Virus detection and its association with symptoms during influenza-like illness in a sample of healthy adults enrolled in a randomised controlled vaccine trial

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Background Viral respiratory infections are associated with significant morbidity and mortality. Many new aetiological agents have been described recently.

Objectives We looked for respiratory viruses in a population-based sample of healthy adults with influenza-like illness (ILI). We investigated host and spatio-temporal associations with virus isolation and host, spatio-temporal and virus associations with self-reported symptoms.

Patients/Methods We recruited 586 participants experiencing 651 illness episodes from a population of healthy adults enrolled in an influenza vaccine effectiveness trial. At ILI assessment visits, a respiratory swab was collected and tested for viruses using a combination of polymerase chain reaction (PCR) assays. Participants also completed a questionnaire detailing their clinical course in 336 episodes.

Results Of 643 samples analysed, a virus was identified in 44%. Half were picornaviruses, with influenza and coronaviruses the next most common. Individuals with influenza were significantly less likely to have been immunised than the reference (virus negative) population (OR = 0·52 (0·31, 0·87) $P = 0·01$).
The mean symptom score (95% CI) reported by individuals with influenza was significantly higher than in all other episodes [Influenza: 10·2 (9·4, 10·9); Other: 7·4 (7·2, 7·7); Difference (95% CI): 2·5 (1·5, 3·5); $P < 0·001$]. In an analysis restricted to influenza-positive cases, the symptom score was not attenuated by vaccination.

Conclusions Our findings indicate that a greater number of symptoms are displayed by individuals presenting with influenza confirmed ILI compared with other agents that cause ILI. While influenza vaccination reduced the probability of influenza virus detection, symptom score for influenza-positive ILI was not attenuated.

Keywords Epidemiology, human, influenza, influenza vaccines, picornavirus infection, respiratory tract infection.

Introduction

Viral infections of the respiratory tract are responsible for significant morbidity and mortality worldwide.[1] Influenza viruses, rhinoviruses (HRVs), adenoviruses, respiratory syncytial virus (RSV) and parainfluenza viruses (PIVs) are the most common aetiological agents in such infections.[2,3] Most of the burden of disease caused by these viruses occurs at the extremes of age, with hospitalisation rates among preschool-aged children similar to those in the elderly.[4] The annual estimated cost of influenza alone to the Australian healthcare system is $115 million.[5] Increased recognition of the burden of influenza morbidity and mortality has resulted in broadening of recommendations for influenza vaccination in the United States[6] and Canada.[7,8]

In recent decades, extensive studies have identified hitherto unknown agents that are aetiological in acute respiratory tract infections (ARIs). Associated developments in technologies for virus detection[9] have facilitated their epidemiological characterisation. Despite these advances, it

remains the case that no known respiratory virus can be identified in 30–40% of all ARI episodes, suggesting that additional respiratory pathogens are likely to exist.[10,11] In support of this notion, a number of previously undescribed viruses have been identified by analysis of clinical specimens from the human respiratory tract since 2001: human metapneumovirus,[12] SARS coronavirus,[13] coronavirus NL63,[14] coronavirus HKU1,[15] human bocaviruses,[16] novel rhinoviruses[3] and the recently described KI and WU polyomaviruses.[17,18]

Several of the authors have previously reported on the community epidemiology of viral acute respiratory infections in a cohort of children <5 years of age.[19] Within a large, industry-sponsored vaccine trial, we conducted a study of the viral aetiology of respiratory tract infections experienced by healthy, community dwelling adults reporting symptoms of influenza-like illness (ILI).

