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ORIGINAL ARTICLE

# Influenza and other respiratory viruses in children: prevalence and clinical features

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## Abstract

With the COVID-19 pandemic still ongoing, the annual season of influenza and other respiratory virus epidemics has arrived. Specimens from patients suspected of respiratory viruses infection were collected. Viral detection was performed following RNA extraction and real-time RT-PCR. During the study period, we received and tested a total of 606 specimens. Rhinovirus virus was the viral type most prevalent, detected in 186 (45.47%) specimens. The age range of patients positive for influenza A, influenza A (H1N1), and influenza B was 18 days to 13 years. With female prevalence for this viral type, cough and asthma were the main clinical manifestations presented by this viral type. Our results indicate that rhinoviruses, adenoviruses, metapneumoviruses, and influenza are among the most important agents of ARI in pediatrics. The epidemic period of respiratory infections observed in Goiânia can be useful for planning and implementing some prevention strategies.

**Keywords** Influenza B · Influenza A · Surveillance · Epidemiology · Respiratory viruses · ICU

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## Introduction

With the COVID-19 pandemic still ongoing, the anual season of influenza and other respiratory virus epidemics has arrived. Previous studies have reported that viral interference among influenza virus and other respiratory viruses can affect viral infections at the host and population level [1, 2]. The influenza virus continues to be one of the greatest public health challenges in the world, being responsible for recurrent annual epidemics, causing acute respiratory infection ranging from mild symptoms such as rhinopharyngitis to viral pneumonia with fatal complications [3].

According to the World Health Organization (WHO), each year, there are an estimated one billion cases worldwide, resulting in about three to five million severe cases and 290,000 to 650,000 deaths from respiratory-related diseases. Influenza, with types A and B, is the most clinically important [3].

The human respiratory tract hosts a diverse community of co-circulating viruses that are responsible for acute respiratory infections. This shared niche provides the opportunity for virus-virus interactions that have the potential to affect individual infection risks and, in turn, influence infection dynamics at population scales [1].

Clinical diagnosis of viral respiratory diseases is a challenge for clinicians, due to the possibility of overlapping signs and symptoms of different viral diseases, which makes accurate clinical diagnosis difficult. It stands out although bacterial agents can also cause respiratory diseases with viral-like symptoms, often leading to overuse of inappropriate antibiotics [4].

The rapid and accurate diagnosis of infections is important for the correct prescription of the antiviral and better prognosis of the disease since the initiation of early treatment, with adequate antivirals, contributes to the reduction of symptoms and severity of the disease, which consequently reduces the extent of illness [4].

To investigate the prevalence of influenza virus and its subtypes, with other respiratory viruses, including COVID-19, we analyzed clinical samples collected from patients in five hospitals in Brazil with respiratory illnesses from May 2020 to April 2022.

## Materials and methods

### Ethical aspects

All procedures and protocols for sample collection and processing were submitted and approved, under registration number 33540320.7.0000.5078 by the Research Ethics Committee of Hospital das Clínicas—GO (HC-UFG), located in Goiânia-Goiás, Brazil. All parents of sick patients and voluntary donors signed the informed consent form.

### Target population

This study included 606 patients with suspected respiratory virus infection. The samples were collect in five hospitals in a capital in the Center-West region of Brazil. In the period from May 2020 to April 2022, we considered the following eligibility criteria: patients admitted to emergencies, intensive care units (ICU), admissions to pediatric wards and wards of hospitals participating in the project. In addition, all subjects were tested by RT-qPCR (TaqMan – Thermo Fisher) for rhinovirus, adenovirus, metapneumovirus, parainfluenza 1, parainfluenza 2, parainfluenza 3, influenza A, influenza A (H1N1), influenza B, bocavirus, human syncytial virus, and novel coronavirus.

### Collection and processing of samples

Peripheral blood samples were collected for molecular analysis by RT-qPCR, in order to evaluate the presence of viruses from the aforementioned viral panel. All samples collected were kept at 4 °C and sent to the Patologia Tropical e Saúde Pública Institute from Universidade Federal de Goiás. All samples were processed and stored at − 20 °C. To obtain peripheral blood samples, 5-mL venous blood samples were collected using the vacuum system through Vacutainer tubes (Beckton & Dickinson, USA) and then homogenized for 10 min and stored at 4 °C. For sample processing, 5 mL of whole blood was subjected to centrifugation at 500 × *g* for 30 min at 20 °C. Plasma was collected and transferred to 1.5-mL tubes. All processed samples were stored at − 20 °C until the analysis time.

