CASE REPORT

In September 2012, a 19-month-old girl with severe acute respiratory distress was admitted to the pediatric intensive care unit (ICU). The child was born at term and healthy but had a history of repeated episodes of bronchiolitis that required hospitalization when she was 5, 6, and 8 months old. Viral diagnosis of these episodes had never been performed. No allergic sensitization was known, and she had received no treatment for asthma nor any inhaled corticosteroids. Her parents had no known history of asthma or atopy. The child had no known underlying condition. Thirty-six hours before hospitalization, the child suffered from rhinitis, respiratory symptoms, and wheezing, suggesting bronchiolitis. Upon admission, she was unconscious and cyanotic with a pulse at 155 beats/min, oxygen saturation at 48%, blood pressure at 125/74 mm Hg, and a temperature of 37.2°C. Plastic bronchitis was suspected, and the patient was treated with high-output oxygenation, intravenous glucocorticoid (1 mg/kg methylprednisolone), and inhaled adrenaline and terbutaline, but her condition did not improve significantly. Laboratory tests revealed a leukocyte count of 31 × 10⁹ cells/liter (75% segmented neutrophils, 17% lymphocytes, and 7% monocytes) and a serum C-reactive protein level of 19.2 mg/liter. A chest X-ray showed a compressive pneumothorax that was immediately treated. Despite orotracheal intubation and institution of mechanical ventilation, profound bradycardia occurred, followed by cardiac arrest. Resuscitation attempts failed and the child died a few hours after hospitalization.

An autopsy was conducted with the parents’ consent, and microbiological investigations were performed on specimens collected just after the patient’s death. None of the microbiological cultures and investigations yielded any pathogenic bacteria in the patient’s blood, cerebrospinal fluid (CSF), urine, stool, throat, tracheal aspiration, or lung biopsy specimens (Table 1). Culture from lung biopsy specimens was negative for mycobacteria. Nucleic acids were extracted with the NucliSSENS easyMAG automated system according to the manufacturer’s instructions (bioMérieux, Lyon, France). The different specimens were tested for human enterovirus (HEV), human herpesviruses (HHV) 1 to 6, Pneumocystis jirovecii, and a panel of 22 respiratory pathogens, including human rhinovirus (HRV), using PCR (Table 1). Cephelic HEV-specific PCR (Cepheid, Sunnyvale, CA, USA) was positive for blood and stool specimens and at the lower limit of detection for the CSF sample, while multiplex PCR (RespFinder SMART 22 assay; Pathofinder, Maastricht, Netherlands) was positive for HEV and HRV in nasopharyngeal aspirate specimens (Table 1). For genotyping, RNA extracts from serum, stool, and nasopharyngeal aspirate specimens were reverse transcribed. A generic nested PCR assay, which amplifies a partial sequence of the HEV VP1 coding region (1), was first attempted but gave negative results on all samples tested, even those with low cycle threshold (Cₚ) values. Therefore, a second nested PCR assay specific for the HRV VP4/VP2 genomic region (2) was performed and allowed virus genotyping in serum, stool, and nasopharyngeal aspirate samples (Table 1). The VP4/VP2 sequences showed a 92% nucleotide homology with the corresponding region of the HRV-C type 8 (HRV-C8) prototype strain (Table 1) (GenBank accession number GQ223227; http://www.picornaviridae.com/enterovirus/rv-c/rv-c_seqs.htm). Phylogenetic analysis confirmed the genotype assignment (data not shown). Globally, these PCR typing results excluded an HEV infection and were in favor of a cross-detection of HRV-C8 by the Cepheid HEV-specific PCR.

The autopsy revealed significant abnormalities only in the lungs and trachea, with acute diffuse alveolitis and tracheobronchitis. Real-time reverse transcription (RT)-PCR and VP4/VP2 sequencing performed on nucleic acids extracted from a frozen...
lungs. The three-dimensional human airway epithelium reconstituted in vitro (4) was used to study HRV-C8 replication and virulence. We inoculated the patient’s serum, as well as the nasopharyngeal aspirate, into reconstituted human airway epithelia (MucilAir; Epithélix Sàrl, Plan-les-Ouates, Geneva, Switzerland) as previously described (3). Efficient viral replication was observed for the two specimens (Fig. 1). The presence of infectious HRV-C8 was confirmed by VP4/VP2 sequencing of the viruses collected from the apical face of the tissue 48 h postinfection (Table 1).

