

# Viral Etiology of Influenza-Like Illnesses During the Influenza Season Between December 2011 and April 2012

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The aim of this study was to determine the frequency of respiratory viruses responsible for respiratory tract infections in Turkish children during the 2011–2012 influenza season. Nasal swabs were obtained from patients with symptoms suggestive of an influenza-like illness between December 2011 and April 2012. Samples were analyzed with multiplex real-time polymerase chain reaction (RT-PCR) to help identify the causative viral pathogen. A total of 200 patients were enrolled in the study. A respiratory virus was detected successfully in 102 (51%) children; influenza A (H3N2) in 39.2%, influenza B in 23.5%, RSV in 15.6%, rhinovirus in 13.7%, bocavirus in 2.9%, coronavirus in 2.9%, and metapneumovirus in 0.9% of patients. Only one patient was co-infected with bocavirus and influenza A virus. A statistically significant difference in the mean age of presentation was observed between the various viral pathogens ( $P < 0.001$ ). Patients with RSV were significantly younger whereas children infected with the influenza viruses were significantly older. Comparison of symptoms revealed that fever and headache occurred more frequently with the influenza viruses than the other viruses combined ( $P < 0.001$ ,  $< 0.05$ ). Durations of symptoms such as fever, cough, nasal congestion, and rhinorrhea were also significantly longer in the influenza group ( $P < 0.001$ ,  $< 0.005$ ,  $< 0.001$ ,  $< 0.005$ , respectively). Demographic analyses revealed that the school/daycare attendance was the only parameter associated with a significantly increased risk for influenza infection. With an overall viral pathogen detection rate of 51%, findings of the present study suggest other respiratory pathogens, whether viral or bacterial, may also lead to hospital visits due to influenza-like illnesses in children.

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**KEY WORDS:** influenza; respiratory viruses; children

## INTRODUCTION

With a wide range of clinical presentations, respiratory tract infections remain a significant cause of morbidity and mortality worldwide, particularly in children [Henrickson et al., 2004; Johansson et al., 2011]. Although many pathogens may cause respiratory tract infections, viruses are the most frequently implicated. Respiratory tract infections have a significant socioeconomic impact due to work and school absenteeism, despite mostly having a mild and self-limiting clinical course [Bramley et al., 2002; Fendrick et al., 2003]. Annual winter epidemics of severe respiratory infections in infants and young children are caused by a range of respiratory viruses. To date, identification of the causative pathogen in respiratory tract infections has mainly been limited to clinical research studies, and routine testing has yet to become common in general practice.

Respiratory syncytial virus (RSV), human rhinovirus (HRV), and seasonal influenza viruses are responsible for a significant majority of viral respiratory illnesses in humans worldwide, which in

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turn place a heavy burden on healthcare systems. RSV causes mainly infections in young children and infants, typically as an epidemic during midwinter, particularly in temperate regions. It is estimated that 70% of children are infected before they reach the age of 1 year, with affected individuals usually presenting with bronchiolitis, pneumonitis, bronchitis, or croup [Glezen et al., 1986]. The influenza viruses, on the other hand, are known to cause yearly epidemics, especially during the winter months. They normally give rise to a systemic illness with high fever, myalgia, and a dry cough which may be life-threatening in high-risk groups such as the elderly or those with chronic cardio-respiratory conditions. Although the initial infection itself can be quite debilitating, there is also the risk of acquiring a secondary bacterial superinfection. The common cold, which is the most frequent form respiratory tract infections, is caused mainly by the HRV. Infection may progress rarely to pneumonia or lead to exacerbation of asthma in children [Pierangeli et al., 2007; Matthew et al., 2009]. The human metapneumovirus (HMPV) is also known to cause respiratory tract infection in children and adults, accounting for approximately 5–10% of cases annually [van den Hoogen et al., 2001]. Other viruses such as human parainfluenza viruses (HPIV), human coronaviruses HCoV-229E and HCoV-OC43 have also been implicated worldwide. Discovered recently, human coronavirus HCoV-NL63 [van der Hoek et al., 2004] and human bocavirus (HBoV) [Allander et al., 2007] have joined the family of viruses that cause respiratory tract infections.

