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Genetic diversity and clinical impact of human rhinoviruses in hospitalized and outpatient children with acute respiratory infection, Argentina

Débora Natalia Marcone[a,*], Andrés Culasso[b], Guadalupe Carballal[a], Rodolfo Campos[b], Marcela Echavarría[a]

[a] Virology Unit and Clinical Virology Laboratory, Hospital Universitario CEMIC, Av. Galván 4102, Buenos Aires, Argentina
[b] Department of Virology, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 954, Buenos Aires, Argentina

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ABSTRACT

Background: Human rhinoviruses (HRV) are recognized as a cause of upper and lower acute respiratory infections (ARI). The circulating species and their clinical impact were not described in Argentina.
Objectives: To describe the molecular epidemiology of HRV in children and to determine the association of HRV species with outcome and severity.

Study design: Hospitalized and outpatients children <6 years old with ARI without comorbidities ($n = 620$) were enrolled (2008–2010). Demographic, clinical data and outcome were analyzed. HRV were identified by RT-PCR. Phylogenetic analysis and demographic reconstruction for HRV were performed in selected samples.
Results: HRV were detected in 252/620 (40.6%) of children; 8.5% in viral coinfection. Bronchiolitis (55%) and pneumonia (13%) were the most frequent clinical diagnosis. Of 202 inpatients with HRV: 72% required oxygen supplementation, 11% intensive care unit and 3% mechanical ventilation. HRV were identified as a risk factor for hospitalization (OR: 2.47).

All three HRV species were detected being HRV-A (55%) and HRV-C (43%) the most frequent; HRV-B was infrequent (2%). Of 44 sequenced HRV, 30 genotypes were detected. Seven of them were the most prevalent and circulated during limited periods of time. The demographic reconstruction revealed a constant population size and a high turnover rate of genotypes. Demographic and clinical outcome were similar for HRV-A and HRV-C infections.
Conclusion: This study highlights the clinical impact of HRV in children without comorbidities as a cause of lower ARI and hospitalization. The high frequency of HRV infections may be associated with the simultaneous circulation of genotypes and their high turnover rate.

1. Background

Human rhinoviruses (HRV) are a frequent cause of upper respiratory tract infections (URTI). However, recently, HRV were found also to be associated with lower respiratory infections (LRTI), such as bronchiolitis [1], pneumonia, exacerbation of asthma or cystic fibrosis [2,3], chronic obstructive pulmonary disease [4] and hospitalization [5].

HRV are non-enveloped positive-sense single strand RNA viruses, with a 7200-base genome, classified into three species within the *Enterovirus* genus of picornaviruses.

By phylogenetic analysis, reference serotypes have been classified in 75 HRV-A and 25 HRV-B genotypes [6] and a new species HRV-C, with 50 accepted genotypes and 13 provisionally assigned types (pat), was described in 2006 [7].

Abbreviations: ARI, acute respiratory infection; LRTI, lower respiratory tract infection; URTI, upper respiratory tract infection; HRV, human rhinoviruses; RSV, respiratory syncytial virus; Flu, influenza; PIV, parainfluenza; AdV, adenovirus; hMPV, human metapneumovirus; 5′NCR, 5′ non-coding region; IF, immunofluorescence; tMRCA, time of the most recent common ancestor; HPD, highest probability density; BSP, Bayesian skyline plot; MCC, maximum clade credibility; BA, Buenos Aires.

* Corresponding author at: Virology Unit and Clinical Virology Laboratory, Hospital Universitario CEMIC, Av. Galván 4102, Buenos Aires C1431FWO, Argentina. Tel.: +54 11 5299 0100x2375; fax: +54 11 5299 1580.
E-mail address: deboramarcone@hotmail.com (D.N. Marcone).

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All 3 HRV species have a global distribution, being HRV-A and HRV-C the most prevalent; a high number of co-circulating genotypes were reported. It remains controversial whether a particular species is associated with higher severity and worse outcome.

2. Objectives

To determine the molecular epidemiology of HRV in hospitalized or outpatient children younger than 6 years old with acute respiratory infection (ARI), without comorbidities. To compare demographic, clinical data, outcome and severity among different HRV species.

3. Study design

3.1. 1-Study population

A cross-sectional, descriptive study was performed in 620 children with ARI, without comorbidities, during two consecutive years (June 1, 2008–May 31, 2010) in Buenos Aires, Argentina. Children hospitalized at either Centro de Educación Médica e Investigaciones Clínicas (CEMIC) University Hospital or Mater Dei Hospital, or outpatients attending the emergency room at CEMIC Hospital were enrolled.

Inclusion criteria were: (a) children <6 years old with LRTI and/or URTI, and symptoms onset ≤3 days; (b) informed consent form signed by parents/tutors; (c) a respiratory sample obtained at admission; (d) demographic and clinical data recorded in a specially designed form. For inpatients, the clinical course including length of stay, oxygen therapy, admission at intensive care unit and mechanical ventilation, was also recorded.

