

REVIEW ARTICLE

The fraction of influenza virus infections that are asymptomatic: a systematic review and meta-analysis

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CONFLICTS OF INTEREST

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ABSTRACT

Background: The fraction of persons with influenza virus infection that do not report any signs or symptoms throughout the course of infection is referred to as the asymptomatic fraction (AF).

Methods: We conducted a systematic review and meta-analysis of published estimates of the AF of influenza virus infections. We found that estimates of the AF were reported from two different types of studies: first, outbreak investigations with short-term follow-up of potentially exposed persons and virologic confirmation of infections; second, studies conducted across epidemics typically evaluating rates of acute respiratory illness among persons with serologic evidence of infection, in some cases adjusting for background rates of illness from other causes.

Results: Most point estimates from studies of outbreak investigations fell in the range 4%-28% with low heterogeneity ($I^2=0\%$) with a pooled mean of 16% (95% CI: 13%, 19%). Estimates from the studies conducted across epidemics without adjustment were very heterogeneous (point estimates 0%-100%; $I^2=97\%$), while estimates from studies that adjusted for background illnesses were more consistent with point estimates in the range 65%-85% and moderate heterogeneity ($I^2=58\%$). Variation in estimates could be partially explained by differences in study design and analysis, and inclusion of mild symptomatic illnesses as asymptomatic in some studies.

Conclusions: Estimates of the AF are affected by the study design, and the definitions of infection and symptomatic illness. Considerable differences between the AF of infections confirmed by virologic versus serologic testing may indicate fundamental differences in the interpretation of these two indicators.

Key words: influenza; asymptomatic; public health

INTRODUCTION

Influenza virus infections lead to a wide range of clinical manifestations, from severe pneumonia through to mild or even asymptomatic disease (1).

Asymptomatic infection is defined as infection without any signs or symptoms of that infection (2). There has been discussion over the proportion of influenza virus infections that are associated with asymptomatic disease, referred to as the asymptomatic fraction (AF). An understanding on the asymptomatic fraction is important in two respects. First, improved estimation of the AF could aid estimation and prediction of incidence of infection from surveillance data on symptomatic illnesses (3). Second, knowledge of the fraction of infections that are asymptomatic and their infectiousness relative to symptomatic infections would be important in optimizing public health control strategies such as contact tracing and quarantine, and characterizing transmission dynamics using mathematical models (4, 5). However, there is currently no consensus on the value of the AF with different studies typically using values from 20%-50% (4, 6-8). Therefore the objective of our study was to describe and summarize published estimates of the AF, and to identify factors in study design or analysis that could contribute to differences in estimates of the AF.

METHODS

Search Strategy

This systematic review was conducted and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (9). We identified publications on 11 April 2014 describing the AF of influenza virus infections in PubMed and Scopus using the following search terms:

asymptomatic[All Fields] AND ("influenza, human"[MeSH Terms] OR ("influenza"[All Fields] AND "human"[All Fields]) OR "human influenza"[All Fields] OR "influenza"[All Fields]). The search was limited to entries created in the database on or before 11 April 2014 but was not limited on publication date. The authors' own databases of full text publications were also searched.

Study Selection

The titles of all articles identified by the search strategy were independently screened by two authors (N.H.L.L. and B.J.C.). Only articles written in English were included, and reviews and articles that did not contain empirical data (i.e. collection of clinical samples) on the number of people with any evidence of laboratory-confirmed infection were excluded (the definition of which is given in the next subsection). Abstracts of potentially relevant papers were then screened, with studies excluded if 1) abstract or full text was not available, 2) participants were taking antiviral prophylaxis, 3) influenza infections were not laboratory-confirmed, 4) symptoms were not reported or 5) the asymptomatic fraction was undetermined. The full texts of the remaining articles were then reviewed for eligibility. Studies were eligible for inclusion if they provided an estimate of the AF defined as laboratory-confirmed infection without any signs or symptoms, or if not, the number of individuals assessed to be infected with laboratory confirmation together with the number of those who have no evidence of symptomatic illness. Volunteer challenge studies (10) were excluded from the present review, which focused on natural infections, because of the potential for mode of inoculation and infectious dose to affect the probability and severity of symptomatic illness (11, 12). Studies that reported the AF as the probability of

influenza virus infection conditional on asymptomatic illness were also excluded (13-15).

Definition of asymptomatic fraction

The AF is defined as the probability of illness without any signs and symptoms, or not fulfilling the criteria of illness as defined by the individual studies, conditional on laboratory-confirmed infection. The estimate of the AF was typically reported in the studies as the proportion of individuals without symptoms (or not fulfilling the study-specific case definition) among all individuals with laboratory-confirmed infection. Laboratory-confirmed influenza virus infection was defined as an infection that was virologically confirmed by reverse transcriptase polymerase chain reaction (PCR) or viral culture on a respiratory specimen such as a nasal swab; or an infection indicated by serologic testing by hemagglutination-inhibition, microneutralization, or complement fixation assay, with a ≥ 4 -fold rise in antibody titer in paired sera across an epidemic, or a titer ≥ 40 in a single serum specimen.

