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WHAT'S KNOWN ON THIS SUBJECT: Human rhinovirus has been known as the common cold agent. Recently, studies have reported that this virus is responsible for severe infections of the lower respiratory tract in children. Reports of factors that increase disease severity have been contradictory.

WHAT THIS STUDY ADDS: This study identifies some of the factors involved in disease severity in HRV infections in children. We expect that children at risk for developing severe disease could be identified sooner and appropriate measures could be taken.

abstract

OBJECTIVE: To evaluate retrospectively human rhinovirus (HRV) infections in children up to 5 years old and factors involved in disease severity.

METHODS: Nasopharyngeal aspirates from 434 children presenting a broad range of respiratory infection symptoms and severity degrees were tested for presence of HRV and 8 other respiratory viruses. Presence of host risk factors was also assessed.

RESULTS: HRV was detected in 181 (41.7%) samples, in 107 of them as the only agent and in 74 as coinfections, mostly with respiratory syncytial virus (RSV; 43.2%). Moderate to severe symptoms were observed in 28.9% (31/107) single infections and in 51.3% (38/74) coinfections (P = .004). Multivariate analyses showed association of coinfections with lower respiratory tract symptoms and some parameters of disease severity, such as hospitalization. In coinfections, RSV was the most important virus associated with severe disease. Prematurity, cardiomyopathies, and noninfectious respiratory diseases were comorbidities that also were associated with disease severity (P = .007).

CONCLUSIONS: Our study showed that HRV was a common pathogen of respiratory disease in children and was also involved in severe cases, causing symptoms of the lower respiratory tract. Severe disease in HRV infections were caused mainly by presence of RSV in coinfections, prematurity, congenital heart disease, and noninfectious respiratory disease. *Pediatrics* 2014;133:e312–e321

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KEY WORDS

human rhinovirus, respiratory disease severity, children, coinfections, comorbidities

ABBREVIATIONS

AdV—adenovirus
ARI—acute respiratory infection
HC-UFU—Hospital de Clínicas of Universidade Federal de Uberlândia
hMPV—human metapneumovirus
HRV—human rhinovirus
ICD-10—*International Classification of Diseases, 10th Revision*
LRTI—lower respiratory tract infection
PIV—parainfluenza virus
RSV—respiratory syncytial virus
URTI—upper respiratory tract infection

Dr Costa conceptualized and designed the study, carried out the experiments, analyzed the results, and drafted the manuscript; Dr Queiróz was responsible for sample collection and helped design the study; Dr Lopes da Silveira carried out the analysis of all clinical cases in regard to comorbidities and clinical status, helped devise the clinical criteria for severity of infections, and revised the manuscript; Dr Bernardino Neto performed the statistical analyses and revised the manuscript; Ms Nayhanne Tizzo de Paula helped carry out the experiments, took part in the discussion, and revised the manuscript; Dr Oliveira and Ms Tolardo helped carry out the experiments and revised the manuscript; Dr Yokosawa helped design the study, oversaw the experimental work and analyses of the results, and revised the manuscript; and all authors approved the final manuscript as submitted.

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Human rhinovirus (HRV) is one of the most important causes of respiratory infections and has been associated mostly with the common cold.[1] In children, this virus is highly common and is responsible for lower respiratory tract infections (LRTIs) with severe symptoms.[2–5] Indeed, HRV infections are one of the main causes of bronchiolitis in young children and an inducer of wheezing episodes.[6–9]

Factors involved in increased disease severity in HRV infections are contradicting in the literature. Some reports have associated coinfection with a second respiratory virus with a more severe disease,[10,11] whereas others have not.[7,9] On the other hand, another study has raised the possibility of a protective role of HRV against other viral infections.[12] Underlying risk conditions may also be involved in disease severity.[9,13] Again, the reports are contradicting; atopic family history has been associated with severe HRV infection, whereas exposure to smoking has not,[11] and the opposite has been reported in another study.[9] Furthermore, congenital heart disease was considered a risk factor in 1 study[3] but not in another.[9] Recently, our group evaluated the role of HRV coinfections in children with LRTI and observed no significant difference in frequencies of severe infections caused by HRV in single or coinfections.[14] However, the investigation was carried out only with LRTI cases. To further evaluate the factors that might be involved in severe illness caused by HRV in young children, we investigated all HRV cases and included in the analyses young children presenting with a wide spectrum of respiratory disease symptoms.

METHODS

Patients and Attendance Settings

This was a retrospective study in which eligible patients were children up to 5 years old presenting with acute respiratory infection (ARI) who were attended by a physician within 5 days from the onset of clinical symptoms, from January 2001 to June 2010. Patients presented with a broad spectrum of respiratory disease symptoms, and 434 inpatients and outpatients were enrolled in the study.

