sex	Female	538	59	179	33 (29, 37)	172 (32)	126 (73)	34 (27)	197	37 (33, 41)	50	9 (7, 12)	28 (22, 35)
	Male	373	41	142	38 (33, 43)	115 (31)	83 (72)	16 (19)	132	35 (31, 41)	24	6 (4, 9)	17 (11, 24)
Ethnicity	Maori	123	14	43	35 (27, 44)	39 (32)	27 (69)	7 (26)	44	36 (27, 45)	11	9 (5, 15)	26 (14, 41)
	Pacific	97	11	45	46 (36, 57)	38 (39)	26 (68)	5 (19)	51	53 (42, 63)	9	9 (4, 17)	20 (10, 35)
	Asian	197	22	78	40 (33, 47)	59 (30)	40 (68)	9 (23)	68	35 (28, 42)	14	7 (4, 12)	18 (10, 28)
	Others*	494	54	155	31 (27, 36)	151 (31)	116 (77)	29 (25)	167	34 (30, 38)	40	8 (6, 11)	26 (19, 33)
NZDep**	1 or 2	206	23	64	31 (25, 38)	62 (30)	48 (77)	15 (31)	70	34 (28, 41)	23	11 (7, 16)	36 (24, 49)
	3 or 4	195	21	60	31 (24, 38)	57 (29)	40 (70)	6 (15)	64	33 (26, 40)	10	5 (3, 9)	17 (8, 29)
	5 or 6	170	19	64	38 (30, 45)	47 (28)	38 (81)	10 (26)	51	30 (23, 38)	13	8 (4, 13)	20 (11, 32)
	7 or 8	164	18	63	38 (31, 46)	63 (38)	45 (71)	13 (29)	73	45 (37, 53)	19	12 (7, 18)	30 (19, 43)
	9 or 10	176	19	70	40 (33, 47)	58 (33)	38 (66)	6 (16)	73	42 (34, 49)	11	6 (3, 11)	16 (8, 26)
Underlying Condition§	No	756	83	275	36 (33, 40)	229 (30)	170 (74)	41 (24)	262	35 (31, 38)	64	9 (7, 11)	23 (18, 29)
	Yes	155	17	46	30 (23, 38)	58 (37)	39 (67)	9 (23)	66	43 (35, 51)	14	9 (5, 15)	30 (18, 46)
Virus	A(H3N2)	911	100	193	21 (19, 24)	-	209 (73)	24 (12)	-		37	4 (3, 6)	19 (14, 25)
	B	911	100	159	18 (15, 20)	-	209 (73)	26 (12)	-		39	4 (3, 6)	25 (18, 32)

Note: ILI refers to influenza-like illness defined as *'an acute respiratory illness with a history of fever or measured temperature of ≥38°C, and cough, and onset within the past 7 days'*. Influenza-PCR-confirmed ILI is defined as PCR-confirmed influenza from those participants with ILI; Influenza infection is defined as haemagglutination inhibition (HAI) seroconversion (4-fold or greater HAI titre rise in paired sera with the second titre at least 1:40) or neuraminidase inhibition (NAI) seroconversion (4-fold or greater NAI titre rise) or influenza RNA detection by rRT-PCR; CI refers to confidence interval.

*Refers to Europeans and others

**NZDep scale measures socioeconomic deprivation on an ordinal scale of 1 to 10 where 1 indicates the individual is living in a household that is in the least socioeconomic deprived decile of all New Zealand households

§Underlying condition includes one or more of the following conditions: asthma, diabetes, chronic respiratory illness, heart disease (angina, myocardial infarction/heart attack, chronic heart failure, atrial fibrillation, rheumatic heart disease, congenital heart condition) and mental illnesses.

