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Human Rhinovirus C Associated with Wheezing in Hospitalized Children in the Middle East

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Abstract

Background—Few studies have investigated the disease burden and genetic diversity of human rhinoviruses (HRV) in developing countries.

Objectives—To assess the burden of HRV in Amman, Jordan and to characterize clinical differences between HRV groups.

Study Design—We prospectively studied children <5 years old hospitalized with respiratory symptoms and/or fever in Amman, Jordan. Viruses were identified by real-time RT-PCR. VP4/VP2 gene sequencing was performed on HRV-positive specimens.

Results—Of 728 enrolled children, 266 (37%) tested positive for picornaviruses, 240 of which were HRV. Of the HRV-positive samples, 62 (26%) were of the recently identified group HRVC, 131 (55%) were HRVA, and 7 (3%) were HRVB. The HRVC strains clustered into at least 19 distinct genotypes. Compared with HRVA-infected children, children with HRVC were more likely to require supplemental oxygen (63% vs. 42%, $p=0.007$) and, when co-infections were excluded, were more likely to have wheezing (100% vs. 82%, $p=0.016$).

Conclusions—There is a significant burden of HRV-associated hospitalizations in young children in Jordan. Infection with the recently identified group HRVC is associated with wheezing and more severe illness.

Keywords

rhinovirus; HRVC; wheezing; hospitalized; children

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Background

Human rhinoviruses (HRV) are members of the family *Picornaviridae* first cultured in 1956, and approximately 100 serotypes have been identified¹. Although once thought to cause only the common cold, it is now known that HRV are associated with lower respiratory tract infections and asthma exacerbations in adults and children²⁻¹¹. Twenty-six percent of hospitalizations for acute respiratory illness (ARI) among children younger than 5 years old were associated with HRV during one year of population-based surveillance in two US cities².

The use of molecular diagnostics substantially improves the detection of HRV^{2, 12-15}. Previously, HRV serotypes were classified into two species (HRVA and HRVB^{1, 16}), but another group of HRV, HRVC, was recently identified by RT-PCR and partial sequence data, first in Australia and subsequently in other regions¹⁷⁻²⁵. Little is known about the epidemiology of the recently identified HRVC group in developing countries, particularly in the Middle East.

Objectives

We determined the frequency, clinical features, and genetic diversity of HRV infections during the peak winter months among children < 5 years of age who were hospitalized with ARI or fever in two major hospitals in Amman, Jordan.

Study Design

Prospective surveillance was conducted among children <5 years of age hospitalized with ARI/fever at Jordan University Hospital (JU) or Al-Basheer in Amman, Jordan from January 18 through March 29, 2007. Institutional Review Boards from Vanderbilt University, Jordan University, and the Jordan Ministry of Health approved this study.

Data collection

Study personnel consented and interviewed the parents or guardians to complete a standardized questionnaire that recorded clinical and demographic information. Medical records were reviewed. Data were entered into handheld PDA devices that were synchronized daily.

Virus testing and sequencing

Nasal/throat swabs were obtained and combined into a single tube of viral transport medium (Beckton-Dickinson). Lysis buffer was added (Roche MagNApure tNA) and samples were stored at -70°C until shipped on dry ice to Vanderbilt.

RNA was extracted from specimens in lysis buffer using an automated platform (MagNApure LC, Roche) as previously described²⁶. Real-time RT-PCR for HRV was performed with the Smart Cycler II (Cepheid) using primers and probe sequences directed at a highly conserved HRV 5'-non-coding region (5'-NCR), allowing detection of all 100 HRV prototype strains²⁷. Specimens were tested for hMPV, RSV, and influenza as described²⁸⁻³⁰.

RT-PCR was performed on HRV-positive samples using primers that amplify a ~548-bp fragment encompassing the VP4/VP2 region and the hypervariable region in the 5'-NCR³¹. Amplified fragments were sequenced bi-directionally on a 3730x1 DNA Analyzer (Applied Biosystems). Sequences were aligned using MacVector (Accelrys) and phylogenetic analyses performed using MEGA 4.0.^{26, 32, 33} HRV87 was used as an outgroup for all phylogenetic analyses³⁴.