Data extraction

Our principal summary measures were the estimates along with 95% confidence intervals (CI) of the AF. We extracted whenever available point estimates and 95% CIs of reported AFs, counts of the number of individuals who had laboratory-confirmed infection, and counts of the number of individuals who were asymptomatic among infected, and documented other features of the studies on a standardized form including study design, age range of participants, influenza types/subtypes recovered, laboratory assays used to identify influenza

virus infection, the definition of influenza virus infection and of asymptomatic illness, and whether estimates of the AF were adjusted for background rates of acute respiratory illnesses not due to influenza virus infection. When estimates and 95% CIs of AF were not reported in the studies they were calculated from the number of individuals infected and the number of those who were asymptomatic assuming a binomial distribution.

Statistical Analysis

A forest plot of the estimates and 95% CIs of AFs was constructed using the estimates reported in the studies or calculated from the counts of number of individuals infected and counts of number of infected individuals who were asymptomatic. Estimates of AFs were classified by type of study and heterogeneity was estimated using the I^2 statistic with a random-effects model (16). I^2 is interpreted as the proportion of total variation in the effect estimates that is due to heterogeneity between studies, with an I^2 of 0% indicating that all variability is due to sampling error within studies and I^2 values of 25%, 50% and 75% indicating low, medium and high degrees of heterogeneity respectively (17, 18). Pooled estimates of the AF would only be made if there was low heterogeneity. All analyses were conducted with R version 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria) and the *metafor* package (19).

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RESULTS

463 titles were identified in the first step, 109 abstracts and 68 full-length articles were then reviewed, and eventually 30 articles were selected for inclusion in this review (Figure 1). The articles could be classified into two types

of study design: outbreak investigations (11 studies) and trans-epidemic studies (19 studies). The characteristics of the 30 included studies are summarized in Table 1.

□

Studies in the group of outbreak/epidemic investigations included eight household transmission studies (20-27) and three studies in other settings (28-30). In these studies, identification of initial laboratory-confirmed cases was followed by intense follow-up of exposed persons that included repeated collection of respiratory specimens or sera regardless of symptomatic illness. The AF could then be estimated among exposed persons (excluding the initial cases) based on the proportion of laboratory-confirmed infections without symptomatic illness. Point estimates of the AF from the studies in this group fell within the range 4%-28% or had wide confidence intervals extending into this range (Figure 2A). Heterogeneity measured by the I^2 statistic was low (0%) with a pooled mean of 16% (95% confidence interval, CI: 13%, 19%). Loeb *et al.* reported that the AF was statistically significantly lower for H3N2 infections compared to infections with H1N1 and B (28), while differences between subtypes were not significant in some other studies (20, 31).

The other 19 studies could be grouped together as serological studies where individuals were followed up across entire epidemics, and testing of single or paired sera was used to identify infections, rarely in combination with virologic testing (32-50). Illness reports in the same individuals could then be used to infer how many influenza virus infections might have been symptomatic. The earliest study we identified was published in 1973 (49). Overall, point estimates

of the AF from this group of studies were spread over a wide range of 0%-100% with very high heterogeneity ($I^2=97\%$) (Figure 2B and 2C).

In one early study, Monto *et al.* defined the “pathogenicity index” as the excess rate of illnesses in individuals with serologic evidence of infection compared to those without (34). In their study, Monto *et al.* subtracted illness rates in individuals without rises in paired titers from illness rates in individuals with titer rises, stratifying by age and then calculating the weighted mean. Assuming that the risk of influenza virus infection was independent of the rate of non-influenza illnesses, the authors estimated that at least 15.1% of influenza A(H3N2) and 33.7% of influenza B virus infections led to symptomatic illness (34). Most studies did not adjust for rates of illness from other non-influenza causes in this way, while one study used a similar approach to the pathogenicity index described above (32), and another study used a regression method (33). The five adjusted estimates of the AF (Figure 2B) were in the range 65%-85% and were higher than most of the unadjusted estimates (Figure 2C). There was less heterogeneity among the studies that reported adjusted estimates, with I^2 statistics of 58% for adjusted versus 97% for unadjusted estimates.

While most studies defined the AF as infection completely without symptoms, some studies presented estimates of the AF in terms of the proportion of infected persons that did not have febrile illness (41, 50), or the proportion of infected persons that did not have an illness which fulfilled a case definition for influenza-like illness that included fever (Table 1) (20, 30, 33, 35, 45).

