The Study

Fecal specimens from 6 clinical cohorts in Salvador, northeastern Brazil, were analyzed (Table 1). Adult cohorts comprised HIV-infected patients with gastroenteritis (105 persons) and without (49 persons) gastroenteritis. Child cohorts included 359 children with gastroenteritis and 204 healthy children from child-care centers. Nasal swab specimens were obtained from controls attending child-care centers and from children with gastroenteritis and concomitant respiratory symptoms. All specimens were stored at −30°C to −80°C until further processing.

Viral RNA was purified from ≈200 mg fecal specimen and 140 μL nasal swab specimen suspended in phosphate-buffered saline by using the Viral RNA Mini kit (QIAGEN, São Paulo, Brazil) as described (7). A nested reverse transcription PCR (3) detected cosavirus. After nucleotide sequencing of all PCR-positive specimens, we developed a specific real-time RT-PCR for quantifying viruses from Brazil (Table 2). Assay optimization and quantification relied on photometrically quantified cRNA in vitro transcripts, as described (8). After assay optimization, sensitivity was 6.8 copies per reaction.

In the adult cohorts, 1 of 105 HIV-infected persons with gastroenteritis had positive results for cosavirus. In the control group of 49 HIV-infected adults without gastroenteritis, none had positive cosavirus results ($\chi^2$ 0.5; p = 0.49).

Considerably higher prevalence was detected among child cohorts. Of children with gastroenteritis, 13 (3.6%) of 359 patients were cosavirus positive. The proportion of cosavirus-positive controls without gastroenteritis, sampled in 2008, was significantly higher: 65 (49.2%) of 132 controls were cosavirus positive ($\chi^2$ 149.1; p<0.0001). On resampling in 2011, 4 (6.5%) of 62 controls were cosavirus positive. Although the difference was not statistically significant ($\chi^2$ 1.1; p = 0.3), this result was almost double that for patients. In another child-care center, none of 10 healthy children were cosavirus positive.

The higher prevalence detected in controls in 2008 could indicate a seasonal infection pattern because specimens were collected in a single weekend in a child-care center. The lower cosavirus-positive results in the same child-care center 3 years after the initial sampling support this idea. However, in sick children, cosavirus-positive specimens were obtained at similarly low rates throughout the year (Figure 1), which might argue against seasonal variation as a generic property of cosavirus infection.

To evaluate whether cosavirus causes disease in ill children, we analyzed co-infections with common viral pathogens causing diarrhea (astrovirus, norovirus, rotavirus, adenovirus) (Figure 2, panel A). In 10 (76.9%) of...
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Table 1. Clinical cohorts tested for cosavirus, Salvador, Brazil

<table>
<thead>
<tr>
<th>Cohort no.</th>
<th>Cohort description†</th>
<th>Sampling site‡</th>
<th>Sampling time</th>
<th>Participant age, mo, mean (SD)</th>
<th>No. participants</th>
<th>No. (%) RT-PCR positive§</th>
<th>Virus concentration, log10 RNA copies/g feces, mean (SD)¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HIV-infected adults with gastroenteritis</td>
<td>Infectious Diseases HIV</td>
<td>2007 Mar–2010 Mar</td>
<td>487.6 (114.4)</td>
<td>105</td>
<td>1 (1.0)</td>
<td>4.43</td>
</tr>
<tr>
<td>2</td>
<td>HIV-infected adults without gastroenteritis</td>
<td>Outpatient Department</td>
<td>2007 Mar–2010 Mar</td>
<td>533.8 (115.9)</td>
<td>49</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>Children with gastroenteritis</td>
<td>Department of Pediatrics</td>
<td>2006 Feb–2007 Sep</td>
<td>19.0 (15.6)</td>
<td>359</td>
<td>13 (3.6)</td>
<td>3.40 (0.93)</td>
</tr>
<tr>
<td>4</td>
<td>Control children without gastroenteritis</td>
<td>Community child-care center**</td>
<td>2008 Dec</td>
<td>29.6 (13.1)</td>
<td>132</td>
<td>65 (49.2)</td>
<td>2.97 (0.97)</td>
</tr>
<tr>
<td>5</td>
<td>Control children without gastroenteritis</td>
<td>Community child-care center**</td>
<td>2011 Nov–2011 Dec</td>
<td>14.3 (5.5)</td>
<td>62</td>
<td>4 (6.5)</td>
<td>3.41 (0.49)</td>
</tr>
<tr>
<td>6</td>
<td>Control children without gastroenteritis</td>
<td>University child-care center**</td>
<td>2011 Oct–2011 Nov</td>
<td>18.6 (4.2)</td>
<td>10</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>717</td>
<td>83 (11.6)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*RT-PCR, reverse transcription PCR; †Gastroenteritis was defined as acute diarrhea with >3 watery stools in the previous 24 h and within 13 d before hospital admission. ‡All hospital units were located within the Hospital Professor Edgard Santos, Federal University of Bahia. All sites were located in Salvador, Bahia, in northeastern Brazil. §Samples only considered if positive in nested RT-PCR as in (3) and in strain-specific real-time RT-PCR. ¶Measured by strain-specific RT-PCR.

