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Contribution of influenza viruses, other respiratory viruses and virus co-infections to influenza-like illness in older adults

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Abstract:	Background Influenza-like illness (ILI) can be caused by a range of respiratory viruses. The present study investigates the contribution of influenza, other respiratory viruses and virus co-infections in older adults presenting with ILI. Methods Following the two previous studies on ILI in the Netherlands, a third prospective

	observational study was performed for influenza season 2014-2015, comprising 2,366 older adults (≥ 60 years). Viruses were identified by multiplex ligation–dependent probe-amplification assay. In addition, viral co-infections among older adults were investigated. Results Influenza virus was the most frequently detected pathogen in ILI cases (39.4%). Other viruses that were often detected: rhinovirus, coronavirus, respiratory syncytial virus, and human metapneumovirus. The proportion of ILI cases was independent of influenza vaccination and vaccine effectiveness. Co-infections of influenza virus with other viruses were rare. Conclusions In line with our previous studies in seasons with high and moderate vaccine effectiveness, we show here that, also in an influenza season with vaccine mismatch, vaccination does not affect the total number of ILI cases in older adults. In addition, the low rate of viral co-infections observed, especially with influenza virus, indicates that influenza virus infection reduces the risk of simultaneous infection with other viruses. Clinical Trials Registration NTR4818
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Cover Letter

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Dear editor,

On behalf of all co-authors, I would like to submit our manuscript entitled "Contribution of influenza viruses, other respiratory viruses and virus co-infections to influenza-like illness in older adults" for consideration in The Journal of Infectious Diseases as a Major Article.

Data on influenza-like illness (ILI) and influenza virus infection in older adults in the community – the target group for influenza vaccination in most countries – is scarce. In the present study among 2,366 older adults (≥ 60 years), influenza virus was the most frequently detected pathogen in ILI cases (39.4%). Other viruses that were often detected: rhinovirus, coronavirus, respiratory syncytial virus, and human metapneumovirus. In line with our previous studies in seasons with high and moderate vaccine effectiveness, we show here that, also in an influenza season with vaccine mismatch, vaccination does not affect the total number of ILI cases in older adults. Furthermore, we found a low rate of viral co-infections, especially with influenza virus, indicating that influenza virus infection reduced the risk of simultaneous infection with other viruses. Interestingly, in light of the current COVID-19 pandemic, our study also showed that co-infections with coronavirus are rare, which may be indicative for the occurrence of viral interference of coronaviruses with other viruses. In the discussion, we speculate that certain respiratory viruses may inhibit or delay circulation of SARS-CoV-2.

The data presented in this manuscript are original and all authors agree with the submission and accept responsibility for the content. The manuscript is not submitted or accepted elsewhere for publication. We kindly ask you to consider this manuscript for publication.
Suggestions for potential reviewers for this manuscript are listed below.

Disclosure: This is an original manuscript of us, and is unpublished work that is not under consideration elsewhere. No writing assistance was provided in the preparation of the manuscript.
All authors contributed significantly, 5.1.2e wrote first draft of the manuscript that was critically reviewed by 5.1.2e designed the clinical tria 5.1.2e repared clinical documents and coordinated the clinical trial, for which 5.1.2e was responsible. 5.1.2e performed laboratory analysis. 5.1.2e nterpreted laboratory results, 5.1.2e conducted statistical analysis.
All authors approved the final version that was submitted for publication.
None of the authors has conflicts of interest to disclose.

If the manuscript is considered suitable for The Journal of Infectious Diseases, we suggest the following reviewers:

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Abstract

Background. Influenza-like illness (ILI) can be caused by a range of respiratory viruses. The present study investigates the contribution of influenza, other respiratory viruses and virus co-infections in older adults presenting with ILI.

Methods. Following the two previous studies on ILI in the Netherlands, a third prospective observational study was performed for influenza season 2014-2015, comprising 2,366 older adults (≥ 60 years). Viruses were identified by multiplex ligation–dependent probe-amplification assay. In addition, viral co-infections among older adults were investigated.

Results. Influenza virus was the most frequently detected pathogen in ILI cases (39.4%). Other viruses that were often detected: rhinovirus, coronavirus, respiratory syncytial virus, and human metapneumovirus. The proportion of ILI cases was independent of influenza vaccination and vaccine effectiveness. Co-infections of influenza virus with other viruses were rare.

Conclusions. In line with our previous studies in seasons with high and moderate vaccine effectiveness, we show here that, also in an influenza season with vaccine mismatch, vaccination does not affect the total number of ILI cases in older adults. In addition, the low rate of viral co-infections observed, especially with influenza virus, indicates that influenza virus infection reduces the risk of simultaneous infection with other viruses.

Clinical Trials Registration. NTR4818

Keywords: influenza virus infection; influenza-like illness; respiratory viruses; viral co-infections; viral interference; older adults

Background

Influenza is responsible for major morbidity and mortality burdens worldwide [1]. Elderly are at increased risk for severe disease and complications upon infection with influenza virus, and vaccination is considered an important measure to prevent this [1]. In the Netherlands, routine seasonal influenza vaccination is offered to all older adults aged ≥ 60 years and to persons with certain chronic comorbidities. In two previous studies, we observed that influenza vaccination reduced the incidence of seasonal influenza virus infections in older adults, but did not reduce the overall incidence of influenza-like illness (ILI) cases in vaccinees [2]. The influenza vaccination reduced the number of ILI cases caused by influenza virus infection is offset by a rise in the number of cases caused by other viruses, such as rhinovirus, coronavirus, human metapneumovirus (hMPV), or respiratory syncytial virus (RSV) [2]. Similar to previous seasons [2], in this third prospective observational study, nasopharyngeal and oropharyngeal swabs were taken from 2,366 participants with self-reported ILI, defined by fever (≥37.8°C) with at least one other symptom of coughing, headache, myalgia, sore throat, rhinitis, or chest pain [3]. Swabs were used to determine the relative contribution of influenza virus versus other respiratory viruses to ILI in influenza season 2014-2015. In addition, to be able to investigate the persistence of viral infections, swabs were taken again two weeks and eight weeks after ILI onset. Some studies have shown a high rate of virus/virus co-infection in children with respiratory illness [4-6], but data on the occurrence of viral co-infections in older adults is scarce. For this reason, we also studied the occurrence of viral co-infections in older adults in both the 2014-2015 and 2012-2013 influenza season.