Most of the studies (24/30) did not report data on age-specific AFs (Table 1), while in 2 studies the estimates of the AF did not allow stratification by age because either all or none of the cases were asymptomatic (41, 46). In the remaining 4 studies where the age group-specific AFs (20, 34, 39) or data at individual level (47) were reported, the estimates of the AF for influenza A tended to be higher in adults than in children or elderly, but Monto *et al.* reported that the pathogenicity index was highest in adults with influenza B virus infection after adjusting for other illnesses (34).

A few of the excluded studies are worthy of mention. Three studies presented the probability of influenza virus infection among asymptomatic persons, which is quite different to the asymptomatic fraction as we defined it above and strongly depends on the prevalence of infection (13-15). We excluded one study that determined laboratory-confirmed cases from both the recovery of viral RNA from intense follow up and from serologic evidence of infection across an epidemic, without providing a breakdown (51). One study measured the prevalence of influenza virus infection among inbound international airline travelers with symptomatic and asymptomatic illness (52), allowing inference on the fraction of infections associated with asymptomatic *or* pre-symptomatic virus shedding although such an estimate was not reported. Another study investigated asymptomatic infection among re-infected individuals, and reported that occurrence of symptoms was prevented during reinfection with a closely related virus even five years later (53).

DISCUSSION

Estimates of the AF are affected by the study design, and the definitions of infection and symptomatic illness. Estimates of the AF based on outbreak investigations and household transmission studies appeared to provide more homogeneity in estimates of the AF, with most point estimates in the range 4%-28% and a pooled mean of 16% (95% CI: 13%, 19%) (Figure 2A). Advantages of outbreak investigations and household transmission studies in determining the AF include the reduced risk of recall bias in symptom reporting with intense prospective follow-up, and the ability to identify the time of infection within a short time frame. However, determining infections based on PCR may underascertain some infections, since it has been reported that some exposed persons can have serologic evidence of infection without PCR-confirmed infection or symptomatic disease. For example, a study in Hong Kong reported that 6/19 (32%) of exposed persons with 4-fold or greater rises in antibody titer did not have PCR-confirmed infection and did not report symptoms (21). In addition, studies of this type might underestimate the asymptomatic fraction if symptomatic illnesses not due to influenza virus infection were mis-attributed to influenza.

We identified considerable variability in estimates of the AF based on cohort studies with point estimates from 0%-100% (Figure 2B and 2C). It is unclear whether this heterogeneity is indicative of real differences in the AF in different studies and settings. It is possible that infections acquired in the community are milder on average than secondary cases in outbreaks in households or other confined settings, because of the less intense exposure from the community so

that lower infection dose might lead to milder illness (10, 54). Infection indicated by serology could be an indicator of adaptive protection which would lead to more asymptomatic infections in individuals with prior exposures or older age (53). On the other hand, it is possible to consider a number of reasons why the heterogeneity might be artefacts of the study design, including variation in the degree of under-reporting of illnesses, and varying definitions of serologic evidence of infection and asymptomatic infection. Regarding the definitions of serologic evidence of infection, most studies used ≥ 4 -fold rise in antibody titer in paired sera to indicate infection, but some studies used less (55) or more stringent (35) criteria. The use of seropositivity in a single serum specimen to indicate infection during the study period could have led to misclassification of some infections in some studies, as individuals might have different baseline titers prior to the study period. Regarding the definitions of “asymptomatic”, many of the studies did not define the AF explicitly. Some studies presented estimates of the AF using a definition that included symptomatic illnesses in the numerator, as individuals not fulfilling the specified case definitions (e.g. influenza-like illness) were considered asymptomatic (33, 38, 41, 45, 50). However, a considerable proportion of persons with influenza virus infection have afebrile but symptomatic disease (24, 36, 39, 42) which could have led to overestimation of the AF.

A few studies adjusted for symptomatic illnesses not caused by influenza (32-34), and some other studies compared rates of disease in persons with versus without evidence of infection without making a single adjusted estimate of the AF (36, 39, 49). The adjusted estimates (32-34) (Figure 2B) were more

consistent with point estimates in the range 65%-85%. Such approaches require the assumption that the risk of non-influenza illnesses is independent of the risk of influenza virus infections, which might not always hold (5, 31, 56). The idea of non-independence is not new (56) and the implication for estimation of the asymptomatic fraction was explicitly discussed by Monto *et al.* who wrote that their approach might underestimate the pathogenicity of the virus in question, “because influenza may replace another illness during a limited time period” (34). This remains controversial.

In the outbreak studies, a reduced AF among H3N2 infections would be consistent with greater seriousness of H3N2 compared to H1N1 and B infections (28, 57). Some studies could not identify significant differences in the AF between types/subtypes (21, 33, 35), and some reported lower estimates of the AF for H1N1 (48) and B (34).