The exclusion criteria were children with cardiopathy, chronic pulmonary disease, metabolic and genetic diseases or immunosuppression.

Nasopharyngeal flocked swabs and aspirates obtained from outpatients and inpatients, respectively, were processed for rapid antigen detection by immunofluorescence (IF) for respiratory syncytial virus (RSV), adenovirus (AdV), influenza A and B (FluA, FluB), parainfluenza (PIV) 1–3 (Chemicon/Millipore) and human metapneumovirus (hMPV) (Argene).

3.2. Rhinovirus detection and typing

Viral RNA/DNA extraction was manually performed from an aliquot of the original sample stored at −70 °C, using the QIAamp MinElute Virus Spin (Qiagen), according to the manufacturer's recommendations.

HRV detection was performed by a real-time RT-PCR targeting 207 nucleotides (nt) of the 5′ non-coding region (5′NCR), using the One step RT-PCR Kit (Qiagen), primers forward (5′-CYA GCC TGC GTG GC-3′) and reverse (5′-GAA ACA CGG ACA CCC AAA GTA-3′), and a Taqman probe (5′-FAM-TCC TCC GGC CCC TGA ATG YGG C-BHQ1-3′) [8].

For HRV genotyping, an RT-PCR that amplifies 549 nt of the 5′NCR/VP4/VP2 region was performed according to a previously published protocol using primer pair 9565-reverse and 9895-forward [9]. PCR products were purified by ethanol precipitation and direct-sequenced by an automatic sequencer 3730XL (Macrogen, Korea).

To type HRV positive cases, 45 samples were selected from both, hospitalized and outpatient children, from every month of the studied period. For inpatients, we selected patients based on their length of stay at the hospital (less or more than 3 days). Strains were named BA (for Buenos Aires), followed by an identification number, a status identification (HL3 stands for hospitalized ≤3 days, HM3 for hospitalized >3 days, and A for outpatients), and the sample collection date (dd-mm-yy).

Sequences corresponding to 420 nt of the VP4/VP2 partial region, were visually inspected and manually edited with BioEdit v7.0.5.3 [10], and aligned with ClustalW v1.81 [11] using GenBank reference sequences for all HRV genotypes and human enterovirus (HEV) species as outgroup.

The obtained HRV sequences were submitted to GenBank under the following accession numbers: KF146656–KF146699.

3.3. Phylogenetic analysis

A maximum likelihood methodology was employed to study the relationship between obtained HRV sequences and reference strains. Phylogenetic trees were obtained by the heuristic search as implemented in PhyML v3.0 program [12]. The models of nucleotide substitution were selected according to the Akaike Information Criterion implemented in ModelTest 3.07 [13]. Branch support was assessed by non-parametric bootstrap (1000 pseudoreplicates). Bootstrap values greater than 70% was used to provide significant evidence for phylogenetic grouping. For recombination analysis, the sequence dataset was examined using bootscan algorithm [14].

3.4. Demographic reconstruction

A Bayesian coalescent analysis was carried out to study the relationship between the different HRV-A and C genotypes. Population dynamics and phylogeny were jointly estimated in a Bayesian framework as implemented in BEAST v1.7.1 [15]. Sampling dates were used to calibrate an uncorrelated log-normal relaxed molecular clock. Population dynamics was modeled by the non-parametric Bayesian skyline plot (BSP) [16]. Results were summarized into maximum clade credibility (MCC) trees with branches scaled in time, using Tree Annotator v1.7.1.

3.5. Statistical analysis

Results were given as percentages for discrete variables and as median, first and third quartile for continuous variables. Bi-variate associations between variables were assessed by χ^2 or Fisher exact test. Continuous variables were compared using Wilcoxon non-parametric test. The odds ratios (OR) with 95% confidence intervals (CI) were calculated. Statistical significance was assumed for p values <0.05. Statistical analysis was performed using STATA 7.0 (Stata Corp.).

4. Results

A HRV diagnosis was achieved in 252/620 (40.6%) children <6 years of age with ARI. Specifically, HRV were detected in 202/434 (46.5%) inpatients, and 50/186 (26.9%) outpatients ($p < 0.01$).

Viral antigen detection by IF was positive in 277/620 (44.7%) children. RSV was detected in 165 (26.6%), hMPV in 53 (8.5%), followed by FluA (3.4%), PIV (3.2%), AdV (2.4%) and FluB (1.9%). A viral coinfection was observed in 61 (9.8%) patients, being HRV detected in 53 (8.5%) of them. A total of 144 (23.2%) children had a negative viral diagnosis.

HRV were significantly more frequent in hospitalized patients and the presence of HRV as a single agent or in coinfection were identified as a risk factor for hospitalization: OR: 2.23 (95% CI: 1.51–3.30) and OR: 2.47 (95% CI: 1.60–4.00), respectively.

