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Respiratory pathogen diversity and co-infections in rural Zambia

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Abstract

Objectives: The role of respiratory co-infections in modulating disease severity remains understudied in southern Africa, particularly in rural areas. This study was performed to characterize the spectrum of respiratory pathogens in rural southern Zambia and the prognostic impact of co-infections.

Methods: Respiratory specimens collected from inpatient and outpatient participants in a viral surveillance program in 2018–2019 were tested for selected viruses and a typical bacteria using the Xpert Xpress Flu/RSV assay and FilmArray Respiratory Panel EZ. Participants were followed

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval

All protocols and materials for this study were approved by the National Institutes of Health/Division of Microbiology and Infectious Diseases (protocol 18–0008), the Johns Hopkins School of Medicine Institutional Review Board, the Macha Research Trust Institutional Review Board (IRB00168163), and the Zambian National Health Research Authority (ZNHRA). All adult participants (≥16 years) and parents or guardians of pediatric participants provided written informed consent for study participation. Children 12–15 years of age provided written assent for participation.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2020.10.054.

for 3–5 weeks to assess their clinical course. Multivariable regression was used to examine the role of co-infections in influencing disease severity.

Results: A respiratory pathogen was detected in 63.2% of samples from 671 participants who presented with influenza-like illness. Common pathogens identified included influenza virus (18.2% of samples), respiratory syncytial virus (RSV) (11.8%), rhinovirus (26.4%), and coronavirus (6.0%). Overall, 6.4% of participants were co-infected with multiple respiratory pathogens. Compared to mono-infections, co-infections were found not to be associated with severe clinical illness either overall (relative risk (RR) 0.72, 95% confidence interval (CI) 0.39–1.32) or specifically with influenza virus (RR 0.80, 95% CI 0.14–4.46) or RSV infections (RR 0.44, 95% CI 0.17–1.11).

Conclusions: Respiratory infections in rural southern Zambia were associated with a wide range of viruses. Respiratory co-infections in this population were not associated with clinical severity.

Keywords

Respiratory viruses; Co-infections; Sub-Saharan; Rural; Severity

Introduction

Southern Africa experiences among the highest mortality rates from influenza virus and respiratory syncytial virus (RSV) infection of any region worldwide (Iuliano et al., 2018; Stein et al., 2017). While it has been suggested that this high mortality is driven in part by a high incidence of infection (Iyengar et al., 2015), recent studies suggest that populations in this region have a heightened risk of severe clinical sequelae associated with viral illness. For both influenza virus (Cohen et al., 2015) and RSV (Moyes et al., 2017; Stein et al., 2017) infections, higher case-fatality rates have been reported from southern Africa than from the United States and Western Europe. These disparities may be due to both differences in care delivery and individual-level vulnerabilities (Duque et al., 2014; Lindsey et al., 2019). Comorbid HIV infection (Cohen et al., 2013; Moyes et al., 2017), tuberculosis (Walaza et al., 2015; Walaza et al., 2019), and malnutrition (Paynter et al., 2014) have been implicated as factors increasing the likelihood of severe viral respiratory disease. Beyond these prevalent conditions, little is known about the drivers of viral respiratory disease severity.

Co-infection with influenza or RSV and another respiratory pathogen may have prognostic implications. While the risk of secondary bacterial infection following primary viral infection has been well-established (Brundage, 2006; Hendaus et al., 2015), less is known about the role of multiple concomitant infections. The recent proliferation of sensitive multiplex PCR assays now allows for the simultaneous detection of a broad range of pathogens from a single upper respiratory specimen at the time of presentation. One recent study from South Africa detected a co-infecting respiratory virus in 49% of RSV cases and 32% of influenza cases (Pretorius et al., 2012). However, the clinical impact of viral co-infections remains unclear: two systematic reviews found no consistent impact of co-infection on respiratory disease severity, but noted substantial heterogeneity by pathogen and study location (Asner et al., 2014; Scotta et al., 2016). In the setting of these knowledge

gaps, the US National Institute of Allergy and Infectious Diseases (NIAID) and the World Health Organization (WHO) have called for research to better characterize the role of co-infecting pathogens in modulating influenza and RSV disease severity, respectively (Erbelding et al., 2018; WHO, 2017).