Given that disease severity is known to vary by age (58-60), and that immunity changes substantially with age (61, 62), it would be reasonable to hypothesize changes in the AF with age. However, most of the studies that we reviewed did not provide sufficient data to allow stratification of the estimates of the AF by age (Table 1). Most studies also did not report on the vaccination status of the infected individuals who did not report symptoms, although given the timing of studies conducted during the first wave of H1N1pdm09, participants in those studies would not have been vaccinated against H1N1pdm09. More data on factors that might affect the estimates of the AF would be valuable, such as larger studies that permit assessment of age-specific AFs.

Knowledge of the AF is important from two perspectives: (1) the fraction of cases that are infected but asymptomatic is important for assessing the severity and the burden of disease; and (2) the fraction of cases that are infectious but asymptomatic is important for optimizing public health control measures. For example, the potential impact to humans of emerging infectious diseases with zoonotic origin and limited human-to-human transmission depends on the fraction of exposed individuals with symptomatic illness (63). On the other hand, entry screening for infectious diseases at borders using health declaration forms and infrared thermal scanners is predicated on the idea that the AF of diseases of interest is low (64), and isolation is only a useful measure if most infectious patients will be symptomatic. The two broad types of studies described above may provide information on each of these interpretations of the asymptomatic fraction. Some individuals with detectable influenza virus shedding do not subsequently have serologic evidence of infection (27, 56, 65), while other individuals with serologic evidence of infection do not have detectable virus shedding (21, 66), suggesting one should exercise caution on the interchangeability and the interpretation of the estimates of AF based on different definitions. Estimates from serologic studies, with a denominator based on serologic evidence of infection, may be more relevant in understanding the severity of illness. Estimates of the AF from outbreak investigations, where the denominator is infections with detectable virus shedding, may be more relevant in understanding the transmission potential of asymptomatic versus symptomatic infections.

Our review was subject to some limitations. First, our search may have missed some published estimates of the AF, and broadening the search would have substantially increased workload. However we believe including any such studies would not change our conclusions substantially. We previously reviewed household transmission studies of H1N1pdm09 (67), and few such studies were conducted before 2009, therefore only a minimal number of such studies might have been missed. On the other hand, inclusion of additional serological studies would not have changed our conclusions as well since the existing studies of this type have already demonstrated a high heterogeneity in estimates of the AF, and including more studies would only increase the heterogeneity further. Second, we did not formally assess the risk of bias in each study, but we did consider how features in the design and analysis of studies could contribute to bias in the estimates of the AF (Figure 1). Selection bias may have affected estimates of the AF if patients included in cohorts or transmission studies were not generalizable to infections in other settings, and this was explicitly discussed above as a potential explanation (i.e. a difference in the intensity of the exposure) for the difference between the estimates of the AF in transmission studies and cohort studies (Figure 2A versus Figure 2B). The cohort studies are particularly likely to be prone to information biases in both assessment of infection and assessment of symptomatic disease. Finally, we did not identify sufficient estimates of the AF to permit meta-regression analysis of the influence of study design characteristics and other factors on the estimates of the AF.

In conclusion, the true AF of influenza virus infections may depend on how infections are identified, and we found quite different estimates of the AF in two

different types of studies. In outbreak investigations where infections were virologically confirmed, we found a pooled mean of 16% (95% CI: 13%, 19%) of infections were asymptomatic, whereas in longitudinal studies in which infections were identified using serology the point estimates of the AF adjusted for illness from other causes fell in the range 65%-85%. We could not fully explain the differences in the scale of estimates from these two types of studies, although features of the respective analyses would have led to under- and over-estimation of the AF respectively. A study in Vietnam did include both of these strategies, estimating the asymptomatic fraction as 45% (17%-77%) in outbreak investigations versus 86% (82%-89%) in the longitudinal serological analysis (27, 35). One potential approach to resolve these differences would be a hybrid study, where intensive follow-up with frequent virologic testing regardless of illness throughout an influenza season is used to ascertain all infections and illnesses in a cohort.

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FIGURE LEGEND

Figure 1: Flow diagram of the process and results of study selection.