Turkey is a Eurasian country, which is considered a crossroad between Western Asia and Southeastern Europe. Very few studies on the viral etiology of respiratory illness have been reported from Turkey [Hatipoğlu et al., 2011; Ceyhan et al., 2012]. The aim of this prospective clinical study was determine the spectrum of viral etiologies of acute respiratory tract infections in an outpatient Turkish population of children presenting with influenza-like illnesses. Viral respiratory pathogens were tested using multiplex real-time reverse transcription polymerase chain reaction (RT-PCR) assays which allow for the simultaneous detection of a variety of respiratory viruses.

## MATERIALS AND METHODS

### Patient Selection and Initial Evaluation

This study was conducted at Hacettepe University Medical Faculty Ihsan Dogramaci's Child Hospital, Ankara with the approval of the Ethical Committee. The parent or legal guardians of children aged <18 years presenting between December 2011 and April 2012 with a suspicion of a respiratory tract infection were approached, and following informed consent their children were screened for eligibility before being enrolled in the study. A diagnosis of acute respiratory illness was made in the presence of at least two of the following signs or symptoms for

duration of at least one day; (1) temperature  $\geq 37.8^{\circ}\text{C}$ , (2) cough, (3) headache, (4) sore throat, (5) myalgia, (6) nasal congestion, and (7) rhinorrhea [Cowling et al., 2010]. Patients with influenza-like illnesses, defined as a temperature  $\geq 37.8^{\circ}\text{C}$  plus cough or a sore throat by the US Centers for Disease Control and Prevention (CDC), were also included in the study [Babcock et al., 2006]. A surveillance questionnaire was completed for each patient on whom information regarding patient age, onset of symptoms, influenza vaccination status, presence of an underlying chronic disorder, daycare or school attendance, presence of older siblings in daycare or school, family size, and smoking at home was recorded by a pediatrician.

On their initial visit, all patients were subjected to a thorough physical examination during which their body temperatures were recorded. Nasal swab specimens were then collected from all participants according to a standard operating protocol put forth by the CDC. The procedure involves insertion of a sterile cotton swab into a nostril and each specimen obtained at a depth 2–3 cm is then inoculated into a vial containing M4 viral transport medium (Medical Wire & Equipment, Corsham, UK).

### Follow-Up

Patients/legal guardians and older patients were informed in detail regarding the study protocol and were requested to measure and record body temperatures at least three times a day throughout the duration of the study. Standard protocol required participants to attend follow-up visits on the third, fifth, and tenth day from the onset of symptoms. For patients who were not able to visit the hospital, parents were contacted by phone. All temperature readings were recorded for each participant, and the highest body temperature during the course of the disease was noted. Parents were also questioned regarding the use of medications (antipyretics, antibiotics, antivirals) during the course of the disease. Resolution times of symptoms that were present on first presentation (e.g., fever, cough, sore throat, myalgia, congestion and rhinorrhea, vomiting, and diarrhea) were also noted. A patient was considered afebrile when his/her body temperature remained  $< 37.5^{\circ}\text{C}$  for more than 24 hr without the use of antipyretics. Patients who developed complications or required hospitalization were identified and relevant information was recorded.

### Viral Assays

Specimens obtained from each patient were stored and transported to the Virology Laboratory of Istanbul University at room temperature to be tested for the presence of an influenza virus by RT-PCR within 72 hr of collection. Specimens were tested for the presence of non-influenza viruses using a multiplex one-step real time PCR method following nucleic

acid extraction (Purelink viral RNA/DNA Mini Kit, Carlsbad, CA). Testing was done using an available commercially primers-probe set and enzyme mixtures (Superscript III Platinum One Step qRT-PCR Kit with rox, Foster City, CA) compatible with an ABI 7500 RT-PCR device.

