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Rhinovirus detection by real-time RT-PCR in children with acute respiratory infection in Buenos Aires, Argentina

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ABSTRACT

Human rhinoviruses (HRV), the major cause of common colds, have a significant genetic diversity and are classified into 3 species (A, B, C) with more than 100 serotypes. HRV species C, described in 2006, can only be detected using molecular methods. The objectives of this paper were to adapt a real-time reverse transcription-polymerase chain reaction (RT-PCR) assay for HRV detection and to further determine the frequency of HRV in respiratory samples from children under 2 years of age, with acute respiratory infection (ARI), from Buenos Aires, Argentina. Two real-time RT-PCR assays amplifying the 207 base pair of the 5' non-coding region were compared. The original protocol includes locked nucleic acid analogues and a pyrimidine derivative in the forward primer, while the adapted protocol avoided those molecules. Of 67 respiratory samples, 17 (25.4 %) were positive with the original protocol, and 20 (29.9 %) with the adapted one. Discrepant results were confirmed by sequencing analysis. An expanded gold standard was defined to determine the performance of both assays, and was used to describe the clinical characteristics of positive patients. Better sensitivity and specificity were obtained with the adapted protocol. Considering the expanded gold standard, HRV were detected in 23/67 (34.3 %) patients with ARI: 8/18 (44.4%) outpatients and 15/49 (30.6 %) hospitalized. Wheezing episodes were more frequent in HRV positive patients (43.5 %) than in HRV negative patients (18.2 %) (p = 0.041). This study describes the utility and clinical sensitivity of an adapted real-time RT-PCR assay for HRV detection.

Key words: rhinoviruses, real-time RT-PCR, acute respiratory infection, children

RESUMEN

Detección de rinovirus por RT-PCR en tiempo real en muestras respiratorias de niños de Buenos Aires, Argentina. Los rinovirus humanos (RVH) constituyen la principal causa de resfrío común y poseen una gran diversidad genética, con más de 100 serotipos clasificados en tres especies (A, B, C). Los RVH C fueron descritos en 2006 y solo pueden detectarse utilizando métodos moleculares. El objetivo del presente trabajo fue adaptar un protocolo de transcripción reversa seguido de reacción en cadena de polimerasa (RT-PCR) en tiempo real para detectar RVH y posteriormente determinar su frecuencia en muestras de niños menores de 2 años con infección respiratoria aguda (IRA). Se compararon dos protocolos de RT-PCR en tiempo real, que amplifican 207 pares de bases de la región 5' no codificante. El protocolo original incluyó un cebador directo con análogos de nucleótidos bloqueados (LNA) y un derivado pirimidínico en su secuencia, mientras que el protocolo adaptado no los incluyó. De 67 muestras, 17 (25,4 %) fueron positivas con el protocolo original y 20 (29,9 %) con el protocolo adaptado; los resultados discrepantes se confirmaron por secuenciación. Se definió un gold standard expandido para determinar el desempeño de ambos ensayos y describir las características clínicas de los pacientes RVH positivos. La mejor sensibilidad y especificidad se obtuvo con el protocolo adaptado. Considerando el gold standard expandido, se detectó RVH en 23/67 (34,3 %) pacientes con IRA: 44,4 % (8/18) ambulatorios y 30,6 % (15/49) internados. Los episodios de sibilancias fueron más frecuentes en pacientes RVH positivos (43,5 %) que en RVH negativos (18,2 %) (p = 0,041). El presente estudio describe la utilidad y la sensibilidad clínica de esta RT-PCR en tiempo real adaptada para detectar RVH.

Palabras clave: rinovirus, RT-PCR en tiempo real, infección respiratoria aguda, niños

INTRODUCTION

Human rhinoviruses (HRV) belong to the Picornaviridae family, genus Enterovirus (25) and were formerly classified in the genus Rhinovirus. To date, more than 100 serotypes have been described and classified into 3 species: A, B and C (22, 23). Their genome is a single 7, 2-kb positive RNA strand
with a single open-reading frame (31).

HRVs are the most frequent cause of common colds and are also associated with acute otitis media in children and sinusitis in adults (27, 28).