Methods

Study design

This prospective observational study in community-dwelling older adults, aged ≥ 60 years, was conducted from October 2014 through April 2015 in the Netherlands. Participants of the previous study (2012-2013) were re-invited and additional participants were recruited through the Civil Registry to reach the sample size of approximately 200 ILI cases [2]. Participants were part of the study for the entire duration of the season. Study was performed according to Good Clinical Practice, the Declaration of Helsinki and written informed consent was obtained from all participants. The study was approved by the ethical committee (http://www.trialregister.nl; NTR4818).

The study design was similar to the previous studies [2]. Participants were instructed about ILI symptoms according to the Dutch Pel criteria, as defined by fever (≥37.8°C) with at least one other symptom of coughing, headache, myalgia, sore throat, rhinitis, or chest pain [3], and were requested to report ILI as soon as possible after onset. A research nurse performed a home visit within 72 hours of fever onset (acute phase). A second and third visit were performed 14 days and 8 weeks (+/- 1 week) later to collect samples at the peak of the immune response and after recovery respectively. If a new ILI episode was reported, participants were visited again. In addition, every month of the study period a fixed number of asymptomatic participants, equally distributed over the different age groups, were invited for swab sampling. A consequence of this practical approach was that the mean age as well as vaccination coverage was slightly higher in the asymptomatic subset compared to the overall group (71.4 years; 80.5% versus 70.9 years; 68.2%). Additional information on influenza vaccination status and demographics was recorded from all participants, data on chronic illnesses was collected from participants who had a home visit.

Nasopharyngeal and oropharyngeal swabs

Naso- and oropharyngeal samples were obtained with a flocked nylon tipped swab and stored in modified liquid Amies transport medium (Eswab, Copan, Brescia, Italy) at -80°C within <8 hours after sampling.

Analysis of viruses by MLPA

Viruses were detected by analysis of DNA isolated from the swabs by Multiplex Ligation-dependent Probe Amplification (MLPA) assay (RespiFinder® Smart 22 kit (Pathofinder, The Netherlands)), as described before [2]. Influenza virus-positive samples were subtyped by real-time RT-PCR using the Roche LightCycler 480 system with slightly modified protocols [7, 8]. The gene coding for HA of influenza A(H3N2) viruses were Sanger-sequenced using universal influenza virus type A primers (available on request) directly from the clinical specimens. HA sequences were used to identify the phylogenetic clade to which the viruses belong. As reference for clade designation following phylogenetic analysis, guidance of WHO CC, London, UK is used (https://www.crick.ac.uk/partnerships/worldwide-influenza-centre/annual-and-interim-reports).

Statistical analysis

In order to compare baseline characteristics, such as sex, vaccination status and having ILI, or chronic illness, between the different groups Pearson's $\chi 2$ testing was applied. Differences in age was assessed using an independent samples T test of the means, and the male/female distribution in infections with a given pathogen was evaluated using the two-way Fisher's exact test.

The level of significance was set to P value $\leq .05$. These statistical analyses were performed using IBM SPSS Statistics version 24.0.0.1 (IBM Corporation, Armonk, New York, USA).

Vaccine-effectiveness (VE) was determined by test-negative design analysis of ILI positive participants in the influenza-active period in the Netherlands in 2014-2015 as previously described [2, 9]. The VE is calculated as (1 – odds ratio [OR]) × 100% with 95% confidence interval (CI) and is calculated per influenza virus subtype or lineage. Period in the season (early and late season), sex, smoking, comorbidity, and age were regarded as potential confounders and their association with influenza virus positivity was analyzed with univariate logistic regression. Variables with P value <

.20 were considered in the multivariable analysis. VE analysis was performed with SAS version 9.4 software.

In order to analyze the presence and frequency of viral co-infections at the genus level in the cohort, all virus variables were assessed in sets of two-virus combinations with the two-way Fisher's exact test that was adjusted for multiple testing using the Benjamini-Hochberg procedure. Results of these analyses are presented as the OR of the odds of being infected with pathogen A when already infected with pathogen B compared to the odds of being infected with a singular pathogen A. The 95% CI is used to estimate the precision of the OR. Adjusted P-values (P_{adj}) were calculated after multiple testing correction, with the false discovery rate (FDR) set to 10%. To determine the frequencies of three-virus combinations, two-virus combinations with a significant result ($P_{adj} < .10$) were tested against remaining pathogens using the two-way Fisher's exact test as described above. Statistical analysis for viral co-infections was performed using R version 3.4.3 (www.r-project.org).

Results

Study cohort and ILI incidence

In this prospective study, data was collected from influenza season 2014-2015 (from October 2014 to April 2015), a season with a vaccine mismatch . From season 2012-2013, a longer season than average, data on single respiratory infections were previously described, but unpublished data on viral co-infections from 2012-2013 were included in this study for comparison with season 2014-2015 [2]. A flow diagram for participants of seasonal cohort 2012-2013 was published previously [2], and is presented for 2014-2015 (Figure 1). A total of 2,366 older adults from season 2014-2015 were included for statistical analysis, i.e. persons with ILI (n =252), persons without ILI (2,114) as well as a subgroup of asymptomatic controls (n = 205). Two persons presented with two ILI episodes with a fever-free period of at least 2 weeks in between, resulting in 254 acute ILI samples. The ILI incidence of season 2014-2015 was 10.7% (252/2,366) and the vaccination coverage was 68.2% (Table 1). ILI incidence was not significantly different between vaccinated (10.4% (168/1.6614)) and unvaccinated persons (11.2% (84/752)). Average age of vaccinated individuals was significantly higher than the age of unvaccinated individuals (72.0 versus 68.5 years; $P < .0001$), and average age of ILI cases was slightly lower than participants without ILI (69.6 versus 71.1 years; $P = .001$). Participants with chronic illness were vaccinated significantly more often than participants without chronic illness (ILI cases: 79.6% versus 56.1%; $P < .0001$; asymptomatic controls: 88.2% versus 75%, $P = .02$). The percentage of participants with chronic illness was comparable in the ILI and control group (Table 2).