Rural southern Africa presents a unique environment for the study of multiple respiratory pathogens. Compared to urban populations, those in rural areas have higher rates of malnutrition, largerfamilysizes, andless access tocare(Central Statistical Office CSO Zambia et al., 2014). However, there have been few effortsto characterize the spectrum of respiratory pathogens in rural settings, where most of the region's population resides (World Bank, 2018). The objective of this study was to describe the diversity of pathogens in the nasopharynx among patients with respiratory infections presenting for care in rural Zambia and the prognostic implications of co-infection.

Methods

Study participants and setting

This study was nested within an ongoing surveillance program supported by the National Institutes of Health (Centers for Influenza Surveillance and Research) at Macha Hospital in Southern Province, Zambia. Macha Hospital is a 208-bed district-level hospital, located in a rural area, that serves a catchment population of approximately 150 000. Southern Province historically experiences three seasons: a rainy season from November to April, a cool dry season from May to August, and a warm dry season from September to November (Sutcliffe et al., 2012). Routine vaccination against *Streptococcus pneumoniae* and *Haemophilus influenzae* type b has been introduced nationwide, and 91% of children 12–23 months of age in Southern Province were estimated to have received three doses of these vaccines in 2018 (Zambia Statistics Agency et al., 2019). In Southern Province, influenza vaccines are not routinely administered, and prior to the initiation of this surveillance program, there had been no ongoingviral surveillance.

Surveillance for influenza-like illness (ILI), influenza, and RSV was initiated on December 10, 2018. All patients presenting for care to the outpatient department were screened for ILI. ILI was defined as documented (≥38 °C) or reported fever with either cough or sore throat, with onset or clinical worsening within 7 days prior to hospital presentation (Dugas et al., 2020). An age-stratified sample of patients with ILI were approached on a weekly basis for enrollment in the study. Additionally, all inpatients newly admitted with respiratory complaints were screened, and those with ILI were approached for enrollment. At enrollment, trained study staff collected information regarding clinical symptoms and exposures using a structured data collection form and performed an examination to measure height, weight, respiratory rate, and peripheral capillary oxygen saturation using a handheld pulse oximeter (CMI Health, Alpharetta, GA, USA). In addition, a nasopharyngeal swab specimen was collected and stored in viral transport medium (Xpert Nasopharyngeal Sample Collection Kit; Cepheid Inc., Sunnyvale, CA, USA). Participants were followed-up within 3–5 weeks to ascertain vital status and clinical course.

Specimen selection, transport, and testing

Nasopharyngeal specimens were tested on the day of enrollment at the Macha Research Trust Clinical Research Laboratory using the Cepheid GeneXpert Xpress Flu/RSV assay (Cepheid Inc., Sunnyvale, CA, USA) to detect the presence of influenza A/B viruses and RSV. RT-PCR cycle threshold (Ct) values were recorded for all positive tests. The remaining specimen volume was aliquoted and the samples stored at −80 °C. To investigate respiratory co-infections and characterize the broader diversity of respiratory pathogens, participant samples were transported to Johns Hopkins University on dry ice and underwent testing using the BioFire FilmArray Respiratory Panel EZ (BioFire Diagnostics, Salt Lake City, UT, USA). This multiplex PCR panel identifies the following set of viral and atypical bacterial targets: adenovirus, coronavirus (nonsevere acute respiratory syndrome coronavirus 2 (SARS-CoV-2) species: HKU1, 229E, OC43, and NL63 subtypes), human meta-pneumovirus, rhinovirus/enterovirus, influenza A virus, influenza B virus, parainfluenza virus, RSV, *Bordetella pertussis, Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*.

Statistical analyses

As results were available for influenza A/B virus and RSV from both the Xpert Xpress Flu/RSV and FilmArray Respiratory Panel assays, an analysis was conducted to assess concordance between these platforms. The inter-test agreement between the Xpert Xpress Flu/RSV and FilmArray Respiratory Panel assays was assessed using Cohen's kappa statistic separately for influenza A virus, influenza B virus, and RSV (McHugh, 2012). To assess the impact of viral RNA concentration on test discordance, the Student *t*-test was used to compare the mean Ct values from Xpert testing for samples with concordant versus discordant results. All Ct values were centered by subtracting the mean Ct value for the virus detected. For the remainder of the analysis, samples testing positive for a given pathogen (influenza A virus, influenza B virus, or RSV) by either the Xpert Xpress Flu/RSV or FilmArray Respiratory Panel EZ were considered positive for that pathogen.