### Statistical Analysis

Statistical analyses were performed using the commercial package SPSS for Windows version 15.0 (Chicago, IL). Values for numerical variables were provided as mean  $\pm$  standard deviation or median (minimum–maximum), depending on normality of distribution. Categorical variables were given as numbers and total percentages. For numerical variables, two-group comparisons were made using the Mann–Whitney *U*-test, whereas the Kruskal–Wallis test with Conover post hoc analysis was preferred for multiple comparisons. Comparisons between groups for categorical variables were made using the Chi-square ( $\chi^2$ ) test. A *P*-value of less than 0.05 was considered indicative of statistical significance.

## RESULTS

### Demographic, Clinical, and Laboratory Characteristics of the Study Population

A total of 200 patients (49% male, 51% female, and a median age of 48.5 months) with influenza-like illnesses were enrolled in this study. With regard to age distribution, 4 patients (2%) were aged  $\leq 3$  months, 26 patients (13%) were aged between 4 and 12 months, 90 patients (45%) were aged between 13 and 60 months, and 80 patients (40%) were aged  $\geq 61$  months. A history of vaccination with the seasonal influenza vaccine was present in 10 patients (5%). Overall, 89 patients (44.5%) were attending school/daycare. A history of another household member having flu-like symptoms was present in 106 patients (53%), whereas 101 of participants (50.5%) had a history of regular exposure to second-hand smoke at home.

In terms of medication use prior to presentation, 168 patients (84%) had used antipyretics, while 47 patients (23.5%) reported antibiotic use. The most commonly used antipyretic was paracetamol (76.1%), and amoxicillin–clavulanic acid was the most frequently used antibiotic (68%). Although six patients (3%) were hospitalized during follow-up due to breathing difficulties and/or feeding problems, none of them required intensive care.

The median duration between onset of symptoms and acquisition of nasopharyngeal swabs was 3 days (1–15). The presence of at least one respiratory virus was detected by RT-PCR in 102 (51%) patients, while in 94 patients (46%) samples were negative for viral pathogens. Five specimens (2.5%) were deemed inadequate for evaluation by RT-PCR. The positivity rate of RT-PCR for the detection of a respiratory virus was 51% (95% confidence interval [CI] 45.2–59.3).

A comparison of demographic and clinical findings in groups with or without virus detection revealed that virally infected patients had fever more frequently and for a longer mean duration ( $P < 0.05$ ). Although the duration of cough was longer in the virally infected group, this difference did not reach statistical significance ( $P = 0.07$ ). In this group, nasopharyngeal hyperemia (55.7%), rhonchi and/or rales on chest auscultation (23%), postnasal serous discharge (15.3%), and hyperemia of the tympanic membrane (5.7%) were the most common physical examination findings documented during the first visit to a physician. A summary of demographic and clinical findings of children with influenza-like illnesses is provided in Table I.

In terms of types/subtypes of respiratory viruses observed in the virally infected group, 40 patients (39.2%) had influenza A (H3N2), 24 patients (23.5%) had influenza B, 16 patients (15.6%) had RSV, 14 patients (13.7%) had HRV, 3 patients (2.9%) had HBoV, 3 patients (2.9%) had HCoV (2 had OC43, 1 had NL63), 1 patient (0.9%) had MPV, and 1 patient (0.9%) had HBoV plus influenza A H3N2 virus. A significant difference in age distribution according to viral pathogen was observed ( $P < 0.001$ ), with influenza A patients having a median age of presentation of 55 months (11–168) compared to 72.5 months (15–183) in influenza B patients, 14.5 months (1–67) in RSV patients, and 62 months (11–205) in HRV patients. The median age for the other detected viruses was 39 months (8–74). Patients with RSV were strikingly younger than those affected by other viruses, whereas the influenza viruses affected older children (Fig. 1). The difference in median age between patients with RSV and the other viruses (HRV, influenza A, and influenza B) was statistically significant ( $P < 0.05$ ).