Patients were attended at Hospital de Clínicas of Universidade Federal de Uberlândia (HC-UFU) and public health centers from 3 different districts of the city of Uberlândia, Minas Gerais State, Brazil. Medical and demographic data were collected through questionnaires filled out by the physician and included age, day of onset of symptoms before attendance, clinical presentation, comorbidities, household smoking, and atopic diseases.

This study was approved by the Ethics and Research Committee of UFU under protocol 877/11, and a signed informed consent was obtained from each child's parent or foster parent before sample collection.

Clinical Criteria

Characteristic ARI symptoms and signs were assigned by the physician and included runny nose, coughing, wheezing, and difficulty breathing, with or without fever. The clinical diagnosis was in accordance with the *International Classification of Diseases, 10th Revision* (*ICD-10*; World Health Organization, 1994). Symptoms of upper respiratory tract infection (URTI) included croup, tracheobronchitis, no sibilant bronchitis, common cold, flu, otitis media, rhinopharyngitis, laryngotracheitis, and laryngitis; LRTI symptoms included pneumonia, bronchopneumonia, bronchiolitis, sibilant bronchitis, and bronchospasm. If a patient had mixed symptoms of URTI and LRTI, we considered the latter.

Mild diseases consisted of URTI or were considered so by the attending physician, following *ICD-10*, or occurred in patient who did not need hospitalization for the respiratory illness. Moderate to severe symptoms encompassed most LRTI cases, and were those considered by the attending physician as moderate or severe, following *ICD-10*, or hospitalization exclusively due to the respiratory infection. All children with pneumonia and those who needed supplemental oxygen or mechanical ventilation were grouped in this category.

Bronchiolitis was classified as mild, moderate, or severe according to the Clinical Score of Respiratory Failure in Bronchiolitis.[15] Clinical scores described by Taussig et al[16] were used to classify other LRTI cases.

Underlying Risk Conditions

We identified comorbidities retrospectively by consulting the clinical medical records from HC-UFU or through the clinical questionnaires administered by the attending physician at the moment of sample collection.

Nasopharyngeal aspirates were collected as described[17] and tested by immunofluorescence assay for detection of respiratory syncytial virus (RSV), influenza viruses A and B, parainfluenza viruses (PIVs) 1, 2, and 3, and adenovirus (AdV) with the Respiratory Panel I Viral Screening and Identification Kit (Merck Millipore, Billerica, MA). Additionally, reverse transcription polymerase chain reaction was used to detect RSV and influenza virus,[18] PIV 1–3,[19] and human metapneumovirus (hMPV),[20] and polymerase chain reaction was used for AdV.[21] RT-PCR for HRV was performed by using a combination of 2 protocols.[22,23]

Detection of pathogens other than viruses was not carried out.

Statistical Analysis

Analyses were performed by using χ^2 to compare the frequency of groups of infections according to severity of symptoms. χ^2 and P values were adjusted through Yates correction using BioEstat software version 5.0.[24] The

median age differences were calculated by using nonparametric Mann–Whitney *t* test. For multiple correlation analyses, canonical correlation was used to evaluate the impact of coinfections on disease severity and to identify which coinfection caused more severe symptoms. For all tests, calculations were also performed by using BioEstat, and $P < .05$ was considered statistically significant.

RESULTS

Nasopharyngeal aspirates were collected from 434 children 0 to 5 years old (median age = 7.0 months) who presented with ARI, and at least 1 respiratory virus was detected in 383 (88.2%) samples (Table 1), with more cases of children <6 months of age and male patients. More severe respiratory disease predominated in younger children, as observed by the higher frequencies in this age group of LRTI, of hospitalizations, and of moderate- to high-complexity clinical attendances at pediatric ward, ICU, nursery ward, and day care ward.

HRV and RSV were the most common agents detected, in 41.7% and 35.9% of samples, respectively (Fig 1). Among HRV-positive cases, this virus was the only agent detected in 59.1% (107/181) samples, and a second virus was detected in 40.9% (74/181) samples, predominantly RSV. The median age of children with HRV single infections was higher than that of RSV single infections (9 months and 2 months, respectively; $P < .001$).

The majority of HRV single infections resulted in mild symptoms (71.0%; Fig 2A). However, in coinfection cases, the frequency of moderate to severe symptoms was higher (51.4%) than that of single infections (29.0%). This higher frequency was probably caused by presence of RSV, because exclusion of the RSV cases from the coinfection group led the frequencies of mild and moderate to severe cases in this group to become similar to those of HRV single infections. Furthermore, frequencies of cases according to severity of symptoms were also comparable between HRV+RSV coinfections and RSV single infections.