The testing results were summarized using descriptive statistics. The monthly prevalence of each respiratory infection among the population with ILI was estimated through direct standardization using the age distribution of outpatients with ILI and the age-specific prevalence of the given infection among outpatient participants. To facilitate visual comparisons of seasonality, monthly trends in age-adjusted prevalence were presented using locally weighted scatterplot smoothing (LOWESS) techniques.

To assess demographic and clinical factors associated with the presence of co-infections, univariable and age-adjusted log-binomial regression were performed. Separate models were used to assess co-infection (any two or more pathogens detected) among all participants and co-infection (one or more additional pathogens detected) among influenza-infected and RSV-infected participants. The association of co-infection with severe clinical illness was explored with an age-adjusted log-binomial analysis. Severe disease was defined as a composite outcome including at least one of the following: inpatient admission at enrollment or during follow-up, oxygen saturation (SpO_2) ≤92% at enrollment, and death during follow-up. Additional analyses were performed evaluating the association of co-infection with each

component of the composite outcome. Where log-binomial regression models failed to converge, a Poisson model with robust variance estimation was employed.

Tachypnea was defined based on age-specific cut-offs: 60 breaths/min for children under 2 months of age, 50 breaths/min for children between 2 months and 1 year, 40 breaths/min for children between 1 and 5 years, and 20 breaths/min for participants over 5 years of age (WHO, 2008). For children ≤18 years, underweight was defined as a body mass index (BMI) more than two standard deviations below the age- and sex-specific mean, using the WHO Child Growth Standard (WHO, 2006). For adults, underweight was defined as a BMI of less than 18.5 kg/m^2.

Ethical considerations

All adult participants (≥16 years) and parents or guardians of pediatric participants provided written informed consent for study participation. Children 12–15 years of age provided written assent for participation. All protocols and materials for the study were approved by the National Institutes of Health/Division of Microbiology and Infectious Diseases (protocol 18–0008), Johns Hopkins Institutional Review Board, the Macha Research Trust Institutional Review Board (IRB00168163), and the Zambian National Health Research Authority (ZNHRA). A Material Transfer Agreement was obtained from the ZNHRA to export samples for further testing.

Results

Study population

Between December 10, 2018 and December 9, 2019, 671 patients with ILI were enrolled and underwent testing with the GeneXpert Xpress Flu/RSV assay and the BioFire FilmArray Respiratory Panel EZ (for the comparison of influenza A/B virus and RSV testing results between the two assays, see Supplementary Material Figure S1). The median age of the study population was 3.2 years (interquartile range 0.8–19 years). The prevalence of HIV infection was 4% and the prevalence of low body weight was 10% (Table 1). During the follow-up period, 33% of participants experienced severe clinical illness based on the composite study outcome.

Pathogen diversity

Overall, the median number of pathogens detected from participant specimens using both the BioFire FilmArray EZ panel and Xpert Xpress Flu/RSV was 1 (range 0–3). At least one respiratory pathogen was detected in 63% (424/671) of these samples, including 75% of samples from children under 5 years of age and 46% among participants over 5 years. Eighty-six participants (13% of participants) tested positive for influenza A virus, 36 (5% of participants) for influenza B virus, and 79 (12% of participants) for RSV. Across all patient specimens, the most commonly detected pathogen was rhinovirus (n = 177; 26%of samples; Table 1). Only a single nasopharyngeal specimen tested positive for *Bordetella pertussis*. Characteristics of the study population by testing result are presented in Table 1.

Several pathogens demonstrated marked seasonality among outpatients presenting for care (Figure 1). Influenza Avirus (April–September), influenza B virus (August–November), RSV (January–April), and coronavirus (September–February) presented with a single annual prevalence peak, while adenovirus was detected at low prevalence throughout the year. Rhinovirus was detected across the study period, with a peak in prevalence in June. The peak prevalence of both influenza A virus and rhinovirus occurred during the cold dry season, while the peaks for influenza B virus and coronavirus occurred in the warm dry season.