Comparison of symptoms revealed fever and headache to occur more frequently with the influenza viruses compared to the other viruses combined ( $P < 0.001$  and  $< 0.05$ , respectively). Durations of symptoms such as fever, cough, nasal congestion, and rhinorrhea were also significantly longer in the influenza group ( $P < 0.001$ ,  $< 0.005$ ,  $< 0.001$ , and  $< 0.005$ , respectively). Distribution and frequency of symptoms according to viral pathogen are summarized in Table II. Among the demographic characteristics evaluated, only school/daycare attendance was found to be associated with a significantly increased risk for influenza infection, with no significant difference in association with family size, history of household member with similar symptoms, presence of a smoker in the household ( $P > 0.05$  for all parameters).

### Seasonal Variability

Between December 2011 and April 2012, all the cases in whom influenza A was detected presented in December and January, whereas influenza B cases started presenting in January, peaking in March,

TABLE I. Comparison of the Demographic and Clinical Findings Between Virus Detected and Not Detected Groups Presenting With Influenza-Like Illnesses

	RT-PCR positive (n = 102)	RT-PCR negative (n = 92)	P-Value
<b>Demographic characteristics</b>			
Age, months <sup>a</sup>	53 (1–205)	43.5 (2–193)	0.366
Male gender <sup>b</sup>	53 (51.9)	45 (48.9)	0.208
Duration between onset of symptoms and nasopharyngeal sampling, days <sup>c</sup>	3 ± 1.95	3.4 ± 2.57	0.554
School/day care attendance <sup>b</sup>	51 (50)	38 (41.3)	0.071
Vaccinated against influenza <sup>b</sup>	2 (1.9)	8 (8.6)	
Family size <sup>a</sup>	4.3 ± 1.27	3.97 ± 0.84	0.092
Family member with reported ILI <sup>b</sup>	55 (53.9)	51 (55.4)	0.553
Smoking at home <sup>b</sup>	49 (48)	52 (56.5)	0.254
Older sibling(s) in day care or school <sup>b</sup>	47 (46)	48 (52.1)	0.432
<b>Clinical symptoms</b>			
Fever <sup>b</sup>	86 (84.3)	66 (71.7)	0.011
Cough <sup>b</sup>	94 (92.1)	82 (89.1)	0.117
Nasal congestion <sup>b</sup>	85 (83.3)	90 (97.8)	0.085
Rhinorrhea <sup>b</sup>	94 (92.1)	87 (94.5)	0.650
Sore throat <sup>b</sup>	3 (2.9)	12 (13)	0.899
Myalgia <sup>b</sup>	25 (24.5)	21 (22.8)	0.744
Headache <sup>b</sup>	26 (25.4)	22 (23.9)	0.759
Breathing difficulties <sup>b</sup>	13 (12.7)	17 (18.4)	0.658
Gastrointestinal symptoms <sup>b</sup>	9 (8.8)	3 (3.2)	0.220
Positive PE finding <sup>b</sup>	52 (50.9)	42 (45.6)	0.203
Complications <sup>b</sup>	25 (24.5)	27 (29.3)	0.380
Hospitalization <sup>b</sup>	3 (2.9)	2 (2.2)	0.746

PE, physical examination.

<sup>a</sup>Values are given as median and range.

<sup>b</sup>Values are given as percentage.

<sup>c</sup>Values are given as mean ± standard deviation.

with cases also presenting in April. The influenza viruses were the most frequently detected viruses (62.7%) during the influenza season, while the frequency of other viral pathogens was 37.2% (Fig. 2).