Influenza virus infection in ILI cases and influenza vaccine effectiveness

The 2014/2015 influenza vaccine contained A/California/7/2009 (H1N1)pdm09-like virus, A/Texas/50/2012 (H3N2 clade 3c.1), and B/Massachusetts/2/2012-like virus (Yamagata lineage) strains. Influenza viruses were detected in 39.4% of the acute ILI samples (Figure 2, Supplementary Table 1). Of the influenza viruses, 76% were of type A, of which 10.5% was subtype A(H1N1)pdm09 and 89.5% A(H3N2) (belonging to A(H3N2) clade 3C.2a (57.4%), A(H3N2) clade 3C.3b (32.4%), and 10.3% could not be determined) and 24% were of type B, all belonged to the B/Yamagata lineage (Supplementary Table 1). These circulating H3N2 strains mismatched with the vaccine A/Texas/50/2012 (H3N2 clade 3c.1) strain. In 9% (9/100) of the influenza virus-infected ILI cases the same influenza virus strain was still detectable 14 days after ILI onset. Influenza viruses were detected in only 1.2% of the recovery samples collected at 8 weeks post ILI onset which is similar to in the influenza positive samples of asymptomatic controls. Men were more often infected with influenza viruses than women during acute ILI phase: 47% versus 33% (P = .028). Vaccination status and age did not differ among male and female ILI cases. No gender preference was found for any specific influenza virus subtype.

Next, we evaluated whether influenza vaccination reduced the percentages of influenza virus infections or ILI cases. The percentages of overall influenza virus infections (Table 3) and ILI cases (Table 1) were not lower in vaccinated compared to unvaccinated individuals.

After correcting the data for potential confounders, the overall adjusted VE for all influenza vaccine strains of season 2014/2015 was -1% (95% CI, -88% to 46%). The VE for predominant influenza virus subtype A (H3N2) strain was -26% (95% CI, -161% to 39%), whereas the VE was highest for influenza B/Yamagata-like strain, i.e. 49% (95% CI, -39% to 81%). However, the point estimates of VE were not statistically significant. (Table 4). Additional sensitivity analyses for multiple ILIs, households with ILI, and the presence or absence of other virus infections did not affect data on vaccine effectiveness (data not shown).

Other respiratory viruses detected in ILI cases from season 2014-2015

For the influenza season 2014-2015, in 78.7% (200/254) of the acute ILI samples at least one respiratory virus was identified (Figure 2A). Fourteen days after ILI onset, this percentage was reduced to 28.7% (73/254), although the same respiratory virus as in the acute ILI samples was detected in 19.3% (49/254) of the samples at 14 days. The same viruses, including influenza viruses, were more often detected 14 days after ILI onset in participants with underlying chronic illness (P = .014). However, no severe infectious disease was reported in any of the ILI cases were the same virus persisted beyond 14 days. At the recovery time point, at 8 weeks after ILI onset, in 14.6% of the samples a respiratory virus was detected (Figure 2A), but in only 3% (8/254) of these ILI samples the same virus was detected as at ILI onset. In the asymptomatic controls: in 13.7% (Figure 2B), and in 14.1% of the swabs from asymptomatic controls respiratory viruses were detected in samples taken 14 days apart (Supplementary Table 1, Figure 2). Apart from influenza virus (39.4%), other viruses that were detected in >5% of the ILI cases were human rhinoviruses (17.3%), coronaviruses (9.8%), respiratory syncytial virus (RSV) (6.7%), and human metapneumovirus (hMPV) (6.3%). The same viruses, i.e. similar strains if subtyping was performed, remained detectable 14 days after ILI onset in 47.7% (21/44) of the rhinovirus cases, in 20% (6/25) of the coronavirus cases, in 23.5% (5//17) of the RSV cases and in 18.8% (3/16) of the cases where hMPV was detected at ILI onset. These viruses were present at lower frequency in recovery and asymptomatic control samples, although in recovery samples of the ILI cases rhinoviruses (6.3%) and coronaviruses (5.1%) were found in a considerable part, and in 8.3% and 3.9% of the asymptomatic control samples respectively (Figure 2, Supplementary Table 1). Only rhinoviruses were detected in both ILI and recovery samples of the same individual, in 9.1% (4/44) of the cases. However, rhinoviruses were not subtyped and therefore it cannot be excluded that these were of different subtypes.

Viral co-infections

In season 2014-2015, in 4.8% (12/252 ILI cases) of the samples from the acute ILI phase more than one respiratory virus was detected in the nasopharyngeal and/or oropharyngeal swabs (Supplementary Table 2). In one ILI case more than two viruses were detected, i.e. influenza virus, coronavirus and

RSV. There were no hospital admissions among the ILI cases with viral co-infections, and even the general practitioner was not visited.

A calculation was made of how often a specific co-infection could be expected based on the frequency of the individual viruses detected per season. We assumed independence of the occurrence of the individual viruses when a joint occurrence of two viruses was observed. During ILI episodes the co-infections of influenza virus with rhinovirus, coronavirus, hMPV or parainfluenza virus appeared to occur less often than expected based on the frequency of the detected single pathogens ($P_{adj} < .10$) (Figure 3).

For comparison, the occurrence of viral co-infections in 2012-2013 was studied as well. In 7.6% (21 out of 275 ILI cases) of the samples viral co-infections were detected. In line with the findings from season 2014-2015, in 2012-2013 co-infections of influenza virus with rhinovirus, coronavirus, hMPV or parainfluenza virus were observed less often ($P_{adj} < .10$). In addition, co-infections of influenza virus with RSV, and co-infections with rhinovirus and coronavirus, occurred less frequently than expected based on the frequency of these single pathogens detected in swabs from ILI cases ($P_{adj} < .10$) (Figure 3).