Recent studies have established that HRV can infect the lower airways causing pneumonia and bronchiolitis in children (12, 26), asthma exacerbation in school-aged children and adults, exacerbation of cystic fibrosis (32) and chronic obstructive pulmonary disease in adults (24).

HRVs have been identified as an important predictor of recurrent wheezing (14) and asthma development in high-risk children (10). Asymptomatic HRV infection can also occur in infants, children and adults (11, 34, 36).

Isolation of HRV in cell culture is difficult, insensitive and time consuming (36). The development of molecular methods has increased the feasibility of HRV detection. Several reverse transcription-polymerase chain reaction (RT-PCR) assays have been developed for a sensitive detection and differentiation of HRV. Some of them target gene regions that are common for both HRV and enteroviruses (HEV) (1, 7, 13, 15, 30), but other RT-PCR assays are only specific for HRV (8, 16, 33). The 5' non-coding region (5'NCR) of the viral genome contains highly conserved sequences, thus presenting a convenient area for amplification. However, part of this region is shared with HEV.

There is a significant sequence variation between HRV strains due to the imprecise replication of the RNA by the virus-encoded RNA dependent RNA polymerase and frequent intra and interspecies recombination events (25, 31).

The frequency of HRV detected by molecular methods in hospitalized children with acute respiratory infection (ARI) ranges from 6% to 35% (2, 5, 6, 17).

Although HRV is frequently detected in coinfection with other respiratory viruses, the role of this simultaneous presence is not yet established: some authors have proposed that viral coinfection increases the severity of disease, while others have not found differences between coinfection and single infections (2).

The goal of this study was to adapt a real-time RT-PCR for HRV detection described by Lu et al. in 2008 (16). This method allows detection of all HRV species and showed high sensitivity and specificity. In our study, we avoided the use of locked nucleic acid analogues and a pyrimidine derivative (molecules difficult to obtained in Argentina) in the forward primer, while maintaining a good performance of HRV detection. The frequency of HRV, viral coinfection and clinical features was determined in hospitalized and outpatient children under 2 years of age with ARI in Buenos Aires, Argentina.

MATERIALS AND METHODS

Samples
Nasopharyngeal aspirates (NPAs) from 67 patients under 2 years of age with ARI who attended the emergency room or were hospitalized at CEMIC University Hospital, Buenos Aires, Argentina, from June to November, 2007, were studied. NPAs were sent in viral transport media to the Clinical Virology Laboratory at CEMIC for viral diagnosis. Remaining NPAs were anonymized and stored at -70 ºC until the HRV studies were performed. This study was approved by the Institutional Review Board at CEMIC.

Stored data included: age, clinical characteristics such as upper respiratory tract infection (URTI), recurrent wheezing episodes, bronchiolitis, pneumonia, hospitalization days, oxygen therapy, mechanical ventilation requirement, and admission to intensive care unit (ICU). In addition, the results of other viral tests such as respiratory syncytial virus (RSV), influenza (Flu), adenovirus (AdV) parainfluenza (PIV) by indirect immunofluorescence and human bocavirus (hBoV) and human metapneumovirus (hMPV) by PCR were included (3).

PCR controls
Serial 10-fold dilutions of a positive control (HRV 31, ATCC® VR-506), kindly provided by Dr. Freymuth (Caen University Hospital, France), were used in two different runs to determine the limit of detection of each RT-PCR protocol. Positive HEV controls: serotype 1, 2 and 3 poliovirus 2006 vaccine strains and HEV 68 (highly similar to HRV) were used to test the specificity of both RT-PCR assays to detect HRV.

RNA extraction
Viral RNA was manually extracted from 140 μl of the NPA using the QiAamp® Viral RNA kit (Qiagen GmbH, Hilden, Germany), following manufacturer’s instructions.

RT-PCR assays
Two real-time RT-PCR assays amplifying 207 base pair (bp) of the 5' non-coding region (5'NCR) of HRV were compared.

