Clinical and Epidemiological Features of Respiratory Virus Infections in Preschool Children Over Two Consecutive Influenza Seasons in Southern Brazil

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This study reports the results of a systematic screening for respiratory viruses in pediatric outpatients from an emergency department (ED) in southern Brazil during two consecutive influenza seasons. Children eligible for enrollment in this study were aged 24–59 months and presented with acute respiratory symptoms and fever. Naso- and oropharyngeal swabs were collected and multiplex reverse transcription PCR (RT-PCR) was performed to identify the respiratory viruses involved. In total, 492 children were included in this study: 248 in 2010 and 244 in 2011. In 2010, 136 samples (55%) were found to be positive for at least one virus and the most frequently detected viruses were human rhinovirus (HRV) (18%), adenovirus (AdV) (13%), and human coronavirus (CoV) (5%). In 2011, 158 samples (65%) were found to be positive for at least one virus, and the most frequently detected were HRV (29%), AdV (12%), and enterovirus (9%). Further, the presence of asthma (OR, 3.17; 95% CI, 1.86–5.46) was independently associated with HRV infection, whereas fever was associated with AdV (OR, 3.86; 95% CI, 1.31–16.52) and influenza infections (OR, 3.74; 95% CI, 1.26–16.06). Ten patients (2%) were diagnosed with pneumonia, and six of these tested positive for viral infection (4 HRV, 1 RSV, and 1 AdV). Thus, this study identified the most common respiratory viruses found in preschool children in the study region and demonstrated their high frequency, highlighting the need for improved data collection, and case management in order to stimulate preventive measures against these infections.
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INTRODUCTION

Viral acute respiratory infections (ARIs) in pediatric outpatients represent a significant burden on emergency departments (EDs) and the patients' families, mainly during influenza seasons, being associated with around 20% of all deaths in pre-school children worldwide, with 90% of these deaths due to pneumonia. [Izurieta et al., 2000; Fiore et al., 2009; Bezerra et al., 2011; Gessner et al., 2011; Munywoki et al., 2011]. However, information about the etiology of viral ARIs in preschool children, particularly in outpatients, is limited, especially in Latin America. Community respiratory viruses (CRVs) are frequently found in this population and could be involved in severe infections in young children and patients with comorbid conditions [Ampofo et al., 2006; Ampofo et al., 2008; Bender et al., 2009]. Therefore, understanding the dynamics of their circulation among various age groups is essential, not only to help pediatricians in their diagnosis but also for developing preventive measures against these infections.

Curitiba city in southern Brazil has a temperate climate with low temperatures and high humidity in winter. Previous studies have shown a seasonal pattern

of prevalence of respiratory viruses in Curitiba, with an increased incidence in winter. During this period, influenza and respiratory syncytial viruses are most frequently associated with increased respiratory disease morbidity and lead to an extensive respiratory disease burden [Tsuchiya et al., 2005; Coelho et al, 2007; Debur et al., 2010; Raboni et al., 2011]. However, the majority of these studies have been conducted in hospitalized patients or outpatients screened under an influenza surveillance program. Although reports that a high number of outpatients consistently present with ARIs, few of them have evaluated the pathogens involved in ARIs in this group of patients [Pavia, 2013; Adams et al., 2015].

This study reports, the results of a laboratory-based surveillance for respiratory viruses in preschool children who were treated in the ED of a pediatric referral hospital during two consecutive influenza seasons.

METHODS

Study Design, Participating Recruitment, and Setting

A cross-sectional study was carried out from July to November in both 2010 and 2011. Children considered eligible for this study were outpatients who were aged 24–59 months and attended the ED of Pequeno Príncipe Hospital (PPH), Curitiba, Brazil; these children had been diagnosed with acute upper or lower respiratory infections or asthma-related conditions. Patients whose parents signed the consent form, completed the form (demographic, clinical, and influenza vaccine data), and met all of the following inclusion criteria were enrolled in the study: (i) ARIs with or without fever ($>100°F/38°C$); (ii) that occurred <7 days before the ED visit; and (iii) for which samples had been obtained. Each patient was included only once. Patients exhibiting symptoms that began >7 days before the ED visit, patients who were administered oseltamivir during the 3 days (6 doses) prior to enrollment, and those from whom samples could not be obtained were excluded from the study. Detailed demographic information was obtained for all children enrolled by using medical charts and brief interviews. In addition, clinical data, outcomes, comorbidities, and the time spent in school or a day-care center were assessed for each child.