Of 252 HRV positive patients, 123 (48.8%) were <12 months, while 195 (77.4%) were <2 years old. Most were males (60.3%) and breastfed (89.7%) (Table 1). Cough was observed in 90.1% and fever in 57.8% of patients, while tachypnea, wheezing and retraction were

Table 1
Demographic and clinical characteristics of 252 children with HRV, and comparison between species A and C.

	Total HRV ($n=252$)		HRV-A[b] ($n=24$)		HRV-C[b] ($n=19$)		p[b]
	n	%	n	%	n	%	
Age, median (p25–p75)[a]	12 (6–22)		8 (4–16)		12 (3–26)		0.658
Male sex	152	60.3	17	70.8	9	47.4	0.341
Hospitalized	202	80.2	16	66.7	10	52.6	0.230
Demographic characteristics							
Breastfeeding	226	89.7	21	87.5	19	100.0	0.242
Bronchial hyperreactivity	128	50.8	13	54.2	12	63.2	0.756
Familiar bronchial hyperreactivity	120	47.6	9	37.5	7	36.8	0.999
Atopy	42	16.7	6	25.0	6	31.6	0.737
Familiar atopy	76	30.2	7	29.2	5	26.3	0.736
Viral exposure	135	53.6	9	37.5	11	57.9	0.228
School siblings	146	57.9	15	62.5	8	42.1	0.228
Day care	111	44.0	6	25.0	7	36.8	0.509
Passive smoking	45	17.9	7	29.2	7	36.8	0.521
Signs and symptoms							
Fever	146	57.9	12	50.0	8	42.1	0.760
Tachypnea	182	72.2	16	66.7	14	73.7	0.743
Cough	227	90.1	23	95.8	17	89.5	0.576
Wheezing	164	65.1	17	70.8	12	63.2	0.745
Retraction	157	62.3	15	62.5	13	68.4	0.755
Apnea	8	3.2	1	4.2	1	5.3	0.999
Cyanosis	17	6.7	3	12.5	0	0.0	0.243
Vomiting	47	18.7	6	25.0	3	15.8	0.999
Clinical diagnosis							
URTI	77	30.5	8	33.3	7	36.8	0.999
LRTI	175	69.5	16	66.7	12	63.2	
Bronchiolitis	138	54.8	14	58.3	11	57.9	0.999
Bronchitis	5	2.0	0	0.0	0	0.0	[c]
Pneumonia	32	12.7	2	8.3	1	5.3	0.999

HRV: human rhinovirus.
[a] Age, median and percentiles 25 and 75 (p25–p75) are given in months.
[b] Comparison according to HRV-A or HRV-C detection. Wilcoxon test was used for median comparison. Fisher test was used for all the other demographic and clinical characteristics. Statistical significance was assumed for $p \leq 0.05$.
[c] This value could not be calculated.

recorded in 72.2%, 65.1% and 62.3%, respectively. Almost 70% of children had LRTI, being bronchiolitis the most frequent diagnosis.

Eighty percent of HRV positive patients were hospitalized: 62.9% had a length of stay of ≤3 days, 72.3% required oxygen supplementation and 10.9% were in intensive care unit (ICU) (Table 2). Of 22 patients in ICU, 5 (22.7%) required mechanical ventilation. None of the children died.

Phylogenetic analysis of 45 BA sequences revealed well-supported groups for all HRV species. HRV-A was the most frequent (55%), followed by HRV-C (43%). Only one strain (2%) grouped with species B. One sample initially diagnosed as HRV, was genotyped as HEV-D68. Thus, HRV typing was performed in 44 patients.

A phylogenetic analysis per species was performed and genotypes were assigned according to the clustering of BA strains with reference sequences (Fig. 1). Thirty different genotypes were detected: 14 HRV-A, 1 HRV-B and 15 HRV-C. The seven most frequent genotypes were observed as monophyletic clusters, named A1–A5 and C1–C2, based on the time of detection. Clusters were genotyped as HRV-A78 (A1), HRV-A55 (A3), HRV-A101 (A4), HRV-A49 (A5), HRV-C10 (C1) and HRV-C40 (C2). Cluster A2 could not be genotyped because the pairwise p-distance in VP4/VP2 obtained with the closest reference genotype (HRV-A78) was higher than 12.5%. No recombination events were observed in the analyzed fragment.

Demographic, clinical characteristics and outcome were similar with either HRV-A ($n=24$) or HRV-C ($n=19$) (Table 1). Of 17 outpatients, 47% had HRV-A and 53% had HRV-C. URTI was observed in 47%, bronchiolitis in 29% and wheezing in 24%. Regarding 27 inpatients, 60% had HRV-A, 37% HRV-C and 1 case HRV-B. Of these, 82% had bronchiolitis, 11% pneumonia and 7% URTI. The clinical outcome, including length of stay, oxygen supplementation, intensive care or mechanical ventilation, was similar with either HRV-A or C (Table 2).

