# Patient characteristics and severity of human rhinovirus infections in children

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## ARTICLE INFO

## ABSTRACT

*Background:* It is increasingly recognized that human rhinoviruses (HRV) can be associated with severe infections. However, conflicting results have been reported on the relative prevalence and severity of the three HRV species.
*Objectives:* The relative prevalence and clinical characteristics of HRV-A, B and C, in children attending a South London teaching hospital were investigated retrospectively.
*Study design:* Children aged <16 years with episodes of respiratory tract infections and detectable entero/rhinovirus RNA in respiratory samples between November 2009 and December 2010 were investigated. Retrospective case review was performed and patients' characteristics recorded.
*Results:* Entero/rhinoviruses were the commonest viral pathogens (498/2316; 21.5%). Amongst 204 infection episodes associated with entero/rhinovirus, 167 were typed HRV, HRV-C was the most prevalent (99/167, 59.3%) followed by HRV-A (60/167; 35.9%) and HRV-B (8/167, 4.8%). The severity spectrum of HRV-A and HRV-C infections were similar and affected all parts of the respiratory tract. Co-pathogens were observed in 54 (26.5%) episodes. Severity was increased in patients with non-viral co-pathogens and those with an underlying respiratory condition. Univariate and multiple regression analyses of potential prognostic variables including age, co-pathogens and underlying respiratory illnesses showed that mono-infection with HRV-C, as compared with other HRV species, was associated with more severe disease in young children <3 years.
*Conclusions:* HRV-C was the most prevalent species and on its own was associated with severe disease in children <3 years. The association between infection with HRV species and clinical presentation is complex and affected by many confounding factors.

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## 1. Background

Human rhinoviruses (HRV) are respiratory picornaviruses under the extended genus of enterovirus. They have been most frequently implicated as the causative agent of common cold. However, there were recent suggestions that HRV could be associated with severe respiratory tract infections (RTI), acute asthma exacerbations [1,2], recurring wheeze [3] and lower RTI (LRTI) [4–7]. Dependent on the study, the reported incidence of HRV infections in children ranges from <10% in RTI [8] to 90% in acute asthma [9].

There are three HRV species: HRV-A, HRV-B and the newly identified HRV-C, each with multiple types (77 HRV-A, 26 HRV-B and 63 HRV-C) [10]. New types continue to be described, especially for the novel HRV-C, and typing of the new species remains challenging since very few full genomes for the proposed types are available for comparison. The results of many clinical and epidemiological studies on HRV are contradictory with no consensus on the significance of its role.

## 2. Objectives

In this study, we retrospectively investigated episodes of RTI with respiratory samples positive for entero/rhinoviruses over a

one-year period to determine the clinical characteristics of RTI associated with different HRV species in children.

## 3. Study design

### 3.1. Patients

Respiratory samples were obtained routinely from all paediatric patients (<16 years of age) with respiratory symptoms attending the Evelina Children's Hospital in London, UK. Samples submitted between November 2009 and December 2010 were investigated for viral pathogens using a multiplex nucleic acid amplification panel (ResPlex II v2, Qiagen, November 2009–June 2010 or xTAG RVP FAST v1, Luminex, from July 2010). The nucleic acid targets of the multiplex panel consisted of influenza A and B viruses, parainfluenzaviruses 1–4 (PIV), respiratory syncytial viruses A and B (RSV), human metapneumovirus (HMPV), adenoviruses, coronaviruses, bocavirus and entero/rhinoviruses. Respiratory specimens used include nasopharyngeal aspirate, nasal swab, throat swab and bronchoalveolar lavage. Samples that tested positive for entero/rhinovirus RNA were further classified into individual enterovirus and HRV types by direct sequencing.