Discussion

The present data confirmed our previous data of two other seasons that vaccination against influenza virus in a cohort of community-dwelling older adults (≥ 60 years) had no influence on the frequency of total ILI when compared to the unvaccinated group. In addition, we found that co-infections of influenza virus with other viruses were rare.

In this third prospective observational study performed in 2014-2015, an influenza season with vaccine mismatch, in 78.7% of the acute ILI samples at least one potential respiratory virus was identified. Influenza virus was involved in 39.4% of the ILI cases, comparable to the 34.2% observed in the study performed in season 2012-2013 [2]. Also other respiratory viruses, i.e., rhinovirus (17.3%), coronavirus (9.8%), RSV (6.7%) and hMPV (6.3%) were detected at a similar level as in season 2012-2013. In the current study, we also show that most of these viruses were cleared 14 days after ILI onset, i.e., in approximately 80% of the cases. In participants with underlying chronic illness the same viruses persisted more often 14 days after ILI onset (P = .014) than in participants without underlying disease. After 8 weeks, i.e. after the person was recovered, only rhinovirus could still be detected in 9.1% of the ILI cases and thus may have persisted longer, however, we cannot exclude occurrence of reinfections since data on molecular subtyping of rhinovirus was not available. Moreover, rhinovirus was detected in 8.3% of the samples taken from asymptomatic controls as well.

ILI incidence of season 2014-2015 was 10.7% (252/2,366) and vaccination coverage was 68.2%, in comparison in season 2011-2012 and in 2012-2013 ILI incidence was respectively, 7.2% (143/1,992) and 11.6% (275/2,368), and vaccination coverage was 75.9% and 68.5% [2].
The number of influenza virus infections was not affected by vaccination. The overall adjusted VE for all influenza vaccine strains of season 2014-2015 was low and the point value estimate of VE was not statistically significant. Low VE against influenza A(H3N2) infection due to vaccine mismatch was also reported elsewhere in Europe in 2014-2015 and has been attributed to circulation of a drifted influenza A(H3N2) strain [10, 11]. For the seasons 2011-2012 and in 2012-2013, with respectively high and moderate vaccine effectiveness (VE of 73% and 51%), we showed that although vaccination reduced the number of influenza virus infections, the overall number of ILI episodes was not reduced

[2]. In line with this, we show here that, also in an influenza season with vaccine mismatch, vaccination did not affect the total number of ILI cases in a cohort of older adults.

Studies in children showed high rate of viral/viral co-infections (18%-34%) [4-6], but such data on older adults is scarce. We used data from the influenza season 2012-2013 and 2014-2015 to examine the occurrence of viral co-infections in older adults. In respectively 7.6% and 4.8% of the ILI cases more than one respiratory pathogen was detected, which is considerably lower than has been reported from studies in children [4-6]. Strikingly, co-infections were not random in our study: infections of influenza virus together with rhinovirus, coronavirus, hMPV or parainfluenza virus were observed less frequently than expected based on the frequency of the detected single viruses. These findings are in agreement with a recent study that was based on virological data of 44,230 episodes of respiratory illness over a 9-year time frame, that provided strong statistical evidence for the existence of a negative interaction between seasonal influenza A virus and rhinovirus, at both the population and individual host scale [12].

The reduced frequency of certain combinations of viruses may indicate the occurrence viral interference. Viral interference is a form of resistance that may occur after a host becomes infected with one virus, and prevents infection and replication of a second virus. It has been speculated that upon infection by the first virus, either through the cell-mediated response, or more likely, through the innate immune response a rapid state of immune activation that protects against simultaneous infections by other viruses is induced. Production of interferons or other cytokines have been indicated to induce this temporarily 'antiviral state' [12-15]. Other causes of exclusion may be: timing at the single cell level, where one virus can block another virus by simply being the first to infect the available host cells [16], climatic factors, differences in reproduction rates of the viruses, host availability and/or host susceptibility [15-17]. It has also been described that a fast-growing virus may reduce replication of slow-growing viruses during a co-infection [16]. Several epidemiological studies have supported the occurrence of viral interference as well, in particular during 2009 influenza pandemic [17-20].

In agreement with our finding that co-infections of influenza virus with rhinoviruses occurred less frequently than expected, several studies indicated that rhinovirus may have inhibited

the circulation of A(H1N1)pdm09 virus in Norway and France [18, 19]. In our study, in season 2012-2013, but not 2014-2015, influenza virus infections were also found to occur less frequently in combination with RSV. In line with this finding, early epidemics of influenza virus infection have been observed to delay RSV epidemics. In France, RSV emerged late at the end of December 2009, when A(H1N1)pdm09 started to decline, which was an unusual pattern compared with previous years [20]. Also in Israel, RSV infections were delayed and started after A(H1N1)pdm09 had declined [21].

Interestingly, these epidemiological findings match with the replication parameters for these respiratory viruses. The grow rate and infection time of rhinovirus is higher than that of influenza virus [16], which may explain why rhinovirus may have inhibited influenza virus infections, whereas the grow rate and infection time of influenza virus is higher than that of RSV [16], which may explain why the influenza pandemic in turn delayed the RSV epidemic. In light of the novel coronavirus disease 2019 (COVID-19) pandemic, it might be interesting to notice that in our study also co-infections with coronavirus and influenza virus, and coronavirus with rhinovirus occurred less often than expected, which may be indicative for the occurrence of viral interference. Considering the incubation time of COVID-19, it might be speculated that both rhinovirus and influenza virus with an estimated shorter incubation time, infects faster and may inhibit or delay circulation of SARS-CoV-2.