Protocol A: the real-time RT-PCR protocol recommended by Lu et al. in 2008 (16). Primers (forward: 5'-OPX GCC GZG GTG GC-3'; reverse: 5'-GAA ACA CGG ACA CCC AAA GTA-3') and probe (5'-FAM-TCC TCC GGC CCC TGA ATG YGG C-BHQ1-3') were kindly provided by Dr. Erdman (CDC, Atlanta, USA). The forward primer included locked nucleic acid (LNA) analogues (X = LNA-dA; Z = LNA-dT) and a pyrimidine derivative (P is a degenerate base mimicking a C/T mix). This protocol was performed using iScript One-Step RT-PCR Kit for probes (Bio-Rad, CA, USA); each 25 μl reaction mixture containing 12.5 μl of 2X reaction mix, 0.25 μl of 100 μM forward and reverse primers, 0.25 μl of 10 μM probe, 0.5 μl of iScript reverse transcriptase, 6.25 μl of nuclelease free water, and 5 μl of nucleic acid extract. RT-PCR cycling conditions were the following: an initial reverse transcription at 48 °C for 10 min, 95 °C for 5 min for polymerase activation, and then 45 cycles of 95 °C for 15 s and 55 °C for 1 min, on a SmartCycler II (Cepheid, CA, USA).
Protocol B: was an adaptation of protocol A. The sequence of the forward primer was: 5'-CYA GCC TGC GTG GC-3', avoiding the use of LNA analogues and the pyrimidine derivative. The reverse primer and probe were the same as in protocol A. This protocol was performed using the One-step RT-PCR kit (Qiagen) that includes two enzymes (Omniscript and Sensiscript) for the RT and a HotStart Taq DNA polymerase for amplification. The RT-PCR cycling conditions were the following: an initial reverse transcription at 50 °C for 30 min, 95 °C for 15 min for polymerase activation, and then 45 cycles of 95 °C for 15 s and 55 °C for 1 min, on a SmartCycler II (20).

In addition, protocol B was evaluated with the iScript One-Step RT-PCR Kit for probes (Bio-Rad) and SuperScrip III platinum one-step quantitative RT-PCR kit (Invitrogen, Brazil) to test the performance of different commercial kits. Both kits include an MMLV reverse transcriptase for the RT step and a Hot-Start Taq polymerase for the amplification.

Protocol for HEV: a nested RT-PCR for HEV detection was performed in samples with discrepant results with protocol A and B (4). This RT-PCR amplifies a region of 306 bp to 311 bp of the 5' NCR. The RT was performed using an MMLV reverse transcriptase and a recombinant RNasin ribonuclease inhibitor (Promega, WI, USA), and a Taq DNA polymerase recombinant (Invitrogen) for PCR amplification.

Sequencing

Sequencing was performed in samples that were positive with only one protocol in order to confirm the presence of HRV. The 207 bp product (obtained with protocol A or B) was purified using ethanol precipitation and sequenced in both senses using Authomatic Sequencer 3730XL (Macrogen, Seoul, Korea). Alignment and analysis of the sequences were performed using Blast 2.2.24 (37).

Expanded Gold Standard

A true positive for HRV was defined as a sample that was positive with both, protocol A and B, or with only one protocol, but further confirmed as HRV by sequencing. A true negative for HRV was defined as a sample that was negative with both protocols or with a positive test for HRV with only one protocol but further confirmed as HEV by PCR. This expanded gold standard was used to determine the performance of protocols A and B and for the evaluation of the clinical characteristics in children with ARI.

Statistical analysis

Performance of protocols A and B, including sensitivity (SE), specificity (SP), positive (PPV), negative predictive value (NPV) and receiver operating curves (ROC) with their respective 95% confidence interval (CI95%) were calculated using the expanded gold standard. Rocgold was used to independently test the equality of the ROC area of each method against a gold standard curve. For each comparison, Rocgold reports the raw and the Bonferroni adjusted significance probability. Fisher’s Exact Test was used to analyze patients’ clinical and epidemiological data. Mann-Whitney test was used to compare medians. Statistical significance was assumed for p values less than 0.05. Statistical analyses were performed using STATA 7.0 (StataCorp).