RTI were classified as an upper RTI (URTI) when only upper respiratory tract symptoms were present with no radiological evidence of LRTI; as an airway disease when the predominant clinical finding was of an obstructive airway disease such as asthma or bronchiolitis without evidence of pneumonia on the chest radiographs; and as LRTI when symptoms were associated with evidence of radiological changes in the chest X-ray. Disease was classified as severe when the respiratory condition warranted admission to Intensive Care Unit (ICU) or High Dependency Unit (HDU), and not severe when admitted to the general paediatric wards. To avoid clinician bias, the case note of each patient was reviewed by the authors retrospectively before the entero/rhinovirus typing results were known. Cases in which the contribution of RTI to disease severity was uncertain were further reviewed and categorized after extensive discussions between the authors. Chronic respiratory diseases, chronic heart conditions, neurological conditions, prematurity, immunosupression and other underlying medical conditions were recorded. Bacterial and fungal organisms detected in respiratory samples by culture or immunofluorescence were considered as co-pathogens if case review concluded that they were significant and not due to colonization. Organisms detected in normally sterile sites, such as blood or cerebrospinal fluid, were considered as significant unless specimen contamination was suspected. Co-infection was defined as the detection of a co-pathogen 7 days before or after the entero/rhinovirus positive sample.

### 3.2. Molecular analysis

RNA extraction and cDNA synthesis was performed as previously described [11]. Samples were screened by PCR with the HotStarTaq Master Mix Kit (Qiagen) targeting the 5′noncoding region (5′NCR) using primers DK001 [12] and DK004 [13] under the following conditions: 15 min at 95 °C, 30 s at 94 °C, 30 s at 53.4 °C, 30 s at 72 °C (45 cycles) and 10 min at 72 °C. Amplification of the VP4/VP2 region for typing was performed as above with primers VP4/2 F and VP4/2 R [14] or RCV556F (ACT ACT TTG GGT GTC CGT GTT TC) and RCV886R (TTT CCR ATA GTG ATT TGC TTK AGC C) with 60 °C annealing and 40 cycles or 52 °C and 40 cycles, respectively. Bidirectional sequencing was performed by LGC Genomics GmbH (Berlin, Germany) or in house (PCR product cleaning with microClean (Microzone), cycle sequencing with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) on the automated sequencer 3130xl Genetic Analyzer (Applied
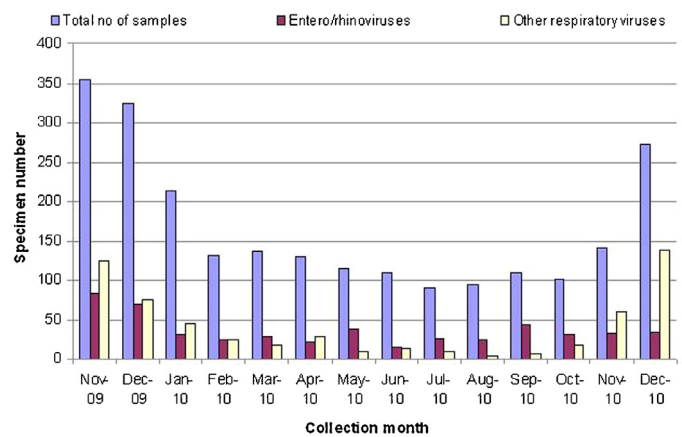


**Fig. 1.** Respiratory samples received during the study period and samples positive for entero/rhinoviruses and other respiratory viruses.

Biosystems)). Sequence analysis was performed using the software BioEdit (version 7.0.9) and MEGA5 (version 5.03).

### 3.3. Statistical analysis

We used generalized linear models with binomial errors and logit link function for univariate and multiple logistic regression analysis of binary response data. The association between 11 independent variables representing clinical and demographical patient characteristics and presence of severe infection, HRV-C or co-infection (viral, non-viral or either) was explored by means of univariate analyses. We conducted multiple regression analysis to assess the significance of age (< or ≥3 years), underlying respiratory conditions, virus type (HRV-C or not) and the interaction between any two independent terms as potentially prognostic variables of severity. The significance of model terms was assessed through deletion tests. All analyses were performed in R version 2.13.2 [15]. In order to determine the importance of HRV species, all enteroviruses and untyped entero/rhinovirus episodes were excluded from statistical analyses. A $P$-value of <0.05 was considered as statistically significant.

## 4. Results

Of 2316 respiratory specimens tested in the study period (1510 ResPlex II, 806 RVP FAST), at least one respiratory virus was detected in 1065 (46.0%). The most commonly detected virus was entero/rhinoviruses (498, 21.5%), followed by RSV (267, 11.5%), influenza A viruses (88, 3.8%), bocaviruses (85, 3.7%), PIV (83, 3.6%), HMPV (71, 3.1%), coronaviruses (54, 2.3%), adenoviruses (47, 2.0%) and influenza B viruses (13, 0.6%). While most seasonal viruses like RSV and influenza A and B viruses were found mainly during winter months, entero/rhinoviruses were detected in similar numbers throughout the study period (Fig. 1).