Table 2. Multivariate analysis for risk of influenza infection and disease among the cohort

Risk factors		Influenza infection			Influenza-PCR-confirmed ILI			%Influenza-PCR-confirmed ILI among seroconverters		
		Corrected No.	Adjusted attack rate ratio (CI)	P-value	Corrected No.	Adjusted attack rate ratio (CI)	P-value	Corrected %	Adjusted attack rate ratio (CI)	P-value
Age (years)	0-4	77	1.8 (1.4, 2.5)	**0.000**	22	2.5 (1.3, 4.8)	**0.009**	28.6	1.3 (0.7, 2.6)	0.395
	5-19	117	1.7 (1.3, 2.2)	**0.000**	27	1.4 (0.7, 2.8)	0.334	23.1	0.8 (0.4, 1.6)	0.585
	20-64	116	ref		25	ref		21.6	ref	
	65+	11	1.0 (0.5, 1.8)	0.947	1	0.6 (0.1, 4.3)	0.589	9.1	0.6 (0.1, 4.4)	0.603
Sex	Female	179	ref		50	ref		27.9	ref	
	Male	142	1.1 (0.9, 1.4)	0.231	24	0.7 (0.4, 1.2)	0.201	16.9	0.6 (0.3, 1.1)	0.085
Ethnicity	Maori	43	1.1 (0.8, 1.6)	0.530	11	1.0 (0.4, 2.2)	0.941	25.6	0.9 (0.4, 2.0)	0.741
	Pacific	45	1.5 (1.1, 2.1)	**0.021**	9	0.9 (0.3, 2.3)	0.788	20.0	0.6 (0.2, 1.5)	0.282
	Asian	78	1.3 (1.0, 1.7)	0.094	14	0.8 (0.4, 1.6)	0.511	17.9	0.6 (0.3, 1.3)	0.205
	Other*	155	ref		40	ref		25.8	ref	
NZDep**	1 or 2	64	ref		23	ref		35.9	ref	
	3 or 4	60	1.0 (0.7, 1.4)	0.957	10	0.4 (0.2, 1.1)	0.075	16.7	0.4 (0.2, 1.1)	0.078
	5 or 6	64	1.2 (0.9, 1.7)	0.277	13	0.8 (0.4, 1.8)	0.601	20.3	0.7 (0.3, 1.5)	0.321
	7 or 8	63	1.2 (0.9, 1.8)	0.232	19	1.1 (0.5, 2.3)	0.823	30.2	0.9 (0.4, 1.9)	0.737
	9 or 10	70	1.3 (0.9, 1.8)	0.153	11	0.5 (0.2, 1.2)	0.116	15.7	0.4 (0.1, 0.9)	**0.037**
Underlying Condition[§]	No	275	ref		64	ref		23.3	ref	
	Yes	46	0.8 (0.6, 1.1)	0.201	14	1.1 (0.5, 2.2)	0.853	30.4	1.3 (0.6, 2.7)	0.460

Note: Influenza infection is defined as haemagglutination inhibition (HAI) seroconversion (4-fold or greater HAI titre rise in paired sera with the second titre at least 1:40) or neuraminidase inhibition (NAI) seroconversion (4-fold or greater NAI titre rise) or influenza RNA detection

by PCR; Influenza-PCR-confirmed ILI is defined as PCR-confirmed influenza among those symptomatic ILI participants; %Infection leading to illness is defined as proportion of influenza-PCR-confirmed ILI among influenza infection. CI refers to 95% confidence interval; statistically significant P value is indicated in bold.

The risks of influenza infection and of developing ILI once infected were examined for five potential risk factors (age, sex, ethnicity, social deprivation, underlying medical conditions). Univariate and multivariate analyses were performed for these variables using rate ratios from Poisson regression generalised linear models.

*Refers to Europeans and others

**NZDep scale measures deprivation on an ordinal scale of 1 to 10 where 1 indicates the individual is living in a household that is in the least deprived decile of all New Zealand households

[§]Underlying condition includes one or more of the following conditions: asthma, diabetes, chronic respiratory illness, heart disease (angina, myocardial infarction/heart attack, chronic heart failure, atrial fibrillation, rheumatic heart disease, congenital heart condition) and mental illnesses.

List Figures

Figure 1 – Serology and PCR testing flow and results among the cohort.

Note: Haemagglutination inhibition (HAI) and neuraminidase inhibition (NAI) assays were performed for the 911 unvaccinated participants of all ages. Of them, 221 had HAI & NAI seroconversion, 46 HAI alone seroconversion, 100 NAI alone seroconversion. Among the 321 seroconverters, 156 had ILI symptoms and 109 (70%, 109/156) had swabs taken for influenza PCR with 50 being positive (46%, 50/109); The rate of ILI was 48.6% (156/321) among HAI or NAI seroconverters and 22.2% (131/590) among non-seroconverters.