Co-infections

Overall, 6.4% of study participants were infected with multiple respiratory pathogens (Table 2). There was heterogeneity in the number of pathogens detected by age; children under 1 year of age experienced the highest prevalence of co-infection(Supplementary Material Figure S2; Table 2). The difference in the prevalence of coinfection comparing infants less than 1 year old with those older than 1 year of age was statistically significant among all participants (prevalence ratio (PR) 0.30, 95% confidence interval (CI) 0.17–0.54) and among participants without influenza or RSV infection (PR0.19, 95% CI 0.08–0.46), but not among influenza or RSV-positive participants. Co-infections were less common for influenza B virus (0.0% of positive specimens) than for influenza A virus (5.8%); however, this difference was not statistically significant ($p = 0.14$). Rhinovirus was the most common co-infecting pathogen detected, including among specimens positive for either influenza virus or RSV (2.5% and 16.5% of specimens, respectively; Table 3).

Among participants with at least one pathogen detected, there were few differences in presenting symptoms between those with single-pathogen infections and those with respiratory co-infections (Supplementary Material Table S1). Headache was associated with co-infection with any pathogen (age-adjusted prevalence ratio (adj PR) 3.67, 95% CI 1.36–9.88). Among participants with a diagnosed RSV infection, diarrhea (adj PR 3.23, 95% CI 1.31–7.96) and longer duration of ILI symptoms (adj PR per additional day of symptoms 1.35, 95% CI 1.02–1.78) were associated with the presence of a co-infecting species. After adjusting for age, no significant patient or household-level factors were found to be associated with co-infection compared to monoinfection Supplementary Material Tables S2 and S3).

The number of concurrent respiratory pathogens detected was not significantly associated with clinical severity (Table 4), although the likelihood ofsevere clinical disease was non-significantly lower for any respiratory co-infection (adjusted relative risk (adj RR) 0.72, 95% CI 0.39–1.32), as well as for co-infections with influenza virus (adj RR 0.80, 95% CI 0.14–4.46) and RSV (adj RR 0.44, 95% CI 0.17–1.11). There were no significant differences in the likelihood of antibiotic administration by co-infection status (adj RR 0.86, 95% CI 0.70–1.05).

Discussion

In this study, multiplexed PCR testing was used to describe the spectrum of pathogens infecting patients with acute respiratory illness in rural southern Zambia. In this setting, a

wide diversity of respiratory viruses associated with ILI and a sizeable prevalence of viral co-infections were found. This study provides valuable context regarding the pathogen landscape in a historically under-surveilled region. An appreciation of pathogen diversity and seasonality may provide key insights in understanding trends in acute respiratory illness in the region, particularly in the context of the ongoing spread of SARS-CoV-2 and minimal diagnostic testing.

Overall, a respiratory virus was detected in 63% of study participants. This is in line with estimates from both Lusaka, Zambia (Simusika et al., 2015) and South Africa (Pretorius et al., 2012). Respiratory pathogens were more commonly detected in young children, which is consistent with the literature for the pathogens identified (Zhang et al., 2014). In this rural area without access to seasonal influenza vaccination or mechanical ventilation, a substantial prevalence of both influenza viruses and RSV was found among acutely ill patients. These pathogens have well-established roles in driving respiratory disease and severe illness (PERCH, 2019; Pretorius et al., 2016). We also report a high prevalence of other viral infections, including rhinovirus and coronavirus. Many of these respiratory viruses presented with distinct seasonality during the study period in this rural setting. For many pathogens, the observed seasonality differed greatly from that reported in the only published Zambian study describing pathogens other than influenza viruses or RSV from the urban center of Lusaka (Simusika et al., 2015). While there may be year-to-year heterogeneity in viral prevalence, the present study's findings may suggest important in-country heterogeneity and highlight the importance of broad-based surveillance that extends beyond urban centers. Interventions that account for the local epidemiology and patterns of infection are needed to address both seasonal respiratory infections and pandemic spread.

In this rural setting, respiratory viral co-infections were identified in 6.4% of patients with ILI. Published studies report a wide range of prevalence of co-infection. The study estimates are similar to the prevalence reported in other low and middle income country (LMIC) settings including China (3.4%) (Zhang et al., 2014) and Cambodia (6%) (Guerrier et al., 2013), but are lower than those reported from studies in Vietnam (27%) (Do et al., 2011), Brazil (44%) (Nascimento et al., 2010), and South Africa (17%) (Pretorius et al., 2012). Setting-specific epidemiology likely underlies much of this difference; many of the published studies were conducted in hospitalized patients with severe disease in whom nosocomial viral infections may be frequently observed (Goldmann, 2001). Further, the ILI case definition in the present study required the presence of fever, which may have excluded patients with mild disease caused by multiple pathogens.