Despite the predominance of symptoms involving the upper respiratory tract (63.6%), more than one-third of the HRV single infections included symptoms of LRTIs, with many cases of bronchiolitis and sibilant bronchitis or bronchospasm (Fig 2B). The frequencies of URTIs and LRTIs (Fig 2B) were similar to those of mild and moderate to severe cases (Fig 2A), respectively, indicating an association between the anatomic structure affected and the severity of the infection. For other groups (HRV coinfections, HRV coinfections excluding RSV, HRV+RSV coinfections, and RSV single infections), the frequencies observed for moderate to severe symptoms were also similar to those affecting the lower respiratory tract.

Because these results showed involvement of RSV with disease severity in coinfections, a multivariable analysis was performed to evaluate the association of factors in severe illness (Table 2). For groups of HRV single infections and coinfections excluding RSV, a direct association was observed between these groups and most URTIs, as indicated by the variables with same sign. In addition, the association of the common cold, bronchitis, and tracheobronchitis was stronger for the group of HRV single infection, as indicated by the high values (strengths). For the HRV+RSV and RSV single infection groups, the opposite signs indicated that no association exists with most URTIs. On the other hand, a direct association was observed between most LRTIs, especially bronchiolitis and pneumonia, and RSV infections, in both single infections and coinfections with HRV. In clinical parameters of disease severity, the main associations were between RSV infection groups and hospitalization or moderate to severe clinical symptoms. Early age (<6 months) was associated mainly with RSV infections, whereas HRV single infection and HRV coinfections excluding RSV groups were associated with infections in older children.

Furthermore, in HRV coinfection cases, a strong and significant association was found with LRTI symptoms, clinical parameters of disease severity, and patient age (Table 3). In this analysis, HRV+RSV coinfection was the most

TABLE 1 Demographic Data on Children <5 Years Old With ARIs

Age Group, mo	*N* (%)	Viral Infection Cases					
		No. of Cases	Male/Female	LRTI (%)[a]	URTI (%)[a]	Patients Hospitalized (%)[a]	Moderate- to High-Complexity Clinical Attendance (%)[a]
0–6	195 (44.9)	175	99/76	119 (68.0)	56 (32.0)	129 (73.7)	140 (80.0)
6–12	68 (15.7)	61	37/24	35 (57.4)	26 (42.6)	15 (24.6)	21 (34.4)
12–24	92 (21.2)	80	45/35	22 (27.5)	58 (72.5)	15 (18.7)	15 (18.7)
≥24	79 (18.2)	67	36/31	17 (25.4)	50 (74.6)	10 (14.9)	12 (17.9)
Total	434 (100.0)	383	217/166	193 (50.4)	190 (49.6)	169 (44.1)	188 (49.1)

[a] Percentages relative to viral infection cases in each age group.