As in our previous study [2], a limitation of the present study is that, due to our method of recruitment, the more frail institutionalized elderly are not included. Consequently, our data acquired from the generally healthy community-dwelling older adults do not necessarily apply to the group of more frail elderly, in which viral exposure and susceptibility to infections may be different. Furthermore, the Dutch Pel criteria were used for the definition of ILI [3]. It can be speculated that the specific ILI definition used in this study, although most other ILI definitions also include fever and cough [22-24], may have affected the number and type of pathogens that were detected.

Summarizing, in line with our previous studies, data of this third prospective observational study show that influenza virus is the most frequently detected pathogen in older adults presenting with ILI. Furthermore, we show that, also in an influenza season with vaccine mismatch, vaccination did not affect the total number of ILI cases. It is tempting to speculate that viral interference by influenza virus may be involved here. When vaccination is effective and influenza virus infection is

prevented, viral interference by influenza virus will not take place and other viruses may therefore have more opportunities to infect and cause ILI.

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Competing interests

All authors have completed the ICMJE uniform disclosure form. EAS declares to have received research grants from Pfizer and GSK and fees paid to the institution for advisory boards and participation in independent data monitoring committees for Pfizer and GSK. Other authors declare no competing interests.

Figures

Figure 1.

Flow diagram of enrollments and influenza-like illness (ILI) cases (2014–2015) (A) and the subgroup of asymptomatic controls (B). A subject could have multiple ILI episodes per season. Per protocol was defined when the sample was taken <72 hours after start of fever. For the recovery visit, the window was 7–9 weeks after ILI onset. Subjects were considered lost to follow-up if they did not respond to the end of study mailing and had no ILI visit. Every month of the study period a fixed number of asymptomatic participants, equally distributed over the different age groups, were invited for swab sampling (B). Subjects were considered lost to follow-up if they did not respond to the end of study mailing and had no ILI visit.

Figure 2.

Incidence per virus that were detected in naso- and oropharyngeal swabs of influenza-like illness (ILI) cases (A) and of asymptomatic controls (B) in influenza season 2014-2015. The percentages were calculated per ILI event. Multiple pathogens could be detected in a single event and therefore contribute to the incidence for multiple pathogens. Abbreviations: hMPV, human metapneumovirus; ILI, influenza-like illness; RSV, respiratory syncytial virus.

Figure 3.

A calculation was made whether a specific virus co-infection appeared to occur less often than expected based on the frequency of the detected single pathogens in season 2014-2015 (squares). For comparison, the occurrence of viral co-infections in 2012-2013 was studied as well (circles). Results of these analyses are presented as the OR of the odds of being infected with pathogen A when already infected with pathogen B compared to the odds of being infected with a singular pathogen A. The 95% CI, presented as bars, was used to estimate the precision of the OR. Adjusted P-values (P_{adj}) were calculated , and $P_{adj} < .10$ was considered as significantly different (indicated with black lines).

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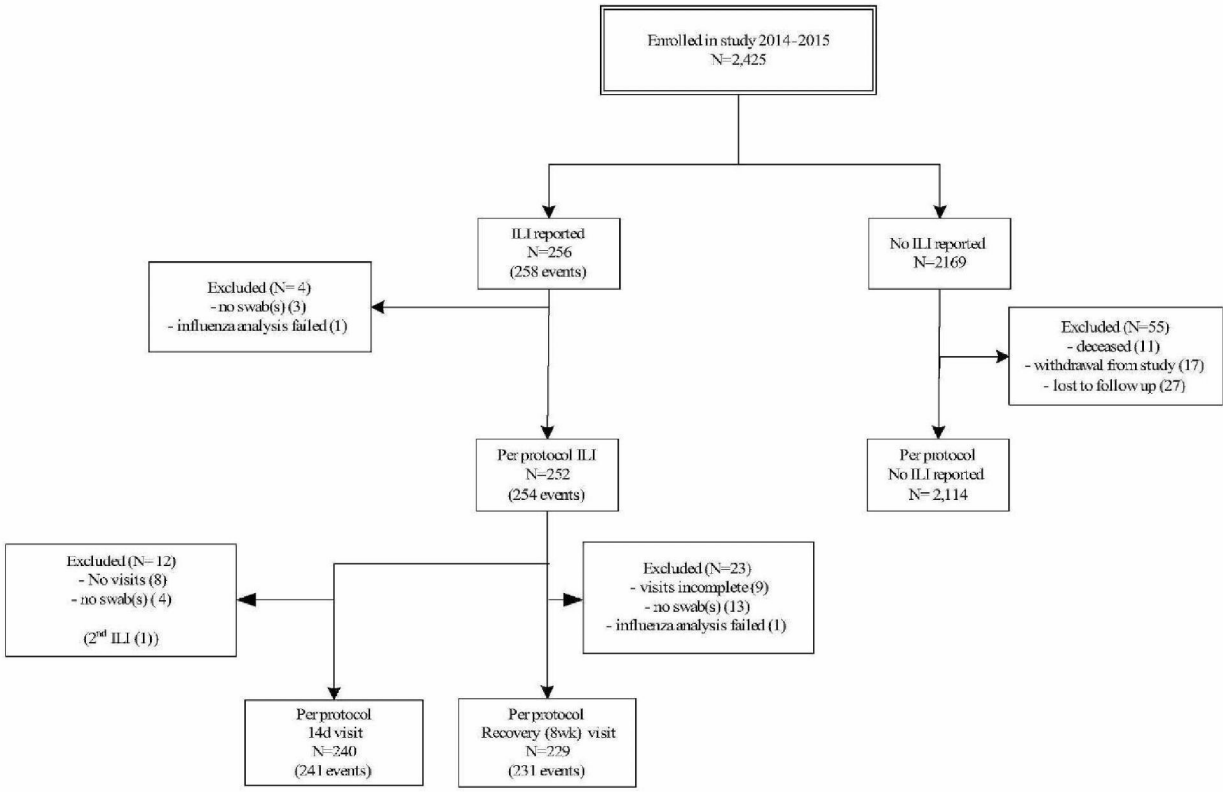
Figure 1