HRV circulation occurred throughout the whole year in both years (June 2008–May 2010), with peaks at the beginning of autumn and spring. A variable frequency was observed during winter and a low frequency in summer (Fig. 2). HRV-A and C strains were detected throughout the whole year, and the only HRV-B strain was detected in June, 2009. Several HRV genotypes co-circulate: seven monophyletic clusters, including genetically close strains, predominated during certain months, simultaneously with others genotypes that were detected only once.

Table 2
Clinical course of hospitalization among 202 children with human rhinoviruses and comparison between species A and C.

	Total HRV ($n=202$)		HRV-A* ($n=16$)		HRV-C* ($n=10$)		p*
	n	%	n	%	n	%	
Length of stay ≤3 days	127	62.9	7	43.8	5	50.0	0.688
Length of stay >3 days	75	37.1	9	56.3	5	50.0	
Oxygen supplementation	146	72.3	13	81.3	9	90.0	0.280
Intensive care requirement	22	10.9	3	18.8	2	20.0	0.999
Mechanical ventilation	5	2.5	2	12.5	1	10.0	0.999

HRV: human rhinovirus.
* Fisher test was used for the comparison between children with HRV-A and those with HRV-C. Statistical significance was assumed for $p \leq 0.05$.

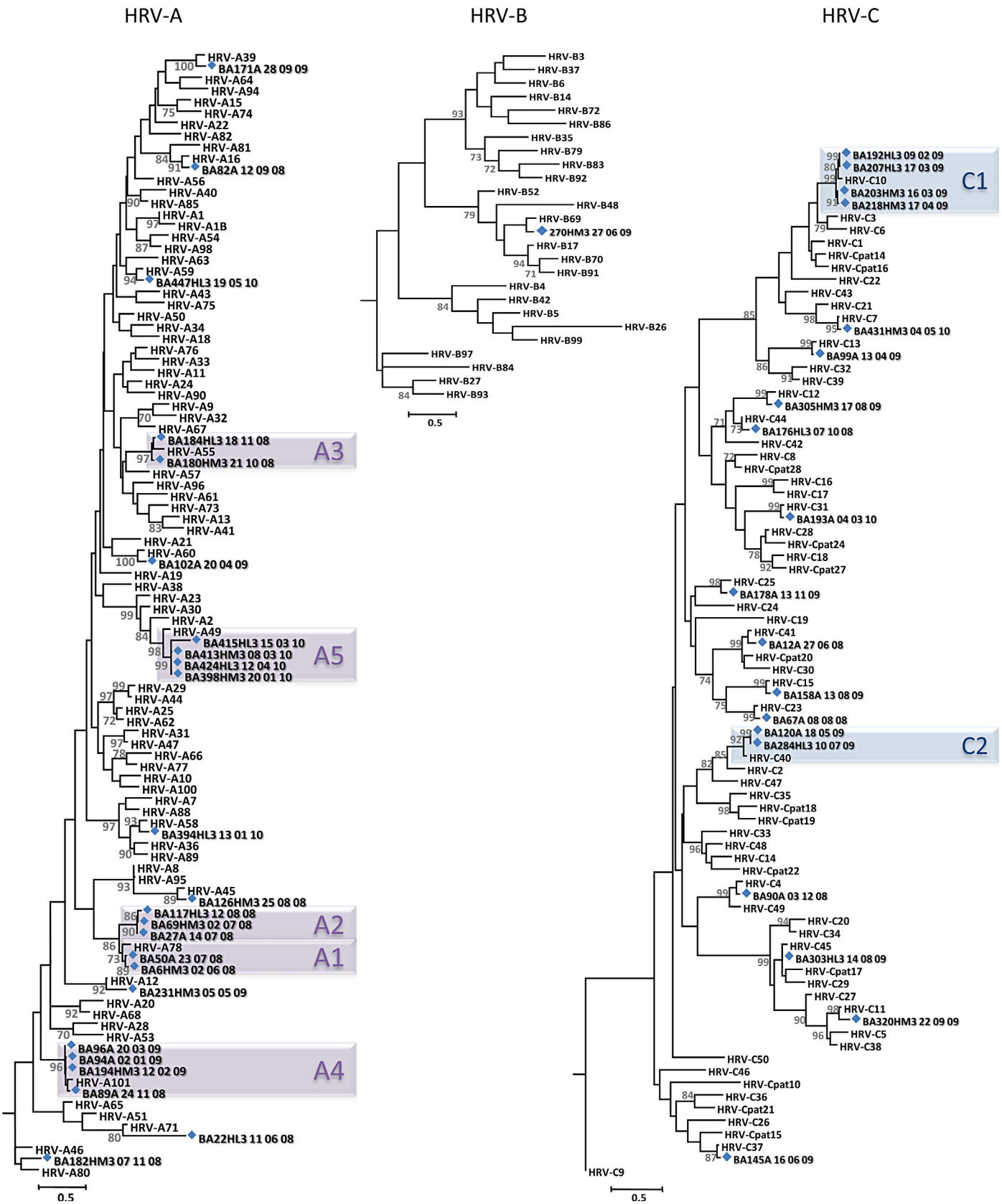

Fig. 1. Phylogenetic analysis of Buenos Aires (BA) strains of HRV (◆) species A, B and C identified in hospitalized and outpatient children, from June 2008 to May 2010. BA strains are in bold and were named BA followed by an identification number, a status identification (HL3 stands for hospitalized less than 3 days, HM3 hospitalized more than 3 days, and A outpatients), and the sample collection date (dd-mm-yy). Reference sequences of all HRV species and genotypes were downloaded from GenBank. Phylogenetic tree was constructed by maximum likelihood, using the GTR + I + Γ model for HRV-A, TIM2 + I+ Γ model for HRV-B, and TIM1 + I+ Γ model for HRV-C, using HEV-C to root the tree. Branch support was assessed by bootstrap (1000 pseudoreplicates); values higher than 70% are shown. Purple boxes represent HRV-A clusters, blue boxes represent HRV-C clusters. Branch distance is indicated by the scale bars at the bottom of the trees. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