Of the 498 entero/rhinovirus positive respiratory specimens, 248 (50%) had residual sample available for typing. In several instances multiple specimens were collected from one patient. In order to account for this, specimens belonging to the same infection episode (defined as identical virus by sequencing) in a patient were excluded. This resulted in a total of 204 episodes of entero/rhinoviral infection in 195 patients (median age 0.98 year, interquartile range (IQR) 1.79 years) of which 163 (79.9%) were in children under the age of 3 (median age 0.67 year, IQR 0.91 years). The main virus was HRV-C with 99 infection episodes (48.5%) followed by 60 HRV-A (29.4%), 15 enteroviruses (7.3%) and 8 HRV-B (3.9%). In 22 episodes (10.8%), the sequencing of VP4/VP2 failed despite the use of degenerate primers. Although 5′NCR sequences were available, they were not suitable for typing [16] and were

**Table 1**
Univariate analysis of respiratory viral co-pathogens and non-viral co-pathogens.

| | Co-infections with respiratory viruses | | | Non-viral co-infections | | |
|---|---|---|---|---|---|---|
| | Odds ratio | 95% CI | P-value | Odds ratio | 95% CI | P-value |
| Gender (male) | 1.04 | (0.48–2.37) | 0.92 | 0.93 | (0.32–2.89) | 0.89 |
| Age under 3 | 1.36 | (0.47–4.94) | 0.59 | 0.71 | (0.21–3.30) | 0.63 |
| Underlying risk factor | **0.35** | **(0.16–0.76)** | **0.008** | 3.48 | (0.92–22.79) | 0.07 |
| - Chronic respiratory diseases | **0.35** | **(0.10–0.97)** | **0.04** | 1.05 | (0.28–3.28) | 0.93 |
| - Asthma | 0.20 | (0.01–1.01) | 0.05 | 0.50 | (0.03–2.71) | 0.48 |
| - Chronic heart diseases | 0.53 | (0.17–1.38) | 0.20 | 1.67 | (0.49–5.04) | 0.39 |
| - Prematurity | 0.77 | (0.17–2.50) | 0.69 | 1.22 | (0.18–4.94) | 0.81 |
| - Chronic neurological diseases | 0.36 | (0.06–1.33) | 0.14 | 0.42 | (0.02–2.27) | 0.36 |
| - Immunocompromised | 1.06 | (0.15–4.49) | 0.95 | **8.85** | **(2.02–36.03)** | **0.005** |
| - LRTI | 1.00 | – | 0.20[a] | 1.00 | – | |
| - Airway disease | 0.37 | (0.10–1.12) | | **0.13** | **(0.01–0.69)** | **0.02[a]** |
| - URTI | 0.88 | (0.38–2.05) | | **0.25** | **(0.05–0.85)** | |
| HRV-C | 1.00 | – | 0.20[b] | 1.00 | – | |
| HRV-A | 1.70 | (0.75–3.86) | | 1.50 | (0.50–4.42) | 0.35[b] |
| HRV-B | 3.36 | (0.64–15.24) | | 0.00 | (NA – 2e+35) | |
| Severe disease | 0.83 | (0.37–1.80) | 0.64 | **5.81** | **(1.76–26.26)** | **0.003** |

Significant results with P < 0.05 in bold.
[a] Comparing LRTI with Airway disease and URTI.
[b] Comparing HRV-C with HRV-A and HRV-B.

considered as untyped. Since the aim of this study was to define the characteristics associated with individual HRV species, episodes caused by enteroviruses or untyped viruses were excluded from further analyses.

