Title

Extensive multiplex PCR diagnostics reveals new insights into the epidemiology of viral respiratory infections

Running title

Epidemiology of respiratory viruses

Author names

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Summary

Viral respiratory infections continue to pose a major global healthcare burden. At the community level, the co-circulation of respiratory viruses is common and yet studies generally focus on single aetiologies. We conducted the first comprehensive epidemiological analysis that encompasses all major respiratory viruses in a single population. Using extensive multiplex PCR diagnostic data generated by the largest NHS board in Scotland, we analysed 44,230 patient episodes of respiratory illness that were simultaneously tested for eleven virus groups between 2005 and 2013, spanning the 2009 influenza A pandemic. We measured viral infection prevalence, described co-infections, and identified factors independently associated with viral infection using multivariable logistic regression. Our study provides baseline measures and reveals new insights that will direct future research into the epidemiological consequences of virus co-circulation. In particular, our study shows that (i) human Coronavirus infections are more common during influenza seasons and in co-infections than previously recognised, (ii) factors associated with co-infection differ from those associated with viral infection overall, (iii) virus prevalence has increased over time especially in infants <1, and (iv) viral infection risk is greater in the post-2009 pandemic era, likely reflecting a widespread change in the viral population that warrants further investigation.

Main text

Introduction

Acute respiratory infections are the commonest cause of illness in all ages, and a leading cause of mortality in children under five, creating a significant global healthcare burden [1-3]. Various aetiological pathogens (viruses, bacteria and some fungi) are recognised, causing largely indistinguishable symptoms. In most settings, viruses are the most frequently detected agent [4, 5]. Although most infections are mild, respiratory viruses have the potential to cause severe illness in high risk groups.

Although influenza is a major research focus [6], the advent of PCR technology has led to improved awareness that non-influenza viruses are also important contributors to disease burden, and of the role of viral subtype in clinical severity [7-9]. The use of PCR testing as part of routine diagnostics provides an important resource for monitoring respiratory viruses as part of national surveillance [10].

Multiplex PCR methods in particular provide a valuable resource for epidemiological enquiry [11]. All patients requiring microbiological diagnosis are tested for all pathogens included in the panel, ensuring consistency in testing across patients. The collation of multiplex diagnostic data from a large patient population and over an extended timeframe therefore enables robust comparisons of infection trends temporally and across patient subgroups. Furthermore, when testing is implemented over multiple

years, sufficient data can be accrued to investigate the clinical relevance of co-infections and their epidemiological patterns [12].

Although the utility of diagnostic data in the epidemiology of respiratory infections has been demonstrated [11, 13-16], studies that cover all major viruses, patient age and illness severity groups, and that span multiple years, are lacking. The largest NHS health board in Scotland, Greater Glasgow and Clyde (NHSGGC), has used multiplex PCR testing as part of their routine diagnostic services since 2005. This health board serves ~1.1 million people, representing ~1.7% of the total UK population [17]. The resultant accumulation of data provides a novel opportunity to investigate viral respiratory infections in a more comprehensive fashion than previously possible. These data also provide a unique opportunity to compare the periods before and after the introduction of the novel pandemic influenza virus (A(H1N1)pdm09) into Scotland [see 18].

We analysed diagnostic data generated by NHSGGC using multiplex PCR from 2005 to 2013 with the objectives to (i) describe testing and virus prevalence trends, (ii) examine temporal and patient subgroup distributions for each individual virus, and (iii) compare factors associated with overall viral infection and co-infection using statistical modelling, in order to provide robust and timely estimates of who is most at risk of viral-associated respiratory illness, and when, within a major urban UK population.

Methods

Virological data

In this study we used virological diagnostic data generated by the West of Scotland Specialist Virology Centre (WoSSVC) for NHSGGC during 2005 to 2013 [19]. During this period, a total of 61,427 clinical samples were received from 40,962 patients attending primary and secondary healthcare services for respiratory diagnostic purposes (i.e. excluding pathology-origin samples). The large majority of clinical samples (98%) were taken from the upper or lower respiratory tract: primarily nasal and/or throat swabs (67%), gargles (13%), nasopharyngeal aspirates (7%), sputum (5%), bronchoalveolar lavage (3%) and nasopharyngeal/endotracheal secretions (2%). In a minority of cases (n=142 samples), plasma was additionally taken for follow-up investigation; most of these samples (89%) related to the 2009 influenza A pandemic period which was excluded from statistical modelling analyses.

Each sample was tested by real-time RT-PCR for eleven groups of respiratory viruses: human Rhinovirus (RV); Influenza A virus (IAV; a generic assay detecting seasonal H3N2 and H1N1 subtypes and one specific to A(H1N1)pdm09), Influenza B virus (IBV), human Respiratory syncytial virus (RSV), human Coronavirus (CoV; aggregating 229E, NL63, HKU1 and OC43 species), Adenovirus (AdV), human Metapneumovirus (MPV) and human Parainfluenza types 1-4 (PIV1-4). Details of nucleic acid extraction methods and the real-time PCR assays are provided elsewhere [20].

Complete testing coverage across viruses was largely maintained throughout the study period. However, high frequencies of partial testing did arise due to the burden placed on laboratory resources during the major waves of A(H1N1)pdm09 virus circulation. The

laboratory protocols were consistent throughout the study period, with the exception of the RV assay which was modified during 2009 to detect a wider array of RV and enteroviruses (including D68), and the CoV-HKU1 assay which was discontinued in 2012.

Data preparation and descriptive analysis

For each of the 61,427 clinical samples, positive/negative PCR test results were recorded by the laboratory for each virus group. Information was also provided on the sampling date, patient age at sampling, gender, and the origin of the sample (whether the patient had attended a General Practice (GP), hospital outpatient or non-critical care inpatient services, or was admitted to a critical care ward). In the case of inconclusive/absent test results or other patient information, the corresponding data were coded as missing. All patient identifiers were anonymised.

Of the 40,962 patients, 8394 had multiple samples submitted for virological testing during the study period (range=1-37 samples, median=1, SD=1.22). For 70% of these patients, the samples were received within a 30-day window. We aggregated the PCR-test results to within this timeframe generating single “episodes” of respiratory illness, using the collection date of the first sample when assigning temporal information. Episodes were classified as positive for a given virus if at least one sample tested positive. Following data exclusions, 44,230 patient episodes, representing 36,157 individual patients, were retained for analysis of temporal distributions. We conducted descriptive statistical analyses of viral infection prevalence among the patient population providing time and age-stratified estimates.

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145 By the end of April 2009, Scotland was afflicted by the influenza pandemic [20]. Figure
146 1a highlights the resultant upsurge in testing frequencies during the summer and autumn
147 waves of 2009, and during a third wave of A(H1N1)pdm09 virus circulation in the winter
148 of 2010/11. During these periods, testing was primarily directed towards IAV and only
149 subsets of IAV-negative patients were tested for other viruses. Due to this disruption in
150 regular testing procedures, we focused our description of viral infection distributions
151 across patient subgroups on the 26,974 patient episodes tested out with this period, and
152 refer readers to a previous report for details of viruses detected during the 2009
153 pandemic [20].

154

155 **Co-infection analyses**

156 For each virus group, we compared the frequency of mono-infection episodes (one virus
157 group detected) and co-infection episodes (more than one virus group detected). To
158 correctly classify episodes into these subgroups, we excluded all partially tested
159 patients. In more detailed analyses, we counted the frequency of each possible virus
160 pair and quantified the statistical correlation between mono-infection and co-infection
161 frequencies across viruses.