Methods

Study population

A total of 7544 healthy, non-pregnant adults aged from at least 18 years to <65 years were enrolled in a placebo-controlled phase IV efficacy trial of a licensed seasonal trivalent influenza vaccine (Fluvax®, CSL Ltd, Parkville, Vic., Australia), conducted between March and November 2008 at 21 study sites in Australia and New Zealand (Clinicaltrials.gov, #NCT00562484). Main exclusion criteria were the following: allergy to any of the vaccine components; medically unstable clinical condition; planned or current pregnancy; lactation; history of Guillain–Barré Syndrome; confirmed or suspected immunosuppressive condition; current or recent immunosuppressive therapy; concurrent participation in a clinical trial or use of an investigational compound; or recommended for seasonal influenza vaccination according to guidelines in Australia[20] or New Zealand.[21] Following provision of written informed consent, participants were randomly assigned to receive vaccine or placebo in a 2:1 ratio.

They were reminded weekly to report symptoms of ILI, commencing 14 days after vaccination and ending on the 30 November 2008. Participants meeting the ILI case definition of at least one respiratory symptom (cough, sore throat, runny nose or nasal congestion) and at least one systemic symptom (fever ≥37·8°C, feverishness, chills or myalgia) were asked to present for collection of respiratory swabs for influenza detection.

Within the industry-sponsored RCT, we nested a cohort for this study, involving a denominator population of 4868 participants from 12 Australian study centres (Figure 1). All participants from these sites presenting for ILI assessment were invited to consent to participation in this 'virus substudy', which involved collection of one extra respiratory swab.

Both the primary phase IV efficacy trial and the substudy were ethically approved at each of the 12 participating substudy sites (as listed in the Acknowledgements). At some substudy sites, participants were reimbursed for time and inconvenience according to approved practices by their respective ethics committees.

Study procedures

At the ILI visit, a nose or throat specimen was collected using a COPAN™ dry flocked swab and initially transported to a local holding laboratory where it was frozen. Specimens were returned, together with those collected for the primary study, to CSL Ltd's laboratories (COPAN Diagnostics Inc., Murrieta, CA, USA) in Parkville and shipped on dry ice in several consignments to the Queensland Paediatric Infectious Diseases Laboratory. Samples were tested for viruses including: influenza A and B, RSV, parainfluenza viruses (1,2,3), adenoviruses, human metapneumovirus (hMPV)[22] picornaviruses,[23,24] bocaviruses,[25] coronaviruses (OC43, 229E, NL63 and HKU1)[26,27] and KI and WU polyomaviruses [28] using a combination of multiplexed and uniplexed conventional and real-time polymerase chain reaction (PCR) assays.

Participants were given a questionnaire requesting information about the clinical course of their illness including: symptoms experienced along with any complications, medical treatment sought, interruption to usual activities and duration of the episode. A reply paid envelope was provided within which to return the questionnaire to the Vaccine and Immunisation Research Group in Melbourne.

Outcome measures

The primary unit of analysis was the ILI episode, with potential for individuals to have repeated illness presentations over the course of the study. All events were assumed to be independent. For each episode, we considered:

Virus detection

The presence or absence of detectable virus in a respiratory specimen was categorised as a binary outcome measure. Associations were sought between virus detection and a number of participant (age, sex, race, smoking and vaccine status) and spatio-temporal (month and study site) variables, using multivariate logistic regression modelling.