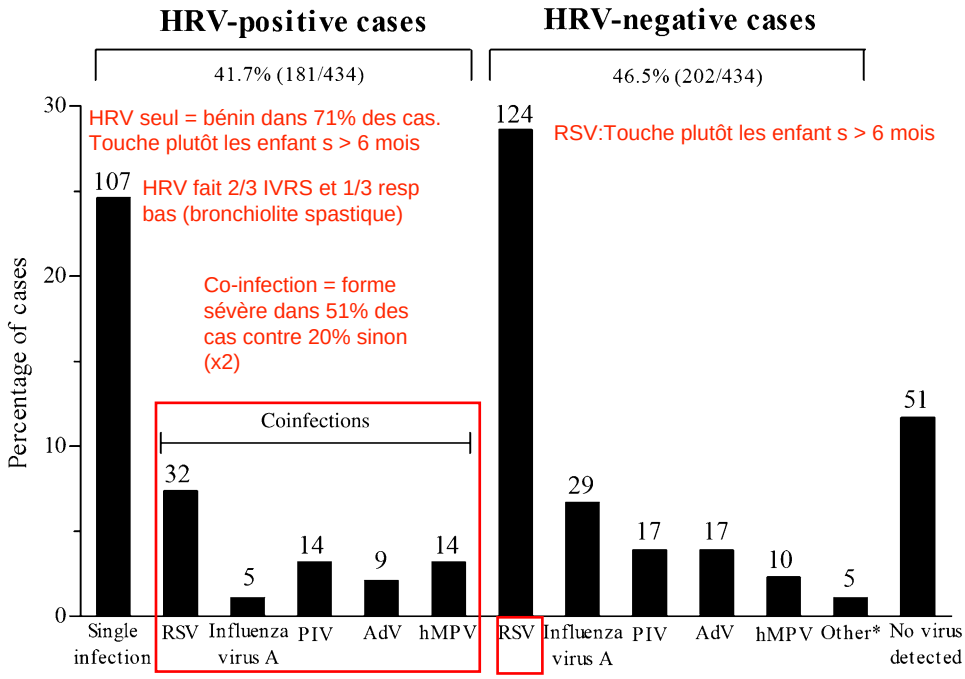

FIGURE 1

Distribution of cases according to the respiratory viruses detected by immunofluorescence assay or reverse transcription polymerase chain reaction in nasopharyngeal aspirates collected from 0- to 5-year-old children presenting with ARI. Numbers shown above bars represent the corresponding numbers of cases. * One case of influenza virus B and 1 case each of RSV+AdV, RSV+PIV, AdV+PIV, and AdV+influenza virus A.

important variable in the correlation analysis, showing stronger associations with bronchiolitis, hospitalization, and early age. This analysis showed that RSV was the main contributor in the association with disease severity and LRTIs, confirming the results shown in Fig 2. Coinfections with PIV also showed some involvement in disease severity, but with a lower strength compared with HRV+RSV coinfection.

Underlying risk conditions that could be associated with increased disease severity were also evaluated (Fig 3). In HRV single infections, the frequency of moderate to severe cases was higher in children who had such conditions than in those who did not have them (24.3% and 4.7%, respectively). In coinfections, the frequency of moderate to severe cases in the presence of these risk factors was even higher (43.2%). However, when RSV cases were excluded from the coinfections, the frequency of moderate to severe cases in children who had comorbidities decreased to a rate (28.6%) comparable to those in HRV single infections. Furthermore, frequencies of moderate to severe cases in HRV+RSV coinfections and RSV single infections were similar (59.4% and 58.9%, respectively). These results indicated that comorbidities and RSV in coinfections may be important factors associated with increases in disease severity of HRV infections.

Because early age is a known factor in disease severity of RSV infections, it was evaluated as a factor in HRV single infections as well (Fig 4A). The frequency of moderate to severe cases was higher in younger children and decreased with age. However, 12 of 19 moderate to severe cases of the group of children up to 6 months of age presented with another risk factor, mostly congenital heart disease or prematurity. Thus, when we carried out the analysis with early age as the only factor, there was no significant association with severe symptoms in comparison with children who did not have any risk conditions (Fig 4B). On the other hand, prematurity, congenital heart diseases, and noninfectious respiratory diseases were important underlying conditions associated with moderate to severe infections. Still, the median age of patients in each of these groups was lower in moderate to severe cases than in mild cases, although slight significance was observed only in the prematurity and congenital heart disease groups.

DISCUSSION

Viruses may have constituted the major group of pathogens involved in ARI in children up to 5 years old in this study, because at least 1 virus was detected in 88.2% samples. Other studies also reported a high percentage of viral infection cases, even though a higher number of viruses were tested in those studies than in ours.[7,25,26] Still, the high frequency of viral infections we detected