RESULTS

Both RT-PCR protocols (A and B) were able to detect HRV in respiratory samples. Of 67 samples, 17 (25.4 %) were positive with protocol A, and 20 (29.9 %) with protocol B. All three commercial RT-PCR reagent kits (Qiagen one-step RT-PCR kit, iScript One-Step RT-PCR Kit for probes and SuperScrip III platinum one-step quantitative RT-PCR kit) proved to be adequate for protocol B.

The limit of detection for HRV was the same with both protocols, and a dilution of 10^5 was achieved of the HRV control. Protocols A and B did not detect poliovirus serotypes 1-3; however, both protocols detected the HEV 68 control.

When testing clinical samples, 1 of 17 HRV positive samples detected with protocol A was negative for HRV with protocol B, and was later confirmed as HEV by a specific RT-PCR. Of the 17 positive samples detected with protocol A, 3 were negative for HRV with protocol B, but were later confirmed as HRV by sequencing. Seven of 20 HRV positive samples detected with protocol B were negative for HRV with protocol A. These 7 samples were later confirmed as HRV by sequencing (Table 1).

Table 1. Comparison of two real-time RT-PCR vs. the Expanded Gold Standard for HRV detection, in 67 NPAs from children with ARI. Buenos Aires, Argentina

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Expanded Gold Standard</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (n = 23)</td>
<td>Negative (n = 44)</td>
</tr>
<tr>
<td>A</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>44</td>
</tr>
</tbody>
</table>

A true positive for HRV was defined as a sample that was positive with both, protocol A and B, or with only one protocol, but further confirmed as HRV by sequencing. A true negative for HRV was defined as a sample that was negative with both protocols or with a positive test for HRV with only one protocol but further confirmed as HEV by PCR. This expanded gold standard was used to determine the performance of protocols A and B and for the evaluation of clinical characteristics in children with ARI.

When the expanded gold standard was applied, protocol A missed 7 samples and protocol B 3 samples. Therefore, sensitivity (SE) was 70 % and 87 %, respectively. Specificity (SP) was 98 % for protocol A and 100 % for protocol B. The performance
of both protocols including SE, SP, PPV and NPV are shown in Table 2. Protocol B performed better in the detection of HRV. However, no statistically significant differences were observed in the ROC Area between both protocols.

The clinical characteristics of children with ARI and true positive HRV cases (expanded gold standard) are shown in Table 3. The overall frequency of HRV was 23/67 (34.3 %); 8/18 (44.4 %) outpatients and 15/49 (30.6 %) hospitalized children were HRV positive. The median length of stay was 5 days. All 15 children received oxygen therapy with a median of 4 days. Of 49 hospitalized patients, 1 (2.0 %) was admitted to the ICU due to respiratory illness for 4 days and received mechanical ventilation during 3 days; he was only diagnosed HRV.

Median age was 10 months (range 1-48 months) for HRV positive patients, and 8 months (range 1-36 months) for HRV negative patients. Clinical characteristics associated with HRV infection include: URTI, wheezing, bronchiolitis and pneumonia. All HRV positive patients had rhinitis, 70 % had difficulty breathing, and 30 % fever.

HRV was statistically associated with recurrent episodes of wheezing, which was observed in 10 of 23 children (43.5 %), compared to 8 of 44 (18.2 %) children with wheezing and without HRV (p = 0.041). Fever and bronchiolitis were statistically more frequent in HRV negative patients.

HRV were detected throughout the studied period (June to November 2007).

Of 67 patients studied, 32 (47.7 %) were negative for HRV but positive for other respiratory viruses, and 12 (17.9 %) patients were negative for any respiratory viruses studied. The frequency for each respiratory virus is shown in Table 4.

**DISCUSSION**

Over the last years, HRV have gained wider recognition as clinically relevant pathogens causing not only mild respiratory infections, but also severe respiratory disease. Association with recurrent episodes of wheezing, asthma and severe lower respiratory disease has also been reported (10, 12, 14, 24, 26).

The diagnosis of the classical respiratory viruses was usually done by IF using specific monoclonal antibodies. However, given the high diversity of HRV, there are no specific monoclonal antibodies available for their diagnosis. The development and use of molecular methods for all HRV have improved the diagnosis.