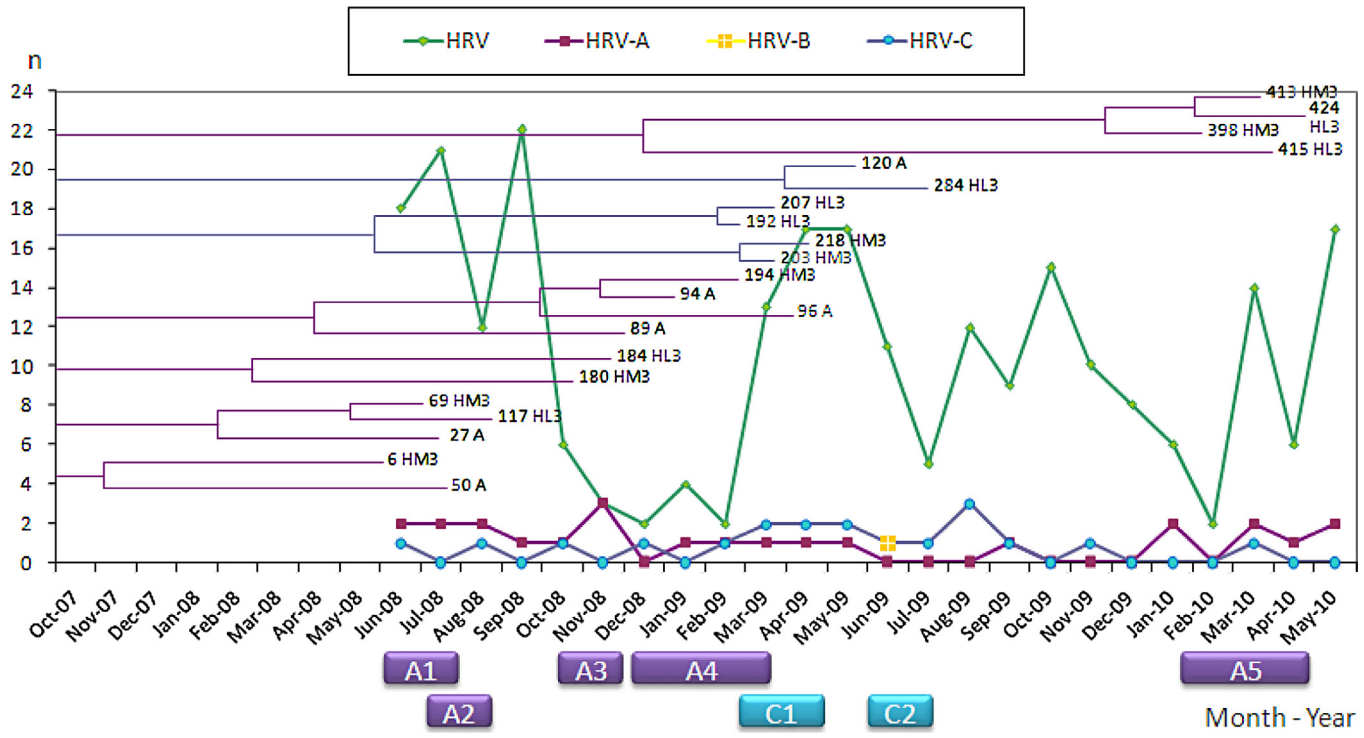

Fig. 2. Seasonality and demographic reconstruction of HRV in Buenos Aires City, Argentina, from June 2008 to May 2010. Total rhinoviruses (green), species A (purple), species B (yellow) and species C (blue). The demographic reconstruction of the 7 monophyletic clusters (A1–A5 and C1–C2) corresponding to 7 different genotypes is represented in the temporal scale showing different common ancestors. BA strains were named by an identification number followed by a status identification (HL3 stands for hospitalized less than 3 days, HM3 hospitalized more than 3 days, and A outpatients). Purple boxes represent the time of detection of the five HRV-A clusters, and blue boxes, the two HRV-C clusters. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The estimated time of the most recent common ancestor (tMRCA) for the 24 BA strains HRV-A was 18.5 years (highest probability density (HPD) 95%: 5–91), and for the 19 strains HRV-C was 84.8 years (HPD 95%: 11.8–703.0). The substitution rate (expressed in nucleotide substitutions/site/year (s/s/year)) for the VP4/VP2 partial region was also estimated, and was similar for both HRV species: 2.3×10^{-2} (HPD 95%: 1.4×10^{-8}–5.3×10^{-2}) for HRV-A and 1.9×10^{-2} (HPD 95%: 1.1×10^{-5}–5.8×10^{-2}) for HRV-C. In addition, the BSP analysis showed a constant demographic profile throughout almost 100 years.

Demographic reconstruction of clusters (A1–A5 and C1–C2) obtained in the MCC trees are shown on a time scale, in order to describe the detection time of the BA strains and the association to their corresponding ancestor, each one belonging to a different HRV genotype (Fig. 2). From June 2008 to March 2009, all BA clusters detected were HRV-A (A1–A4). In March and June two HRV-C clusters (C1–C2) appeared, while by the end of the study a different HRV-A cluster (A5) was observed.

5. Discussion

Our two-year study in children <6 years presenting ARI reveals that HRV were the most frequently detected viruses. Surprisingly, this frequency was significantly higher in inpatients than in outpatients. Remarkably, all studied patients had no underlying disease [17]. In the literature, HRV frequencies in children with ARI range from 17% to 44%, depending on the age, onset of disease, season and the RT-PCR employed [18–20]. However, higher frequencies are usually detected (45–58%) when premature infants and children with wheezing or asthma exacerbation are studied [2,21,22].

Regarding the rest of the respiratory viruses studied, RSV was the second in frequency, followed by hMPV, and lower frequencies of the rest. Frequencies for each virus were different in both populations studied: RSV and hMPV were most frequent in hospitalized patients, while FluB and PIV were most frequent in outpatients, as previously described in our hospital [17]. Although coinfection was observed in a low number of cases, HRV was the most frequently detected. Nosocomial acquisition of these viruses was ruled out since respiratory samples were collected at admission.