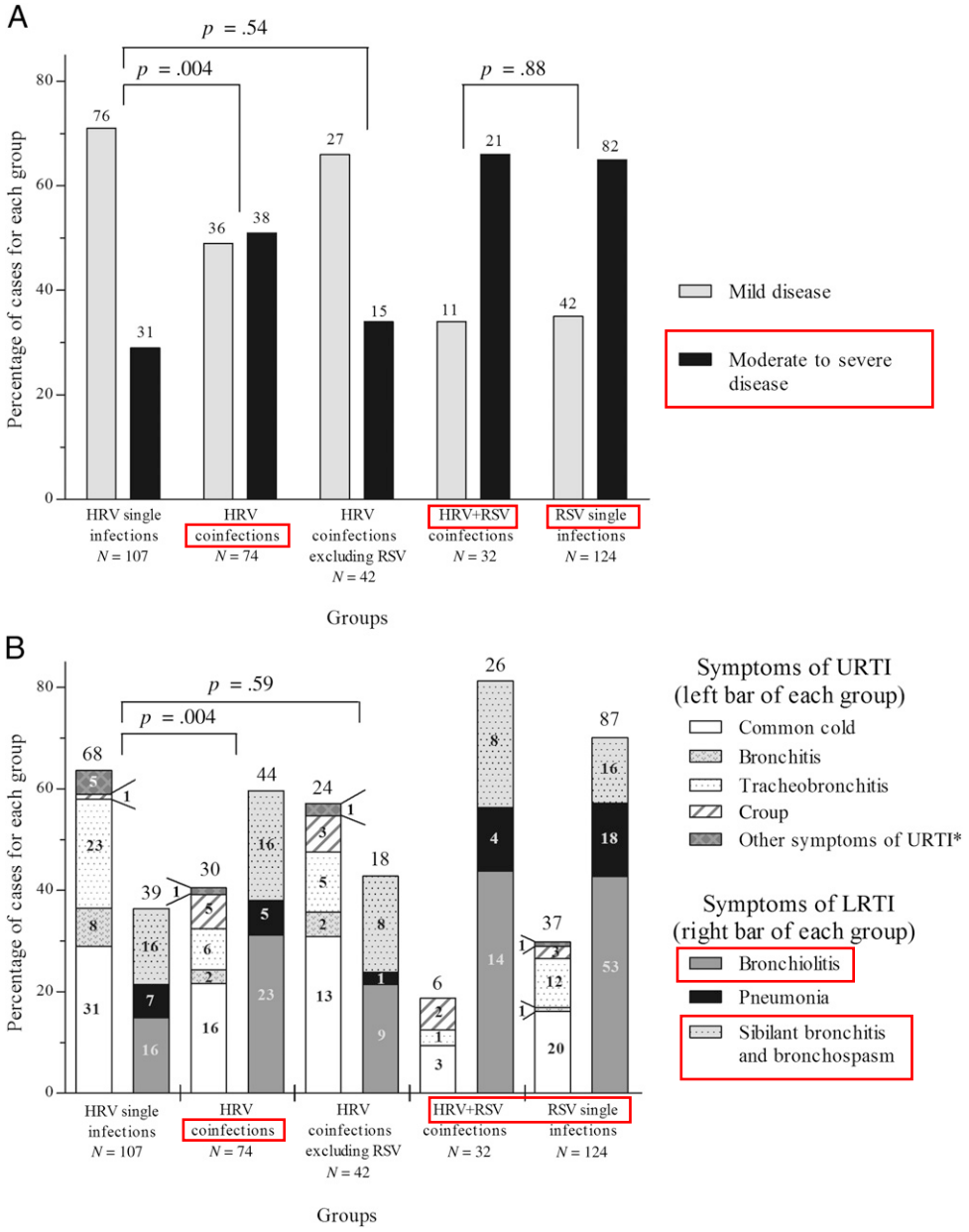

FIGURE 2

Distribution of cases according to (A) etiology and severity and (B) clinical symptoms in children up to 5 years old with respiratory infection. The numbers above and inside bars represent the corresponding numbers of cases. * Laryngitis and rhinopharyngitis.

could reflect the testing for the main viruses responsible for ARI in children and the application of 2 detection methods.

Although some studies reported RSV as the predominant virus detected in ARI followed by HRV, with detection of the latter ranging from 7.2% to 30.0% in single or coinfection cases,[7,9,25] in our study HRV was predominant, although it was followed closely by RSV. In those reports, the studies were carried out only with children who were hospitalized and had bronchiolitis. In our study, on the other hand, samples were collected from children presenting with respiratory disease symptoms ranging from mild to severe, and in all seasons. This difference may account for the high percentage of HRV cases we detected, because this virus was detected during all seasons[27] and caused infections with a broad clinical presentation. Still, the number of mild HRV cases could be higher because parents of children with mild symptoms are less likely to seek medical care.

In this study, a second respiratory virus was detected in many of the patients with HRV infections. The HRV+RSV

TABLE 2 First Canonical Correlation Analysis Between Types of Infections and Symptoms of URTIs, LRTIs, Clinical Parameters of Disease Severity, and Patient Age

Groups of Variables	Groups According to Virus Found
URTIs	(r = 0.366; $P < .001$)
Common cold: (−) 0.484	HRV single infection: (−) 0.857
Bronchitis: (−) 0.458	HRV coinfections excluding RSV: (−) 0.203
Tracheobronchitis: (−) 0.481	HRV+RSV: (+) 0.507
Acute otitis media: (−) 0.178	RSV single infections: (+) 0.659
Croup: (+) 0.141	
Laryngotracheitis: (−) 0.161	
LRTIs	(r = 0.382; $P < .001$)
Bronchiolitis: (+) 0.785	HRV single infections: (−) 0.787
Pneumonia: (+) 0.356	HRV coinfections excluding RSV: (−) 0.353
Sibilant bronchitis: (−) 0.014	HRV+RSV: (+) 0.410
Bronchospasm: (+) 0.094	RSV single infections: (+) 0.756
Clinical parameters of disease severity	(r = 0.346; $P = .003$)
Orotracheal intubation: (−) 0.009	HRV single infection: (−) 0.923
Mechanical ventilation: (−) 0.164	HRV coinfections excluding RSV: (−) 0.095
Supplementary O_2: (−) 0.049	HRV+RSV: (+) 0.347
Hospitalization: (+) 0.685	RSV single infections: (+) 0.747
Hospitalization >7 d: (+) 0.480	
Moderate to severe symptoms: (+) 0.930	
Patient age, mo	(r = 0.380; $P < .001$)
0–6: (−) 0.925	HRV single infections: (+) 0.466
6–12: (+) 0.109	HRV coinfections excluding RSV: (+) 0.709
12–24: (+) 0.768	HRV+RSV: (−) 0.315
	RSV single infections: (−) 0.753