Virological results were then organised into broad groupings: none (no virus detected), picornaviruses, influenza viruses (A and B), coronaviruses and other (hMPV, parainfluenza viruses, RSV, adenoviruses, bocaviruses and polyomaviruses). For each virus group, associations with host and external variables were described.
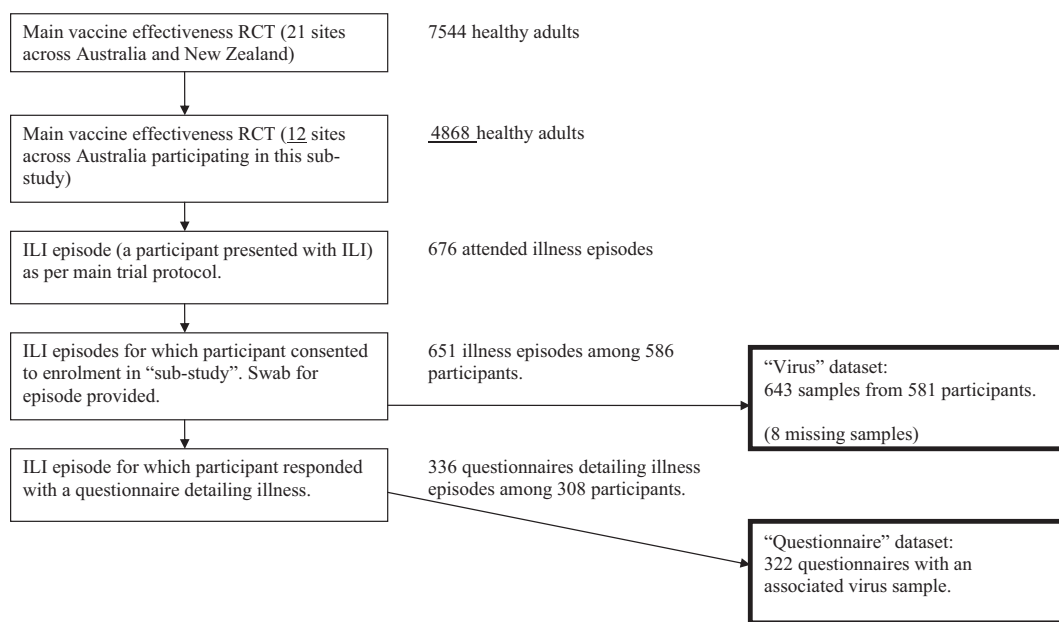
Figure 1. Flow diagram for influenza-like illness episode (by participant) inclusion in the substudy.

Symptom score

The symptom scores developed by Hayden *et al.*[29] in trials of the influenza drug Oseltamivir, while ideal, were considered too labour intensive to be applied in this protocol. We therefore sought to encapsulate the most comprehensive range of symptoms possible for characterisation and comparison of illness episodes. For participants who returned the questionnaire, a 'symptom score' was calculated by scoring one point for each symptom, ranging from a minimum of 2 (necessary to meet the case definition) to a maximum of 16. Associations between this score and the virus isolated (by group), host factors (age, sex, race and smoking) or spatio-temporal (month and study site) variables were considered using multivariate linear regression models. Vaccination status of participants was not (and cannot be) included in the regression models because of its known mitigating effect on the likelihood of influenza acquisition (seeResults).

Measures of episode severity including medically diagnosed complications, medical attendance (and practitioner type), prescription of antiviral agents, duration of episode and number of days away from usual activity as a result of illness were reported.

All statistical analyses were conducted using stata/ic 11·1 (StataCorp LP, College Station, TX, USA).

Results

Study population

Consent to participate in the substudy was obtained during 651 of 676 attended illness episodes in the main industry-sponsored RCT. The 651 episodes occurred in 586 participants. As eight specimens were missing, only 643 samples from 581 participants were included in the analysis of virus isolation (Virology Data set). Self-completed questionnaires detailing the clinical course of 336 illness episodes were returned by 308 participants. A virus sample was associated with 322 of these questionnaires (Questionnaire Data set) (Figure 1).

Demographic characteristics of individual participants in each of these two study populations are summarised in Table 1. A number of participant factors were associated with increased probability of completion of the questionnaire: female sex (RR = 1·13 (0·99, 1·30), $P = 0·07$), older age ($P < 0·001$ in an omnibus test across age categories versus questionnaire completion) and vaccination (RR = 1·13 (1·00, 1·28), $P = 0·05$). Similarly, an omnibus test for an association with smoking is statistically significant ($P = 0·001$) but the proportion who have 'never' smoked is very similar (160 of 274 (58·4%) versus 186 of 307 (60·6%)) in the two data sets.