![Image](https://via.placeholder.com/150)

**Table 2. Performance parameters of two real-time RT-PCR for HRV detection**

<table>
<thead>
<tr>
<th>Protocol</th>
<th>SE (CI95%)</th>
<th>SP (CI95%)</th>
<th>PPV (CI95%)</th>
<th>NPV (CI95%)</th>
<th>ROC Area (CI95%)</th>
<th>X² (1gl)</th>
<th>p</th>
<th>X²</th>
<th>p Bonferroni</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>69.6 (58.6-80.6)</td>
<td>97.7 (94.2-100.0)</td>
<td>94.1 (88.5-99.8)</td>
<td>86.0 (77.7-94.3)</td>
<td>0.837 (0.7-0.9)</td>
<td>2.05</td>
<td>0.153</td>
<td>0.458</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>87.0 (78.9-95.0)</td>
<td>100.0 (100.0-100.0)</td>
<td>100.0 (100.0-100.0)</td>
<td>93.6 (87.8-99.5)</td>
<td>0.935 (0.9-1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SE: Sensitivity; SP: Specificity; PPV: positive predictive value; NPV: negative predictive value; CI95%: confidence interval 95%
Table 3. Clinical characteristics of 67 children under 2 years of age with ARI

<table>
<thead>
<tr>
<th></th>
<th>HRV Positive (n = 23)</th>
<th>HRV Negative (n = 44)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Median age. month (range)</td>
<td>10 (1-48)</td>
<td>8 (1-36)</td>
<td>0.074</td>
</tr>
<tr>
<td>Gender, Male</td>
<td>15 (65.2)</td>
<td>29 (65.9)</td>
<td>0.999</td>
</tr>
<tr>
<td>Hospitalized</td>
<td>15 (65.2)</td>
<td>34 (77.3)</td>
<td>0.385</td>
</tr>
<tr>
<td>Outpatient</td>
<td>8 (34.8)</td>
<td>10 (22.7)</td>
<td></td>
</tr>
<tr>
<td>Clinical diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>3 (13.0)</td>
<td>6 (13.6)</td>
<td>0.999</td>
</tr>
<tr>
<td>Bronchiolitis</td>
<td>5 (21.7)</td>
<td>22 (50.0)</td>
<td>0.036</td>
</tr>
<tr>
<td>Wheezing episodes</td>
<td>10 (43.5)</td>
<td>8 (18.2)</td>
<td>0.041</td>
</tr>
<tr>
<td>URTI</td>
<td>5 (21.7)</td>
<td>8 (18.2)</td>
<td>0.753</td>
</tr>
<tr>
<td>Clinical findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhinitis</td>
<td>23 (100.0)</td>
<td>44 (100.0)</td>
<td>0.999</td>
</tr>
<tr>
<td>Fever</td>
<td>7 (30.4)</td>
<td>26 (59.1)</td>
<td>0.039</td>
</tr>
<tr>
<td>Difficulty breathing</td>
<td>16 (69.6)</td>
<td>35 (79.5)</td>
<td>0.381</td>
</tr>
<tr>
<td>Acute otitis media</td>
<td>2 (8.7)</td>
<td>6 (13.6)</td>
<td>0.705</td>
</tr>
<tr>
<td>Clinical severity of Hospitalization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median Days</td>
<td>5</td>
<td>6</td>
<td>0.034</td>
</tr>
<tr>
<td>ICU admissions</td>
<td>1 (4.3)</td>
<td>7 (15.9)</td>
<td>0.406</td>
</tr>
<tr>
<td>Median Days in ICU</td>
<td>4</td>
<td>14</td>
<td>0.149</td>
</tr>
<tr>
<td>Requiring mechanical ventilation</td>
<td>1 (4.3)</td>
<td>5 (11.4)</td>
<td>0.652</td>
</tr>
<tr>
<td>Median Days under mechanical ventilation</td>
<td>3</td>
<td>9.5</td>
<td>0.218</td>
</tr>
<tr>
<td>Requiring oxygen</td>
<td>15 (65.2)</td>
<td>30 (68.2)</td>
<td>0.298</td>
</tr>
<tr>
<td>Median Days of oxygen requirement</td>
<td>4</td>
<td>4</td>
<td>0.244</td>
</tr>
</tbody>
</table>

URTI: Upper respiratory tract infection; ICU: Intensive care unit. 
(1) Mann-Whitney test was used. 
(2) Fisher bilateral test was used.