coinfection predominance could reflect the high frequency of these 2 infections in children with ARIs, which was also reported by others,[7,9,11,13,25] and the overlap in time periods when occurrence of respiratory infections caused by both viruses was high, from February to June.[27]

TABLE 3 First Canonical Correlation Analysis Between HRV Coinfections and Symptoms of LRTIs, Parameters of Disease Severity, Comorbidities, and Patient Age

Groups of Variables	Coinfecting Virus
LRTIs	(r = 0.388; $P = .03$)
Bronchiolitis: (+) 0.813	RSV: (+) 0.923
Pneumonia: (+) 0.134	Influenza virus A: (−) 0.032
Sibilant bronchitis: (+) 0.292	PIV: (+) 0.235
Bronchospasm: (−) 0.155	AdV: (−) 0.224
	hMPV: (−) 0.093
Clinical parameters of disease severity	(r = 0.539; $P < .001$)
Orotracheal intubation: (+) 0.235	RSV: (+) 0.377
Mechanical ventilation: (+) 0.564	Influenza virus A: (−) 0.147
Supplementary O_2: (+) 0.167	PIV: (+) 0.052
Hospitalization: (+) 0.956	AdV: (−) 0.194
Moderate to severe symptoms: (+) 0.649	hMPV: (−) 0.101
	No underlying diseases: (−) 0.901
Patient age, mo	(r = 0.397; $P = .03$)
0–6: (−) 0.790	RSV: (−) 0.657
6–12: (−) 0.153	Influenza virus A: (+) 0.381
12–24: (+) 0.889	PIV: (+) 0.099
≥24: (+) 0.144	AdV: (+) 0.713
	hMPV: (+) 0.106

We had shown previously that coinfection with HRV and another respiratory virus was not associated with disease severity.[14] However, that study was limited to LRTI cases. To obtain a better evaluation of factors that might be involved in increased disease severity, in this study we included patients who attended different settings in public health service units and presented with varying degrees of severity of symptoms, with or without the need for hospitalization. By doing this, we attempted to decrease bias caused by factors that may have affected the results reported previously, including ours.[7,9,14,25]

By performing multivariate analysis, we found that coinfections were associated with greater disease severity in comparison with HRV single infections. However, this association resulted mainly from the presence of RSV in the coinfections, because removal of RSV cases from the coinfection group revealed that the severity and frequency of clinical symptoms were similar to those in HRV single infections. Investigating the first episode of bronchiolitis in hospitalized infants up to 1 year old, Marguet et al[7] also observed more severe disease in infections caused by RSV and HRV+RSV. However, comparative analysis with coinfections caused by HRV and other viruses was not shown in that study.

Although the majority of the HRV single infections caused mild URTI symptoms, approximately one-third of these cases involved LRTIs, which was consistent with the findings reported by others[7,9,13,25] and was reflected directly in the number of severe cases.

In moderate to severe cases, at least 1 underlying risk condition was present in 83.9% (26/31) of HRV single infections, a finding similar to those reported by others,[9,11] whereas in mild cases the presence of comorbidities was observed in only 43.4% (33/76).