Figure 2. Temporal distribution of influenza-like illness (ILI) and influenza-PCR-confirmed ILI and no ILI among the cohort during 27 April to 27 September 2015.

Note: Influenza viruses were detected continuously during 27 June to 27 September with two distinct circulation patterns: A(H3N2) predominated during weeks 26-33, whereas influenza B (mainly B/Victoria lineage) predominated during weeks 34-39. No influenza A(H1N1)pdm09 was detected. ILI refers to influenza-like illness defined as *'an acute respiratory illness with a history of fever or measured temperature of ≥38°C, and cough, and onset within the past 7 days'*. No-ILI refers those participants indicated not having ILI in the weekly text/email 'Have you had cough and fever in the previous 7 days' and also those indicated having ILI but not verified by nurses.

Figure 3 Proportions of HAI or NAI seroconversion by age groups and by viruses.

Panel A – all influenza viruses

Panel B – A(H3N2) virus

Panel C – influenza B virus

Note: %HAI refers to proportion of individuals with haemagglutination inhibition (HAI) seroconversion (4-fold HAI titre rise in paired sera with the second titre at least 1:40); %NAI refers to proportion of individuals with neuraminidase inhibition (NAI) seroconversion (4-fold NAI titre rise in paired sera); %NAI-%HAI refers to the difference between NAI and HAI seroconversion rates by subtraction; Yo refers to year(s) old.

Figure 1.