Virus detection

A known virus was identified from 284 of 643 (44·2%) specimens in the Virology Data set. These viruses are listed in Table 2, together with groupings for the purpose of further analysis given small numbers in many instances. The overall distribution of viruses isolated (by group) varied substantially by month of collection, with picornaviruses the most common in May, coronaviruses isolated predominantly in June–August and influenza viruses detected primarily in August–September (Figure 2).

Table 1. Characteristics of study participants

Characteristics	Virology data set n (%)	Questionnaire data set n (%)
Total participants	581 (100)	308 (100)
Sex (female)	343 (59·0)	193 (62·7)
Race		
Caucasian	525 (90·4)	279 (90·6)
Asian	40 (6·9)	20 (6·5)
Other/Unknown	16 (2·8)	9 (2·9)
Smoking status		
Current	76 (13·1)	26 (8·4)
Ever	149 (25·7)	93 (30·2)
Never	346 (59·6)	184 (59·7)
Unknown	10 (1·7)	5 (1·6)
Vaccine recipients	374 (64·4)	211 (68·5)
Age (years)		
18–24	192 (33·0)	89 (28·9)
25–34	115 (19·8)	39 (12·7)
35–44	110 (18·9)	70 (22·7)
45–54	94 (16·2)	59 (19·2)
55–64	70 (12·0)	51 (16·6)

Table 2. Viruses identified from influenza-like illness swabs, by PCR

Virus group	PCR result	Virology data set (n = 643 specimens)	
None	No virus detected	359	55·8
Picorna	Picornaviruses	144	22·4
Flu	Influenza A	29	4·5
	Influenza B	39	6·1
Corona	Coronaviruses	35	5·4
Other	Human metapneumovirus	11	1·7
	Parainfluenza viruses	11	1·7
	Respiratory syncytial virus	8	1·2
	Adenovirus	3	0·5
	Other viruses*	4	0·6

*Other viruses include bocaviruses, polyomaviruses.

Associations were sought between a range of participant variables and positive virus identification considering the illness episode as the primary unit of analysis, adjusted for month of collection and study site (Table 3). Adults aged 25–34 years and 45–54 years were significantly less likely to have a virus detected by PCR than the reference age group of 18–24 years. Specimen positivity was not associated with the time gap between ILI onset and specimen collection and was consistent throughout the study period, with no effect of month observed. Of note, three of the four highest recruiting sites reported significantly lower adjusted odds ratios for a detectable virus, with between 35·6% and 41·6% of specimens returning a result compared with the fourth (the reference site) where 53 of 103 (51·5%) of specimens tested positive (data not shown).

As can be seen from Table 4, participant characteristics were not associated with the detection of one virus group over another, with the predictable exception of influenza vaccination status. In a univariate logistic regression model with vaccination status as the outcome variable, individuals testing positive for influenza viruses were significantly less likely to have been immunised than the reference (virus negative) population (OR = 0·52 (0·31, 0·87) P = 0·01), indicating a protective effect (against ILI) of the vaccine. There was also a trend towards higher immunisation coverage among those in whom picornaviruses were detected (OR = 1·5 (0·99, 2·3) P = 0·06). Given no association between the reference (virus negative) and vaccination [OR = 0·92 (0·67, 1·3) P = 0·6], this finding indicates an association between picornavirus detection and vaccinated participants. Prior to the influenza season (defined as months August and September), this association between picornavirus detection and influenza vaccination was not evident [OR = 1·3 (0·73, 2·3) P = 0·38], while during and following the season the association was stronger [OR = 2·0. (1·1, 3·6) P = 0·02].