Statistical analysis

Comparisons of demographic and clinical data between groups were made using Chi-square or Fisher's exact test for categorical variables and Wilcoxon rank sum test or Kruskal-Wallis test for continuous variables. All analyses were performed using R software (<http://r-projects.org>, v2.6.1).

Results

Study Population

Of 803 eligible children hospitalized with respiratory symptoms and/or fever between January 18 and March 29, 2007, 743(92.5%) were enrolled. Analyses included the 728(98%) of enrolled children for whom clinical and laboratory data were available. Forty-four(6%) children were enrolled from Jordan University. The mean age of the subjects was 7.85 months, the median age was 4.34 months, and 58.4% were male. Of all children enrolled, 638/728 (88%) tested positive for at least one virus. There were 266/728(37%) patients who tested positive for picornaviruses: 240(33%) for HRV and 26(4%) for human enteroviruses(HEV). There were 467(64%) who tested positive for RSV, 44(6%) who tested positive for hMPV, and 6(0.8%) who tested positive for influenza A or B.

Demographic and Clinical Characteristics of Rhinovirus-infected Children

Demographic and presenting symptoms of children with HRV vs RSV vs no study virus are presented in Table 1. Compared to children with RSV, those with HRV were more likely to be older (5.4 vs 3.8 months, $p=0.04$) and were less likely to have poor appetite(64%vs77%, $p=0.009$), or require supplemental oxygen(43%vs74%, $p<0.001$). Children infected with HRV or RSV were more likely to have wheezing (HRV 87%, RSV 89%, no virus 73%, $p<0.001$) and nasal congestion than children who had no study virus detected (HRV 68%, RSV 74%, no virus 54%, $p=0.001$).

The age distribution of children with HRV, RSV, and hMPV is illustrated in Figure 1. The age distribution of HRV and RSV were similar. Of the HRV-positive children, 64% were younger than 6 months old. Of the 240 HRV-positive children, 128 were coinfecting with another virus studied: 110(46%) with RSV, 17(7%) with hMPV, and 1(0.4%) with influenza A. Compared with children infected with HRV alone, children coinfecting with HRV and RSV were younger (2.5vs5.4 months, $p<0.001$), more likely to require supplemental oxygen (65%vs43%, $p=0.001$), more likely to vomit (55%vs38%, $p=0.011$), and more likely to stay an extra day in the hospital (5vs4 days, $p=0.007$) (Table 2).

Two children with HRV died. The first was an 18-month-old male with low birth weight and a history of mental retardation/developmental delay. This child was infected with HRVA alone and was admitted with a diagnosis of ARI and pneumonia. The second fatal case was a 1.4-month-old male term infant with congenital heart disease. This child was coinfecting with HRVC and RSV, required supplemental oxygen on admission, and died on the 11th day of hospitalization. Other bacterial or parasitic culture data on these two subjects were not available.

Clinical Features of HRVA Compared with HRVC

Of the 240 HRV positive samples, 62(26%) were HRVC, 131(55%) were HRVA, and 7(3%) were HRVB. We were unable to amplify sequences from the remaining 40(17%) specimens. In January, 52% of HRV-positive specimens were HRVA and 20% were HRVC; in February, 40% were HRVA and 36% were HRVC; and in March, 70% were HRVA and 18% were HRVC. The median ages of children infected with HRVA or HRVC did not differ. Children with HRVC were more likely to require supplemental oxygen than those with HRVA(63%

vs42%, $p=0.007$) and were less likely to have otalgia (11% vs24%, $p=0.047$). Excluding coinfections, children with HRVC were still more likely to require supplemental oxygen than children with HRVA (57% vs34%, $p=0.04$). Compared to children infected with HRVA, those infected with HRVC alone were more likely to report wheezing/noisy breathing (100% vs82%, $p=0.02$). (**Table 3**).