162

163 **Statistical associations**

164 We investigated statistical associations between time period, season, patient age,
165 gender, and GP/general hospital/critical care origin (a proxy for illness severity), and two
166 outcomes: (i) virus-positive versus virus-negative episodes, and (ii) co-infection versus
167 mono-infection episodes. With respect to time, we split sampling dates into two major

periods either side of the influenza pandemic and periods of high partial testing: pre-pandemic (prior to May 2009 when the A(H1N1)pdm09 virus was established in Scotland) and post-pandemic (following subsidence of the third major wave of the A(H1N1)pdm09 virus in January 2011).

Associations with each factor were first assessed by crude unadjusted odds ratios, and then adjusted for confounding using multivariable logistic regression models that included all factors to assess their independence. Statistical interactions were examined using Mantel-Haenszel (MH) stratification methods (based on a p-value <0.05, results not shown). The potential interactions were added to the main effects models and their significance assessed based on an interaction parameter p-value <0.05. Model fit was assessed by le Cessie van Houwelingen global goodness of fit tests [21]. All statistical analyses were carried out in R v.3.1.1 [22].

To correctly classify patients into outcome groups, all partially tested patients were excluded. Of the 36,157 fully tested patients, 90% sought healthcare facilities once during the study period thereby contributing a single episode. However, 4218 patients had attended healthcare facilities more than once, providing information for multiple episodes (range 2-26 episodes; median=2; SD=2.04). We retained the first observed episode per patient in the statistical analyses to ensure the patient-level interpretation of statistical associations was not influenced by the non-independence of data relating to the same individual. See supplementary Figure S1 for full details of data preparation.

Results

Episodes of illness and viral infection frequencies

We analysed 44,230 episodes of respiratory illness tested by WoSSVC during 2005 to 2013. Full details of patient distributions across subgroups and per study year are provided in supplementary Table S1. The median patient age was 27 years (range=0-98 years, SD=25.5 years) and 49% were male. Excluding the three major waves of Influenza A(H1N1)pdm09 virus circulation, episode frequencies increased year-by-year from 2472 cases tested in 2005 to 6149 cases tested in 2013. However, the age patterns were not consistent over this period; the percentage of adult episodes was greater in 2013 than in 2005 (e.g. 21% vs. 8% in patients aged ≥65 years), whilst the percentage of child episodes was less in 2013 than 2005 (e.g. 16% vs. 26% in patients aged 1-5 years) (Figure 1b).

At least one virus was detected in 35% (15,302/44,230) of tested patients; these patients had a median age of 17 years (range=0-96 years, SD=25 years) and 49% were male. The prevalence of confirmed viral infection among the patient population was greater in the 2013 influenza season than in 2005 among all age groups (Figure 1c); the absolute difference in prevalences were 22% (infants <1 year old), 12% (1-5 year olds), 14% (6-16 year olds), 18% (17-45 year olds), 12% (46-64 year olds) and 17% (≥65 year olds). Overall virus-specific prevalences among the patient population were ranked as follows: RV (14%, n=4847); IAV (9.7%, n=4244); RSV (4.9%, n=1786); CoV (4.1%, n=1339); AdV (3.6%, n=1221); IBV (3%, n=1019); MPV (2.6%, n=345); PIV-3 (2.2%, n=757); PIV-4 (0.86%, n=286) ; PIV-1 (0.84%, n=295) and PIV-2 (0.35%, n=122). Age distributions for each viral infection group are presented in supplementary Table S2. The most

common infection in each six-month period (excluding 2009) was RV, constituting a low of 19% of infections during the typical influenza period of 2005/06, to a high of 59% during the typical non-influenza period of 2010 (Figure 1d).

For most virus groups, detections were most frequent in 1-5 year olds (with the exception of IAV, IBV and CoV), males, and hospital-attendees not admitted to a critical care ward (Figure 2). Seasonally, virus detections were most common in December (45% among GP-attendees and 43% among hospital-attendees) and least common in August (11% among GP-attendees and 22% among hospital-attendees) (Figure 3a,c). The most commonly detected viral infection in each month was RV, peaking in September among both GP and hospital-attendees (Figure 3b,d). Influenza A and B were the most common detections in December-March among GP-attendees (combined proportion ranging 31% - 45%), and in January-February among hospital-attendees (combined proportion of 30%). Of the remaining non-influenza viral infections, a large proportion was attributed to RSV, RV and CoV during periods of high influenza activity; their combined proportions ranged 39% - 52% among GP attendees (December-March) and 51% - 55% among hospital-attendees (January-February).

Of 9094 positive patients (among 26,974 patients out with the pandemic period), 1952 were GP-attendees, 6560 were general hospital-attendees (outpatients and non-critical care inpatients), and 1282 were inpatients admitted to a critical care ward (an intensive care unit (ICU), intensive therapy unit (ITU), high dependency unit (HDU), or coronary care unit (CCU)). The latter group provided a proxy for classifying episodes of severe respiratory illness. Eighty-eight percent (n=4443) of GP-attendees and 69% (n=15,027)

of hospital-attendees were over 5 years of age. As shown in Figure 4, the prevalence of severe episodes among all virus-positive patients, regardless of origin, was greater among patients with RV (7.5%), RSV (7.5%), PIV1 (11.8%) and PIV4 (7.4%) infections than among virus-negative patients or other viral infections including IAV (5.5%) and IBV (4.1%). Investigating further the RV/ IAV and RV/PIV1 comparisons, we found the observed difference in prevalence was statistically significant based on Pearson's Chi squared tests (p=0.036 and p=0.05 respectively). Age-specific prevalence of severe episodes was greatest at the extremes of age (under-fives and adults over 65) for all viruses except hPIV2 (we note the particularly small sample size for this virus group).

Co-infections and virus mixing patterns

Of 9654 virus-positive patients, among 27,284 episodes tested for all eleven viruses, 11% (1086/9654) had a co-infection. The median age among co-infected patients was three years (range=0-91 years, SD=22 years) and 58% were male. Co-infections were more commonly detected among under-fives overall (18% compared to 7% among over fives) and for each viral infection, particularly RV, RSV, AdV and CoV (detected in 6%, 3%, 3% and 2% of these infections respectively in under-fives) (Figures 5a-b).

A total of 1389 virus pairs were detected among 1086 episodes of co-infection; most episodes involved two viruses (87%; 964/1086), the remaining involved three (n=105), four (n=15) and five (n=2) viruses. All viruses were detected with most others at least once (Figure 5c); however, a clustering pattern was evident in which RV, AdV, RSV and CoV were frequently detected with one another. The most common virus detection in a co-infection was RV (56% of co-infections), the majority of which were with AdV (n=195,

25%) and RSV (n=181, 23%). Other viruses relatively frequently detected in co-infections were AdV, RSV and CoV; constituting 31%, 30% and 28% of co-infections respectively.

We found a significant positive correlation between virus detection frequencies in mono-infections and co-infections; Pearson's product-moment correlation = 0.88 (95% CI = 0.60 - 0.97, $p<0.001$) and fitted linear regression model slope = 0.85, $p<0.001$ (Figure 5d). However, IAV and IBV were identified in co-infections at relatively low frequencies (n=121 and n=68 respectively) compared to non-influenza viruses (e.g. RV, n=678) (Figure 5d).