Recorded symptoms

Individual symptoms reported by participants at each episode are summarised in Table 5, along with medically diagnosed complications of ILI. Twenty-eight participants experienced repeated infections over the study period (two episodes in 25, three episodes in three subjects). The same virus group (picornaviruses) was only isolated on more than one occasion from two participants, in each instance more than 6 weeks apart, justifying their consideration as independent events. The median duration of illness was 6 days (range 1, 51) with no significant variation by virus type (data not shown). The median time spent away from usual activity was 2 days (range 0, 14), with an increase in the mean of 1·3 days (0·5, 2·2; P = 0·001) in episodes associated with isolation of influenza compared to episodes with virus-negative isolates in a univariate linear regression model. In all, 94 episodes were medically attended, of which 83 were visits to the general practitioner. No association with virus type was observed (logistic regression, data not shown). Thirteen participants were prescribed antiviral medication; none were hospitalised. Small numbers of recorded medically diagnosed complications (Table 5) prevented any detailed analysis of associations with virus isolation, but we note a weak suggestion that bronchitis (9%) and 'chest infection' (12%) were more common in participants with influenza than overall (cf, 2·4% and 7·4%, Table 5).
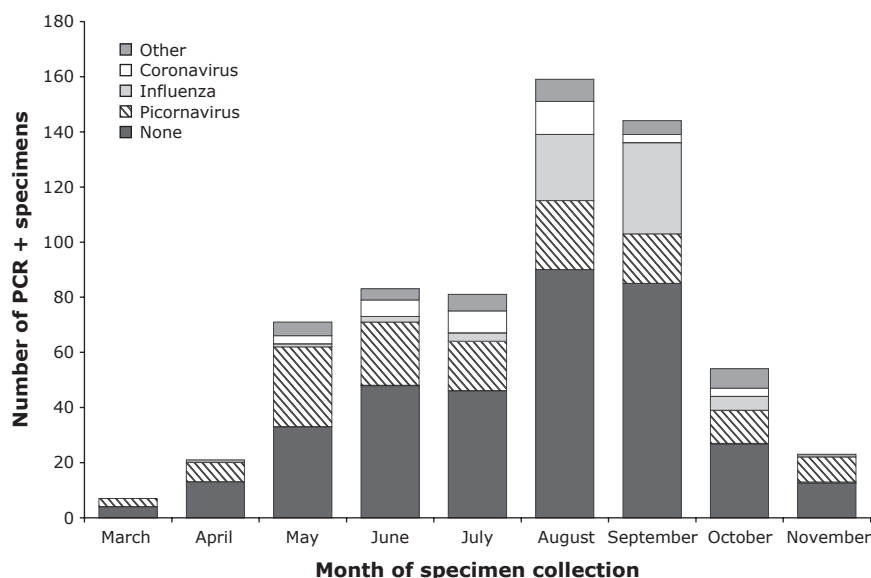
Figure 2. Viruses identified, by month of specimen collection.

Table 3. Multivariate logistic regression model, reporting associations with positive virus detection in respiratory specimens

Variable	Adjusted OR* (95% CI)	*P*-value	Virus not detected *n* (%)	Virus detected *n* (%)
Age (ref: 18–24 years)				
18–24 years	1	–	113 (31·5)	103 (36·2)
25–34 years	**0·51 (0·31, 0·83)**	**0·01**	**84 (23·4)**	**47 (16·5)**
35–44 years	0·93 (0·57, 1·5)	0·8	60 (16·7)	63 (22·2)
45–54 years	**0·52 (0·30, 0·90)**	**0·02**	**60 (16·7)**	**40 (14·1)**
55–64 years	0·61 (0·33, 1·1)	0·1	42 (11·7)	31 (10·9)
Sex (ref: male)				
Male	1	–	155 (43·2)	112 (39·4)
Female	1·2 (0·88, 1·7)	0·2	204 (56·8)	172 (60·6)
Race (ref: Caucasian)				
Caucasian	1	–	327 (91·1)	252 (88·7)
Asian	1·4 (0·72, 2·6)	0·3	23 (6·4)	23 (8·1)
Other	1·5 (0·56, 4·2)	0·4	9 (2·5)	9 (3·2)
Smoking status (ref: Current)				
Current	1	–	43 (12·0)	36 (12·7)
Ever	0·88 (0·49, 1·6)	0·6	91 (25·3)	77 (27·1)
Never	0·70 (0·41, 1·2)	0·2	217 (60·4)	169 (59·5)
Unknown	0·16 (0·03, 0·88)	0·04	8 (2·2)	2 (0·7)
Vaccinated (ref: placebo)				
Placebo	1	–	132 (36·8)	99 (34·9)
Vaccine	1·1 (0·77, 1·55)	0·6	227 (63·2)	185 (65·1)