A number of studies have found increased clinical severity with influenza virus (Esper et al., 2011) and RSV co-infections (Aberle et al., 2005; Calvo et al., 2008) and this remains a controversial topic. Our observations support the results of recent systematic reviews that have reported that the presence of viral co-infections do not impact clinical severity (Asner et al., 2014; Scotta et al., 2016). Importantly, systematic reviews to date have included very few studies from southern Africa, and none from the region's rural areas. Here, we found a non-significantly lower risk of severe disease with both influenza virus and RSV co-infections as compared to viral mono-infections. While the reasons for this are unclear, this may suggest successive infections with ongoing influenza or RSV shedding in the context of

a new, mild viral respiratory infection. This study was not powered to detect differences in severity for specific co-infecting species. Additionally, we did not test for common bacterial pathogens that may have profound immunological interactions with viral causes of respiratory illness (Almand et al., 2017). Further work is needed to understand the clinical impact of pathogen-specific interactions in this setting.

Concordance between the Xpert Xpress Flu/RSV assay and BioFire FilmArray Respiratory Panel EZ ttest results was found to be lower than has been reported previously in studies performed in the United States (Banerjee et al., 2018; Wahrenbrock et al., 2016). Specifically, a number of specimens determined to be positive for viruses by the Xpert assay were not confirmed with the FilmArray EZ panel. It was found that these discrepant testing results were more likely to occur with specimens with low viral loads of detected virus (i.e., high Ct values), thus likely reflecting higher sensitivity of the Xpert Flu/RSV assay. The Xpert test has three influenza A virus target genes (M, PB2, PA) and two influenza B virus target genes (M, NSP), while the BioFire has two influenza A virus target genes (M and HA) and one influenza B virus target gene (HA) (Kanwar et al., 2020). These differences in target number and type may underlie some of the testing discrepancies observed. However, we cannot exclude the possibility of false-positive results from the Xpert assay, which have been reported previously in studies comparing its performance to that of the gold standard CDC Flu A/B PCR test (Azar and Landry, 2018).

This study is not without limitations. The evaluation of co-infections was limited to the pathogens included in the multiplex diagnostic tests used. While this included 11 viruses and three atypical bacteria, other pathogens causing respiratory infections were not evaluated, including common pneumonia-associated bacterial species such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type B. Additionally, the high sensitivity and broad targets of the multiplex diagnostic assays present two distinct challenges. First, the clinical relevance of detected pathogens can be difficult to discern. While the causal role of influenza viruses, RSV, and human metapneumoviruses in causing disease has been highlighted consistently, the attribution of clinical disease to other detected species has been less clear and consistently supported (Pretorius et al., 2016; Shi et al., 2019; Shi et al., 2015). Asymptomatic controls were not tested in this study, making context-specific disease attribution challenging. Secondly, RT-PCR-based diagnostics allow for the detection of viral nucleic acids after symptom resolution and without ongoing viral replication (Inagaki et al.,2016). This means that the observation of multiple infections might, in fact, be sequentially occurring rather than co-occurring infections, leading to misclassification of co-infections and resulting bias. Finally, while the BioFire Respiratory EZ panel is able to detect atypical bacterial infections, nasopharyngeal specimens are less sensitive than sputum samples for their diagnosis. The prevalence of atypical bacteria may therefore have been underestimated (Cho et al., 2012).

In summary, in rural southern Zambia there is a high prevalence of viral infection among individuals with acute respiratory illness. While a broad array of viruses were detected, there was no association of viral co-infections with increased clinical severity. This study highlights the regional role of respiratory viruses as causative agents of ILI and also demonstrates the profound seasonality associated with specific viruses. Continued