Of the 167 typed HRV episodes, co-infections with one or more other respiratory viruses were observed in 29 (17%): 12 RSV, 6 adenoviruses, 3 bocaviruses, 4 PIV, 1 HMPV, 1 coronavirus, 1 RSV + bocavirus and 1 RSV + HMPV. Other co-pathogens were identified by case review and included 1 parechovirus (as triple infection with coronavirus), 14 bacteria (1 *Bordetella pertussis*, 3 *Streptococcus pneumoniae* (1 as triple infection with bocavirus), 1 *Haemophilus influenzae*, 2 *Klebsiella pneumoniae*, 1 *Moraxella catarrhalis*, 1 *Mycobacterium tuberculosis*, 1 *Neisseria meningitidis*, 4 *Pseudomonas aeruginosa* (1 as triple infection with PIV)) and 1 fungus (*Pneumocystis jiroveci*). Thus, a total of 40 co-infections and 5 triple-infections were observed; the majority of these cases were found in children <3 years (38/45, 85%).

Co-infections were not associated with a particular HRV species (P = 0.41), nor severity (P = 0.16). However, episodes associated with non-viral co-pathogens were significantly more likely to be severe (12/15 (80%); odds ratio [OR]: 5.81; 95% confidence interval [CI]: 1.76–26.26; P = 0.003) (Table 1). Viral respiratory co-pathogens were less likely to occur in patients with underlying medical conditions (OR: 0.35; 95% CI: 0.16–0.76; P = 0.008) and more specifically in patients with chronic respiratory conditions (OR: 0.35; 95% CI: 0.10–0.97; P = 0.04). Immunocompromised patients (P = 0.005) and patients with LRTI (P = 0.02) were more likely to have non-viral co-pathogens (Table 1). Thus, the presence of co-pathogen is a major confounding factor. In order to study the effect of HRV mono-infection, all co-infections were excluded from further analysis, resulting in 122 episodes of HRV mono-infections in 116 patients. The patient characteristics of all episodes of entero/rhinovirus infection and mono-infection of HRV-A, B and C were listed in Table 2.

In this cohort of HRV mono-infections, the HRV diagnosis was considered as not causing severe illness in 72 episodes and as severe in 50. There were 5 deaths but retrospective case reviews suggested that HRV infection was a significant contributing factor in only two (one HRV-A and one HRV-C) of the deaths (Table 2).

Most patients with an HRV mono-infection episode (81/116, 70%) had at least one underlying medical condition, with chronic respiratory (29%) and heart diseases (23%) being the most common.

Patients with underlying chronic respiratory conditions were more likely to have severe illness (OR: 2.75; 95% CI: 1.25–6.22; P = 0.01). In particular, those with underlying asthma were more likely to have severe illness (OR: 3.47; 95% CI: 1.24–10.68; P = 0.02) (Table 3). On the other hand, children with a chronic heart condition were less likely to have severe disease episodes (OR: 0.35; 95% CI: 0.13–0.85; P = 0.02). One explanation could be that in this patient group HRV were often incidental findings as a result of intense monitoring after heart surgery.

HRV-C was the dominant species in severe episodes (35/50, 70%) followed by HRV-A (15/50, 30%). None of the HRV-B mono-infections were associated with a severe disease.

No overall significant difference in the severity of disease was found between HRV-A, B and C (Table 3). However, majority of the severe episodes in children under the age of 3 (31/41, 76%) were associated with HRV-C, compared to 44% in older children (4/9 episodes). Multiple regression analysis showed that the odds of severity in children under 3 years associated with HRV-C is more than 13-fold that of children infected with other HRV species (OR: 13.62; 95% CI: 1.63–151.33; P = 0.02). An underlying respiratory condition or asthma on its own, were associated with an at least 2- to 3-fold increased odds ratio of having a severe episode, but this was independent of the HRV species type (Table 3).

## 5. Discussion

HRV-C was found to be the dominant HRV species (49%) in this study, which was even more pronounced when all infections with co-pathogens were excluded (62%). This is in agreement with several other studies investigating hospitalised children [9,17,18]. Other reports found HRV-A as the main species [19–22]. These discrepancies could be due to a combination of local distribution of species, selection criteria of the study population, collection time, duration of the study and variation in the assays used.