*Adjusted for month of collection and study site. Bold text indicates *P* < 0.05.

Observed values for the symptom score were normally distributed, with a mean of 7·7 (SD 2·5) and median of 7 (range 2–15). Associations were sought between this continuous outcome and virus and participant characteristics in a multivariate linear regression model, adjusted for month and site of specimen collection (Table 6).

The mean symptom score reported by individuals with influenza was significantly higher than that observed in ILIs attributed to other infectious causes, or where no virus was detected. This score was not attenuated by vaccination status, in a post hoc subgroup analysis restricted to influenza-positive episodes [Vaccinated mean score: 9·9,

Table 4. Characteristics of participants, by virus group

Variable	No virus detected (n = 359)	Picornaviruses (n = 144)	Influenza A&B (n = 68)	Coronaviruses (n = 35)	Other viruses (n = 37)
Mean age (SD) in years	35·5 (13·7)	34·0 (13·2)	33·6 (13·1)	37·9 (16·3)	38·8 (14·2)
Sex (% female)	56·8	61·8	53·0	60·0	70·3
Race					
Caucasian	91·1%	87·5%	94·1%	85·7%	86·5%
Asian	6·4%	9·0%	3·0%	8·6%	13·5%
Other	2·5%	3·5%	2·9%	5·7%	0%
Smoking status					
Current	12·0%	11·8%	11·8%	11·4%	18·9%
Ever	25·4%	23·6%	30·9%	28·6%	32·4%
Never	60·4%	63·9%	55·9%	60·0%	48·7%
Unknown	2·2%	0·7%	1·5%	0%	0%
% Vaccinated	63·2%	72·2%	47·1%*	60·0%	75·7%

*$P < 0.05$ as assessed by univariate regression (linear, ordinal or logistic as appropriate).

Table 5. Reported symptoms and complications in 336 illness episodes

Symptoms	n (%)
Fever (≥37·8°C)	136 (40·4)
Feverish	214 (63·5)
Chills	199 (59·1)
Myalgia	272 (80·7)
Headache	253 (75·1)
Less active	241 (71·5)
Irritability	89 (26·4)
Cough	234 (69·4)
Productive cough	106 (31·5)
Shortness of breath	64 (19·0)
Wheeze	64 (19·0)
Sore throat	261 (77·4)
Blocked nose	196 (58·2)
Runny nose	241 (71·5)
Vomiting	20 (5·9)
'Pneumonia'	1 (0·3)
Complications	
Otitis media	4 (1·2)
Sinusitis	12 (3·6)
Bronchitis	8 (2·4)
Chest infection	25 (7·4)
Pneumonia	1 (0·3)

Unvaccinated mean score: 10·3; Difference (95% CI): −0·38 (−1·9, 1·1); $P = 0.61$]. Asian participants reported significantly fewer symptoms than Caucasians for non-influenza presentations [Asian mean score: 6·30, Caucasian mean score: 7·52; Difference (95% CI):−1·2 (−2·2, 0·20); $P = 0.02$]. No Asian participants who had influenza com-

pleted the questionnaire, preventing further analysis. Symptom scores were consistent throughout the year, with no effect of month of presentation observed. Participants at two study sites reported significantly higher symptom scores than at other centres – on further inspection, these results were likely due to small numbers, involving a total of 25 participants, of whom three had influenza.