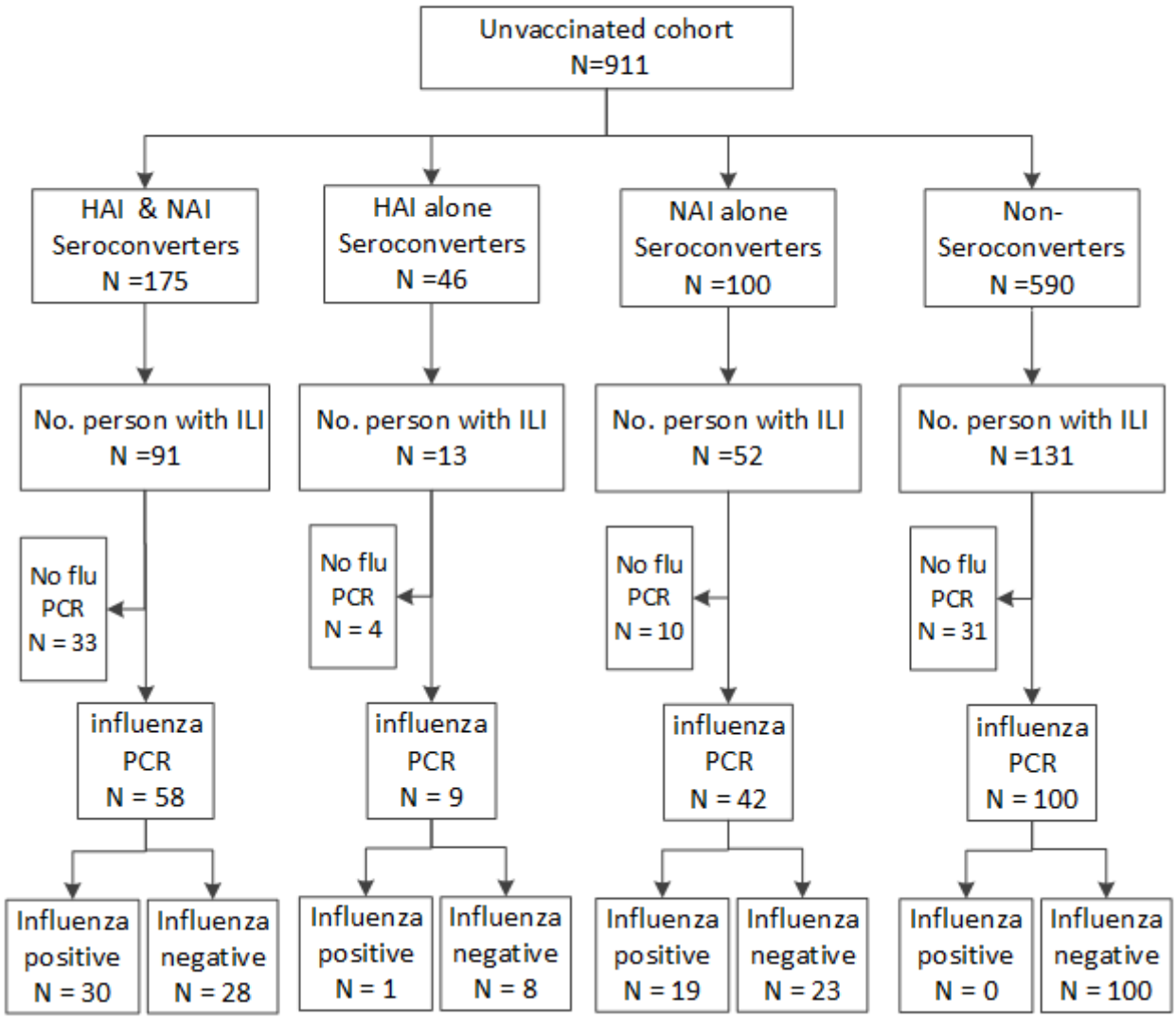


Figure 2.