Table 4. Detection of respiratory viruses in 67 children with ARI and HRV diagnosis

<table>
<thead>
<tr>
<th>Respiratory virus</th>
<th>HRV positive n (%)</th>
<th>HRV negative n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV</td>
<td>0 -</td>
<td>14 (31.8)</td>
<td>14 (28.4)</td>
</tr>
<tr>
<td>hBoV</td>
<td>6 (26.1)</td>
<td>3 (6.8)</td>
<td>9 (20.9)</td>
</tr>
<tr>
<td>hMPV</td>
<td>3 (13.0)</td>
<td>4 (0.9)</td>
<td>7 (13.4)</td>
</tr>
<tr>
<td>AdV</td>
<td>0 -</td>
<td>3 (6.8)</td>
<td>3 (4.5)</td>
</tr>
<tr>
<td>Flu</td>
<td>0 -</td>
<td>2 (4.5)</td>
<td>2 (3.0)</td>
</tr>
<tr>
<td>PIV</td>
<td>0 -</td>
<td>1 (2.3)</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>RSV + hBoV</td>
<td>1 (4.3)</td>
<td>4 (0.9)</td>
<td>5 (1.5)</td>
</tr>
<tr>
<td>hBoV + hMPV</td>
<td>0 -</td>
<td>1 (2.3)</td>
<td>1 (3.0)</td>
</tr>
<tr>
<td>Negative</td>
<td>13 (56.5)</td>
<td>12 (27.3)</td>
<td>25 (17.9)</td>
</tr>
<tr>
<td>Total (n)</td>
<td>23</td>
<td>44</td>
<td>67</td>
</tr>
</tbody>
</table>

HRV: human rhinovirus; RSV: respiratory syncytial virus; hBoV: human bocavirus; hMPV: human metapneumovirus; AdV: adenovirus; Flu: influenza; PIV: parainfluenza.
when using the SuperScript III platinum kit. We chose the Qiagen OneStep RT-PCR kit to perform protocol B because it includes two Reverse Transcriptases: an Omniscript and a Sensiscrip, which are optimized for high and low amounts of RNA, respectively. Both enzymes exhibit high affinity for RNA, thus facilitating transcription through secondary structures, such as IRES, present in the 5'NCR of HRV.

In our study, the overall frequency of HRV in children with ARI was 34.3 %: 30.6 % in hospitalized patients and 44.4 % in outpatients. These results are similar to those previously reported in other countries (2, 5).

Two previous publications on HRV in hospitalized children with recurrent wheezing from Argentina reported frequencies of 23 % - 25 % (18, 19), with a conventional RT-PCR. The higher frequency detected in our study may be due to differences in the studied population, or to the use of a more sensitive RT-PCR assay. Some RT-PCR may be less sensitive in detecting certain HRV strains; the use of more specific and sensitive real-time RT-PCR protocols may be necessary to better establish the frequency and epidemiology of these viruses.

Protocol B has been recently performed in our laboratory to study 347 children under 6 years of age with ARI, and enrolled throughout a whole year (June 2008 to May 2009). Results showed an HRV frequency of 43 % in hospitalized and 26 % in outpatient children (21, 35).

In the present study, the only sign statistically associated with HRV was recurrent wheezing episodes (p = 0.041). Likewise, Piotrowska et al. (29) conclude that HRV are the major cause of wheezing among children under 2 years of age.

Of the 23 patients with HRV, 43.5 % were diagnosed to be coinfected with other respiratory virus, and 7 (30.4 %) of them were hospitalized. HRV coinfection was most frequently with hBoV, followed by hMPV and RSV. One hospitalized patient, who required oxygen therapy, had a triple coinfection: HRV, RSV and hBoV.

In conclusion, the adapted protocol B proved to properly detect HRV in respiratory samples. Further studies including a larger series of patients with ARI and sequencing HRV samples to identify HRV types are needed to better determine the epidemiology and impact of these viruses in Argentina.

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Conflict of interest
Competing interest: None declared
Ethical approval: Yes

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