Factors associated with viral infection and co-infection

Table 1 summarises the results of univariable and multivariable logistic regression analyses for associations with viral infection. Season, age group, and patient origin were significantly associated with the odds of viral infection based on unadjusted odds ratio estimates. In the multivariable analysis, several independently significant factors were identified based on the adjusted odds ratios. Viral respiratory infections were more likely detected in winter, in children aged 1-5 years, and among GP-attendees, irrespective of the other factors. Following adjustment for multiple factors, time period was also a significant predictor (because of a negative confounding by age): the odds of viral infection were significantly greater post-pandemic than pre-pandemic.

Significant statistical interactions (based on $p<0.05$) revealed that the effect of age was not homogeneous across gender or patient origin subgroups. This variation in age

association across other factors is shown in Figure 6a-b where age-specific infection prevalences are stratified by the third factor. These figures show that the age distribution of infection differed according to gender and patient origin subgroups.

Table 2 summarises the results of univariable and multivariable logistic regression analyses for associations with co-infection. Several differences were found in comparison with viral infections overall. Based on unadjusted odds ratio estimates, time period, season (autumn only), age group, gender and patient origin were significantly associated with co-infection. However, in the multivariable analysis time period and gender were confounded by age and were therefore not identified as significant independent factors. In contrast to viral infection overall, co-infections were equally likely to be detected in spring and winter, were less likely detected among 1-5 year olds than infants, and were more likely detected among general hospital-attendees (outpatients and those not admitted to critical care wards) than GP-attendees.

Significant statistical interactions (based on $p<0.05$) revealed that the effect of age on co-infection status was not homogeneous across gender and patient origin groups. In contrast to viral infection overall, co-infections were relatively more common in males than females among 46-64 year olds and among hospital-attendees in all age groups (Figures 6c-d).

There was no evidence of a poor model fit based on the global goodness of fit tests: (i) p-values=0.147, 0.07, 0.07 for the main effect model and two models with interaction terms respectively for associations with viral infection overall, and (ii) p-values=0.940,

0.985, 0.746 for the main effect model and two models with interaction terms respectively for associations with co-infection.

Discussion

The advent of multiplex-PCR as part of routine diagnostics provides an unprecedented opportunity for studying the epidemiology of multiple respiratory viruses simultaneously within a single population. Previous UK-based studies have highlighted the utility of laboratory-based surveillance for monitoring respiratory infection trends, and in comparing the relative burdens between viruses [10, 13, 23]. Our study is the first to compare the epidemiologies of different respiratory virus groups utilising extensive diagnostic data generated by multiplex RT-PCR from patients attending both primary and secondary healthcare services.

The collation of test negative results by diagnostic laboratories provides valuable denominator information for measuring disease occurrence, to estimate the relative contribution of different pathogens to healthcare usage (such as GP consultations) and to provide an early warning for periods of increased healthcare pressures. Importantly, the diagnostic test data utilised in this study were generated by a single laboratory, permitting a more consistent comparison of trends across patient and virus groups because testing methods were on the whole standardised throughout the study.

Our study has revealed changes in the frequency of virological testing of respiratory illnesses in the NHSGGC health board during 2005 to 2013, with adults representing an

increasingly greater percentage of episodes. However, age-specific prevalences were greater in the 2013 influenza season than in 2005 for all age groups. It is possible that there is raised awareness among the public and/or clinicians, and consequently greater healthcare seeking and/or sampling behaviour in adults. Alternatively these results could reflect a true increase in non-viral causes of respiratory illness among this age group. We note that a shift in the demography of the Glasgow population has been reported [24]. Our observations might indicate the impact of an aging population on respiratory-related healthcare services, through an increase in GP/hospital consultations, or a genuine increase in the incidence of adult respiratory infections.

Rhinovirus was the most prevalent virus overall, corroborating previous UK-based studies that include patients attending both primary and secondary healthcare services [10, 12]. The clinical significance of RV is disputed, although severe cases of disease are recognised depending on virus species, patient subgroups, and season [7, 25-27]. In additional analyses (Figure 4) we found the prevalence of severe respiratory illness (patients located in critical care wards) was significantly greater among RV infections than IAV, supporting the proposition that RV is associated with more severe disease than traditionally accepted.

Of the other non-influenza viruses, RSV and CoV were relatively highly prevalent. We note that the extent of research into the commonly circulating coronaviruses is small when compared to IAV and RSV, although severe clinical cases are recognised [28]. Our study is the first comparative analysis in the UK to include CoV, providing an important opportunity to quantify its temporal and patient subgroup distributions and co-

infection patterns in comparison to the other common virus groups. We confirm that CoV contributes a large fraction of infections during periods of high influenza activity and that CoV is relatively frequently co-detected with other viruses. The contribution of different respiratory viruses to the healthcare burden in Scotland has previously been studied [23]. Further investigation on a seasonal basis is needed to help elucidate the public health relevance of RV and CoV, particularly since CoV has a similar age distribution as the influenza viruses. The remaining viruses (AdV, MPV, and PIV1-4) were detected in comparatively smaller numbers on a yearly basis and during months of high influenza activity.

The nine-year study period provided a novel opportunity to compare the epidemiology of respiratory viruses before and after the 2009 influenza A pandemic [18]. In our multivariable statistical analysis we found viral infections to be more likely in the post-pandemic era. This result was independent of other factors such as patient age implying non-patient factors, such as a change in the underlying virus population, have increased the likelihood that a patient seeking healthcare services will have a viral infection (as opposed to non-viral causes). Whether this is a direct consequence of the pandemic virus, its impact on the epidemiologies of others viruses, or a consequence of long-term changes in the non-influenza virus population, remains to be elucidated. Seasonal and patient-related factors corroborate existing knowledge and were independent of time, indicating the generality of these factors as predictors of viral infection.

It is well recognised that the burden of viral respiratory illness lies predominantly among young children [29]. We found that among patients with respiratory illness attending

healthcare facilities, 1-5 year olds were more likely than other age groups to have a viral infection independent of season or time period. The most commonly detected viruses among this age group were RV, RSV, AdV and MPV (20%, 9.3%, 9.1% and 4.7% of infections respectively) corroborating previous reports [23, 30]. Together with a recent study that found bacterial-viral co-infections were relatively uncommon in children with pneumonia [31], these findings support the concern regarding the over-prescription of antibiotics in children [32]. That the increasing trend in virus prevalence was most notable among infants (<1 year) also warrants further attention. Whilst it is possible that these findings are influenced by changes in clinical testing decisions, we note that this trend is particularly pertinent in relation to recent European outbreaks of enterovirus D68 in children [33]; investigation into the contribution of individual viruses will be the focus of future work. We further note that, based on the multivariable statistical analyses, the increasing trend in prevalence among children explained why co-infections were more likely detected in the post-2009 pandemic era.

There are very few studies describing co-infection patterns among respiratory viruses. Our study provides the largest examination to date, confirming that around 11% of viral infections among patients attending healthcare services in an urban setting involve more than one virus, similar to the 10.4% reported by a previous UK-based study [12]. That nearly all respiratory viruses were co-detected with all others highlights the sufficient opportunities for co-infections. We would expect co-infection frequencies to reflect individual virus prevalences. Indeed, in line with the aforementioned study [12], RV was the most common detection among co-infections, RV/RSV was a frequent pairing, and most co-infections were in children under five. Our study also reveals that CoV are

relatively frequently involved in co-infections. However, co-infections with influenza viruses were relatively few, perhaps explained by differences in their age and seasonal distributions, or an inter-viral interference [34].