Rhinovirus Diversity

While several clinical differences were seen between patients infected with HRVA versus HRVC, there were also significant genetic differences among viruses. The VP4/VP2 sequence regions correlate with the serotype classification of HRV^{1, 16, 31}. HRVC strains were defined using a nucleotide identity of <90%, based on the calculated genetic relatedness of established serotypes as previously described²⁶. Applying this criterion, 19 distinct HRVC genotypes were identified, comprising 63 distinct strains that did not cluster into either the HRVA or HRVB groups (Figure 2). The HRVC genotypes had a mean nucleotide identity of 72.7% (minimum 66.4% and maximum 91.4%), compared to HRVA (mean 77.9%, minimum 70.5% and maximum 91.3%) and HRVB (mean 80%, minimum 78.6% and maximum 82.9%). Thus, HRVC viruses exhibited greater diversity among themselves than did HRVA. Based on comparisons of nucleotide identity, 3 of the HRVC genotypes were >95% identical with strains HRVQPM, HRVNY, and HRVX, and likely represent the same viruses¹⁹⁻²³. To our knowledge, the other 16 HRVC genotypes we identified have not been reported. Of the 131 HRVA strains sequenced, 10 were <90% identical to previously identified HRVA, including 5 that were <85% identical; these may represent novel HRVA types. Two of these potentially novel HRVA types were detected in 9 and 7 distinct subjects, respectively, demonstrating that these were common viruses in the study population. All but one of the few HRVB viruses were ≥90% identical with previously described strains; the remaining strain was 88.8% identical.

Discussion

In this study, we found a significant burden of HRV-related illness among young children hospitalized with ARI or fever in Amman, Jordan. Few studies in the Middle East have reported HRV infections. Rashid detected HRV in 13% (19/150) of UK pilgrims and 3% (3/110) of domestic pilgrims with URI in Mecca, Saudi Arabia³⁵. Kaplan identified HRV in 11% (36/325) of hospitalized young children in Jordan^{35, 36}. We enrolled a larger cohort than these studies and discovered a greater burden of HRV-associated illness in young children, likely because we used highly sensitive real-time RT-PCR. In addition, we found a trend in children with HRV/RSV coinfection to have more severe disease compared with HRV alone, evidenced by increased use of supplemental oxygen and duration of hospitalization. Papadopoulos *et al.* reported that the presence of HRV increases the risk for disease severity in acute bronchiolitis by approximately 5-fold³⁷.

Investigators have detected the newly described HRVC in several countries. Of the HRV-positive samples sequenced in this study, 26% were HRVC. These findings are the first report of HRVC disease in the Middle East. We observed several clinically significant differences between patients infected with HRVC compared to HRVA. Children with HRVC were more likely to require supplemental oxygen and less likely to report otalgia, suggesting that HRVC may be more virulent than HRVA. Excluding coinfections, children with HRVC were more likely to wheeze than children with HRVA. A recent multi-year population-based study of HRVC in two U.S. locations found that HRVC was associated with medically diagnosed wheezing and asthma²⁶. These data, as well as other reports, suggest that viruses in the HRVC group are more likely to be associated with wheezing¹⁹⁻²⁴.

In prior studies^{24, 26}, HRVC accounted for about half of the sequenced HRV, compared with the 26% reported here. This discrepancy may be attributable to the apparent seasonality of

HRVC: HRV circulate year-round with peaks in the spring and fall ³⁸. In our previous study, HRVC was significantly more prominent in the fall months compared with HRVA, which predominated in the spring. Lau et al also identified an HRVC peak in the fall/winter, although they only detected 5 HRVC strains ²³. Thus, in this study during three winter months, we may have underestimated the true burden of illness of HRVC. Almost 70% of HRV detected in March were HRVA, consistent with our prior study. These seasonal variations underscore the importance of conducting studies over multiple seasons in diverse locations to understand fully the epidemiology of HRV.

We found a surprising amount of genetic diversity among the 63 HRVC strains detected with clustering into 19 genotypes. Although these are genotypes and do not prove distinct serotypes, phylogenetic analysis of the VP4/VP2 protein-coding region correlates with serotyping for known HRV ³¹. The genetic diversity seen amongst HRVC strains was greater than that seen amongst HRVA strains when analyzed by percent sequence identity or phylogenetic distance, consistent with other studies to date. All of the recently described HRV for which the VP4/VP2 sequences are available fell into the same group, including the QPM strains from Queensland that were originally classified as a subgroup of HRVA ¹⁷⁻²⁶.