## DISCUSSION

The aim of this study was to determine the prevalence of respiratory viruses and epidemiological characteristics of patients with influenza-like illnesses during the 2011–2012 influenza season. The overall detection rate of a respiratory virus in our

study population was 52%, a value that is slightly higher than previously reported rates of 37% and 48% [Druce et al., 2005; Brittain-Long et al., 2008]. This difference could be attributed to variations in age, seasonality, environmental factors, and socio-economic status. Patients enrolled in this study were indeed younger, and nasopharyngeal swabs were obtained during the peak season for respiratory tract infections, both of which are factors known to increase diagnostic yield. Furthermore, the PCR assays performed in this study allowed for the detection of a wider variety of viruses, including

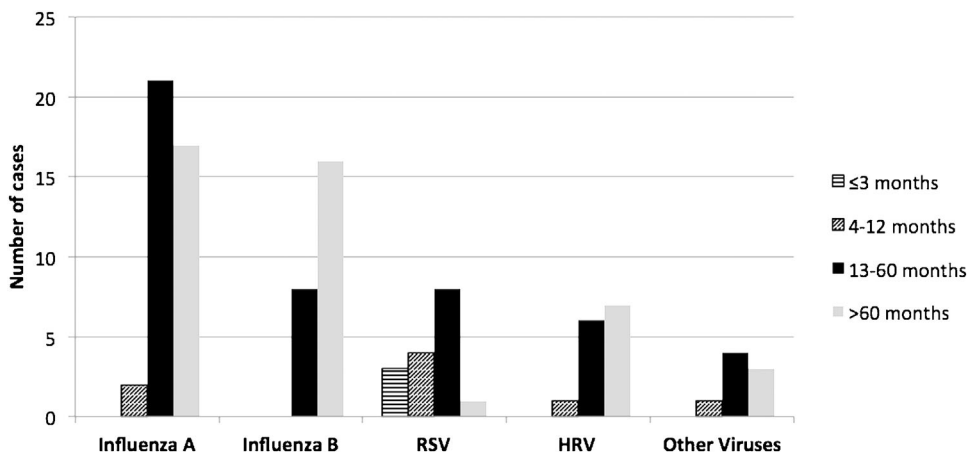


Fig. 1. Age distribution according to viral pathogen.

TABLE II. Distribution and Frequency of Symptoms According to Viral Pathogens

	Influenza viruses (n = 64)	Other viruses (n = 38)	P-Value
Age, months <sup>a</sup>	61 (11–183)	19 (1–205)	<0.001
Male gender	32 (50)	21 (55.2)	0.599
Clinical symptoms			
Fever <sup>b</sup>	62 (96.8)	24 (63.1)	<0.001
Duration of fever, days <sup>c</sup>	5.50 ± 1.29	3.34 ± 1.40	<0.001
Cough <sup>b</sup>	62 (96.8)	32 (84.2)	0.135
Duration of cough, days <sup>c</sup>	9.82 ± 2.46	8.40 ± 2.10	<0.005
Nasal congestion <sup>b</sup>	54 (84.3)	31 (81.5)	1
Duration of congestion, days <sup>c</sup>	8.09 ± 2.05	6.25 ± 1.56	<0.001
Rhinorrhea <sup>b</sup>	58 (90.6)	36 (94.7)	0.253
Duration of rhinorrhea, days <sup>c</sup>	7.94 ± 2.08	6.61 ± 1.60	<0.005
Myalgia <sup>b</sup>	18 (28.1)	7 (18.4)	0.453
Duration of myalgia, days <sup>c</sup>	3.16 ± 1.04	2.57 ± 0.78	0.198
Headache <sup>b</sup>	22 (34.3)	4 (10.5)	0.020
Duration of headache, days <sup>c</sup>	3.13 ± 1.03	3.50 ± 0.57	0.429
Breathing difficulties <sup>b</sup>	6 (9.3)	7 (18.4)	0.063
Gastrointestinal symptoms <sup>b</sup>	8 (12.5)	1 (2.6)	0.150
Positive PE finding <sup>b</sup>	35 (54.6)	17 (44.7)	0.575
Complications <sup>b</sup>	14 (21.8)	11 (28.9)	0.493
Hospitalization <sup>b</sup>	2 (3.1)	1 (2.6)	—

PE, physical examination.

<sup>a</sup>Values are given as median (minimum–maximum).

<sup>b</sup>Values are given as percentage.

<sup>c</sup>Values are given as mean ± standard deviation.