surveillance in urban and rural settings in southern Africa is needed to verify differences in seasonality and viral epidemiology and to assess their impact on disease presentation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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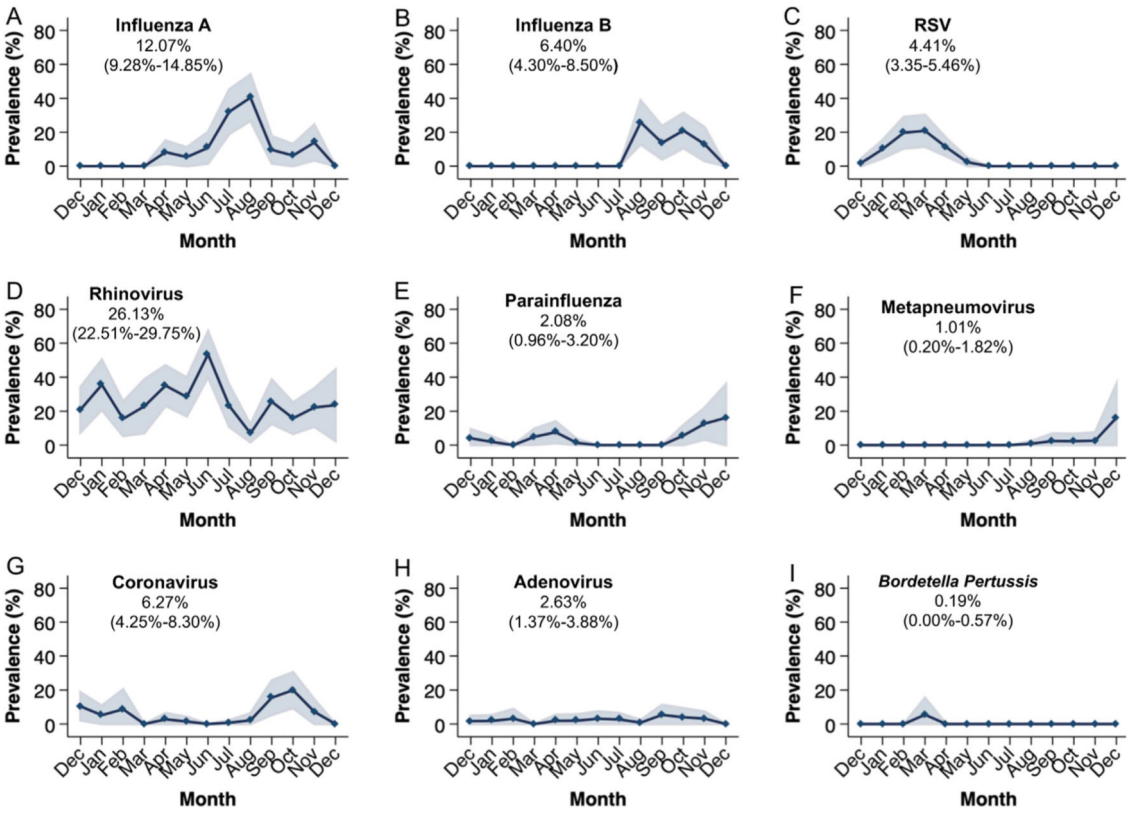

Figure 1.

Seasonal trends by respiratory pathogen; Macha, Zambia—December 2018 to December 2019. Estimated pathogen prevalence among outpatients with influenza-like illness (ILI) over the study period for (A) influenza A virus, (B) influenza B virus, (C) respiratory syncytial virus (RSV), (D) rhinovirus, (E) parainfluenza virus, (F) metapneumovirus, (G) coronavirus, (H) adenovirus, and (I) *Bordetella pertussis*. Percentages represent estimated pathogen prevalence overall among all outpatients with ILI. Lines represent estimated monthly prevalence and gray bands represent associated 95% confidence intervals.

Table 1

Characteristics of the study population by testing result.