Click here to access/download;Figure;Kaaijk2020_ILI3_Fig1_FlowDiagr.eps

Figure 1

A



B

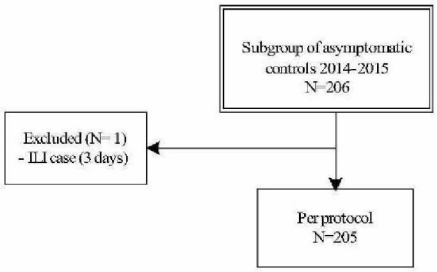


Figure A

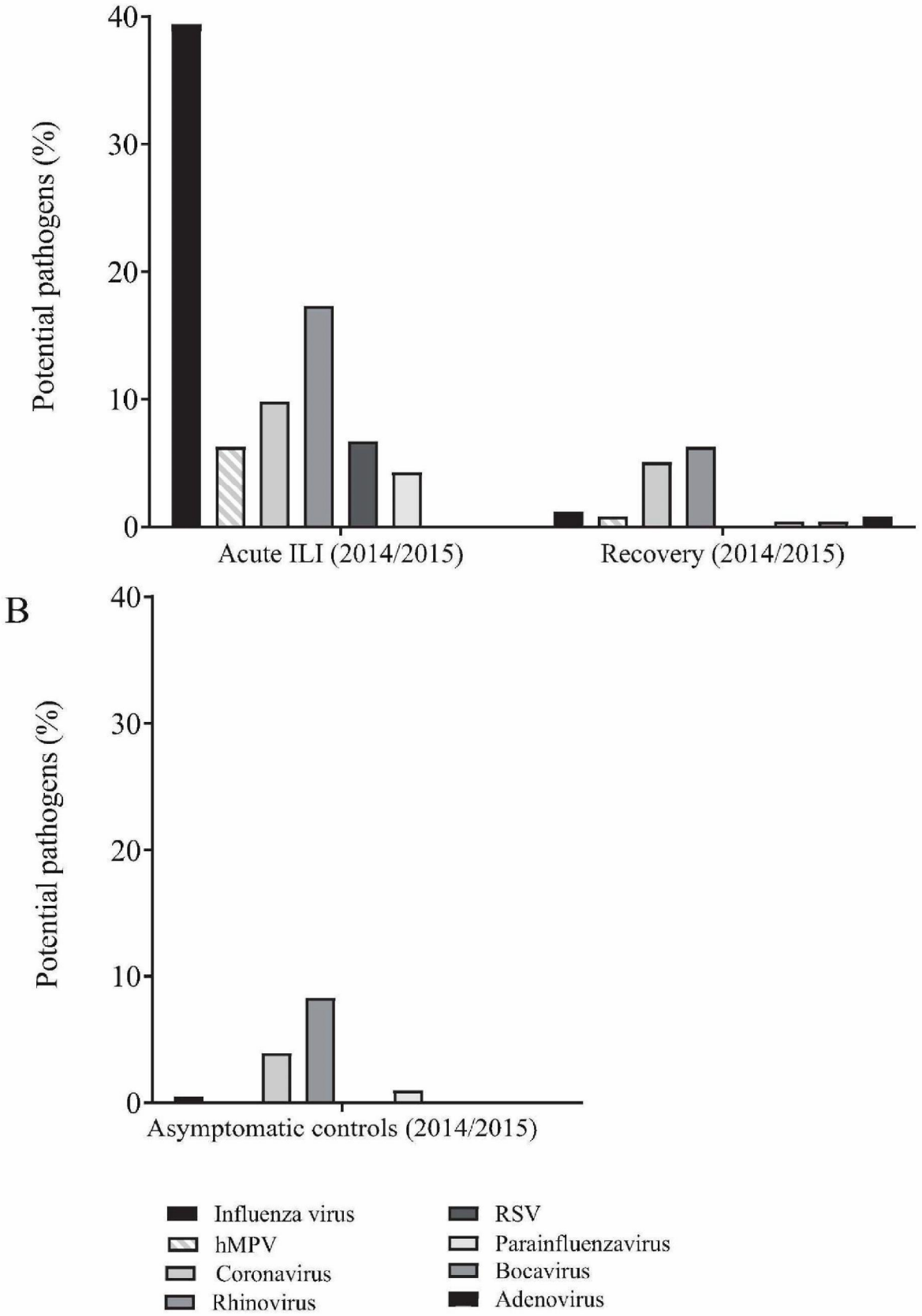


Figure 3

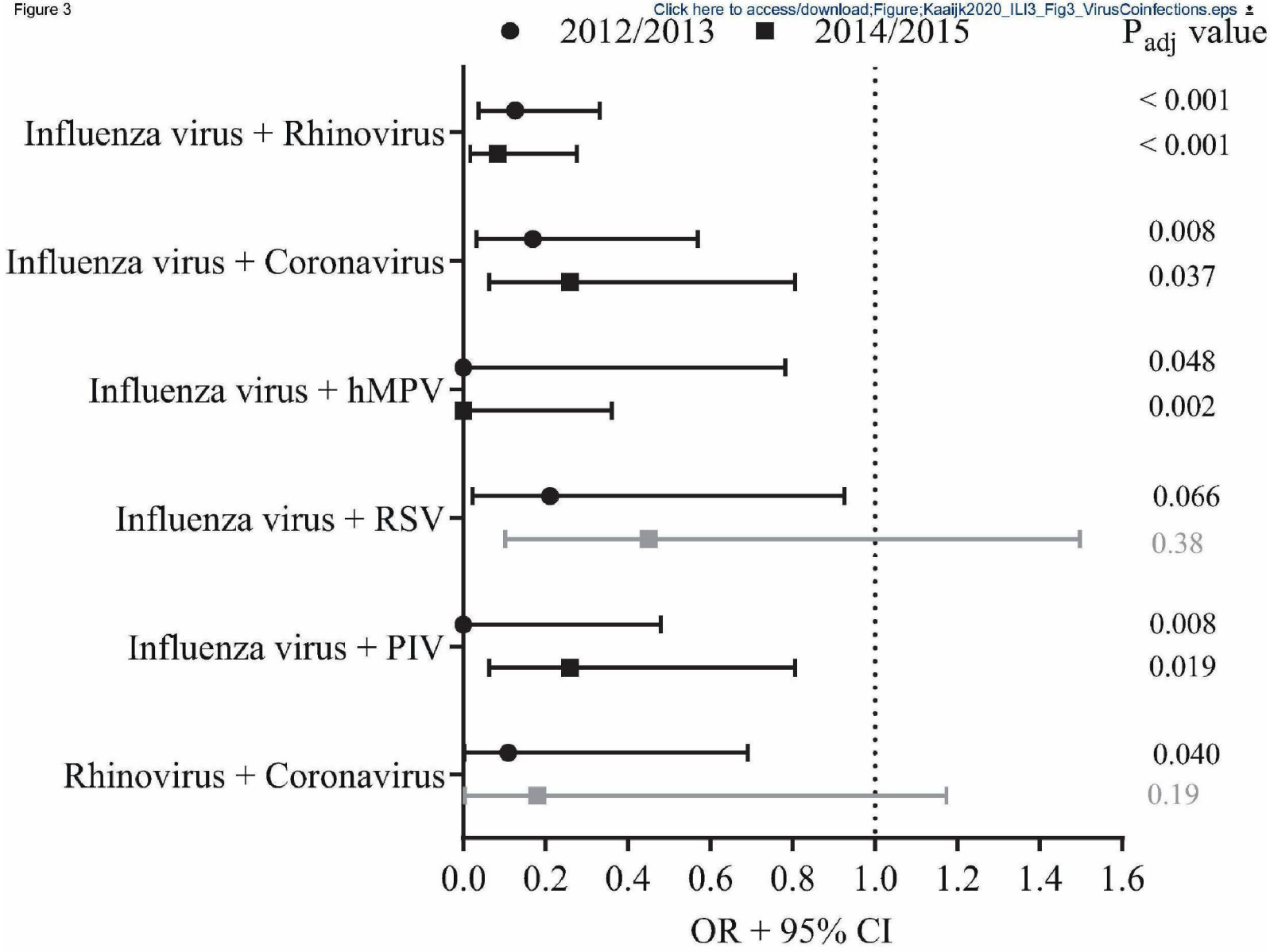


Table 1

Table 1. Demographic characteristics of participants

2014/2015

	All (n= 2,366)	ILI (n= 252[a])	No ILI (n= 2,114)	asymptomatic controls (n = 205)	*P* value ILI vs No ILI	*P* value ILI vs asymptomic controls
Male sex	1205 (50.9%)	121 (48.0%)	1,084 (51.3%)	107 (52.2%)	NS[a]	NS[a]
Age, y, mean (range)	70.9 (60-94)	69.6 (60-88)	71.1 (60-94)	71.4 (60-88)	.001[b]	.001[b]
Influenza vaccination 2014/2015	1,614 (68.2%)	168 (66.7%)	1,446 (68.4%)	165 (80.5%)	NS[a]	.001[a]

Data are presented as No. (%)

Abbreviations: ILI, influenza-like illness; NS, not significant.

[a]Pearson χ^2 test.

[b]Independent samples t test of the means.

Individuals in the asymptomatic control group were selected to be evenly distributed over the different age groups; therefore, the overall vaccination level was higher in the asymptomatic control group compared to the ILI and non-ILI groups

Two persons had two ILI events during season 2014/2015 (ILI events, n = 254)

Table 2

Table 2. Occurrence of chronic illness in combination with vaccination status