Commercial kits follow the following steps: (1) RNA extraction, (2) reverse transcription to obtain complementary DNA (cDNA), and (3) Thermo Fisher® TaqMan real-time polymerase chain reaction. The detection of amplification of genetic material is performed in real time by measuring the fluorescence emitted by the fluorophore. The steps are described below:

In more detail, reverse transcription is followed by the PCR phase, which consisted of a 3–10-s denaturation step at 95 °C, during which the DNA strands separate into single strands, and a hybridization step and 15–45-s polymerization at 55–60 °C, during which the amplification primers (and detection probes) hybridize to the single-stranded DNA templates and allow the polymerase to replicate the template, creating double-stranded DNA. During successful polymerization, the probe is displaced and hydrolyzed, releasing fluorescence. This process was usually repeated about 40–45 times (cycles). A typical real-time RT-PCR run, as exemplified here, completes in about an hour and 30 min.

Cycle threshold between 8 and 35 had considered a detected virus (positive) and greater than 40 undetected viruses (negative) between 35 and 40 required confirmation.

### Statistical analysis

Data were analyzed using Excel, Prism, and word software.

## Results

Of the 606 patients tested in the five hospitals, 186 rhinovirus, 19 influenza A (H1N1), 9 influenza A, 8 influenza B, and 197 negative, as described in Fig. 1.

We observed the presence of both viral and bacterial co-infection (Table 1), with greater numbers of patients with influenza A virus infection with rhinovirus; influenza A with bacterial co-infection; and influenza A (H1N1) with bacterial co-infection. Patients with influenza A virus had no co-infection with parainfluenza virus (1–3) or HRSV. In addition, patients with influenza B virus did not have co-infection with metapneumovirus, HRSV, and adenovirus. This and other information is described in Table 1.

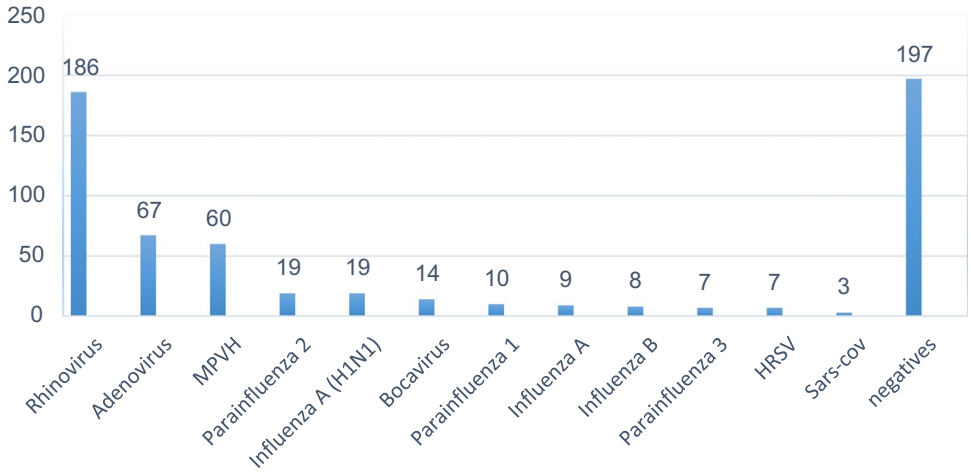


**Fig. 1** Molecular screening of the main respiratory viruses in hospitals in a capital city in the Midwest region of Brazil. MPVH, metapneumovirus; HRSV, human respiratory syncytial virus; Sars-Cov, novel coronavirus

**Table 1** Total results of simple infections and co-infections

| | Influenza A | Influenza A (H1N1) | Influenza B | Total |
|---|---|---|---|---|
| Influenza A | **7** | - | 2 | 9 |
| Influenza A (H1N1) | 1 | **17** | 1 | 19 |
| Influenza B | 2 | **-** | **6** | 8 |
| MPVH | 1 | 2 | - | 3 |
| Parainfluenza (1–3) | - | 1 | 1 | 2 |
| Rhinovirus | 4 | 1 | 2 | 7 |
| HRSV | - | 1 | - | 1 |
| Adenovirus | 2 | 1 | - | 3 |
| Bacterial co-infection | 5 | 6 | 1 | 12 |

Number of samples containing each virus set (row × column). Simple infections in bold. *MPVH*, metapneumovirus; *HRSV*, human respiratory syncytial virus

Table 2 describes the main taxonomic characteristics of the viruses addressed and the main symptoms of the viruses that cause respiratory infections found in the literature.