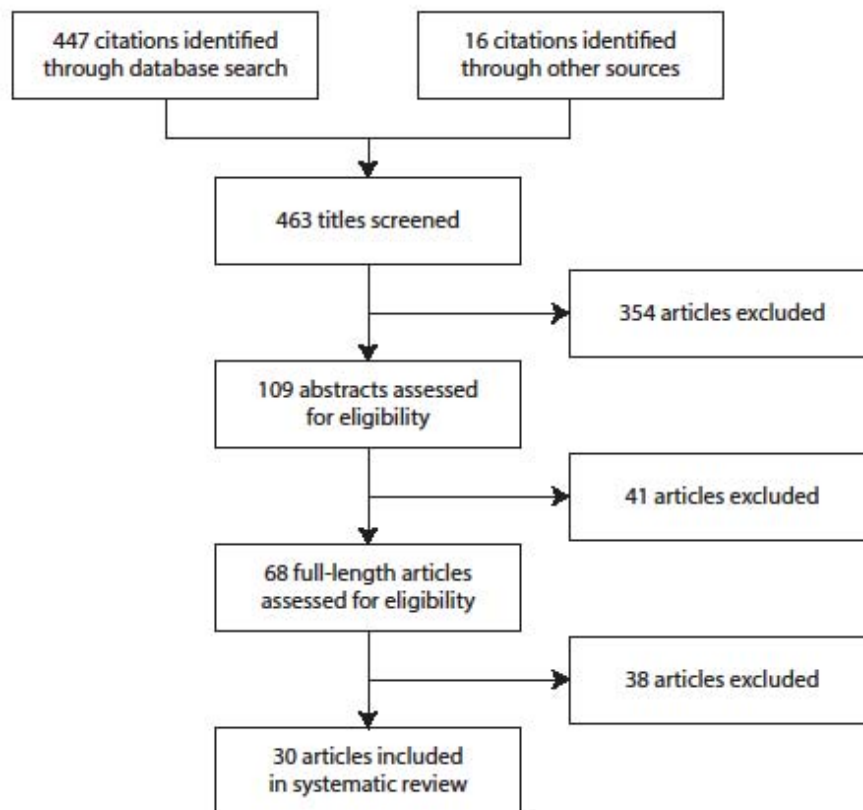
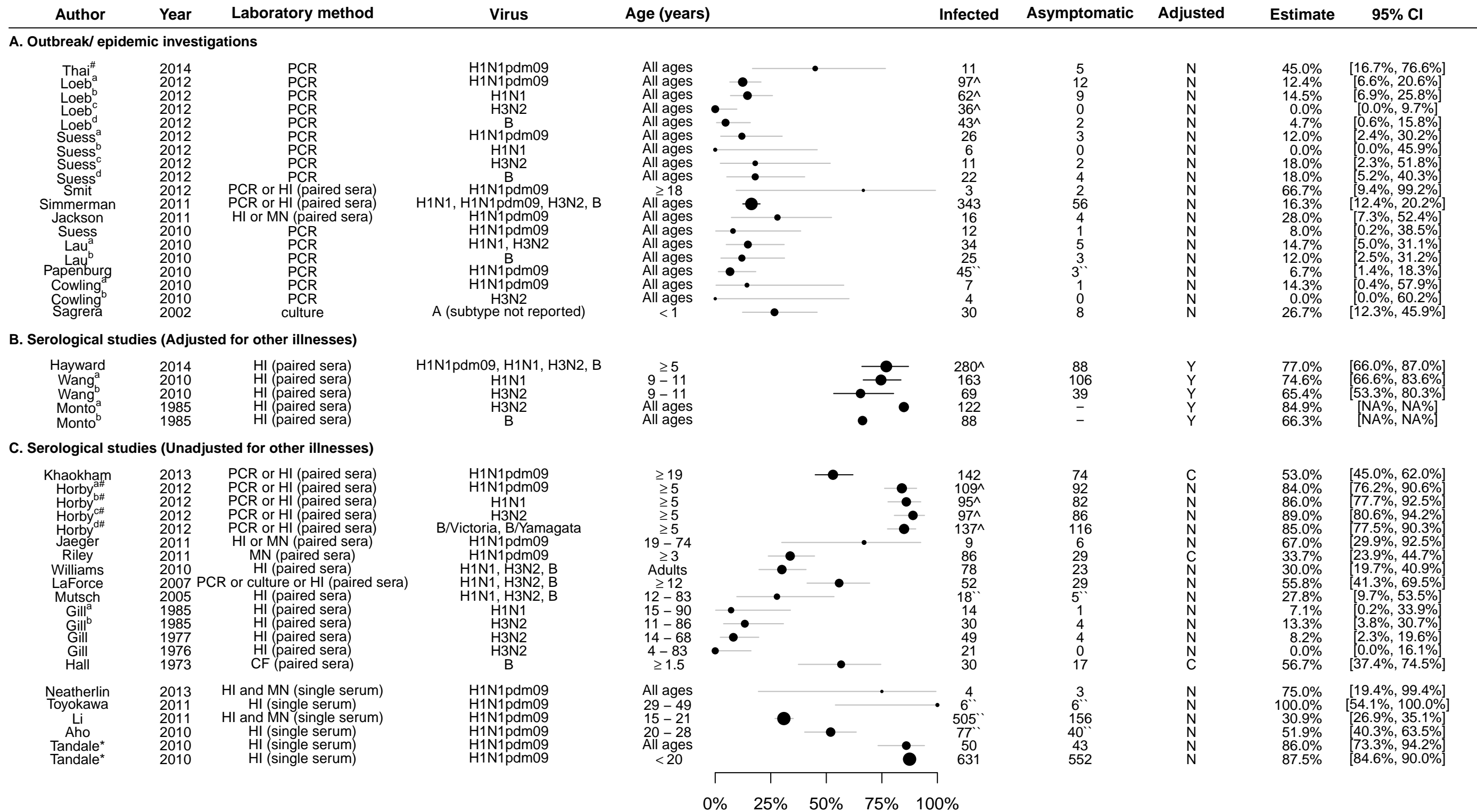


Figure 2: Forest plot of estimates of the asymptomatic fraction ('Estimate'), stratified by study design. Panel A: estimates from outbreak investigations in which potentially exposed individuals were followed intensively for a short time and infections were typically confirmed by virologic methods. Panel B and C: estimates from cohort studies in which individuals were followed across entire influenza seasons, and numbers of illnesses assessed in individuals with serologic evidence of infection. Estimates in Panel B were adjusted for rates of symptomatic illness in uninfected persons, and not adjusted in Panel C.