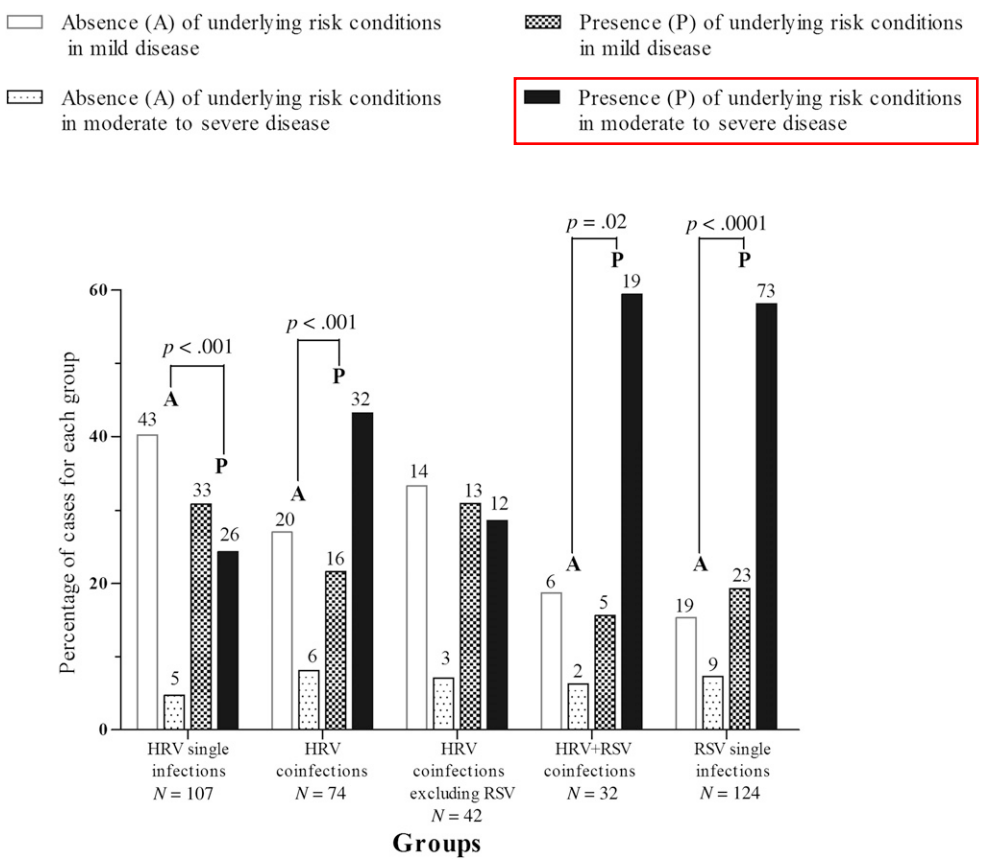

FIGURE 3

Distribution of cases according to severity of symptoms and presence or absence of underlying risk conditions. The values above bars represent the corresponding number of cases. *P* values were calculated by comparing the number of patients who did not present (A) with those who presented (P) with underlying risk conditions, according to the severity of symptoms in each group.

Brand et al[9] suggested that comorbidities, not coinfections, might be important factors associated with severity in children.

Prematurity alters normal lung development and results in chronic lung diseases.[28] Thus, the premature child may be at higher risk of developing a more severe respiratory disease regardless of whether the virus causing the infection is HRV or RSV.[29] This possibility was supported by our study, because we also observed an association between prematurity and disease severity in HRV single infection. Similar to our findings, Watanabe et al[3] showed involvement of congenital heart diseases in severe HRV infection. In addition, we also observed the involvement of noninfectious respiratory diseases with more severe disease.

Our data also showed that the number of viral infections was highest in children <6 months old who had a more severe prognosis, which was indicated by higher frequencies of hospitalizations, LRTIs, and cases that necessitated moderate- to high-complexity clinical interventions. Children in this age group may be at higher risk of having more severe disease, probably because of their immature immune systems.

In HRV single infections, moderate to severe cases occurred mainly in patients up to 1 year old, suggesting that young children may be more susceptible to severe respiratory infections caused by HRV, as also proposed by others.[25,30] Bronchiolitis, sibilant bronchitis, and bronchospasm cases were observed in nearly 30% of all HRV single infections, indicating that HRV might be 1 of the main causes of wheezing in infants.[31] However, in our analyses early age as the only factor presented by the child did not show an association with moderate to severe infection. Instead, a combination with underlying conditions might be needed to cause more severe disease.

Many of the cases we evaluated were of children presenting with moderate to severe symptoms, and we observed a significant difference in frequency of more severe cases in those who presented with comorbidities (Fig 3). However, children with mild respiratory disease usually do not need medical care, and in 1 study,[32] physician consultations, as an indicator of disease severity, were associated with 25.4% of respiratory illnesses in the