When we approached the main clinical manifestations presented by patients who had infection and co-infection by the influenza A, influenza A (H1N1), and influenza B viruses, we observed a variation in age from 18 days to 13 years, with a predominance of females with the highest number of affected. The main complaints presented by the patients were fever, cough, asthma, bronchiolitis, and flu, and there were no reports of headache. The hospitalization rate ranged from 26.3 to 73.6% among the three viral types, with a predominance of patients admitted to hospital outpatient clinics. For these viral types, no patient died and all results were obtained are described in detail in Table 3.

## Discussion

This study presented an initial knowledge about the etiology of acute respiratory infection (ARI) in children admitted to five hospitals in Goiânia—GO. Rhinovirus was the most common cause of ARI detected in 45.47% of positive samples, followed by adenovirus which was detected in 16.38% of positive samples and metapneumovirus which represented 14.66% of positive samples. Other studies show the incidence of metapneumovirus can vary from year to year, sometimes competing with or exceeding the incidence of HRSV [5, 6]. Epidemiological data from tropical regions have demonstrated an association between HRSV outbreaks and the rainy season [7]. Goiânia is located in the Center-West of Brazil and has a tropical climate. Our data demonstrate the positivity of 1.71% of these patients. This pattern of HRSV infection is similar to that observed by other authors [8, 9] and to that observed in South American countries of the extreme south such as Chile, Uruguay, and Argentina [10, 11].

Among the parainfluenza viruses, the most detected were type 2, found in 19 samples, being 4.64%. Parainfluenza types 1 (2.44%) and 3 (1.71%) were found in lower percentages. In the literature, type 3 is the most frequent among parainfluenzas and types 1 and 2 were not detected or were detected in only a few samples [12, 13].

Influenza B virus was detected in 8 (1.95%) samples, while influenza A virus was detected in 9 (2.2%) samples and the influenza A (H1N1) subtype was detected in 19 (4.64%) of the samples. These results are in line with surveillance studies done by the Centers for Disease Control and Prevention (CDC), which indicated that 99.8% of influenza viruses isolated were type A and 0.2% were type B [14].

To complete the epidemiological profile of the main respiratory viruses in 2020–2022, bocavirus were 14 positive samples and Sars-Cov were 3 positive samples. The

**Table 2** Taxonomic characteristics, most common symptoms of the viruses that cause the most common respiratory infections

| Virus | Family | Genome | Subtypes | Symptoms of disease |
|---|---|---|---|---|
| Adenovirus | *Adenoviridae* | Linear dsDNA | > 50 serotypes with different disease presentations | Respiratory tract infection, gastroenteritis, conjunctivitis, hemorrhagic cystitis, hepatitis, hemorrhagic colitis |
| Bocavirus | *Parvoviridae* | Linear ssDNA | Serotypes 1–4 | Lower respiratory tract infection (HBoV 1), gastrointestinal infections (HBoV2-4), asthma |
| Coronavirus | *Coronaviridae* | Linear ssRNA | 229E, Oc43, NL63.HKU1, MERS, SARS | Atypical pneumonia |
| Influenza | *Orthorryxoviridae* | Linear ssRNA or segmented | A, B, C | High fever with headache and body ache, gastrointestinal symptoms, bacterial infections, rarely encephalitis, myositis, and myocarditis |
| Metapneumovirus humano | *Paramyxoviridae* | Linear ssRNA | Subtypes A and B | Second leading cause of bronchiolitis (after RSV), pneumonia |
| Parainfluenza | *Paramyxoviridae* | Linear ssRNA | 1–4 with different seasonal patterns | Febrile upper and lower respiratory tract infections |
| Rinovirus | *Picornaviridae* | Linear ssRNA | More than 100 serotypes | Coryza, sore throat, cough |
| Virus sincicial respiratório humano | *Paramyxoviridae* | Linear ssRNA | Subtypes A and B | Bronchiolitis, pneumonia, upper respiratory tract infection |