Footnotes: The values for 95% confidence interval ("95% CI") were either supplied from the articles (black) or derived from the point estimates (grey). We cannot derive the 95% CI for Monto *et al.* (34) as the number of individuals who were asymptomatic among infected was not provided. If individual estimates for different subtypes (a-d) or populations (*) from the same study were provided, they were presented separately. Studies by Thai *et al.* (27) and Horby *et al.* (35) were conducted in the same cohort of subjects (#). For some of the studies estimates of the AFs and counts were extracted differently from what have been reported (``) and justifications were given in Table 1. Some studies reported estimates of the AFs with denominator based on person-season of follow up (^). The column "Adjusted" indicates whether estimate of the asymptomatic fraction was adjusted (Y) for rates of symptomatic illness in uninfected persons or not (N), or although not adjusted a separate estimate of the AF was reported for individuals without evidence of laboratory-confirmed influenza virus infections (C). Remarks for each individual study are included in Table 1.

Abbreviations. PCR: reverse transcriptase polymerase chain reaction; HI: hemagglutination-inhibition assay; MN: microneutralization assay; CF: complement fixation assay; culture: viral culture; paired sera: the corresponding serological assay (HI, MN or CF as indicated) was conducted in baseline and convalescent sera; single serum: the corresponding serological assay was conducted in a single serum specimen; +ve: positive.

Table 1. Summary of characteristics of studies included in the review of the asymptomatic fraction (AF) of influenza virus infections