HBoV, HCoV-OC43, NL63, and HMP, which improves detection rate. Underdeveloped immunity and prolonged viral shedding are believed to be the main contributing factors for the higher incidence of viral respiratory tract infections in children [Jartti et al., 2004; Naghipour et al., 2009; To et al., 2010]. Furthermore, unlike adults, young children tend to be brought to medical attention earlier in the course of disease, and it has been established that the detection rate with RT-PCR is correlated inversely with the duration of symptoms [Brittain-Long et al., 2010].

In this study, 60% of children presenting to the outpatient clinic with influenza-like illnesses were younger than 5 years of age. A comparison of presenting age between viral pathogens revealed patients with RSV to be significantly younger than patients with HRV, influenza A, and influenza B ( $P < 0.05$  for all comparisons). Khamis et al. [2012] observed in community-based study that RSV infec-

tion occurred solely in children aged less than 2 years of age, with 57% of infections occurring in infants younger than 6 months. The oldest patient with RSV in the present study was a 67-month-old child, and only 4 (25%) out of 16 patients with documented RSV were older than 2 years of age. Additionally, an influenza virus was detected in nearly two-thirds of patients who were included in the virally infected group, with an overall frequency in children with influenza-like illnesses of 32%. In this research group's study from last influenza season, influenza viruses were detected in 129 of 300 patients (43%) with influenza-like illnesses [Ceyhan et al., 2012]. While the H1N1 strain of influenza A was predominant last season, it has been replaced this season by the H3N2 strain as well as influenza B. Other viruses that were detected this season were RSV, HRV, HBoV, HCoV, and MPV. The present study shows that it may not be possible to distinguish between influenza viruses and other respiratory viruses in

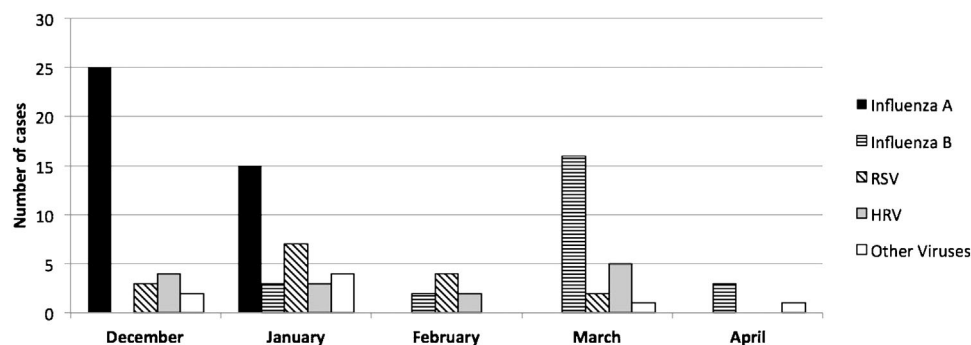


Fig. 2. Distribution of viral pathogen according to month.

patients with influenza-like illnesses based on symptoms alone, since all the respiratory pathogens evaluated have the potential to cause a febrile illness in affected children during the influenza season.

Respiratory virus infections of early childhood are confined mostly to the upper respiratory tract resulting in common cold-like symptoms such as coryza, cough and sore throat. On the contrary, nearly one-third of infants with respiratory viral infections present with symptoms such as tachypnea, wheezing, severe cough, breathlessness, and respiratory distress, suggestive of lower respiratory tract involvement [Pavia, 2011]. In this study, 23% of patients who had a positive RT-PCR result had lower respiratory symptoms, whereas the most frequently encountered symptoms were cough (92.1%), rhinorrhea (92.1%), fever (84.3%), nasal congestion (83.3%), headache (25.3%), and myalgia (24.5%). The presence of a significant difference between viral pathogens in terms of frequency and duration of symptoms is also demonstrated. Fever and headache were observed more commonly in patients with infections due to the influenza viruses compared to other viruses, while the duration of symptoms such as fever, cough, rhinorrhea and nasal congestion was also significantly longer with influenza virus infections. In this group's recent study during the last influenza season [Ceyhan et al., 2012], it was also observed that the duration of symptoms such as cough, nasal congestion, and rhinorrhea in influenza positive patients were longer than those reported in the previous season, with no significant difference in the duration of fever. The conclusion shared by different studies is that on its own, clinical presentation should not be considered a reliable indicator of the causative viral pathogen.