HRV is the most frequent cause of the common cold and other self-limited upper respiratory tract illnesses, but it can be associated with lower respiratory tract complications, including recurrent wheezing, bronchitis, asthma exacerbation, and pneumonia (4). Fatal cases caused by HRV infections are infrequent and have been described mostly in patients with severe underlying conditions (5–8). As HRV is also commonly detected in asymptomatic individuals, particularly in children, it may be difficult to establish a causal link between viral detection and symptoms. We report a systemic HRV-C type 8 infection as the cause of fatal acute respiratory distress in a 19-month-old girl with a history of repeated episodes of bronchiolitis. Both host and viral factors may have contributed to the disease severity and death. Some hosts, e.g., asthmatic subjects, present a deficient innate immune response to HRV and are predisposed to severe lower respiratory illnesses upon infection (9–11). In our case, we cannot rule out that the patient suffered from an unknown underlying disease, including infantile asthma, that could have predisposed her to a severe and fatal rhinovirus infection. In the absence of ethical authorization, genetic investigations were not carried out to explore a primary immunodeficiency or asthma susceptibility. Viral factors may also have an impact on disease severity. HRV-Cs could present a higher virulence and be associated with asthma exacerbations and pediatric hospitalizations (12–14). In this study, HRV RNA was detected at a low level in the CSF and at higher concentrations in the lung and fecal samples. The latter observation suggests replication in the gastrointestinal tract, although isolation in tissue culture was not attempted due to the limited amount of clinical specimen available. An open question remains whether this HRV-C strain resisted the acidic environment of the gastrointestinal tract or if infants can present a less acidic gastrointestinal environment under some circumstances (15–17). In addition to this potential unusual tropism for the gastrointestinal tract, HRV RNA was identified in blood samples. The presence of HRV nucleic acids in blood has been reported previously. It was shown to be more frequent for HRV-C than for the other two HRV species (17–19) and more common in children with asthma exacerbation (19). However, the presence of HRV nucleic acids is not necessarily associated with infectious virions. To our knowledge, HRV culture from blood samples has only been described once, in 1970 (20). In that study, Urquhart and Stott isolated two HRV-A clinical strains (HRV-A15 and -A22) in cell culture after inoculation with the serum of two children who died of acute respiratory illness. In our case, replication-competent HRV-C virions were successfully isolated from the patient’s serum in human airway epithelia.

In conclusion, HRV-Cs are known to bind to an as-yet-uncharacterized receptor that differs from the HRV-A and -B receptors (4). Thus, the nature of infected cells and the exact tissue

### TABLE 1 Virological findings from patient specimens

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>HHV PCR</th>
<th>HEV PCR (Ct value)</th>
<th>Respiratory virus multiplex PCR</th>
<th>Viral culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasopharyngeal aspirate</td>
<td>Neg</td>
<td>ND</td>
<td>Pos for HEV/HRV</td>
<td>Pos&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Blood (serum)</td>
<td>Neg&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Pos (28)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>Pos&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stool</td>
<td>ND</td>
<td>Pos (29)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>Neg</td>
<td>Pos (45)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Frozen lung tissue from autopsy&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>Pos (24)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> Neg, negative; Pos, positive; ND, not done; HHV PCR, human herpesvirus PCR; HEV PCR, human enterovirus PCR (Cepheid); Ct, cycle threshold. Tests for HHV 1 to 3 were conducted using LightMix kit HSV-1/2 (herpes simplex virus)/VZV (varicella-zoster virus) (TIB Molbiol). Tests for HHV 4 to 6 were conducted using Argene kits (bioMérieux).

<sup>b</sup> Only HHV 4 to 6 were tested in serum.

<sup>c</sup> Tested negative for Pneumocystis jirovecii.

<sup>d</sup> Typing as HRV-C8 by sequencing.

<sup>e</sup> The respiratory virus multiplex PCR assay used was the Respifinder SMART 22, which allows the detection of 18 viruses (rhinovirus/enterovirus, influenza A/B/A H1N1, adenovirus, metapneumovirus, respiratory syncytial viruses A and B, parainfluenza viruses 1 to 4, coronavirus NL63/OC43/229E/HKU1, and bocavirus) and 4 bacteria (Chlamydia pneumoniae, Mycoplasma pneumoniae, Legionella pneumophila, and Bordetella pertussis). This assay was performed on a LightCycler 480.

#### FIG 1

HRV RNA loads measured by real-time RT-PCR at the apical surface of three-dimensional human airway epithelium reconstituted in vitro and inoculated with HRV-A16, the patient’s nasopharyngeal aspirate, or the patient’s serum or not infected (3). Absolute copy numbers present in the input (100 μl) or apical washes (200 μl) collected at different time points are indicated. Error bars show standard deviations calculated from two independent biological replicates. RNA load measured 4 h postinoculation represents the leftover inoculum. NPA, nasopharyngeal aspirate.
tropism of HRV-C types are not yet totally elucidated. Recovery of infectious virus from blood, either as free virions or cell-associated virus, suggests that authentic viremia with HRV-C may occur in given situations and lead to dissemination beyond the respiratory tract. The presence of HRV-C in the bloodstream could affect the clinical outcome of viral infection and cause more severe disease, particularly in immunocompromised hosts or individuals with asthma and other comorbidities.

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REFERENCES