ARIs comprise a large and heterogeneous group of diseases such as the following: flu syndrome; common cold; pharyngitis; laryngitis; tracheitis; asthma and wheezing crisis; bronchiolitis; and pneumonia of bacterial, viral, and other etiologies.

This study was approved by the Institutional Review Board (IRB: #820-10), and written informed consent was obtained from the individuals (parents, tutor, or relatives) responsible for the patients. The Pequeno Príncipe Hospital is the largest pediatric referral hospital of Curitiba and attends children referred from various health units in the city and metropolitan region for tertiary and quaternary medical care. It also has a large number of specialized clinics and emergency rooms for primary and secondary medical care, the study was conducted with these group of patients.

Viral Detection Technique

Samples from naso- and oropharyngeal mucosa were collected with swabs and transferred to virological transport media (tryptose phosphate buffer enriched with gelatin) and shipped at 4°C to the virology laboratory. CRVs were detected using multiplex RT-PCR. The viral genome was extracted using the Viral Gene-Spin™ Kit (Intron Biotechnology Inc., Korea) according to the manufacturer's instructions. The multiplex RT-PCR technology used for the samples from 2010 enables simultaneous detection of 12 viruses (Seeplex® RV 12 ACE detection, Seegene, Korea): human adenovirus (AdV); human coronavirus (CoV) types 229E/NL63 and OC43/HKU1; human metapneumovirus (MPV); parainfluenza virus types 1, 2, and 3 (PIV-1, PIV-2, and PIV-3); influenza A (FLUA) and influenza B (FLUB) viruses; respiratory syncytial virus types A and B (RSV-A, RSV-B); and human rhinovirus types A and B (HRV A/B).

The multiplex RT-PCR technology used for the 2011 samples enables simultaneous detection of 15 viruses (Seeplex® RV 15 ACE detection, Seegene, Korea): the 12 viruses of the Seeplex® RV 12 ACE kit plus human enterovirus (EV), human bocavirus (BoV), and parainfluenza virus type 4 (PIV-4). Subtyping of influenza A viruses was performed by using real time RT-PCR (qRT-PCR) according to the CDC protocol [WHO, 2010].

Sample Size and Data Analysis

Sample size estimation was based on a 95% bilateral confidence interval for the expected proportion of children with infection caused by influenza virus. The expected proportion was assumed to range from 0.07 to 0.40, the number of subjects included was 250, and the maximum confidence interval precision (range) was chosen to be 0.12.

Data were compiled using JMP version 5.2.1 (SAS Institute Inc., Cary, NC) and analyzed using the R package version 3.0.1 (R Core Team; 2014). Descriptive statistics were used to describe the general characteristics of the study population. Quantitative variables with normal and non-normal distributions are presented as means ± standard deviation and medians with interquartile ranges (IQRs, 25th–75th percentiles), respectively, whereas qualitative variables have been expressed as numbers and percentages with 95% confidence intervals (CIs). Chi-square or Fisher's test were used for comparison of qualitative variables. The Student's t-test or the Mann–Whitney U-test was applied to compare quantitative variables, as appropriate. Five viruses (HRV, AdV, PIV, FLU, and CoV) were selected for a binomial analysis to assess the impact of viruses on the health of the children. The

presence or absence of the virus was compared with different variables (demographic, clinical features, and host characteristics). The selection of these viruses was based on either the high frequency of infections or previously reported severity of infections caused by them. Only cases with mono-detection were included in this evaluation. Variables with an associated P-value < 0.2 in the univariate analysis were subjected to multivariate logistic regression to identify independent predictors of these viruses infection. Odds ratios (ORs) and 95% CIs were calculated. All P-values were based on two-tailed comparisons, and the level of significance was set at $P < 0.05$.