Previous studies on disease severity have shown conflicting results. One study suggested that HRV-C was associated with more severe LRTI in children [23], whereas others found it either caused less severe infections [24], or no correlation with severity or hospitalisation [5]. One study suggested no difference in severity and clinical characteristics between the three HRV species [22], contradictory to another study that associated HRV-B with prolonged hospitalisation [25]. In our study, HRV-C was found to be associated with a wide spectrum of disease severity. After multiple regression

**Table 2**
Patient characteristics of all episodes of entero/rhinovirus infection and mono-infection of HRV-A, B and C.

| | All episodes of entero/rhinovirus infection n (%) | HRV-A mono-infection n (%) | HRV-B mono-infection n (%) | HRV-C mono-infection n (%) |
|---|---|---|---|---|
| Number | 204 | 41 (20.1) | 5 (2.5) | 76 (37.3) |
| Median age in years | 0.98 | 0.74 | 0.38 | 0.89 |
| Male gender | 129 (63.2) | 29 (70.7) | 3 (60) | 44 (57.9) |
| Site of infection | | | | |
| URTI | 76 (37.3) | 14 (34.1) | 5 (100) | 28 (36.8) |
| Airway disease | 50 (24.5) | 11 (26.8) | 0 | 24 (31.5) |
| LRTI | 78 (38.2) | 16 (39) | 0 | 24 (31.6) |
| Severity | | | | |
| Mild disease | 115 (56.4) | 26 (63.4) | 5 (100) | 41 (53.9) |
| Severe disease | 89 (43.6) | 15 (36.6) | 0 | 35 (46.1) |
| Death[a] | 9 (4.4) | 2 (4.9) | 1 (20) | 2 (2.6) |
| Underlying risk factors | 136 (66.7) | 27 (65.9) | 1 (20) | 58 (76.3) |
| - Chronic respiratory diseases | 55 (27.0) | 12 (29.3) | | 24 (31.6) |
| - Asthma | 27 (13.2) | 6 (14.6) | | 12 (15.8) |
| - Chronic heart diseases | 48 (23.5) | 11 (26.8) | | 19 (25) |
| - Chronic neurological diseases | 26 (12.7) | 6 (14.6) | 1 (20) | 13 (17.1) |
| - Prematurity | 21 (10.3) | 5 (12.2) | | 10 (13.2) |
| - Immunocompromised | 13 (6.4) | 1 (2.4) | | 3 (3.9) |
| - Multiple risk factors | 44 (21.6) | 8 (19.5) | | 20 (26.3) |

[a] Of the 5 deaths in HRV mono-infection without co-pathogens, HRV was considered as possibly responsible or contributory in only 2.

analysis, a significant risk for increased severity was found in HRV-C infected patients under the age of 3 years and in patients with chronic respiratory diseases or asthma independent of HRV species or age. Variation in patient selection and geographical locations of study cohorts may account for the discrepancies between studies. As the clinical data for other respiratory viruses were not collected, we were not able to compare the relative severity of HRV with those due to other viruses.

As this is a hospital-based study, it is likely that only those with a more severe illness are included in the study. It is possible that HRV-B was associated with milder disease and therefore had a lower frequency of detection in hospital-based studies. In support of this theory are several other studies which reported low numbers of HRV-B in hospitalized patients [8,18,25,26]. However, a recent study also found a low prevalence of HRV-B in the community [27] suggesting that the low frequency of HRV-B may be related to biological fitness rather than selection bias. Studies have

also shown conflicting results for association of HRV-C or HRV-A with asthma [5,9,28–31]. High titre IgE antibody to dust mite antigen in asthmatic children may increase the risk of acute wheezing provoked by HRV [32]. Our study also found an association of HRV infection with severe disease in patients with underlying asthma but did not found any specific association with a specific HRV species.

The proportion of asymptomatic infection in the general population is likely to be important as well. A recent study found a high HRV prevalence in healthy children (37%) who were regularly swabbed [33]. HRV-A accounted for the majority of asymptomatic findings in that study. Our study was a hospital-based study and no asymptomatic healthy children were included. Thus, the finding of this study cannot be extrapolated to RTI in the community without hospitalisation. Due to the retrospective nature of this study, not all residual samples were available for sequencing. This is a limitation and could potentially lead to bias. Nevertheless, up to 50% of all