The highest frequency of HRV was observed in patients with LRTI, being bronchiolitis the main diagnosis. Surprisingly, 80% of HRV positive children were hospitalized due to their ARI. Furthermore, 11% of patients had very severe ARI as they required ICU or mechanical ventilation. In our study, HRV was identified as a risk factor for ARI which required hospitalization. Case–control studies will be necessary to confirm this finding.

The phylogenetic analysis of BA strains, showed the presence of all HRV species in Buenos Aires: HRV-A and C were more prevalent, while HRV-B was infrequent. These results are consistent with data from the Northern hemisphere, where HRV-A and C are more frequent, and species B is observed in less than 20% of cases [22,23]. To date, few data are available on the detection and molecular epidemiology of HRV in Argentina. Only two recent studies carried out in Latin America reported that HRV frequency ranges from 16% to 34%, and described the 3 HRV species in circulation, with HRV-A and C being the most frequent [24,25].

Regarding seasonality, HRV were detected throughout the 2 years, with peaks in the fall and spring. Species A and C were detected during all the study period, while the single HRV-B was observed in winter. This pattern was similar to that reported in temperate countries [1,26].

A significant genetic diversity of HRV was observed during two consecutive years, evidenced by the high number of genotypes detected and their rapid turnover. Of 30 different genotypes, 7 were detected as monophyletic clusters that circulated on

consecutive months. The seasonality pattern of these clusters shows that a frequent HRV genotype may be replaced by a different one. Furthermore, these monophyletic clusters circulated simultaneously with other HRV genotypes detected only once. This observation is consistent with reports describing the simultaneous circulation of a high number of genotypes [27,28].

Interestingly, one HRV-A cluster could neither be genotyped as a known genotype nor as a new recombinant. The pairwise *p*-distance in VP4/VP2 of those strains obtained with the closest reference genotype was higher than 12.5%. According to the proposals for HRV classification, this finding suggests the presence of an undescribed genotype [7,27]. Further studies sequencing the VP1 region are currently ongoing, to determine whether these strains corresponds to a new genotype. One of the sequenced samples was genotyped as HEV-68, usually associated with respiratory diseases [29].