*dsDNA*, double-stranded DNA; *ssDNA*, single-stranded DNA; *ssRNA*, single-stranded RNA. Source: Adapted from Krauze (2014)

**Table 3** Main clinical manifestations presented by patients

| Indicators | Influenza A | Influenza A (H1N1) | Influenza B |
|---|---|---|---|
| Features | $n=9$ | $n=19$ | $n=8$ |
| Age (years) | 3 and 13 | 0.18 and 12 | 4 and 12 |
| Male | 3 | 8 | 5 |
| Female | 6 | 11 | 3 |
| Symptoms | | | |
| Fever | 44.4% (4) | 47.3% (9) | 0% (0) |
| Cough | 11.1% (1) | 15.8% (3) | 25% (2) |
| Asthma | 33.3% (3) | 26.3% (5) | 25% (2) |
| Bronchiolitis | 0% (0) | 10.5% (2) | 0% (0) |
| Headache | 0% (0) | 0% (0) | 0% (0) |
| Outpatient | 55.5% (5) | 73.6% (14) | 37.5% (3) |
| ICU | 44.4% (4) | 26.3% (5) | 62.5% (5) |
| The flu | 0% (0) | 5.2% (1) | 25% (2) |
| Deaths | 0% (0) | 0% (0) | 0% (0) |
| Diagnosis | | | |
| RT-qPCR | 100% | 100% | 100% |

results presented here indicate the feasibility of using an RT-qPCR panel for the detection of RNA contained in viral pathogens in monitoring studies. Through this technique, we were able to detect the twelve most common pathogens of the respiratory tract, demonstrating that this method is very suitable for epidemiological studies and for rapid microbiological studies in clinical practice.

One of the goals of our study was to provide baseline data that health authorities can consider for long-term surveillance plans. Currently, the reported frequency of dual respiratory infections varies considerably and the importance of such an infection remains unresolved [15, 16]. In this study, multiple infections were observed in 22 patients, i.e., dual viral infections were detected in 5.37% of the total positive samples. The rate of multiple infections is higher than that observed in the literature, which can be explained by the greater sensitivity of the PCR panel based on fluorescent techniques when compared to the direct detection of antigen or virus isolation [17, 18] and by the greater number of respiratory viruses included in this test. The analysis of age distribution according to viral infection shows that the largest number of positive cases, whether simple infections or co-infections, occurs in infants younger than 1 year, confirming the findings of the international literature [19–21].

When physical symptoms and clinical diagnosis were compared with etiology, no association was established; therefore, it is impossible to know the type of virus based on clinical signs alone. In addition, there was no significant association between patient sex and viral etiology.

## Conclusions

Our results corroborate the data that indicate that rhinoviruses, adenoviruses, metapneumoviruses, and influenza are among the most important agents of ARI in pediatrics.

The epidemic period of respiratory infections observed in Goiânia can be useful for planning and implementing some prevention strategies. Longitudinal studies should be performed in order to confirm the results obtained in this medium-term study. Efficient strategies, such as the control of nosocomial infections caused by respiratory viruses, the use of antiviral therapy, and the more rational use of antibiotics in viral ARF, may be one of several benefits generated by longitudinal studies of the clinical and epidemiological aspects of these infections.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10096-022-04515-3.

**Author contribution** Conceptualization, Paulo Alex N. Silva; methodology, Célia Regina Malveste Ito and André Luís Elias Moreira; formal analys, Isabela Jubé Wastowski and Mônica Oliveira Santos; software, Lucas Candido Gonçalves Barbosa; writing—review and editing, Lilian Carla Carneiro and Melissa Ameloti Gomes Avelino.

**Data availability** Not applicable.

**Code availability** Not applicable.

## Declarations

**Ethics approval** The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Research Ethics Committee of Hospital das Clínicas—GO (HC-UFG) (33540320.7.0000.5078) for studies involving humans.

**Consent for publication** All participants reviewed the manuscript and accepted publication.

**Informed consent** Informed consent was obtained from all subjects involved in the study.

**Conflict of interest** The authors declare no competing interests.

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