We found that the average age of co-infection was three years, compared to 17 years for viral infections overall, and co-infections were more likely in infants than 1-5 year olds. That co-infections were more likely in young children is likely explained by (i) a greater opportunity for co-infection due to a shorter exposure lifetime and consequently greater susceptibility to a wider array of viruses, and (ii) a greater chance of co-infections being detected because children tend to shed virus for longer periods.

In adults, the age distribution of co-infections differed according to gender and patient origin; the prevalence was greatest in males and among general hospital-attendees not admitted to critical care wards in 46-64 year olds (Figure 6c-d). This result provides insight into an age-dependent factor in co-infection patterns among adults but must be viewed with some caution; it is potentially influenced by a bias in multiple specimens submitted in relation to single episodes of illness among adults, most likely as a result of co-morbidities. Interestingly, co-infections were more likely among general hospital-attendees not admitted to critical care wards than GP-attendees, supporting the potential role of co-infections in illness severity [35].

There are several limitations to our study to be noted. Detection of viral nucleic acid may not represent active infection for all viruses in all cases [36], potentially introducing detection biases temporally and across patient groups. Furthermore, the timing of

infection events, and variation in shedding duration across virus and patient groups [37, 38], could potentially bias the observed co-infection patterns. We also note that our study lacked information on the presence/absence of bacterial pathogens which are also significant contributors to respiratory infections.

One further important consideration is that laboratory diagnostic data cannot inform on the epidemiology of asymptomatic infections in the community, or among symptomatic people who do not attend healthcare services. Furthermore, that viral populations are not static could also impact on the generalisability of the observed trends and associations; the introduction of new strains can alter disease outcomes, and consequently healthcare seeking behaviour, influencing the stability of healthcare consultation rates among patient subgroups. Given the dynamic nature of virus populations, the epidemiological information generated through surveillance must be maintained to ensure future vaccine and antiviral developments are directed to where they are most needed [39, 40].

Conclusions

Our study provides the most comprehensive description of viral respiratory infections in the UK to date, revealing new epidemiological insights with public health relevance. Of particular concern is a greater viral prevalence in 2013 compared with 2005, particularly in infants, and a greater risk of viral infection in the post-2009 pandemic era. Further investigation into the long-term temporal dynamics of individual viruses and the epidemiological consequences of virus co-circulation is needed.

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Conflict of interest

None

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Figure legends

Figure 1. Trends in episodes of respiratory illness and viral infection prevalence among patients seeking healthcare services within NHS Greater Glasgow and Clyde during 2005 to 2013.

(a) Episodes of respiratory illness tested in each month highlighting the three major waves of A(H1N1)pdm09 virus circulation; (b) Distribution of episodes across age groups in each six-month period; (c) Age-specific prevalence of confirned viral infection and virus-negative illness detected in each six-month period; (d) Relative prevalence of each viral infection and virus-negative illness (Neg*) in each six-month period; (A) = typical non-influenza period (April-September) and (B) = typical influenza period (October-March). Note that January-March 2005 and October-December 2013 were excluded from figure (d).

Figure 2. Episodes of viral respiratory infection by patient subgroup

Distribution of each viral infection and virus-negative illness (Neg*) by (a) age group, (b) gender, and (c) patient-origin. These results are based on 26,974 patient episodes of respiratory illness; excluding patients tested during the major waves of Influenza A(H1N1)pdm09 virus circulation. GP = General Practitioners surgery, Hospital: general = outpatients and non-critical care patients; Hospital: critical care = patients admitted to an intensive care, intensive therapy, high dependency, or coronary care unit.

Figure 3. Distribution of virus positive/negative episodes of illness and respiratory infection types detected in each calendar month

(a,b) patients attending primary healthcare services (General Practitioners) and (c,d) patients attending secondary healthcare services (hospital inpatients and outpatients). These results are based on 26,974 patient episodes of respiratory illness; excluding patients tested during the major waves of Influenza A(H1N1)pdm09 virus circulation.

Figure 4. Prevalence of severe cases among patients with confirmed viral infection attending primary and secondary healthcare facilities in NHSGGC during 2005 to 2013

Comparing across viral infection types and virus-negative patients (Neg*). Absolute numbers of severe cases are indicated in parentheses. Severe cases were identified

based on patient admission to intensive care, intensive therapy, high dependency or coronary care units.

Figure 5. Co-infection and virus mixing patterns among patients tested for all virus groups

Comparing mono-infection and co-infection distributions for each virus group among (a) children ≤ 5 years of age, and (b) patients > 5 years of age. (c) A network of co-infections: each node represents a respiratory virus and links between viruses are proportional to the frequency at which each virus pair was observed among co-infected patients. Viruses are coloured according to their prevalence among co-infections (darker=greater prevalence). (d) Correlation between mono-infection and co-infection frequencies across virus groups; solid line=fitted linear regression model with corresponding R^2 value.

Figure 6. Stratification of viral infection and co-infection associations

Age-specific viral infection (a,b) and co-infection (c,d) prevalences stratified by gender and patient origin. Significant interactions with age are indicated by *.

632 Table 1: Investigating factors associated with viral infection using logistic regression

Factor	Level	Summary*	Virus-positive*	Virus-negative*	Unadjusted OR (95% CI, p-value)†	Adjusted OR (95% CI, p-value)‡
Time period	Pre-pandemic	6296 (39)	2090 (39)	4206 (39)	Reference	Reference
	Post-pandemic	9961 (61)	3315 (61)	6646 (61)	1.00 (0.94 – 1.07, p=0.912)	1.31 (1.22 – 1.41, p<0.001)
Season	Winter	5016 (31)	2001 (37)	3015 (28)	Reference	Reference
	Spring	4305 (26)	1541 (29)	2764 (25)	0.84 (0.77 – 0.91, p<0.001)	0.79 (0.73 – 0.87, p<0.001)
	Summer	2952 (18)	667 (12)	2285 (21)	0.44 (0.40 – 0.49, p<0.001)	0.42 (0.38 – 0.47, p<0.001)
	Autumn	3984 (25)	1196 (22)	2788 (26)	0.65 (0.59 – 0.71, p<0.001)	0.61 (0.56 – 0.67, p<0.001)
Age group (years)	< 1	1277 (13)	959 (18)	1218 (11)	Reference	Reference
	1-5	2596 (16)	1327 (25)	1269 (12)	1.33 (1.18 – 1.49, p<0.001)	1.25 (1.11 – 1.41, p<0.001)
	6-16	1722 (11)	564 (10)	1158 (11)	0.62 (0.54 – 0.71, p<0.001)	0.53 (0.46 – 0.60, p<0.001)
	17-45	3782	1035	2747	0.48 (0.43 – 0.53,	0.36 (0.32 – 0.40,

		(23)	(19)	(25)	p<0.001)	p<0.001)
	46-64	3247 (20)	866 (16)	2381 (22)	0.46 (0.41 – 0.52, p<0.001)	0.37 (0.33 – 0.41, p<0.001)
	≥65	2733 (17)	654 (12)	2079 (19)	0.40 (0.35 – 0.45, p<0.001)	0.34 (0.30 – 0.39, p<0.001)
Gender	Female	7941 (49)	2575 (48)	5366 (49)	Reference	Reference
	Male	8316 (51)	5486 (52)	2830 (51)	1.07 (1.01 – 1.15, p=0.03)	1.08 (1.01 – 1.15, p=0.032)
Patient origin[¥]	GP	3012 (19)	1260 (23)	1752 (16)	Reference	Reference
	Hospital: general	11,878 (73)	3725 (69)	8153 (75)	0.64 (0.59 – 0.69, p<0.001)	0.54 (0.49 – 0.59, p<0.001)
	Hospital: critical care	1367 (8)	420 (8)	947 (9)	0.62 (0.54 – 0.71, p<0.001)	0.56 (0.49 – 0.65, p<0.001)

633

634 * Distribution of patient numbers, with corresponding % in parantheses, across factor
635 levels for all patients (summary) and for virus-positive and virus-negative groups; [†]
636 Unadjusted odds ratios (OR) based on univariable logistic regression; [‡] Adjusted OR
637 based on multivariable logistic regression; [¥] Patient location corresponding with first
638 clinical sample: GP = General Practitioners surgery, Hospital: general = outpatients and
639 non-critical care patients; Hospital: critical care = patients admitted to an intensive care,
640 intensive therapy, high dependency, or coronary care unit.