The demographic reconstruction of BA strains showed that the effective population size remained constant for the last one hundred years for HRV-A and for HRV-C. The tMRCA for HRV-A and C was younger, and the estimated mean rates of evolutionary change for both HRV species were higher (10^{-2} s/s/year), than previously reported (10^{-4} s/s/year) [30]. Therefore, the estimated mean rates do not represent the mutation rate of each species, but could represent the turnover rate of HRV genotypes. Further studies including more strains from wider periods of time will be necessary to better determine these observations.

Whether one HRV species could be more severe than others has been a matter of discussion. Some authors suggest that HRV-A and B are associated with higher severity [29,31]; others propose that HRV-C cause more severe ARI [1,32,33]; there are reports that showed species A and C associated with more severe respiratory illness than HRV-B [34], while others found no association between severity and HRV species [5,19,35,36]. Our data showed that all 3 HRV species were detected in severe cases and no differences between HRV-A and HRV-C were observed in the clinical characteristics and outcome.

Our study has some limitations. First, the scarce budget allowed us to genotype only selected and representative HRV strains. Second, the diagnostic procedure used for HRV (real-time RT-PCR) differs from that used for the other respiratory viruses studied (IF). Therefore, due to the lower sensitivity of IF, especially for certain viruses such as AdV or PIV, the frequency of these viruses may be underestimated. Finally, some respiratory viruses such as human coronavirus, bocavirus or PIV-4 were not studied. Therefore, some of the respiratory infections did not get a viral diagnosis.

In summary, this study highlights the clinical impact of HRV in children presenting ARI without comorbidities, as a frequent cause of severe LRTI associated with hospitalization. In Buenos Aires, Argentina, all HRV species were detected, a high number of HRV genotypes co-circulated throughout the whole year, a constant population size and high turnover rate of genotypes was herein estimated. These findings describe the molecular epidemiology of HRV and will help to implement strategies of prevention of HRV infections that will contribute to a better patient's management.

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Competing interests

None.

Ethical approval

This study was approved by CEMIC and Mater Dei Institutional Review Boards (No. 466). IRB00001745-IORG0001315.

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References

[1] McErlean P, Shackelton LA, Lambert SB, Nissen MD, Sloots TP, Mackay IM. Characterisation of a newly identified human rhinovirus, HRV-QPM, discovered in infants with bronchiolitis. J Clin Virol 2007;39:67–75.
[2] Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. Am J Respir Crit Care Med 2008;178:667–72.
[3] de Almeida MB, Zerbinati RM, Tateno AF, Oliveira CM, Romão RM, Rodrigues JC, et al. Rhinovirus C and respiratory exacerbations in children with cystic fibrosis. Emerg Infect Dis 2010;16:996–9.
[4] Seemungal TA, Harper-Owen R, Bhowmik A, Jeffries DJ, Wedzicha JA. Detection of rhinovirus in induced sputum at exacerbation of chronic obstructive pulmonary disease. Eur Respir J 2000;16:677–83.
[5] Piotrowska Z, Vázquez M, Shapiro ED, Weibel C, Ferguson D, Landry ML, et al. Rhinoviruses are a major cause of wheezing and hospitalization in children less than 2 years of age. Pediatr Infect Dis J 2009;28:25–9.
[6] Savolainen C, Blomqvist S, Mulders M, Hovi T. Genetic clustering of all 102 human rhinovirus prototype strains: serotype 87 is close to human enterovirus 70. J Gen Virol 2002;83:333–40.
[7] Simmonds P, McIntyre C, Savolainen-Kopra C, Tapparel C, Mackay IM, Hovi T. Proposals for the classification of human rhinovirus species C into genotypically assigned types. J Gen Virol 2010;91:2409–19.
[8] Marcone DN, Videla C, Ricarte C, Carballal G, Vidaurreta S, Echavarría M. Rhinovirus detection by real-time RT-PCR in children with acute respiratory infection. Rev Argent Microbiol 2012;44:259–65.
[9] Savolainen C, Mulders M, Hovi T. Phylogenetic analysis of rhinovirus isolates collected during successive epidemic seasons. Virus Res 2002;85:41–6.
[10] Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 1999;41:95–8.
[11] Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;22:4673–80.
[12] Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 2003;52:696–704.
[13] Posada D, Crandall KA. Modeltest: testing the model of DNA substitution. Bioinformatics 1998;14:817–8.
[14] Martin DP, Posada D, Crandall KA, Williamson C. A modified bootscan algorithm for automated identification of recombinant sequences and recombination breakpoints. AIDS Res Hum Retrovir 2005;21:98–102.
[15] Drummond AJ, Rambaut A. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 2007;7:214.
[16] Drummond AJ, Rambaut A, Shapiro B, Pybus OG. Bayesian coalescent inference of past population dynamics from molecular sequences. Mol Biol Evol 2005;22:1185–92.
[17] Marcone DN, Ellis A, Videla C, Ekstrom J, Ricarte C, Carballal G, et al. Viral etiology of acute respiratory infections in hospitalized and outpatient children in Buenos Aires, Argentina. Pediatr Infect Dis J 2013;32:e105–10.
[18] Arden KE, McErlean P, Nissen MD, Sloots TP, Mackay IM. Frequent detection of human rhinoviruses, paramyxoviruses, coronaviruses, and bocavirus during acute respiratory tract infections. J Med Virol 2006;78:1232–40.
[19] Iwane MK, Prill MM, Lu X, Miller EK, Edwards KM, Hall CB, et al. Human rhinovirus species associated with hospitalization for acute respiratory illness in young US children. J Infect Dis 2011;201:1702–10.
[20] Cheuk D, Tang I, Chan K, Woo P, Peiris M, Chiu S. Rhinovirus infection in hospitalized children in Hong Kong: a prospective study. Pediatr Infect Dis J 2007;26:995–1000.
[21] Miller EK, Bugna J, Libster R, Shepherd BE, Scalzo PM, Acosta PL, et al. Human rhinoviruses in severe respiratory disease in very low birth weight infants. Pediatrics 2012;129(January (1)):e60–7.
[22] Smuts HE, Workman LJ, Zar HJ. Human rhinovirus infection in young African children with acute wheezing. BMC Infect Dis 2011;11:65.
[23] Lauinger IL, Bible JM, Halligan EP, Bangalore H, Tosas O, Aarons EJ, et al. Patient characteristics and severity of human rhinovirus infections in children. J Clin Virol 2013;58:216–20.