RESULTS

Initially, 505 patients were enrolled, of these 13 were later excluded because: (i) inappropriate sample collection (01); (ii) date of onset of symptoms ≥ 7 days (06); (iii) medical record was not found (01); (iv) not meeting ILI acute condition (03); and (v) age >59 months (02). A total of 492 children between the ages of 24 and 59 months were included in the study during two influenza seasons (248 in 2010 and 244 in 2011). The mean age of the children was 39.1 (± 10.2) months, and 265 (54%) were male individuals. Further, 376 (77%) of the 492 children attended school or daycare centers, of which 218 (58%) attended school or day care on a full-time basis. Comorbid conditions were present in 149 (30%) children, with asthma/bronchitis (n $= 129$, 26%, as evidenced by reporting of previous episodes of wheezing with the use of bronchodilator medication) being most frequently reported (Table I). Fever, cough, and pharyngeal erythema were the most common clinical manifestations. Chest radiography was performed for 44 (9%)

patients, and perihilar infiltrates, and pulmonary consolidation were found in 27/44 (61%) and 10/44 (23%) cases, respectively. Six of the 10 patients (2%) with pulmonary consolidation tested positive for multiple viruses (4 HRV, 1 RSV, and 1 AdV). Nine (2%) patients required non-invasive respiratory support, and antibiotics were prescribed for 34 (7%) children. Nasopharyngitis and influenza-like infection were diagnosed in 71% of the cases (Table II). Clinical complications occurred in eight (1.6%) patients: one had sinusitis, two presented with otitis media, one had tracheobronchitis, and one had pneumonia.

Of the 248 children evaluated in 2010, 136 (55%) samples were positive for at least one respiratory virus. HRV A/B (n $= 46$, 18%), AdV (n $= 33$, 13%), and CoV 229/NL 63 (n $= 13$, 5%) were detected most frequently. Viral co-infections occurred in 18 (7%) patients, mainly with the association of HRV A/B and

TABLE I. Demographic, Clinical, and Epidemiological Data from the 492 Children Enrolled in the Study in Southern Brazil

Data	N $= 492$ (%)
Sex	
Female	227 (46)
Male	265 (54)
Age (months)	
Mean $\pm$ SD	39.1 $\pm$ 10.2
Median (min–max)	38.3 (24.0–60.0)
Enrolled in school/day-care centers	
No	112 (23)
Yes	376 (77)
Part-time school	158 (42)
Full-time school	218 (58)
Comorbid conditions	
No	342 (69.8)
Yes	149 (30)
Unknown	1 (0.2)
Type of comorbidity present	
Asthma and/or bronchitis	129 (26)
Allergic rhinitis	36 (7)
Other	29 (12)
Passive smoking	
No	184 (76)
Yes	57 (24)

TABLE II. Clinical Manifestations, Radiological Findings, and Diagnoses of the Enrolled Subjects

Data	N $= 492$ (%)
Fever	107 (22%)
No	385 (78)
Yes	
Cough	
No	140 (28)
Yes	352 (72)
Coryza	298 (61)
No	194 (39)
Yes	
Pharyngeal erythema	160 (32)
No	332 (68)
Yes	
Wheezing	427 (87)
No	65 (13)
Yes	
Dyspnea	464 (94)
No	28 (6)
Yes	
Antibiotics used	
Yes	34 (7)
Amoxicillin	12 (35)
Amoxicillin clavulanate	6 (15)
Benzathine penicillin	5 (15)
Azithromycin	4 (12)
Ceftriaxone	2 (6)
Others	5 (6)
Respiratory support needed	
No	483 (98)
Yes	9 (2)
Radiological assessment performed	
Yes	44 (9)
Normal	7 (16)
Perihilar infiltrate	27 (61)
Pulmonary consolidation	10 (23)
Most frequent diagnoses	
Nasopharyngitis	255 (52)
Influenza-like infection	96 (19)
Asthma/bronchitis	48 (10)
Tonsillitis	42 (8)
Pharyngitis	24 (5)
Acute laryngitis	14 (3)
Pneumonia	13 (3)
Bronchospasm crisis	12 (2)

AdV (n = 5; 2%). In 2011, 157 out of 244 (65%) samples were positive for at least one respiratory virus. Further, HRV A/B (n = 71, 29%) and AdV (n = 29, 12%) were detected most frequently, followed by enterovirus (n = 21, 9%). Viral co-infections occurred in 26 (11%) patients and mainly involved an association of HRV A/B and AdV (n = 5, 2%), enterovirus and HRV A/B (n = 5, 2%), or HRV A/B and parainfluenza 3 (n = 5, 2%) (Table III, Fig. 1).