Discussion

Over the course of the study period, approximately 10% of participants presented for ILI evaluation and half of these provided additional information on the course of their disease. A known virus was identified in 44% of samples, with varying detection rates by age and site. For isolates with undetectable virus, we cannot distinguish between inadequate sampling from the participant, issues relating to transport of the sample, or presence of an unknown virus. Picornaviruses were the most common viruses observed followed by influenza. While influenza was only confirmed in approximately 2% of the placebo-group participants, the 2008 influenza season in Australia was particularly mild, following on from the notably severe 2007 seasonal epidemic.[30] Given this, however, we do acknowledge that under-ascertainment may have occurred as a result of sub-clinical infections and potential failure of participants to report mild symptoms, despite intensive follow-up.

Consistent with expectations and findings in healthy children in which influenza was associated with higher severity,[31] here influenza was associated with a higher composite 'symptom score'. Vaccination provided partial protection against ILI associated with influenza, but there was no evidence of disease modification (as measured by

Table 6. Multivariate linear regression model, reporting associations between virus group, participant characteristics and symptom score

Variables	Mean Score (95% CI)	Adjusted coefficient* (95% CI)	*P*-value
Virus Group (ref: None)			
None	7·5 (7·1, 7·8)	Ref	–
Picorna	7·4 (6·8, 8·0)	−0·04 (−0·72, 0·65)	0·9
Influenza	**10·2 (9·4, 10·9)**	**2·6 (1·6, 3·6)**	**<0·001**
Corona	7·3 (6·2, 8·3)	−0·11 (−1·5, 1·3)	0·9
Other	7·7 (6·5, 8·9)	0·26 (−0·85, 1·4)	0·6
Age (ref: 18–24 years)			
18–24 years	7·9 (7·4, 8·3)	Ref	–
25–34 years	7·6 (6·9, 8·3)	−0·37 (−1·3, 0·55)	0·4
35–44 years	7·6 (7·0, 8·2)	−0·48 (−1·3, 0·34)	0·3
45–54 years	7·5 (6·9, 8·2)	−0·21 (−1·1, 0·69)	0·6
55–64 years	8·0 (7·1, 8·8)	0·14 (−0·83, 1·1)	0·8
Sex (ref: male)			
Male	7·7 (7·2, 8·1)	Ref	–
Female	7·7 (7·4, 8·1)	0·40 (−0·19, 1·0)	0·2
Race (ref: Caucasian)			
Caucasian	7·8 (7·5, 8·1)	Ref	–
Asian	**6·3 (5·3, 7·3)**	**−1·4 (−2·5, -0·26)**	**0·02**
Other	7·1 (5·5, 8·8)	−0·34 (−2·2, 1·5)	0·7
Smoking status (ref: Current)			
Current	7·6 (6·6, 8·7)	Ref	–
Ever	7·9 (7·4, 8·4)	0·07 (−1·0, 1·2)	0·9
Never	7·6 (7·2, 7·9)	−0·01 (−1·1, 1·0)	1·0
Unknown	9·6 (4·7, 14·5)	−0·43 (−3·4, 2·6)	0·8

*Adjusted for month of collection and study site. Bold text indicates *P* < 0.05.

symptom score) in participants with breakthrough symptomatic infection. We speculate that this may be due to infection with a strain of influenza not covered by the vaccine. In a further study of household transmission (in preparation) using the Questionnaire data set, we find further evidence consistent with this hypothesis, although without further virus typing, cannot subject our hypothesis to further scrutiny.

The observed association between vaccination and isolation of picornaviruses perhaps suggests that picornaviruses are filling the niche vacated by exclusion of influenza owing to vaccination, consistent with the hypothesis of viral interference.[32] Support for the association is present during and following the influenza season but not prior to the season, consistent with an interference effect, although we cannot exclude other explanations, including simple statistical fluctuation.