[24] Espínola EE, Russomando G, Aquino C, Basualdo W. Phylogeny-based classification of human rhinoviruses detected in hospitalized children with acute lower respiratory infection in Paraguay, 2010–2011. J Med Virol 2013;85:1645–51.
[25] Garcia J, Espejo V, Nelson M, Sovero M, Villaran MV, Gomez J, et al. Human rhinoviruses and enteroviruses in influenza-like illness in Latin America. Virol J 2013;10:305.
[26] Bochkov YA, Gern JE. Clinical and molecular features of human rhinovirus C. Microbes Infect 2012;14:485–94.
[27] Wisdom A, Leitch EC, Gaunt E, Harvala H, Simmonds P. Screening respiratory samples for detection of human rhinoviruses (HRVs) and enteroviruses: comprehensive VP4–VP2 typing reveals high incidence and genetic diversity of HRV species C. Clin Microbiol 2009;47:3958–67.
[28] Xiang Z, Gonzalez R, Xie Z, Xiao Y, Chen L, Li Y, et al. Human rhinovirus group C infection in children with lower respiratory tract infection. Emerg Infect Dis 2008;14:1665–7.
[29] Lu QB, Wo Y, Wang HY, Wei MT, Zhang L, Yang H, et al. Detection of enterovirus 68 as one of the commonest types of enterovirus found in patients with acute respiratory tract infection in China. J Med Microbiol 2014;63:408–14.
[30] Briese T, Renwick N, Venter M, Jarman RG, Ghosh D, Köndgen S, et al. Global distribution of novel rhinovirus genotype. Emerg Infect Dis 2008;14:944–7.
[31] Miller EK, Williams JV, Gebretsadik T, Carroll KN, Dupont WD, Mohamed YA, et al. Host and viral factors associated with severity of human rhinovirus-associated infant respiratory tract illness. J Allergy Clin Immunol 2011;127:883–91.
[32] Linsuwanon P, Payungporn S, Samransamruajkit R, Posuwan N, Makkoch J, Theanboonlers A, et al. High prevalence of human rhinovirus C infection in Thai children with acute lower respiratory tract disease. J Infect 2009;59:115–21.
[33] Lau SK, Yip CC, Tsoi HW, Lee RA, So LY, Lau YL, et al. Clinical features and complete genome characterization of a distinct human rhinovirus (HRV) genetic cluster, probably representing a previously undetected HRV species, HRV-C, associated with acute respiratory illness in children. J Clin Microbiol 2007;45:3655–64.
[34] Lee WM, Lemanske Jr RF, Evans MD, Vang F, Pappas T, Gangnon R, et al. Human rhinovirus species and season of infection determine illness severity. Am J Respir Crit Care Med 2012;186:886–91.
[35] Huang T, Wang W, Bessaud M, Ren P, Sheng J, Yan H, et al. Evidence of recombination and genetic diversity in human rhinoviruses in children with acute respiratory infection. PLoS ONE 2009;4:e6355.
[36] Tapparel C, Cordey S, Junier T, Farinelli L, Van Belle S, Soccal PM, et al. Rhinovirus genome variation during chronic upper and lower respiratory tract infections. PLoS ONE 2011;6:e21163.