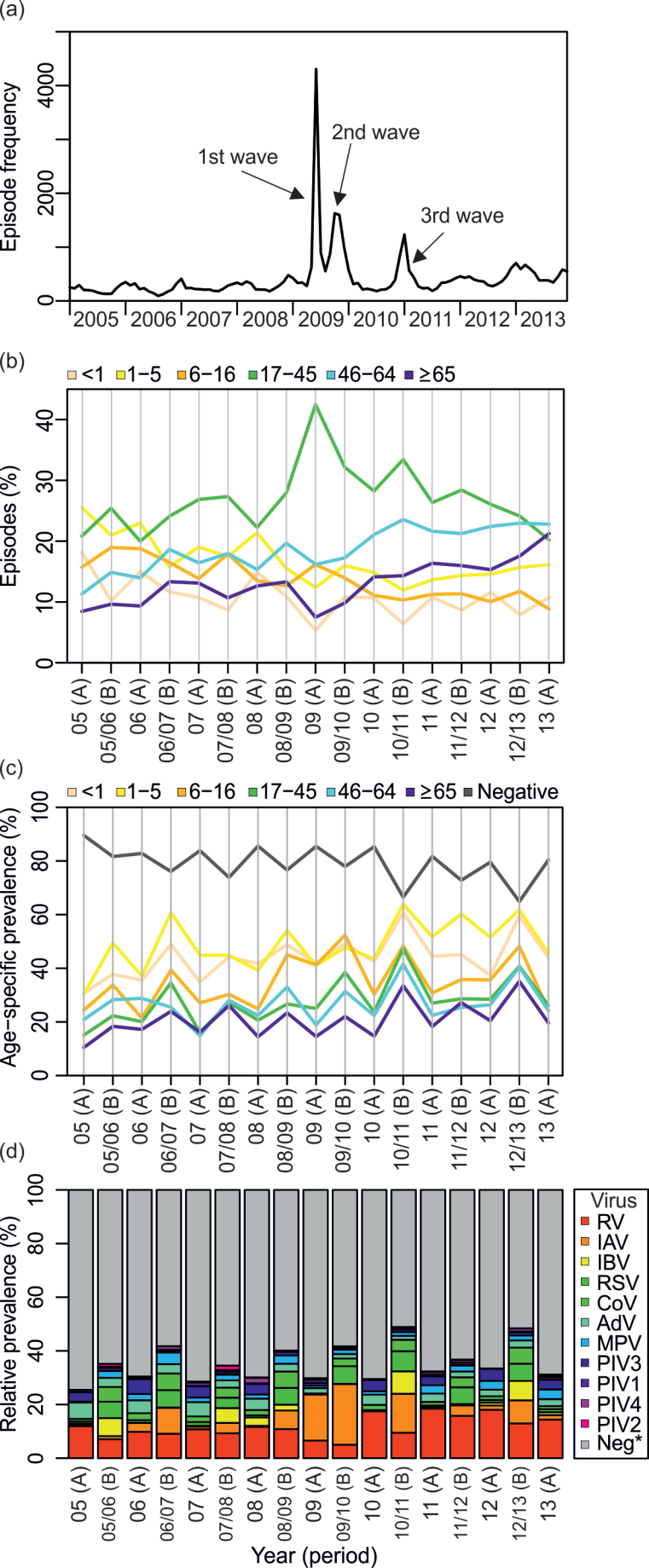
641 Table 2: Investigating factors associated with co-infection using logistic regression

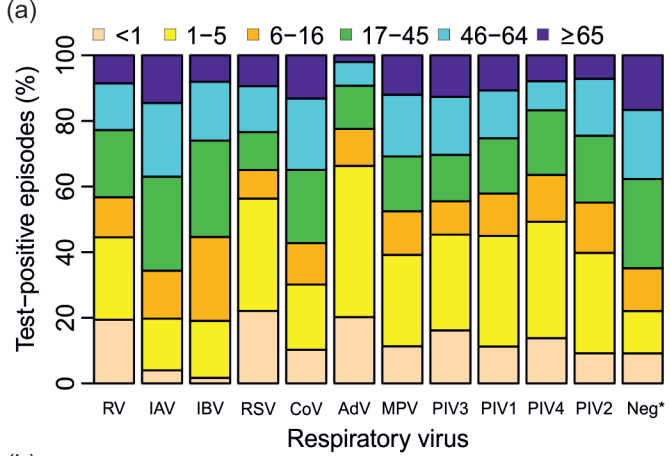
Factor	Level	Summary*	Co-infection*	Mono-infection*	Unadjusted OR (95% CI, p-value) †	Adjusted OR (95% CI, p-value) ‡
Time period	Pre-pandemic	2090 (39)	232 (44)	1858 (38)	Reference	Reference
	Post-pandemic	3315 (61)	293 (56)	3022 (62)	0.78 (0.65 – 0.93, p=0.006)	0.97 (0.80 – 1.18, p=0.774)
Season	Winter	2001 (37)	209 (40)	1792 (37)	Reference	Reference
	Spring	1541 (29)	165 (31)	1376 (28)	1.03 (0.83 – 1.28, p=0.801)	0.94 (0.75 – 1.18, p=0.595)
	Summer	667 (12)	54 (10)	613 (13)	0.76 (0.55 – 1.03, p=0.079)	0.55 (0.40 – 0.76, p<0.001)
	Autumn	1196 (22)	97 (18)	1099 (23)	0.76 (0.59 – 0.97, p=0.03)	0.63 (0.48 – 0.82, p=0.001)
Age group (years)	< 1	959 (18)	184 (35)	775 (16)	Reference	Reference
	1-5	1327 (25)	187 (36)	1140 (23)	0.69 (0.55 – 0.86, p=0.001)	0.67 (0.54 – 0.84, p=0.001)
	6-16	546 (10)	28 (5)	536 (11)	0.22 (0.15 – 0.33, p<0.001)	0.21 (0.14 – 0.32, p<0.001)
	17-45	1035	47	988	0.20 (0.14 – 0.28,	0.21 (0.15 – 0.30,