	ILI (n = 252)	Asymptomatic controls (n = 205)	*P* value[a]
Any chronic illness	113 (44.8%)	85 (41.5%)	NS[a]
% vaccinated with any chronic illness	90 (79.6%)	75 (88.2%)	
% vaccinated without any chronic illness	78 (56.1%)	90 (75%)	
P value[a]	.0001	.02	

[a] Pearson χ^2 test

Table 3

Table 3. Pathogens detected in participants with acute ILI relative to vaccination status
2014/2015

	Vaccinated (N= 168 participants)	Non-vaccinated (N= 84 participants)	*P* value[a]
Influenza virus	69 (41.1%)	31 (36.9%)	NS
Influenza virus A	57 (33.9%)	19 (22.6%)	NS
A(H3N2)	52[b] (31.0%)	16 (19.0%)	NS
- 3C.2a	31 (18.4%)	8 (9.5%)	NS
- 3C.3b	14 (8.3%)	8 (9.5%)	NS
A(H1N1)pdm09	5 (3.0%)	3 (3.6%)	NS
Influenza virus B	12 (7.1%)	12 (14.3%)	NS
- Yamagata-like			
Coronavirus	16 (9.5%)	9 (10.7%)	NS
hMPV	13 (7.7%)	3 (3.6%)	NS
RSV	9 (5.4%)	8 (9.5%)	NS
Rhinoviruses	28 (16.7%)	16 (19.0%)	NS
Parainfluenza virus	6 (3.6%)	5 (6.0%)	NS

Data are presented as No. (%)
Abbreviations: hMPV, human metapneumovirus; RSV, respiratory syncytial virus; NS, not significant (*P* value > .05).
Vaccine strains for season 2014/2015:
A/California/7/2009 (H1N1)pdm09-like virus, A/Texas/50/2012 (H3N2 clade 3c.1), B/Massachusetts/2/2012-like virus (Yamagata lineage)
[a] Pearson $\chi 2$ test
[b] 7 out of these 52 A(H3N2) positive samples could not be subtyped