The distribution of the frequency of the viruses according to the age group showed that most of them were detected in children aged between 24 and 36 months, except for influenza A and human coronaviruses.

Notably, in both years, there was a higher rate of detection of influenza type B (5%; 25/492) than of type A (3.6%; 18/492). While 17 of the 18 samples containing influenza A were of subtype H3N2, one was of an undetermined subtype (0.4%), and none of them belonged to subtype H1N1pdm09. There was a fluctuation in the most frequently detected respiratory viruses according to the epidemiological week during the study, with a higher frequency of detection of influenza B in the first 6 weeks of the study, followed by influenza A H3N2. The proportion of influenza virus infection in the population studied was between 5% and 12% (95% CI, 0.05–0.12).

Further, a significant difference in the coverage rate of influenza vaccination was observed between 2010 and 2011 (88%; 95% CI, 85.9–93.6 vs. 24%; 95% CI, 19.2–30). Over 90% of immunized patients received the monovalent H1N1pdm influenza vaccine in 2010, whereas in 2011, most of the immunized children received the trivalent vaccine (Table IV).

Binomial analysis of the five selected viruses showed that the presence of underlying asthma/bronchospasm was significantly associated with HRV infection (OR, 3.17; 95% CI, 1.86–5.46), while fever and myalgia was less likely to be associated with HRV. On the other hand, the presence of fever was significantly associated with AdV and influenza infections (OR, 3.86; 95% CI, 1.3–16.5 and OR, 3.74; 95% CI, 1.26–16.0, respectively), whereas the presence of underlying conditions did not increase the chance of acquiring these infections. None of the evaluated variables showed significant correlation with the presence of parainfluenza virus and coronavirus infections (Table V).

DISCUSSION

This study showed CRV circulation in a population of preschool children in post-pandemic years. Human rhinovirus and adenovirus were the main viruses found in the 2 years following the pandemic. The profile of detected viruses was different from that previous reported, and viruses such as RSV were less frequent, while adenoviruses, which are generally associated with severe infections in hospitalized patients, were more frequently detected. This is probably due to differences in age groups, because previous studies with pediatric outpatients under 2 years old showed a pathogen profile similar to that of hospitalized children [Milstone et al., 2012; Hara et al, 2014; Adams et al., 2015]. Çiçek et al. [2015] studied respiratory viruses in adult and pediatric patients in a 12-year follow-up study. Similar to our results, in pediatric patients, they found that HRV was detected more frequently in outpatients, and RSV, in inpatients; however, the distribution of the detected viruses was not stratified according to age group. Moreover, diagnosis was based on antigen detection and virus isolation, which are less sensitive than the method used in this study. Likewise, Zimmerman et al. [2015] re-] reported a higher frequency of RSV and influenza A viruses in outpatients, although they were between 6 months and 17 years of age, unlike the group assessed in this study.

When comparing the two periods studied, we observed a higher prevalence of viral coinfection in 2011, due to increased virus detection owing to the use of RV 15 multiplex PCR. However, in both years, the coinfection rates were lower than those in various previous reports, wherein they ranged from 31% to 51% [Lepiller et al., 2013; Luchsinger et al., 2014; Turunen et al., 2014]. Though, most of these studies were carried out in hospitalized children who generally are younger and presented with their first episodes of viral respiratory infection.