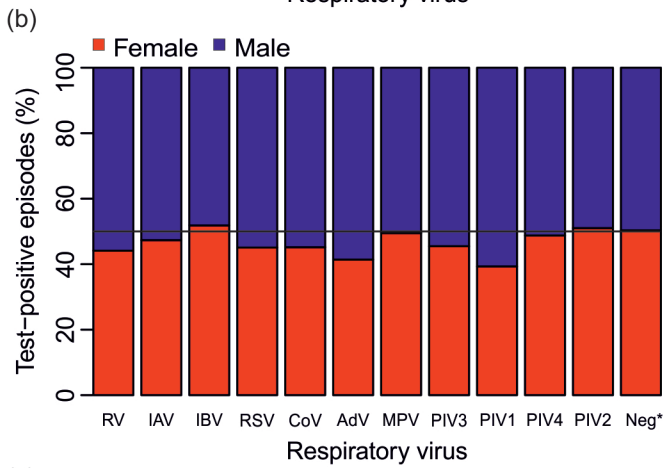
		(19)	(9)	(20)	p<0.001)	p<0.001)
	46-64	866 (16)	47 (9)	819 (17)	0.24 (0.17 – 0.34, p<0.001)	0.24 (0.17 – 0.34, p<0.001)
	≥65	654 (12)	32 (6)	622 (13)	0.22 (0.15 – 0.32, p<0.001)	0.21 (0.14 – 0.31, p<0.001)
Gender	Female	2575 (48)	222 (42)	2353 (48)	Reference	Reference
	Male	2830 (52)	303 (58)	2527 (52)	1.27 (1.06 – 1.52, p=0.01)	1.11 (0.92 – 1.33, p=0.293)
Patient origin[¥]	GP	1260 (23)	72 (14)	1188 (24)	Reference	Reference
	Hospital: general	3725 (69)	418 (80)	3307 (68)	2.09 (1.61 – 2.70, p<0.001)	1.52 (1.15 – 2.00, p=0.003)
	Hospital: critical care	420 (8)	35 (7)	385 (8)	1.50 (0.99 – 2.28, p=0.058)	1.15 (0.75 – 1.79, p=0.521)

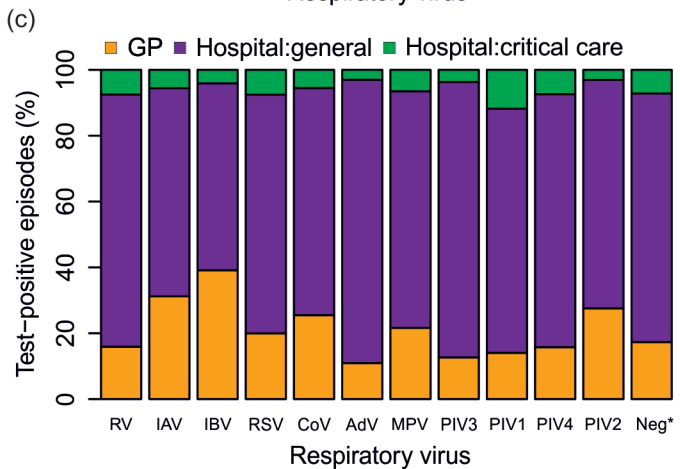
642

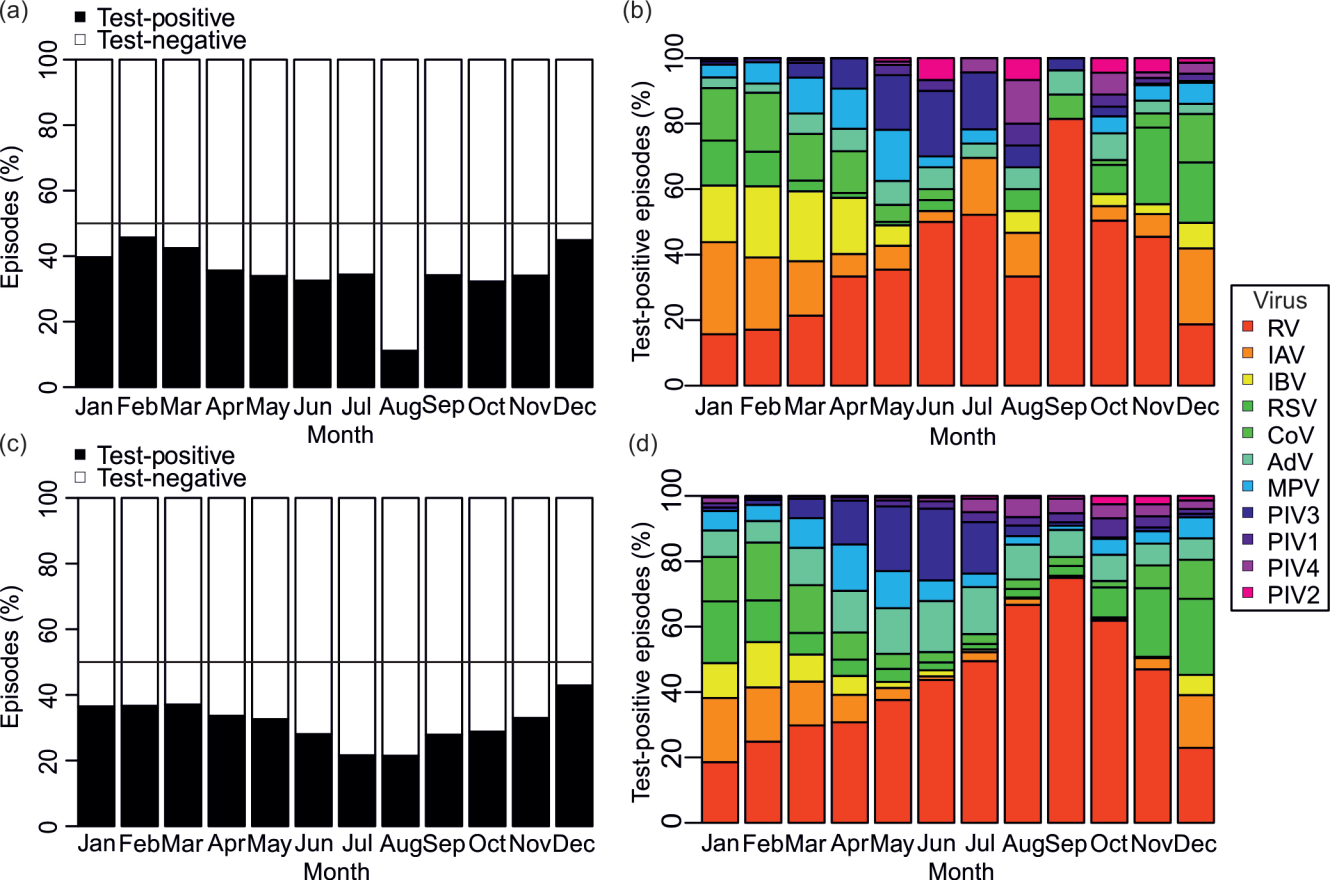
643 * Distribution of patient numbers, with corresponding % in parantheses, across factor
644 levels for all patients (summary) and for co-infection and mono-infection groups; [†]
645 Unadjusted odds ratios (OR) based on univariable logistic regression; [‡] Adjusted OR
646 based on multivariable logistic regression; [¥] Patient location corresponding with first
647 clinical sample: GP = General Practitioners surgery, Hospital: general = outpatients and
648 non-critical care patients; Hospital: critical care = patients admitted to an intensive care,
649 intensive therapy, high dependency, or coronary care unit.