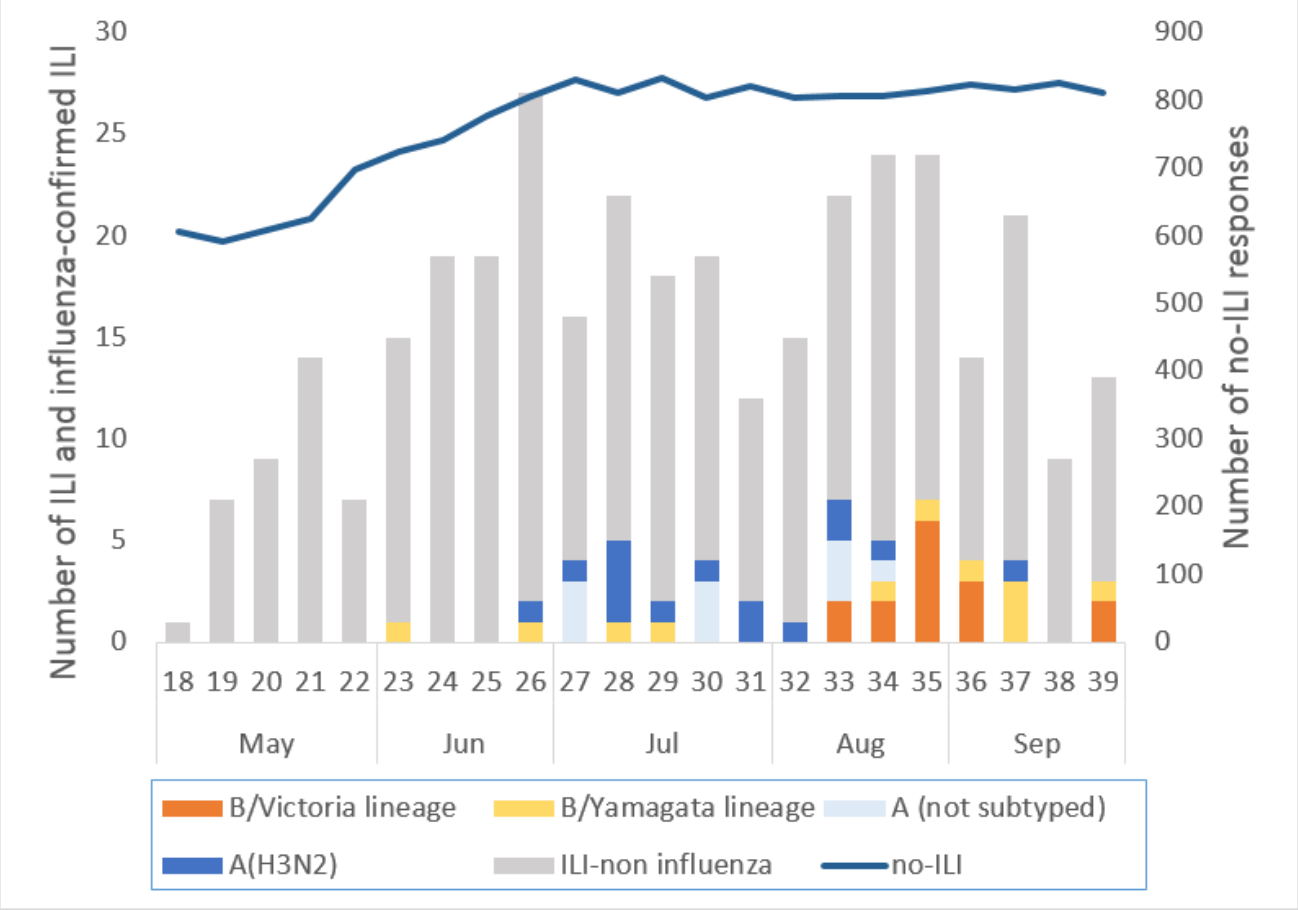
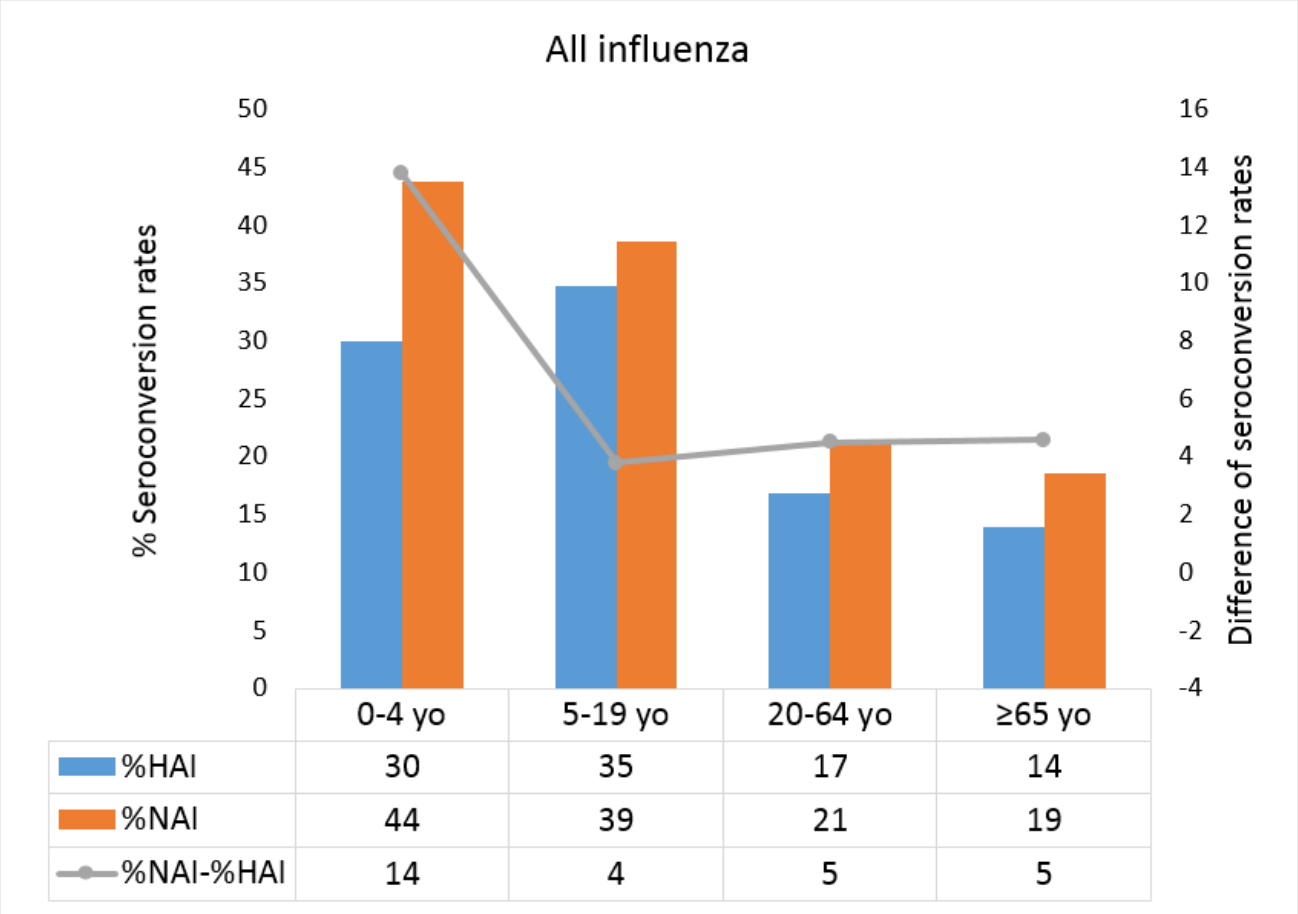