The frequency of infection caused by coronaviruses, which are usually associated with common cold, was 9.5%, a rate higher than that previously reported

TABLE III. Respiratory Viruses Identified in Pediatric Outpatients in Southern Brazil in 2010 and 2011

| Results from nasopharyngeal swab exudates: viral mono- and co-detection	2010 N = 248 (%)	2011 N = 244 (%)
Samples		
Positive	136 (55)	157 (65)
Negative	112 (45)	86 (35)
One virus	118 (48)	132 (54)
Two viruses	17 (7)	26 (11)
Three viruses	1 (0.4)	—
Viruses detected		
Human rhinovirus A/B	46 (18)	71 (29)
Human adenovirus	33 (13)	29 (12)
Human enterovirus	—	21 (9)
Human coronavirus 229/NL 63	13 (5)	3 (1)
Influenza virus B	11 (4)	14 (6)
Parainfluenza virus 3	11 (4)	17 (7)
Human metapneumovirus	11 (4)	8 (3)
Influenza virus A	9 (3)	9 (4)
Human coronavirus OC43/HKU1	9 (3)	3 (1)
Parainfluenza virus 4	—	5 (2)
Parainfluenza virus 1	5 (2)	1 (0.4)
Respiratory syncytial virus B	3 (1)	—
Respiratory syncytial virus A	2 (0.8)	2 (0.8)
Influenza A non-typed	1 (0.4)	—
Parainfluenza virus 2	1 (0.4)	—
Human bocavirus	—	1 (0.4)
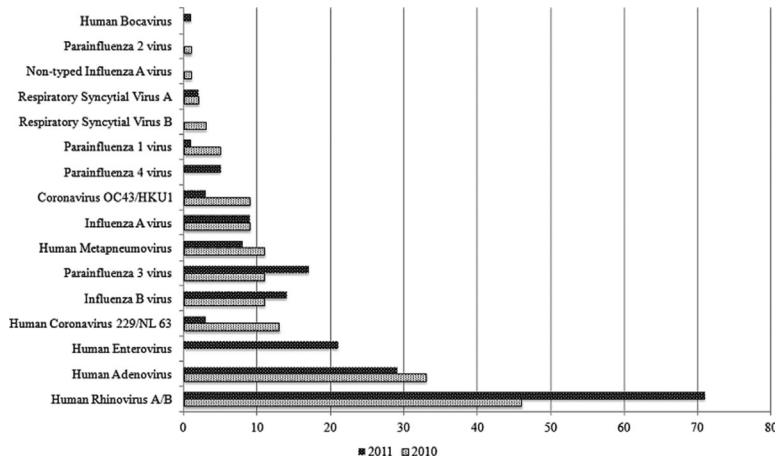
Fig. 1. Respiratory viruses identified in pediatric outpatients in southern Brazil in 2010 and 2011.

[Pilger et al., 2011; Hara and Takao, 2015]. The molecular method used did not allow genotype differentiation, but in general, patients infected with coronavirus showed favorable outcomes, without reports of any complications.

Several factors contributed to the low influenza A H1N1pdm09 circulation in 2010 and 2011. First, vaccination coverage rates were high owing to the public immunization campaigns in 2010 and 2011: vaccination coverage in the studied age group in the city of Curitiba was almost 100% [Brazilian Health Ministry, 2011]. In addition, because of the magnitude of the 2009 pandemic in southern Brazil, the population can be considered sensitized during the study period. Second, the early use of oseltamivir might be a contributing factor. Third, a high rate of influenza A H1N1pdm09 infection was observed in 2010 [Libster et al., 2010], which might have led to a protective effect in many children during the subsequent season.

No gender-specific differences were found in the prevalence of CRV infections, which were generally of low severity. The incidence of CRVs was higher in children attending school and/or day-care centers; however, there was no significant difference in the amount of time spent in these places in the 2 years.

TABLE IV. Influenza Vaccination in Pediatric Outpatients Enrolled in the Study

Immunization history	2010 N = 248 (%)	2011 N = 244 (%)	P value
Yes (two doses of influenza vaccine)	219 (88)	60 (25)	<0.0001
Monovalent influenza vaccine	198 (90)	1 (2)	
Trivalent influenza vaccine	17 (8)	54 (90)	
Unknown	4 (2)	5 (8)	

Similar to previous reports, in both seasons, the most prevalent underlying conditions associated with CRV infection were asthma and wheezing crisis [Tregoning and Schwarze, 2010; Turunen et al., 2014]. X-rays were performed in only 9% of the patients, and perihilar infiltrates were the most common radiologic findings, although none of the patients needed mechanical ventilation or intensive care treatment.