First author, Year	Study Period	Study design	Laboratory method	Infection Definition	Asymptomatic Definition	Influenza types/subtypes	Age	Adjustment/ Comparison with background rates	Remarks
Aho 2010	Nov 2009 - Dec 2009	Serological studies	HI	single serum HI titer ≥ 40	No ARI	H1N1pdm09	20 - 28y	Unadjusted	Reported the estimate of the AF as 50% (=84/169) based on HI titer ≥ 10 as definition for confirmed infection; Estimate of the AF became 51.9% (=40/77) if based on HI titer ≥ 40 as definition for confirmed infection. Therefore we extracted the estimate of the AF as 40/77 (51.9%)
Cowling 2010	Jul 2009 - Aug 2009	Outbreak/ epidemic investigations	PCR	PCR +ve	No ARI	H1N1pdm09, H3N2	All ages	Unadjusted	-
Gill 1976	Apr 1974 - Oct 1974	Serological studies	HI	paired sera HI titer ≥ 4 -fold rise	No ARI	H3N2	4 - 83y	Unadjusted	-
Gill 1977	Apr 1976 - Oct 1976	Serological studies	HI	paired sera HI titer ≥ 4 -fold rise	No symptoms	H3N2	14 - 68y	Unadjusted	Individual-level data on symptoms and age were provided, therefore age-specific AF could be estimated as follows: 0/6 (0%) for 14-19y, 3/26 (11.5%) for 20-49y, 1/17 (5.9%) for $\geq 50y$
Gill 1985	1977/78 and 1980/81 influenza seasons	Serological studies	HI	paired sera HI titer ≥ 4 -fold rise	No symptoms	H1N1(1980/81), H3N2 (1977/78)	11 - 90y	Unadjusted	-
Hall 1973	Nov 1965 - May 1969	Serological studies	CF	paired sera CF titer ≥ 4 -fold rise	No ARI	B	$\geq 1.5y$	Compared	Also reported the estimate of the AF in non-seroconverted individuals was 21/33 (64%)
Hayward 2014	Oct 2006 - Jul 2011	Serological studies	HI	paired sera HI titer ≥ 4 -fold rise	No ARI	H1N1pdm09, H1N1, H3N2, B	$\geq 5y$	Adjusted	Denominator based on person-season of follow-up, with the AF adjusted for background illness: 192 respiratory illnesses over 280 person-seasons of follow-up from 327 participants with serological evidence of infection, versus 623 respiratory illnesses over 1423 person-seasons of follow-up from 1742 participants without serological evidence of infection, i.e. rate of respiratory illness attributable to influenza was 23 (95% CI 13-34) illnesses per 100 person-seasons; Reported insufficient power to test whether the AF varied by age or subtype
Horby 2012	Dec 2007 - Apr 2010	Serological studies	PCR or HI	PCR +ve; OR paired sera HI titer ≥ 4 -fold rise; OR single serum HI titer ≥ 160 (seasonal influenza) or ≥ 80 (H1N1pdm09 in <40y)	No ILI	H1N1pdm09, H1N1, H3N2, B	$\geq 5y$	Unadjusted	Denominator based on person-season of follow-up: 62 respiratory illnesses over 438 person-seasons of follow-up with evidence of infection, from a total of 1,793 person-seasons of follow up from a cohort of 932 participants
Jackson 2011	May 2009 - Jun 2009	Outbreak/ epidemic investigations	HI or MN	paired sera HI/MN titer ≥ 4 -fold rise; OR single serum HI titer ≥ 20 and single serum MN titer ≥ 40	No ARI	H1N1pdm09	All ages	Unadjusted	Each of the 4 asymptomatic infected individuals belonged to each of the 4 age groups (0-9y, 10-18y, 19-54y, $\geq 55y$)
Jaeger 2011	Mar 2009 - May 2009	Serological studies	HI or MN	paired sera HI/MN titer ≥ 4 -fold rise	No ARI	H1N1pdm09	19 - 74y	Unadjusted	Among the 9 seropositive individuals, 6 were identified by seroconversion ≥ 4 -fold, and the remaining 3 were identified by a MN titer of ≥ 40 plus a HI titer of ≥ 20
Khaokham 2013	Feb 2006 - Oct 2009	Serological studies	PCR or HI	PCR +ve; OR paired sera HI titer ≥ 4 -fold rise	No ARI	H1N1pdm09	$\geq 19y$	Compared	Among the 142 confirmed infections, 123 were identified by HI, 16 by PCR, and 3 by both HI and PCR; The reported estimate of the AF was adjusted for age, sex, race, service, rank and shipboard location of exposure to ill persons; Also reported the estimate of the AF in non-infected individuals was 69% (95% CI 64-74%)
LaForce 2007	2000/01 influenza season	Serological studies	PCR or culture or HI	PCR +ve; OR culture; OR paired sera HI titer ≥ 4 -fold rise	No ARI	H1N1, H3N2, B	$\geq 12y$	Unadjusted	Among the 52 confirmed infections, 51 were identified by seroconversion and the remaining case by viral culture in respiratory specimen
Lau 2010	Jan 2008 - Sep 2008	Outbreak/ epidemic investigations	PCR	PCR +ve	No symptoms	H1N1, H3N2, B	All ages	Unadjusted	-
Li 2011	Sep2009 - Oct 2009	Serological studies	HI and MN	single serum HI titer ≥ 40 and single serum MN titer ≥ 20	No symptoms	H1N1pdm09	15 - 21y	Unadjusted	Among the 505 confirmed infections, all were identified by serology except 2 by PCR in throat swab; With 156 asymptomatic patients, in the abstract reported the "asymptomatic infection rate (risk) was 9.9%" with the denominator based on 1570 students who have participated in the survey; but in the full text reported 30.9% with the denominator based on 505 students who have confirmed infection. Therefore we extracted the estimate of the AF as 156/505 (30.9%)
Loeb 2012	Dec 2007 - Jun 2010	Outbreak/ epidemic investigations	PCR	PCR +ve	No symptoms	H1N1, H1N1pdm09, H3N2, B	All ages	Unadjusted	Denominator based on person-season of follow-up: 23 asymptomatic viral shedding episodes among 238 viral shedding episodes from 208 participants; Reported the AF for H3N2 infections was statistically significantly lower than other subtypes (H3N2: 0%, H1N1: 15%, H1N1pdm09: 12%, B: 5%)
Monto 1985	1976/77 and 1977/78 influenza seasons	Serological studies	HI	paired sera HI titer ≥ 4 -fold rise	No symptoms	H3N2 (1977/78), B (1976/77)	All ages	Adjusted	Reported "pathogenicity index", i.e. illness rate in seroconverted individuals minus that in non-seroconverted individuals: 15.1% for influenza A/H3N2 and 33.7% for influenza B. Therefore we extracted the estimates of the AFs as 1-15.1%=84.9% for influenza A/H3N2 and 1-33.7%=66.3% for influenza B. Also reported the age-specific pathogenicity index, and we converted it to AF as below: A/H3N2: 92.9% (0-4y), 75.6% (5-19y), 93.5% (20-49y), 68.6% ($\geq 50y$) B: 90.3% (0-4y), 64.1% (5-19y), 58.6% (20-49y), 86.7% ($\geq 50y$)