Our study was conducted within a large, community dwelling cohort of healthy adults and used a non-specific and sensitive (i.e. inclusive) ILI case definition to ascertain presentations across the full spectrum of symptomatic disease.[33] It therefore complements protocols that have focused on individuals with underlying respiratory disorders presenting for medical care in outpatient or hospital settings,[34] to provide a more comprehensive picture of the spectrum of symptomatic disease. The diagnostic methods (PCR) employed were of high specificity and allowed us to test for a large number of recently described pathogens, reducing the probability of returning a negative result.

As the study was conducted within an industry-sponsored trial of vaccine effectiveness, it was necessarily constrained by the primary protocol to some degree. Participants were selected to be 'healthy', without underlying conditions that would have resulted in a routine recommendation for annual influenza vaccination, given the placebo-controlled study design. The study population varies from the general population, being younger, less likely to smoke and more likely to be women. Furthermore, the study population who returned a questionnaire, enabling an examination of symptom score, was further biased towards women and those 35 years and over. Two-thirds of participants were immunised against influenza, associated with the anticipated reduction in influenza cases in the vaccinated proportion. Swabs were only collected from individuals presenting for ILI evaluation, and not from a 'control' population. This limited our ability to

assert pathogenicity of the viruses detected and restricts our comparison of 'symptom scores' by virus to only those infection events in which participants met (and reported) the ILI case definition.

A study conducted using a General Practice sentinel surveillance scheme in Victoria, Australia in the same year (2008)[35] showed a similar overall rate for virus detection (43%) in those ILI's submitted for swabbing (46% of all ILIs reported by the GPs in the study), despite a smaller number of viruses tested for. In that study, 29% of all specimens were positive for influenza, compared with 11% here. The mix of viruses detected in the 83 participants in our study who presented to a GP was similar to the full data set (data not shown). These discrepancies perhaps reflect the non-specific nature of the ILI definition used in our study or a potential bias in the ILIs chosen to be swabbed by the GPs in the study by Grant et al.[35]

Compared with a case–control study of respiratory viruses involved with exacerbations of chronic obstructive pulmonary disease (COPD) conducted in Melbourne, Australia, our overall rate of virus detection was higher (21% of exacerbations in that study) and the distribution of viruses, with a preponderance of picornaviruses and influenza similar.[36] That study confirmed far greater odds of virus detection in individuals with symptoms than matched controls, providing prior evidence of the aetiological role of viruses in illness exacerbations, as did a similar study in Germany where picornaviruses, influenza and RSV most commonly caused disease.[37] Among patients with COPD in Belfast, rhinoviruses and adenovirus were most frequently associated with increasing respiratory difficulty.[38] A study in a similar patient population in Hong Kong revealed a far lower burden of picornaviruses (rhinovirus), perhaps reflecting true differences in epidemiology, or differing sensitivity of detection methods.[39]

Our findings add to an evolving knowledge base regarding the experience of a range of respiratory viruses in Australia.[19,40] In particular, they confirm a distinction between influenza and other pathogens (as measured by a symptom score) even among healthy individuals, which has led to calls for expansion of funded immunisation recommendations in Australia to reduce the spread of disease.[41] More data such as ours are required to fill existing knowledge gaps regarding the community burden of disease to inform more robust estimates of the likely cost-effectiveness of such strategies.[42]

In further work, we will consider the household-level impact of infection introduction, by examining patterns of secondary transmission described in participant questionnaires. Having identified specimens testing negative for all known viruses, the next phase of this project involves discovery of hitherto unknown respiratory viruses using broad-range viral molecular detection methods.

Authorship addendum

Acknowledgements

Conflict of interest

currently and previously has been the principal investigator for clinical trials sponsored by Abbott, Baxter, CSL, GSK, MedImmune, Merck, Novartis, Sanofi-Pasteur, Wyeth, and Pfizer.

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