The rate of detecting multiple pathogens, the clinical significance of which remains disputed, was much lower in this study (0.9%) compared to previous community-based studies [Brittain-Long et al., 2012; Khamis et al., 2012]. However, it has been postulated that multiple viral infections may be associated with higher fever, longer durations of hospital stay, more frequent use of antibiotics and increased risk of requiring intensive care in a hospital-based study [Semple et al., 2005]. Current evidence suggests that co-infections such as RSV+HRV or RSV+HMPV may lead to a more severe clinical course of disease in infants with bronchiolitis [Semple et al., 2005; Paranhos-Baccala et al., 2008; Richard et al., 2008]. Only one of the patients in the study was coinfecting with HBoV and the H3N2 strain of influenza A, which was not found to be associated with a more severe clinical course than the other virally infected patients. No clear consensus on the effect of co-infection on disease severity is remained.

Risk factors for acquiring a viral respiratory tract infection have been investigated extensively in numerous community and hospital-based studies [Esposito et al., 2011; Khamis et al., 2012; Papenburg

et al., 2012]. Male gender, low socioeconomic status, exposure to cigarette smoke or animals, history of breastfeeding, presence of a co-morbidity such as asthma, chronic heart disease or iron deficiency anemia have all been linked with an increased risk of RSV infection. Data on risk factors for other viral pathogens are limited. In a recent study on an adult population, cigarette smoking was found to be associated with higher mortality in patients with influenza [Wong et al., 2012]. In the present study, younger age and daycare/school attendance were linked with an increased likelihood of having a viral pathogen ( $P < 0.005$ ). In contrast, a history of household smoking, another house member with similar symptoms, and siblings attending school/daycare did not seem to be significantly associated with an increased risk for respiratory infection with a viral etiology ( $P > 0.05$ , for all).

Common respiratory viruses usually show typical seasonal distribution in temperate climates. The best examples are RSV, influenza A, and occasionally HMPV, which usually peak during the winter months [Stensballe et al., 2003; Monto, 2004; du Prel et al., 2009]. In a previous study from India, a similar pattern for influenza A and RSV has been observed [Agrawal et al., 2009]. An age distribution leaning toward a young age for RSV infections during winter may be attributed to an actual high incidence as well as increased parental awareness. It has long been established through several observational studies that the seasonal incidence of many respiratory viruses is influenced by weather conditions. The incidence of RSV infections has been found to be correlated inversely with average temperature, while a significant positive association between the incidence of HRV infections and increased humidity has also been reported [du Prel et al., 2009]. During the 5-month follow-up period in this study, influenza A cases were seen mostly in the months of December and January, with no further cases after February. On the other hand, the first case of influenza B was detected in January, with the number of new cases peaking in March. Cases of RSV and HRV presented in each month of the influenza season studied.

One of the main limitations of this study is that subjects were not randomized which could have led to a certain degree of bias. However, it is believed that potential bias was minimized since the goal was to enroll all patients presenting to the outpatient clinic during the 5-month study period, provided consent was given. Another limitation is that children with respiratory tract infections due to bacterial pathogens such as *Streptococcus pneumoniae* and *Streptococcus pyogenes* were not excluded from the study, which may have affected the calculated detection rates.

The findings of this study suggest that almost all of the major groups of respiratory viruses may be responsible for influenza-like illnesses in children from Ankara, Turkey. Among the patients, the influenza viruses were the most frequently detected

viruses (62.8%), with non-influenza viruses responsible for the remaining 37.2% of cases. Taking the overall viral pathogen detection rate of 51% into consideration, it is obvious to conclude that other respiratory pathogens, whether viral or bacterial, also lead to respiratory tract infections related hospital visits in children.

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