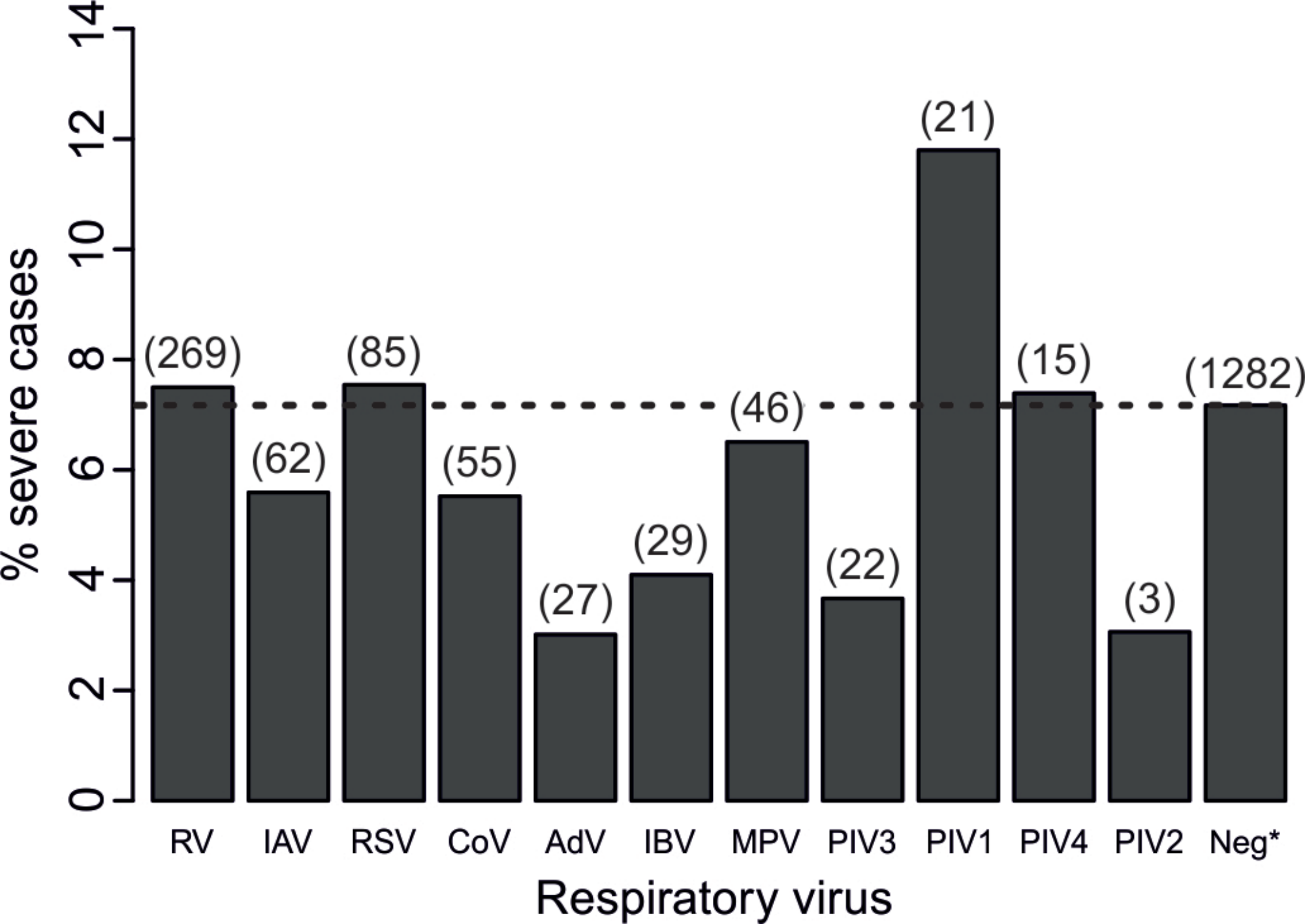

(a)
Episode frequency
0
2000
4000
1st wave
2nd wave
3rd wave
2005
2006
2007
2008
2009
2010
2011
2012
2013
(b)
<1
1–5
6–16
17–45
46–64
≥65
Episodes (%)
0
10
20
30
40
05 (A)
05/06 (B)
06 (A)
06/07 (B)
07 (A)
07/08 (B)
08 (A)
08/09 (B)
09 (A)
09/10 (B)
10 (A)
10/11 (B)
11 (A)
11/12 (B)
12 (A)
12/13 (B)
13 (A)
(c)
<1
1–5
6–16
17–45
46–64
≥65
Negative
Age−specific prevalence (%)
0
20
40
60
80
100
05 (A)
05/06 (B)
06 (A)
06/07 (B)
07 (A)
07/08 (B)
08 (A)
08/09 (B)
09 (A)
09/10 (B)
10 (A)
10/11 (B)
11 (A)
11/12 (B)
12 (A)
12/13 (B)
13 (A)
(d)
Relative prevalence (%)
0
20
40
60
80
100
Virus
RV
IAV
IBV
RSV
CoV
AdV
MPV
PIV3
PIV1
PIV4
PIV2
Neg*
05 (A)
05/06 (B)
06 (A)
06/07 (B)
07 (A)
07/08 (B)
08 (A)
08/09 (B)
09 (A)
09/10 (B)
10 (A)
10/11 (B)
11 (A)
11/12 (B)
12 (A)
12/13 (B)
13 (A)
Year (period)