Study Limitations

Our study has some limitations. First, we did not test concurrent healthy controls. Previous reports suggest that the rate of HRV detection in asymptomatic individuals varies between 4-18% and thus is lower than the rate of HRV we detected in hospitalized children ^{9, 39}. Second, we defined wheezing by parental report. Because other clinical symptoms may be confused with “wheezing”, this could lessen the strength of association of HRVC with wheezing. However, other studies support this association ¹⁹⁻²⁴ ¹⁸. Third, we only evaluated samples during the peak winter months; therefore, we may have underestimated the overall burden of HRVC. We did not collect data on bacterial co-infections or antibiotic usage. However, rates of antibiotic use are high in this population, one study showed that over 60% of RSV-infected children were treated with antibiotics ⁴⁰. Thus, improved viral diagnostic capabilities might limit inappropriate antibiotic use.

In conclusion, there is a significant burden of HRV-associated illness in young children hospitalized during the peak winter months with fever or ARI in Amman, Jordan. The HRVC group is responsible for many of these infections. HRVC is associated with wheezing and increased severity of illness. Further studies are required to confirm these observations and better understand the relationship between HRVC and asthma.

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Abbreviations

HRV

human rhinovirus

hMPV	human metapneumovirus
RSV	respiratory syncytial virus
RT-PCR	reverse transcriptase polymerase chain reaction
JU	Jordan University
ARI	acute respiratory illness

Figure 1.

Age distribution of subjects infected with respiratory syncytial virus (RSV), human metapneumovirus (MPV), or human rhinovirus (HRV).

Figure 2.

Phylogenetic tree depicting relationships between known HRV serotypes and novel HRV. Previously described HRV serotypes are designated by “HRV” and a black circle. Novel sequences identified in this study are designated by numbers. The numbers in parentheses after the label of these sequences indicates how many additional specimens contained each virus. Novel sequences identified in recent studies are designated by “HRV” and a grey circle. Genbank accession numbers for previously published sequences are AY016398–AY016409, AF343583–AF343655, AY040232–AY040243, EF186077, EF077237, EF077281, DQ875929, and EF585385–EF585387. Sequences described in this paper have been deposited with Genbank under accession numbers XXXXXX-2236116.

Table 1

Demographic and clinical characteristics of children with HRV vs RSV vs no virus detected, excluding coinfections.

	HRV+ only (n=112)	RSV+ only (n=341)	No Virus Detected (n=90)
Age in months **	5.4 (2.0, 10.0) *	3.8 (1.5, 8.7) *	6.4 (2.4, 11.9) *
Male	65% (73)	56% (190)	63% (57)
Breastfed	89% (100)	86% (292)	86% (77)
Daycare	4% (4)	3% (9)	4% (4)
Smokers in home	71% (80)	62% (213)	71% (64)
Nasal congestion **	68% (76)	74% (252)	54% (48)
Poor appetite **	64% (72)	77% (262)	72% (65)
Sore throat	34% (30)	42% (119)	35% (25)
Wheezing **	87% (97)	89% (302)	73% (66)
Supplemental oxygen **	43% (48)	74% (252)	44% (40)
Intensive Care Stay	5% (6)	7% (23)	10% (9)
Days Hospitalized **	2.8 4.0 6.0	3.0 4.0 6.3	3.0 4.0 7.0
Mechanical Ventilation	2% (2)	4% (15)	3% (3)

* median (interquartile range)

** p<0.05

Clinical data ascertained from questionnaire.

Table 2

Demographic and clinical characteristics of children with HRV only versus HRV coinfection.

	HRV only (n=112)	HRV coinfection (n=128)
Age in months **	5.4 (2.0, 10.0) *	3.2 (1.5, 7.0) *
Male	65% (73)	57% (73)
Breastfed	89% (100)	93% (119)
Daycare	4% (4)	4% (5)
Smokers in home	71% (80)	76% (97)
Nasal congestion **	68% (76)	55% (70)
Poor appetite	64% (72)	72% (92)
Sore throat	34% (30)	29% (30)
Wheezing	87% (97)	91% (116)
Supplemental oxygen **	43% (48)	61% (78)
Intensive Care Stay	5% (6)	6% (8)
Days Hospitalized	2.8 4.0 6.0	3.0 5.0 7.0
Mechanical Ventilation	2% (2)	2% (2)

* median (interquartile range)

**
p<0.05

Clinical data ascertained from questionnaire or charts.