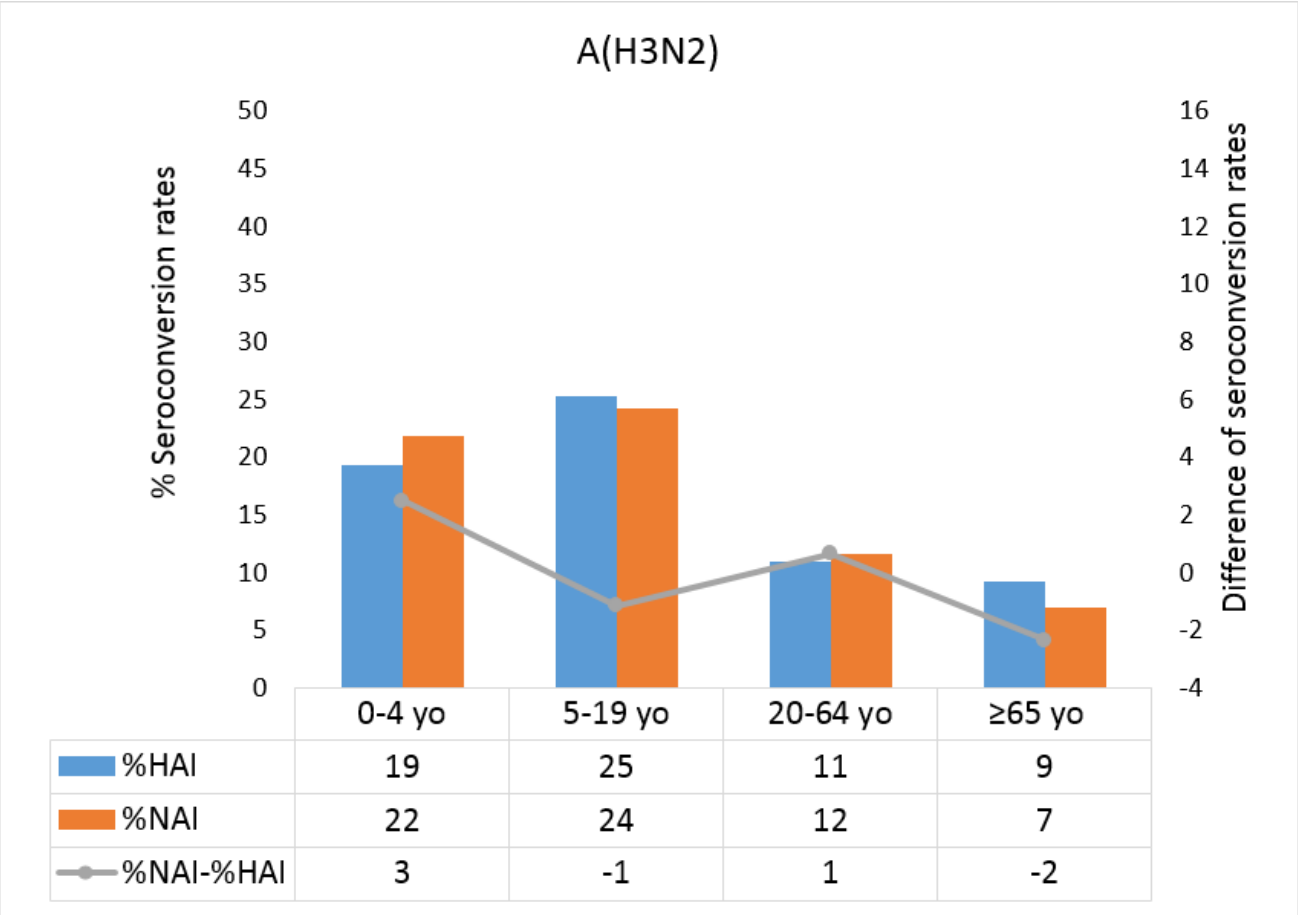
Figure 3.