Mutsch 2005	Jan 1998 - Mar 2000	Serological studies	HI	paired sera HI titer \geq 4-fold rise	No fever	H1N1, H3N2, B	12 - 83y	Unadjusted	Reported "32/40 (80%) were asymptomatic", but the 40 cases were based on 18 confirmed (paired sera HI titer \geq 4-fold rise) and 22 probable (paired sera HI titer 2-3.9 fold rise) cases. Therefore we extracted the estimate of the AF as 5/18 (27.8%)
Neatherlin 2013	Apr 2009 - Jun 2009	Serological studies	HI and MN	single serum HI titer \geq 20 and single serum MN titer \geq 40	No ARI	H1N1pdm09	All ages	Unadjusted	-
Papenburg 2010	May 2009 - July 2009	Outbreak/ epidemic investigations	PCR	PCR +ve	No symptoms	H1N1pdm09	All ages	Unadjusted	Reported the estimate of the AF as 5/53 (9.4%) based on the result from MN in paired sera or/and PCR in nasopharyngeal swabs, but only individuals \geq 7y provided paired sera while all individuals provided swabs. Therefore we extracted the estimate of the AF based on PCR-confirmed infection only which was 3/45 (6.7%); The estimate of the AF based on seroconversion was 3/36 (8.3%)
Riley 2011	Jul 2009 - Feb 2010	Serological studies	MN	paired sera MN titer \geq 4-fold rise	No ARI	H1N1pdm09	\geq 3y	Compared	Also reported the age-specific AF: 10/41 (24.4%) for 0-18y, 19/45 (42.2%) for \geq 19y; Also reported the estimate of the AF in non-seroconverted individuals: 28/57 (49.1%) for 0 - 18y, 423/627 (67.5%) for \geq 19y, i.e. 451/684 (65.9%) for all ages
Sagrera 2002	Feb 1999 - Oct 1999	Outbreak/ epidemic investigations	culture	culture	No symptoms	A (subtype not reported)	<1y	Unadjusted	-
Simmerman 2011	Apr 2008 - Aug 2009	Outbreak/ epidemic investigations	PCR or HI	PCR +ve; OR paired sera HI titer \geq 4-fold rise	No symptoms	H1N1, H1N1pdm09, H3N2, B	All ages	Unadjusted	Among the 343 confirmed infections, 309 were identified by PCR and 34 by HI; Also reported symptomatic cases (mean age 30y) were significantly older than asymptomatic cases (38y)
Smit 2012	Aug 2009 - early 2010	Outbreak/ epidemic investigations	PCR or HI	PCR +ve; OR paired sera HI titer \geq 4-fold rise	No ARI	H1N1pdm09	\geq 18y	Unadjusted	Among 3 seroconverted non-vaccinated individuals, 2 were asymptomatic. The remaining symptomatic individual was also positive by PCR, did not meet the criteria for influenza-like illness but reported some influenza-like signs and symptoms (cough, headache and malaise), therefore we extracted the estimate of the AF as 2/3 (66.7%) using no ARI as the definition for asymptomatic instead of no ILI as was used in the study
Suess 2010	Apr 2009 - Aug 2009	Outbreak/ epidemic investigations	PCR	PCR +ve	No symptoms	H1N1pdm09	All ages	Unadjusted	-
Suess 2012	Jan 2008 - Apr 2011	Outbreak/ epidemic investigations	PCR	PCR +ve	No ILI	H1N1, H1N1pdm09, H3N2, B	All ages	Unadjusted	Also reported the age-specific AF: 0/21 (0%) for children <14y, 9/44 (21%) for adults \geq 14y
Tandale 2010	Sep 2009 - Oct 2009	Serological studies	HI	single serum HI titer \geq 40	No ILI	H1N1pdm09	All ages	Unadjusted	Reported "Almost 90% pandemic H1N1 infections were asymptomatic or mild"
Thai 2014	Sep - Dec 2009	Outbreak/ epidemic investigations	PCR	PCR +ve	No ARI	H1N1pdm09	All ages	Unadjusted	This study included a subset of the subjects from Horby et al. AJE 2012, but in the present study only PCR-confirmed infection (but not by seroconversion) was used for the estimation of the AF
Toyokawa 2011	Jun 2009 - Jul 2009	Serological studies	HI	single serum HI titer \geq 40	No fever	H1N1pdm09	29 - 49y	Unadjusted	Reported most of the seropositive HCWs were asymptomatic (only 1 had fever out of the 14 seropositive), but 8 out of the 14 seropositive had received chemoprophylaxis including the individual with fever. Therefore we extracted the estimate of the AF as 6/6 (100%)
Wang 2010	2005/06 influenza season	Serological studies	HI	paired sera HI titer \geq 4-fold rise	No ILI	H1N1, H3N2	9 - 11y	Adjusted	The estimate of the AF was adjusted by log-linear binomial regression model accounting for the probability of not showing symptoms because of other pathogens
Williams 2010	Jan 2007 - Mar 2007	Serological studies	HI	paired sera HI titer \geq 4-fold rise	No ARI	H1N1, H3N2, B	Adults	Unadjusted	-