		Overall (*n* = 671)	**Adenovirus (*n* = 21)**	**Coronavirus (*n* = 40)**	**Metapneum virus (*n* = 6)**	**Rhinovirus (*n* = 177)**	**Influenza A virus (*n* = 86)**	**Influenza B virus (*n* = 36)**	**Parainfluenza virus (*n* = 25)**	**RSV (*n* = 79)**	***Bordetella pertussis* (*n* = 1)**	**Negative (*n* = 247)**
Age, years	Median (IQR)	3.2 (0.8–19)	0.9 (0.7–1.8)	2.0 (0.5–5.1)	1.1 (0.5–2.5)	1.3 (0.6–7)	4.0 (1.6–11)	3.3 (1.5–8)	2.5 (1.1–9)	1.5 (0.6–3.4)	2.2 (2.2–2.2)	10 (1.5–39)
	0–11 months, *n* (%)	186 (28%)	11 (52%)	18 (45%)	2 (33%)	74 (42%)	13 (15%)	6 (17%)	6 (24%)	31 (39%)	0 (0%)	52 (21%)
	1–4 years, *n* (%)	212 (32%)	6 (29%)	12 (30%)	4 (67%)	54 (31%)	37 (43%)	14 (39%)	10 (40%)	35 (44%)	1 (100%)	47 (19%)
	5–15 years, *n* (%)	86 (13%)	3 (14%)	2 (5%)	0 (0%)	11 (6%)	17 (20%)	12 (33%)	3 (12%)	8 (10%)	0 (0%)	35 (14%)
	16–50 years, *n* (%)	109 (16%)	1 (5%)	5 (12%)	0 (0%)	24 (14%)	10 (12%)	4 (11%)	0 (0%)	2 (3%)	0 (0%)	67 (27%)
	51+ years, *n* (%)	78 (12%)	0 (0%)	3 (8%)	0 (0%)	14 (8%)	9 (10%)	0 (0%)	6 (24%)	3 (4%)	0 (0%)	46 (19%)
Female, *n* (%)		360 (54%)	11 (52%)	19 (48%)	4 (67%)	98 (55%)	42 (49%)	25 (69%)	11 (44%)	36 (46%)	1 (100%)	135 (55%)
Number of individuals in household, median (IQR)		7 (5–9)	8 (6–9)	7 (4.5–10)	6.5 (6–7)	7 (5–10)	6 (5–8)	6 (5–8)	8 (6–10)	6 (5–8)	9 (9–9)	7 (4–9)
Number of individuals sharing sleeping space, median (IQR)		3 (2–3)	3 (3–4)	3 (3–3)	3 (3–3)	3 (3–3)	3 (2–3)	3 (2–3)	2 (2–3)	3 (3–3)	3 (3–3)	3 (2–3)
Underweight, *n* (%)		68 (10%)	1 (5%)	3 (8%)	0 (0%)	16 (9%)	14 (16%)	3 (8%)	3 (12%)	6 (8%)	0 (0%)	25 (10%)
HIV-infected, *n* (%)		29 (4%)	1 (5%)	0 (0%)	0 (0%)	4 (2%)	3 (3%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	21 (9%)
History of tuberculosis, *n* (%)		22 (3%)	1 (5%)	2 (5%)	0 (0%)	3 (2%)	1 (1%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	15 (6%)
Clinical severity[a]	Severe illness, *n* (%)	165 (33%)	6 (40%)	7 (29%)	2 (50%)	32 (25%)	32 (38%)	5 (14%)	6 (30%)	33 (62%)	0 (0%)	53 (29%)
	Admitted to the hospital, *n* (%)	108 (18%)	5 (29%)	3 (9%)	0 (0%)	21 (14%)	23 (27%)	2 (6%)	3 (13%)	17 (26%)	0 (0%)	40 (18%)
	Hypoxemic, *n* (%)	81 (15%)	4 (22%)	5 (17%)	2 (33%)	17 (12%)	13 (16%)	4 (11%)	3 (15%)	18 (35%)	0 (0%)	22 (11%)
	Died during follow-up, *n* (%)	10 (2%)	0 (0%)	0 (0%)	0 (0%)	3 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	7 (3%)

IQR, interquartile range; RSV, respiratory syncytial virus.

[a]Clinical severity was not ascertained for 168 participants due to missing oxygen saturation or follow-up data.

Table 2

Prevalence of respiratory co-infections by age.

Age	Prevalence of co-infections[a]			
	Influenza- and RSV-negative	Influenza-positive	RSV-positive	Overall
0–11 months	11.03% (15/136)	5.26% (1/19)	25.81% (8/31)	12.90%
1–4 years	2.38% (3/126)	1.96% (1/51)	11.43% (4/35)	3.77%
5–15 years	4.08% (2/49)	3.45% (1/29)	25.00% (2/8)	5.81%
16–50 years	2.15% (2/93)	7.14% (1/14)	50.00% (1/2)	3.67%
51+ years	0.00% (0/66)	11.11% (1/9)	33.33% (1/3)	2.56%
Overall	4.68% (22/470)	4.10% (5/122)	20.25% (16/79)	6.41%

RSV, respiratory syncytial virus.

[a]Co-infection refers to the presence of two or more pathogens. For influenza virus- or RSV-positive samples, the presence of a second infecting pathogen defines co-infection.

Table 3

Respiratory co-infections among study participants.