One of the main challenges in the care of patients with ARIs is to determine clinical and demographic factors that can help in the etiological diagnosis of this infection. For this reason, we selected five RVs that are often detected in ARIs and used logistic regression to evaluate correlations between their prevalence and various parameters. An association was observed between HRV and asthma, and between the presence of fever and hAdV and influenza infections. Similarly, previous reports have linked HRV to atopic characteristics, and the susceptibility to HRV-induced wheezing has been recognized as an important risk factor for childhood asthma [Tregoning and Schwarze, 2010; Mackay et al., 2013; Turunen et al., 2014]. The strongest association was observed between fever and adenovirus and influenza infections, suggesting that screening for influenza by rapid tests would be recommended. As previously reported, children maintain the chain of transmission of influenza since they shed viruses for a more prolonged time and in higher concentrations; thus, control of these infections must be based on disease control in pediatric patients [Ploin et al., 2007; D'onise and Raupach, 2008; Fiore et al., 2009].

This study had some limitations: (i) the difference in the number of viruses detected by the multiplex RT-PCR kits used in the 2 years may have influenced the detected virus profile, although no significant difference was found in the positivity rates; and (ii) the predefined sampling period, made it impossible to assess the seasonality of the infections, although this information is already available for the studied region.

TABLE V. Impact of Infection by Human Rhinovirus, Adenovirus, Parainfluenza Virus, Influenza Virus, and Coronavirus in Outpatients Compared to That of Infection by Other Respiratory Viruses (N = 293)

Human rhinovirus, n = 89

Characteristics	Positive HRV (%)	Negative HRV (%)	Unadjusted analysis		Adjusted analysis	
			OR (95% CI)	P value	OR (95% CI)	P value
Gender						
Male/Female	48/41	109/95	1.02 (0.50–1.91)	1.000	—	—
Age (mean ± SD)	36.83 (±0.94)	37.19 (±1.06)	—	0.665	—	—
Underlying medical conditions						
Yes	46 (52)	49 (24)	3.34 (1.77–5.06)	<0.001	3.17 (1.86–5.46)	<0.001
Asthma/bronchospasm						
Yes	34 (38)	30 (15)	3.59 (2.23–7.93)	<0.001	—	—
Clinical signs and symptoms*						
Fever						
Yes	60 (67)	171 (84)	0.4 (0.22–0.71)	0.003	0.43 (0.23–0.79)	0.007
Myalgia						
Yes	1 (1)	15 (7)	0.14 (0.01–1.10)	0.061	—	—
Cough						
Yes	65 (73)	140 (69)	1.24 (0.71–2.15)	0.49	—	—

Human adenovirus, n = 50

Characteristics	Positive AdV (%)	Negative AdV (%)	Unadjusted analysis		Adjusted analysis	
			OR (95% CI)	P value	OR (95% CI)	P value
Gender						
Male/Female	25/25	132/111	0.84 (0.45–1.54)	0.641	—	—
Age (mean ± SD)	39.59 ± 11.7	37.06 ± 11.2	—	0.398	—	—
Underlying medical conditions						
Yes	11 (22)	84 (35)	0.54 (0.26–1.12)	0.132	0.61 (0.28–1.25)	0.199
Asthma/bronchospasm						
Yes	5 (10)	59 (24)	0.35 (0.13–0.91)	0.003	—	—
Clinical signs and symptoms*						
Fever						
Yes	47 (94)	184 (76)	5.02 (1.5–16.7)	0.002	3.86 (1.3–16.5)	0.03
Myalgia						
Yes	6 (12)	10 (4)	3.18 (1.09–9.19)	0.037	2.92 (0.91–8.73)	0.058
Cough						
Yes	27 (54)	178 (73)	0.43 (0.16–0.58)	0.002	0.48 (0.25–0.92)	0.028