Table 4

Table 4. Vaccine effectiveness during influenza active period 2014-2015

			N	Odds ratio (95% CI)	VE (95% CI)[a]
2014/2015					
Influenza virus			210	1.005 [0.538 – 1.878]	-1% [-88% - 46%]
	-A		186	1.170 [0.571 – 2.399]	-17% [-140% - 43%]
		A(H3N2)	178	1.261 [0.609 – 2.610]	-26% [-161% - 39%]
		- 3C.2a	149	1.965 [0.733 – 5.270]	-96% [-427% - 27%]
		- 3C.3b	132	0.400 [0.131 – 1.222]	60% [-22% - 87%]
		A(H1N1)pdm09	118	1.339 [0.274 – 6.555]	-34% [-555% - 73%]
	-B	B/Yamagata-like	134	0.509 [0.187 – 1.389]	49% [-39% - 81%]

Abbreviations: CI, confidence interval; VE, vaccine effectiveness
Vaccine strains for season 2014/2015:
A/California/7/2009 (H1N1)pdm09-like virus, A/Texas/50/2012 (H3N2 clade 3c.1), B/Massachusetts/2/2012-like virus (Yamagata lineage)
[a]Calculated VE was corrected for the possible confounders: age group, comorbidity, sex and smoking.
Data was calculated for the influenza active period in the Netherlands as defined by the Netherlands Institute for Health Services Research

Supplementary Table 1

Supplementary Table 1. Viruses detected by MLPA in naso- and oropharyngeal swabs. Influenza is typed by sequencing

			ILI events		ILI at 14d		ILI recovery events at 8 wk		Asymptomatic control events		Asymptomatic at 14 d	
			n (%)		n (%)		n (%)		n (%)		n (%)	
Subjects			252		252		252		205		205	
Events			254		254		254		205		203	
Missing			-		8 (3.1%)		9 (3.5%)					
No 14 days visit			-		4 (1.6%)						2 (1.0%)	
No recovery visit					-		9 (3.5%)					
No sample: 2nd ILI			-		1 (0.4%)		-					
Any virus[a]			200 (78.7%)		73 (28.7%)		37 (14.60%)		28 (13.7%)		29 ((14.1%)	
Influenza virus			100 (39.4%)		15 (5.9%)		4 (1.2%)		1 (0.5%)		2 (1.0%)	
	-A		76 (76.0%)		14 (93.3%)		3 (75.0%)		0 (0%)		2 (100%)	
		A (H1N1)pdm09		8 (10.5%)		0 (0%)		0 (0%)		-		
		A(H3N2)		68 (89.5%)		14 (100%)		2 (66.6%)		-		2 (100%)
		- 3C.2a		-39 (57.4%)								-2 (100%)
		- 3C.3b		-22 (32.4%)								
		- Not typable[b]		- 7 (10.3%)				1 (33.3%)				
	-B		24 (24%)		1 (6.7%)		1 (25.0%)		1 (100%)		0 (0%)	
		B Victoria-like		0 (0%)						1 (100%)		-
		B Yamagata-like		24 (100%)		1 (100%)				0 (0%)		-
hMPV			16 (6.3%)		3 (1.2%)		2 (0.8%)		0 (0%)		2 (1.0%)	
Coronavirus			25 (9.8%)		15 (5.9%)		13 (5.1%)		8 (3.9%)		5 (2.4%)	
	-C229E			9 (36%)		7 (46.7%)		5 (38.5%)		2 (25%)		1 (20.0%)
	-OC43			12 (48%)		5 (33.3%)		6 (46.2%)		1 (12.5%)		2 (40.0%)
	-NL63			2 (8%)		2 (13.3%)		1 (7.7%)		3 (37.5%)		0 (0%)
	-HKU1			2 (8%)		1 (6.7%)		1 (7.7%)		2 (25%)		2 (40.0%)
Rhinovirus			44 (17.3%)		25 (9.8%)		16 (6.3%)		17 (8.3%)		12 (5.9%)	
RSV			17 (6.7%)		4 (1.6%)		0 (0%)		0 (0%)		3 (1.5%)	
	-A			8 (47.1%)		2 (50%)		0 (0%)		0 (0%)		0 (0%
	-B			9 (52.9%)		2 (50%)		0 (0%)		0 (0%)		3 (100%)
Parainfluenza virus			11 (4.3%)		8 (3.1%)		1 (0.4%)		2 (1.0%)		5 (2.4%)	
	-1			0 (0%)		0 (0%)		0 (0%)		0 (0%)		0 (100%)
	-2			3 (27.3%)		3 (37.5%)		0 (0%)		0 (0%)		0 (100%)
	-3			4 (36.4%)		4 (50%)		1 (100%)		1 (50%)		3 (60.0%)
	-4			4 (36.4%)		1 (12.5%)		0 (0%)		1 (50%)		2 (40%.0)
Bocavirus			0 (0%)		3 (1.2%)		1 (0.4%)		0 (0%)		1 (0.5%)	
Adenovirus			0 (0%)		7 (2.8%)		2 (0.8%)		0(0%)		1 (0.5%)	

[a] Any virus: includes ILI events whereby corresponding samples contained multiple viruses (co-infections)

[b] Not typable: % could not be further characterized due to sequencing failure as a result of low viral load

Abbreviations: 14d, 14 days; 8 wk, 8 weeks; hMPV, human metapneumovirus; ILI, influenza-like illness; RSV, respiratory syncytial virus

Supplementary Table 2

Supplementary Table 2. Occurrence of virus co-infections

	2012-2013 Co-infections (n)	2014-2015 Co-infections (n)
Influenza virus-Rhinovirus	5	3
Influenza virus-Coronavirus	2	3
Influenza virus-hMPV	0	0
Influenza virus-RSV	2	3
Rhinovirus-Coronavirus	1	1
Rhinovirus-RSV	2	0
Rhinovirus- Parainfluenza virus	3	1
Coronavirus-RSV	1	0
Coronavirus-hMPV	1	0
RSV-Parainfluenza virus	1	0
hMPV-Parainfluenza virus	1	0
Coronavirus-Adenovirus	1	0
Influenza virus-Bocavirus	1	0
Influenza virus-Coronavirus-RSV	0	1

Abbreviations: hMPV, human metapneumovirus; RSV, respiratory syncytial virus