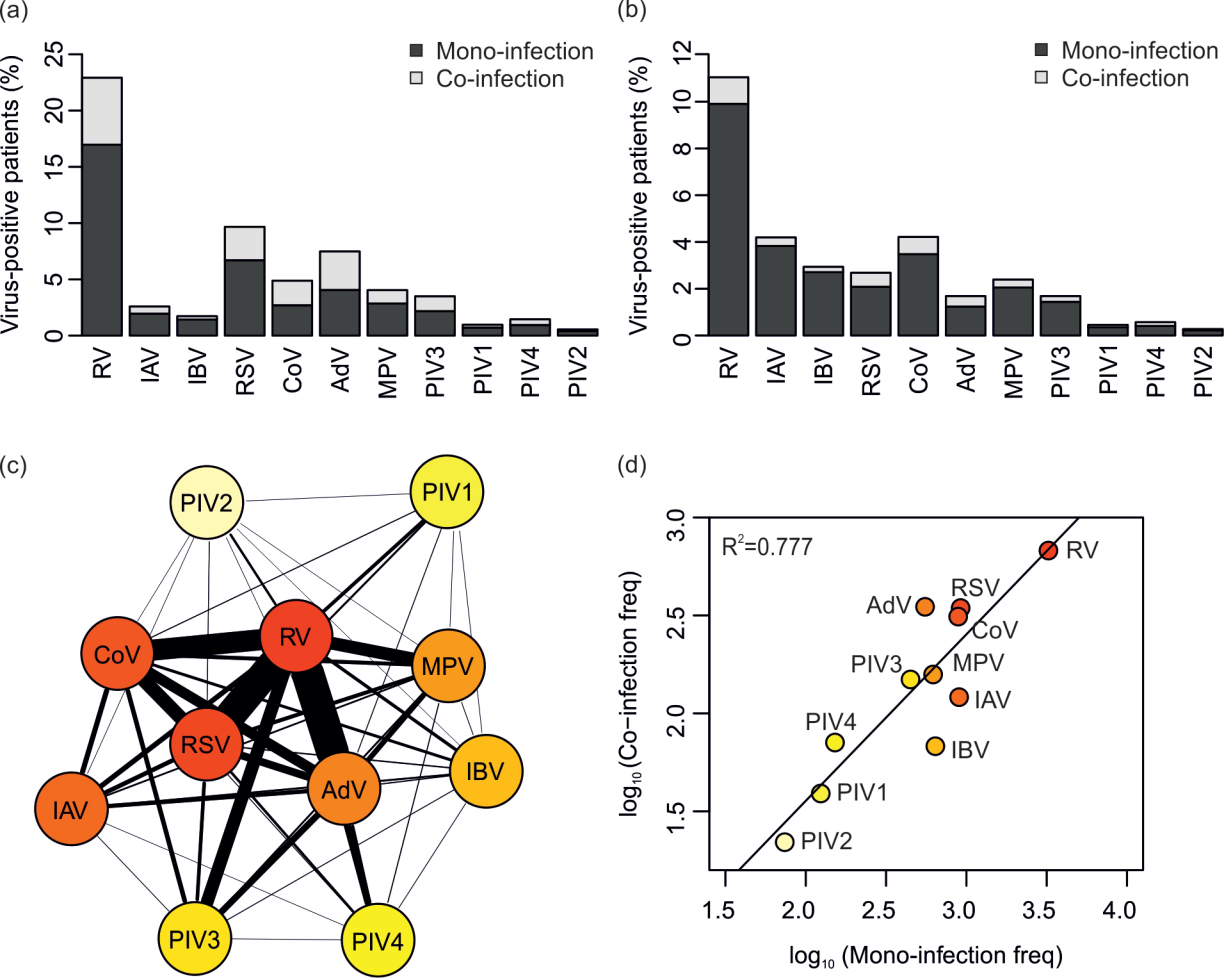

(a)
<1
1–5
6–16
17–45
46–64
≥65
Test–positive episodes (%)
0
20
40
60
80
100
RV
IAV
IBV
RSV
CoV
AdV
MPV
PIV3
PIV1
PIV4
PIV2
Neg*
Respiratory virus

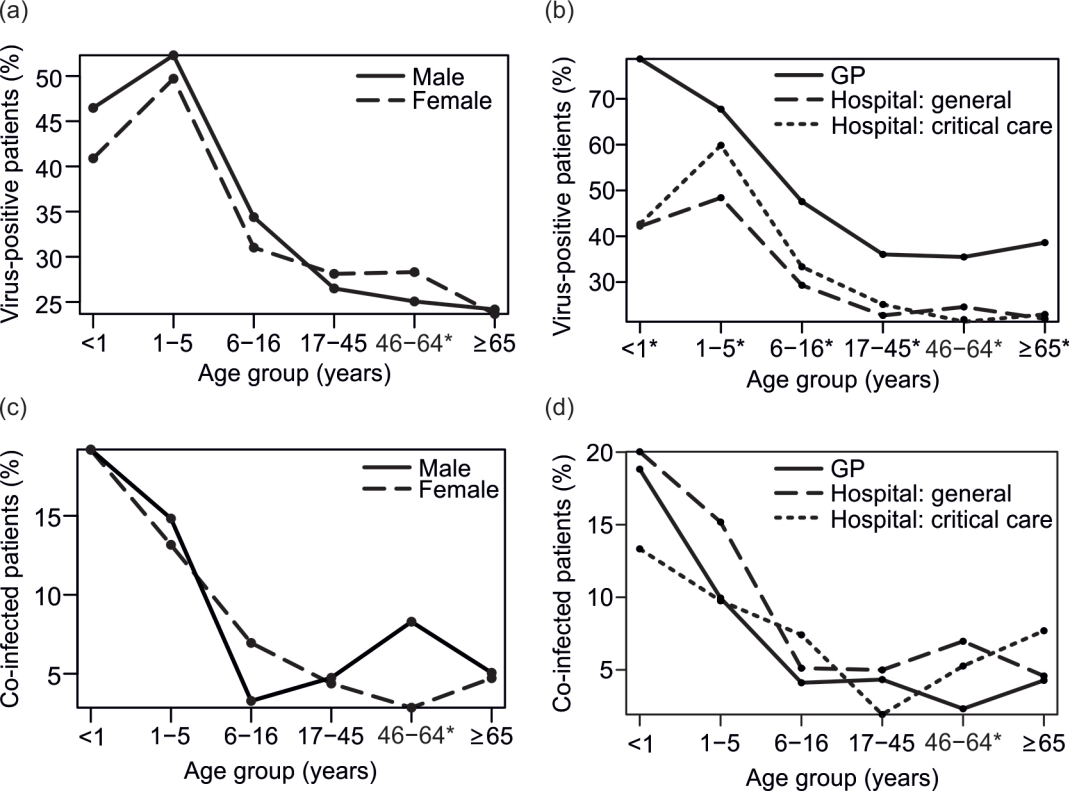

(b)
Female
Male
Test–positive episodes (%)
0
20
40
60
80
100
RV
IAV
IBV
RSV
CoV
AdV
MPV
PIV3
PIV1
PIV4
PIV2
Neg*
Respiratory virus


(c)
GP
Hospital:general
Hospital:critical care
Test–positive episodes (%)
0
20
40
60
80
100
RV
IAV
IBV
RSV
CoV
AdV
MPV
PIV3
PIV1
PIV4
PIV2
Neg*
Respiratory virus


(a)
Test-positive
Test-negative
Episodes (%)
0
20
40
60
80
100
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month
(b)
Test-positive episodes (%)
0
20
40
60
80
100
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month
Virus
RV
IAV
IBV
RSV
CoV
AdV
MPV
PIV3
PIV1
PIV4
PIV2
(c)
Test-positive
Test-negative
Episodes (%)
0
20
40
60
80
100
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month
(d)
Test-positive episodes (%)
0
20
40
60
80
100
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month


% severe cases
0
2
4
6
8
10
12
14
(269)
(62)
(85)
(55)
(27)
(29)
(46)
(22)
(21)
(15)
(3)
(1282)
RV
IAV
RSV
CoV
AdV
IBV
MPV
PIV3
PIV1
PIV4
PIV2
Neg*
Respiratory virus


(a)
Virus-positive patients (%)
0
5
10
15
20
25
Mono-infection
Co-infection
RV
IAV
IBV
RSV
CoV
AdV
MPV
PIV3
PIV1
PIV4
PIV2
(b)
Virus-positive patients (%)
0
2
4
6
8
10
12
Mono-infection
Co-infection
RV
IAV
IBV
RSV
CoV
AdV
MPV
PIV3
PIV1
PIV4
PIV2
(c)
PIV2
PIV1
CoV
RV
MPV
RSV
IBV
IAV
AdV
PIV3
PIV4
(d)
R2=0.777
RV
RSV
AdV
CoV
PIV3
MPV
IAV
PIV4
IBV
PIV1
PIV2
log10 (Co−infection freq)
1.5
2.0
2.5
3.0
log10 (Mono-infection freq)
1.5
2.0
2.5
3.0
3.5
4.0


(a)
Virus-positive patients (%)
50
45
40
35
30
25
Male
Female
<1
1–5
6–16
17–45
46–64*
≥65
Age group (years)
(b)
Virus-positive patients (%)
70
60
50
40
30
GP
Hospital: general
Hospital: critical care
<1*
1–5*
6–16*
17–45*
46–64*
≥65*
Age group (years)
(c)
Co-infected patients (%)
15
10
5
Male
Female
<1
1–5
6–16
17–45
46–64*
≥65
Age group (years)
(d)
Co-infected patients (%)
20
15
10
5
GP
Hospital: general
Hospital: critical care
<1
1–5
6–16
17–45
46–64*
≥65
Age group (years)