Parainfluenza virus, n = 27

Characteristics	Positive PIV (%)	Negative PIV (%)	Unadjusted analysis		Adjusted analysis	
			OR (95% CI)	P value	OR (95% CI)	P value
Gender						
Male/Female	15/12	142/124	1.09 (0.49–2.42)	0.843	—	—
Age (mean ± SD)	35.31 ± 9.69	38.98 ± 11.42	—	0.069	0.97 (0.93–1.01)	0.153

TABLE V. (*Continued*)

Parainfluenza virus, n = 27

Characteristics	Positive PIV (%)	Negative PIV (%)	Unadjusted analysis OR (95% CI)	P value	Adjusted analysis OR (95% CI)	P value
Underlying medical conditions						
Yes	5 (18)	90 (34)	0.44 (0.16–1.19)	0.131	—	—
Asthma/bronchospasm						
Yes	5 (18)	59 (22)	0.8 (0.28–2.19)	0.89	—	—
Clinical signs and symptoms*						
Fever						
Yes	20 (74)	211 (79)	0.74 (0.29–1.85)	0.62	—	—
Myalgia						
Yes	3 (11)	13 (5)	2.43 (0.64–9.13)	0.174	—	—
Cough						
Yes	22 (81)	183 (69)	(0.73–545)	0.194	—	—

Influenza virus, n = 39

Characteristics	Positive FLU (%)	Negative FLU (%)	Unadjusted analysis OR (95% CI)	P value	Adjusted analysis OR (95% CI)	P value
Gender						
Male/Female	20/19	137/117	0.9 (0.45–1.76)	0.863	—	—
Age (mean ± SD)	41.01 ± 14.28	38.28 ± 10.77	—	0.06	—	—
Underlying medical conditions						
Yes	7 (18)	88 (35)	0.41 (0.17–0.96)	**0.043**	0.40 (0.15–0.92)	**0.042**
Asthma/bronchospasm						
Yes	4 (10)	60 (23)	0.37 (0.12–1.08)	0.063	—	—
Clinical signs and symptoms*						
Fever						
Yes	36 (92)	195 (77)	3.63 (1.09–12.2)	**0.033**	3.74 (1.26–16.0)	**0.035**
Myalgia						
Yes	0 (0)	16 (6.3)	NA	—	—	—
Cough						
Yes	27 (69)	178 (70)	0.96 (0.46–1.99)	0.142	—	—

Human coronavirus, n = 20

Characteristics	Positive CoV (%)	Negative CoV (%)	Unadjusted analysis OR (95% CI)	P value	Adjusted analysis OR (95% CI)	P value
Gender						
Male/Female	12/8	145/128	1.32 (0.52–3.34)	0.645	—	—
Age (mean ± SD)	43.85 ± 11.07	38.26 ± 11.25	—	**0.033**	1.05 (1.01–1.10)	**0.012**

(*Continued*)

TABLE V. (Continued)

Characteristics	Human coronavirus, n = 20		Unadjusted analysis		Adjusted analysis	
	Positive CoV (%)	Negative CoV (%)	OR (95% CI)	P value	OR (95% CI)	P value
Underlying medical conditions						
Yes	8 (42)	87 (32)	1.55 (0.6–3.99)	0.448	—	—
Asthma/bronchospasm						
Yes	6 (30)	58 (21)	1.59 (0.58–4.31)	0.4	—	—
Clinical signs and symptoms*						
Fever						
Yes	13 (65)	218 (80)	0.47 (0.17–1.23)	0.152	—	—
Myalgia						
Yes	3 (15)	13 (5)	3.53 (0.57–7.81)	0.086	—	—
Cough						
Yes	11 (55)	194 (71)	0.5 (0.19–1.24)	0.137	—	—

*Only results with significant differences. HRV, human rhinovirus; AdV, human adenovirus; PIV, parainfluenza virus; FLU, influenza virus; CoV, coronavirus; OR, odds ratio; NA, not applicable.
Bold values, statistical differences.

Despite the advances in the establishment of a wide network of information on the circulation of influenza viruses, little is known about vaccination coverage as well as the impact of CRVs in preschool children. Our findings provide novel insights into the epidemiology and clinical impact of respiratory viruses on pediatric health and highlight the need for implementation of surveillance programs for respiratory viral infections, seeking to stimulate vaccination and to guide preventive measures to protect this population.

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