	Influenza virus	RSV	Adenovirus	Coronavirus	Parainfluenza virus	Rhinovirus	*Bordetella pertussis*
Influenza virus	**122**	0 (0%)	0 (0%)	1 (1%)	1 (1%)	3 (2%)	0 (0%)
RSV	0 (0%)	**79**	1 (1%)	2 (3%)	2 (3%)	13 (16%)	0 (0%)
Adenovirus	0 (0%)	1 (5%)	**21**	5 (24%)	0 (0%)	8 (38%)	0 (0%)
Coronavirus	1 (2%)	2 (5%)	5 (12%)	**40**	0 (0%)	7 (18%)	0 (0%)
Parainfluenza virus	1 (4%)	2 (8%)	0 (0%)	0 (0%)	**25**	5 (20%)	0 (0%)
Rhinovirus	3 (2%)	13 (7%)	8 (5%)	7 (4%)	5 (3%)	**177**	0 (0%)
Bordetella pertussis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**1**

RSV, respiratory syncytial virus. Percentages represent the proportion of the total by row. Diagonal cells (bold) represent the total number of positive tests for each pathogen.

Table 4

Clinical outcomes of respiratory co-infections.

	Overall				Influenza				RSV			
	Infection		Relative risk		Infection		Relative risk		Infection		Relative risk	
	Mono- (*n* = 381)	Co-(*n* = 43)	Univariable 95% CI	Age-adjusted 95% CI	Mono-(*n* = 117)	Co-(*n* = 5)	Univariable 95% CI	Age-adjusted 95% CI	Mono-(*n* = 63)	Co-(*n* = 16)	Univariable 95% CI	Age-adjusted 95% CI
Pneumonia diagnosis, *n* (%)	26 (7%)	1 (2%)	0.34 (0.05–2.45)	0.35 (0.05–2.51)	7 (6%)	0 (0%)	N/A	N/A	10 (16%)	1 (6%)	0.39 (0.05–2.85)	0.40 (0.06–2.89)
RTI diagnosis, *n* (%)	293 (77%)	32 (74%)	0.97 (0.81–1.16)	0.96 (0.80–2.51)	91 (78%)	5 (100%)	**1.29 (1.17–1.42)**	**1.28 (1.16–1.43)**	49 (78%)	13 (81%)	1.04 (0.80–1.37)	1.05 (0.82–1.34)
Sepsis diagnosis, *n* (%)	11 (3%)	1 (2%)	0.81 (0.11–6.09)	0.69 (0.09–5.25)	8 (7%)	0 (0%)	N/A	N/A	3 (5%)	0 (0%)	N/A	N/A
Received antibiotics, *n* (%)	307 (81%)	30 (70%)	0.87 (0.71–1.06)	0.86 (0.70–1.05)	99 (85%)	4 (80%)	0.95 (0.60–1.48)	0.96 (0.61–1.50)	58 (92%)	12 (75%)	0.81 (0.61–1.09)	0.82 (0.61–1.09)
Severe illness[a]	107 (36%)	8 (27%)	0.75 (0.41–1.38)	0.72 (0.39–1.32)	36 (31%)	1 (25%)	0.80 (0.14–4.45)	0.80 (0.14–4.46)	30 (70%)	3 (30%)	0.43 (0.16–1.13)	0.44 (0.17–1.11)
Severe end-points												
Inpatient admission	64 (17%)	4 (10%)	0.56 (0.21–1.46)	0.55 (0.21–1.44)	25 (21%)	0 (0%)	N/A	N/A	15 (24%)	1 (7%)	0.28 (0.04–1.93)	0.25 (0.04–1.70)
Hypoxemia	54 (17%)	6 (18%)	1.07 (0.50–2.29)	1.02 (0.47–2.19)	16 (14%)	1 (25%)	1.78 (0.31–10.32)	1.80 (0.31–10.31)	16 (40%)	2 (18%)	0.45 (0.12–1.68)	0.49 (0.13–1.77)

CI, confidence interval; Co-, co-infection; Mono-, mono-infection; N/A, not available; RSV, respiratory syncytial virus; RTI, respiratory tract infection. Bold = $p < 0.05$ from log-binomial regression.

[a]Severe clinical illness defined as a composite outcome including at least one of the following: inpatient admission at enrollment or during follow-up, hypoxemia (SpO_2 ≤92% at enrollment), and death during follow-up. Clinical severity was not ascertained for 121 participants due to missing oxygen saturation or follow-up data.