A:



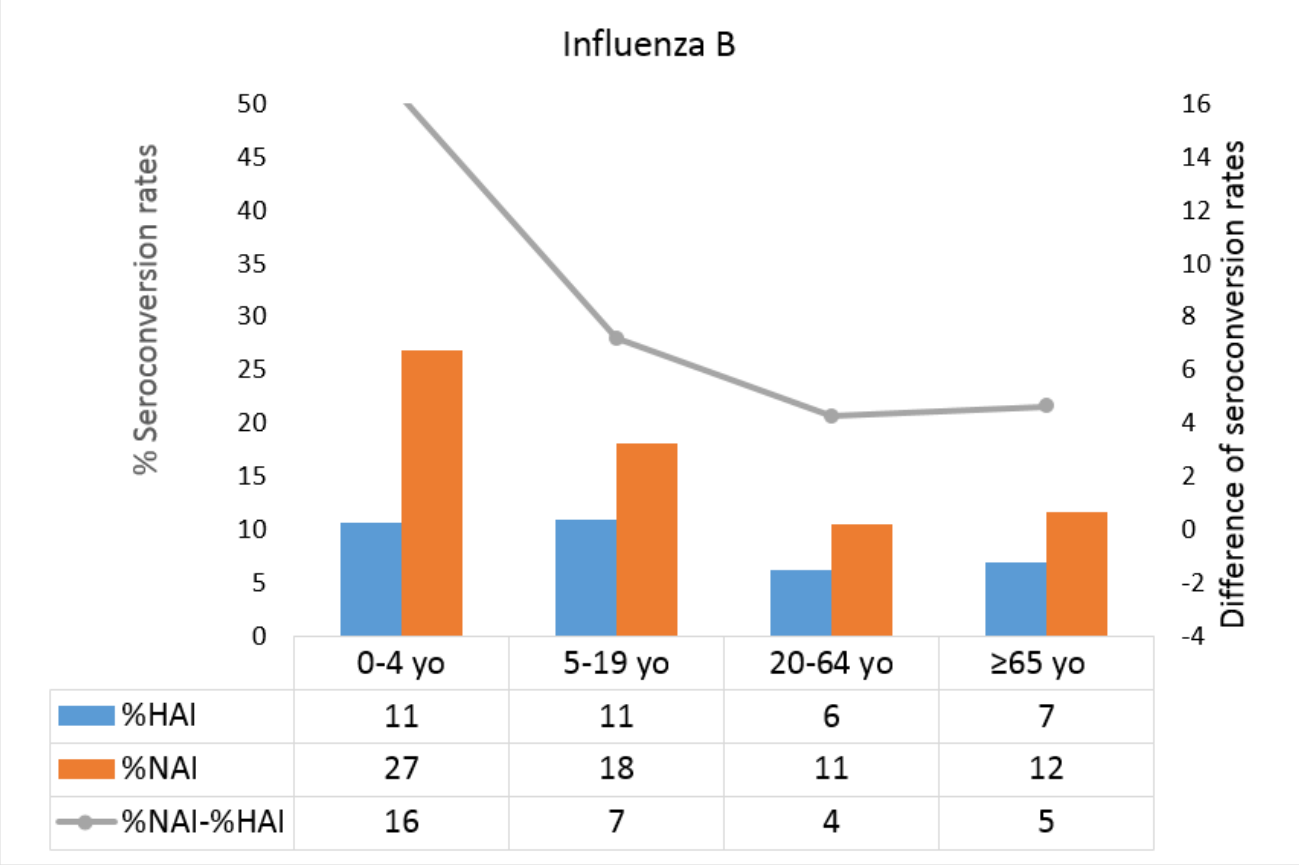
	0-4 yo	5-19 yo	20-64 yo	≥65 yo
%HAI	30	35	17	14
%NAI	44	39	21	19
%NAI-%HAI	14	4	5	5

B:



	0-4 yo	5-19 yo	20-64 yo	≥65 yo
%HAI	19	25	11	9
%NAI	22	24	12	7
%NAI-%HAI	3	-1	1	-2

C:



	0-4 yo	5-19 yo	20-64 yo	≥65 yo
%HAI	11	11	6	7
%NAI	27	18	11	12
%NAI-%HAI	16	7	4	5