**Table 3**
Univariate logistic and multiple regression analyses of disease severity and HRV-C mono-infection.

| | n | Severity | | | HRV-C | | |
|---|---|---|---|---|---|---|---|
| | | Odds ratio | 95% CI | P-value | Odds ratio | 95% CI | P-value |
| Male gender | 76 | 0.98 | (0.47–2.07) | 0.96 | 0.60 | (0.27–1.29) | 0.19 |
| Age <3 years | 103 | 0.73 | (0.27–2.00) | 0.54 | 0.96 | (0.33–2.59) | 0.93 |
| Underlying risk factors: | 86 | 0.96 | (0.44–2.14) | 0.92 | 2.07 | (0.94–4.62) | 0.07 |
| - Chronic respiratory diseases | 36 | **2.75** | **(1.25–6.22)** | **0.01** | 1.31 | (0.59–3.03) | 0.52 |
| - Asthma | 18 | **3.47** | **(1.24–10.68)** | **0.02** | 1.25 | (0.45–3.83) | 0.68 |
| - Chronic heart diseases | 30 | **0.35** | **(0.13–0.85)** | **0.02** | 1.06 | (0.46–2.55) | 0.89 |
| - Prematurity | 15 | 1.30 | (0.43–3.89) | 0.63 | 1.24 | (0.41–4.22) | 0.71 |
| - Chronic neurological diseases | 20 | 0.74 | (0.26–1.96) | 0.55 | 1.15 | (0.43–3.29) | 0.78 |
| - Immunocompromised | 4 | NA[a] | NA[a] | NA[a] | 1.85 | (0.23–38.01) | 0.58 |
| Severe disease | 50 | – | – | – | 1.76 | (0.83–3.85) | 0.14 |
| Severe disease in children <3 years[e] | 41 | – | – | – | **13.62** | **(1.63–151.33)** | **0.02** |
| Site of infection | | | | | | | |
| LRTI | 40 | 1.00 | – | | 1.00 | – | 0.66[b] |
| Airway | 35 | **0.32** | **(0.12–0.82)** | **<0.001**[b] | 1.45 | (0.56–3.84) | |
| URTI | 47 | **0.05** | **(0.01–0.13)** | | 0.98 | (0.41–2.33) | |
| Virus species | | | | | | | |
| HRV-C | 76 | 1.00 | – | | | | |
| HRV-A | 41 | 1.76 | (0.83–3.85) | 0.14[c] | | | |
| HRV-B | 5 | NA[d] | NA[d] | | | | |

Significant results with P < 0.05 in bold.

[a] Excluded from analysis due to low numbers of observations and no episode of severe infection in immunocompromised patients.
[b] Comparing LRTI with Airway and URTI.
[c] Comparing HRV-C with HRV-A.
[d] HRV-B was excluded from the analysis due to low number of observations and no episode of severe infection with this virus.
[e] Multiple regression analysis.

the entero/rhinoviruses positive samples during the study period were analyzed which corresponded to >200 episodes of illnesses. No firm conclusion on the relative prevalence of HRV-A and HRV-C can be drawn as it may vary in different geographical location and from season to season.

Co-infections with other respiratory viruses have been reported in about 20% of HRV infections. An investigation of respiratory viruses in the West Midlands of the UK found more co-infections with HRV than observed in our study [34]. The presence of co-pathogens have been linked to lower quality of life in children with asthma [31] and co-infections in children were suggested to have increased associated severity [35]. Our study highlights the importance of the nature of co-pathogens, as co-infection with respiratory viruses or bacteria/fungi were associated with different clinical characteristics. As the presence of co-pathogens was more likely to be actively sought in severe cases, there may be some bias in ascertainment. Nevertheless, it is quite plausible that in these cases, bacteria/fungi were the dominant infection driving the illness.

The association between HRV species and clinical presentation is complex and affected by age of the patient, presence and type of co-pathogens and underlying medical conditions. HRV-C was common in this hospital-based patient cohort and occurred all year round. Using multiple regression analysis, HRV-C mono-infection was found to be associated with severe disease in children <3 years of age compared to HRV-A and B. Severe diseases were also associated with the presence of underlying chronic respiratory conditions and in patients with asthma, which is independent of the species of HRV.

## Funding

## Competing interest

None declared.

## Ethical approval

This study was considered by the Chairman of the Research Ethics Committee of St. Thomas' Hospital and was advised that ethical review of this study was not required (St Thomas' Research Ethics Committee Reference: 10/08).

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