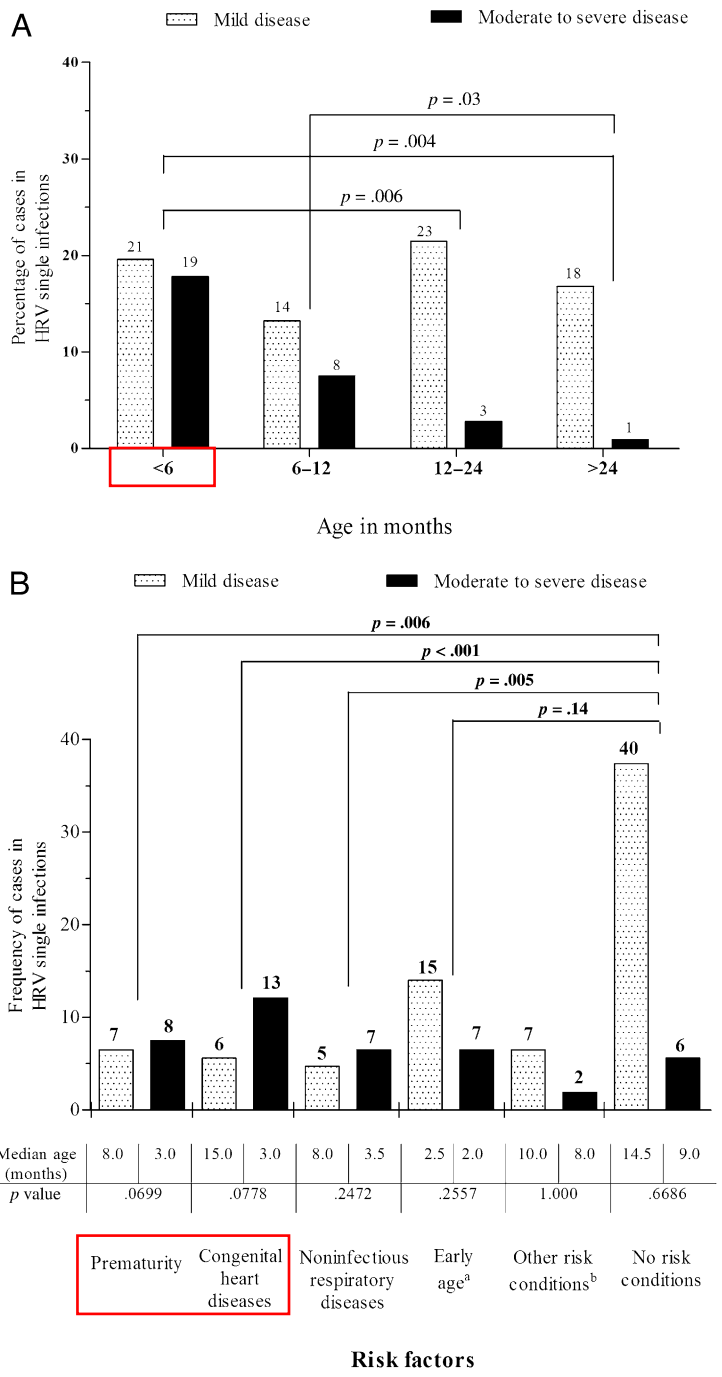

FIGURE 4

Distribution of cases according to underlying risk conditions in children up to 5 years old with HRV single infections ($N = 107$). A, Age factor regardless of the presence of other comorbidities. B, Occurrence of each of the comorbidities and risk factors. The numbers shown above bars represent the corresponding number of cases. *P* values were calculated by comparing the number of cases in a group with patients who presented with a risk factor and a group of patients who did not present with risk factors, and according to the severity of symptoms in each group. [a] Early age exclusively. [b] Smoking during pregnancy, atopic disease, anemia, nervous system diseases, West syndrome, Down syndrome, short bowel syndrome, cholestatic syndrome, biliary atresia, adenomegaly, and nodal polyarthritis.

community, with 17.6% of the HRV cases resulting in medical attention. The impact of risk factors in HRV infections in the community remains to be evaluated.

Although this study assessed the involvement of some factors in severity of HRV infections, we did not investigate the role of bacteria, such as *Streptococcus* and *Haemophilus*, in these cases. It is also possible that viruses that were not tested or are still unknown could have caused more severe disease. On the other hand, detection of a pathogen, especially with the use of polymerase chain reaction, does not indicate that this pathogen is the causative agent of the disease. Studies have shown that respiratory viruses can be detected in up to 36% of samples from asymptomatic children.[33,34] Another contradiction in the literature was the association of the HRV C strain with severe cases.[4,5] Some of the HRV samples we sequenced belonged to the C strain. However, because there was no indication that there was an association with disease severity, the remaining samples were not sequenced (data not shown).

CONCLUSIONS

Our results showed a high incidence of HRV and RSV in ARIs in young children, and the majority of the HRV infections involved mild symptoms. However, we also found HRV in moderate to severe cases, and this study highlighted the importance of the association of this virus with coinfection with RSV, prematurity, congenital heart disease, or noninfectious respiratory disease in symptom severity. Also, early age as the only factor might not have played a role in more severe disease. These important findings may help children who are at higher risk of more severe respiratory disease.

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