Table 2. Oligonucleotides used for detection and quantification of cosaviruses

<table>
<thead>
<tr>
<th>Oligonucleotide identity</th>
<th>Sequence, 5’→3’</th>
<th>Genomic target region, RT-PCR type</th>
<th>Use</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DKV-N5U-F1</td>
<td>CCGCTTTTACAGCTTGGTTTGA (+)</td>
<td>5’-UTR, nested RT-PCR 1st round</td>
<td>Cosavirus detection†</td>
<td>(3)</td>
</tr>
<tr>
<td>DKV-N5U-R2</td>
<td>GTGACCTTACAGCTTGGTTTGA (-)</td>
<td>5’-UTR, nested RT-PCR 2nd round</td>
<td>Brazilian cosavirus quantification†</td>
<td>–</td>
</tr>
<tr>
<td>DKV-N5U-F2</td>
<td>ACCGGTTTGAGACCCACAC (+)</td>
<td>5’-UTR, real time RT-PCR</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DKV-N5U-R3</td>
<td>GCCTTTTGGACAGCATTTTTG (–)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HCoSv-rfF35-1</td>
<td>TTGTAGYGTAGTCTGRTGTGTTG (+)</td>
<td>5’-UTR, nested RT-PCR</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HCoSv-rfP783</td>
<td>FAM-ACCTCCAGAGCCTGGCTGTCG-DQ1 (+, Probe)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HCoSv-rfR827-1</td>
<td>CAAGGGTTGGCTTCCTTCGTC (-)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*RT-PCR, reverse transcription PCR; †Gastroenteritis was defined as acute diarrhea with >3 watery stools in the previous 24 h and within 13 d before hospital admission. ‡All hospital units were located within the Hospital Professor Edgard Santos, Federal University of Bahia. All sites were located in Salvador, Bahia, in northeastern Brazil. §Samples only considered if positive in nested RT-PCR as in (3) and in strain-specific real-time RT-PCR. ¶Measured by strain-specific RT-PCR.

Conclusions

Human cosavirus infections were reported previously from a limited number of persons and geographic areas.
In Brazil, the 3.6% detection rate in children with gastroenteritis was comparable to the 1.8% rate in a cohort study of gastroenteritis patients in China (6). Although the 6.5% detection rate in 1 control cohort in Brazil was compatible with the 1.7% rate in 60 healthy controls in China, the combined 33.8% prevalence detected in controls from 3 different samplings in Brazil was much higher. Nonetheless, the prevalence was comparable to the 43.9% detected in 41 healthy Southeast Asian children in the only other cohort study (3). Detecting cosavirus in 1 of 154 adults in Brazil was compatible with finding a single cosavirus-positive patient among 1,000 adults with gastroenteritis in Scotland, confirming that cosaviruses are rare and probably neither pathogenic nor commensal in adults (3).

The higher prevalence of cosavirus found in controls than in patients, the frequent co-infections with established pathogens, and the unusually low RNA virus concentrations give evidence against cosavirus involvement in human gastroenteritis. Viruses that replicate in the human gut generally reach concentrations 1,000- to 100,000-fold higher than those of cosavirus. This finding is exemplified by genetically related picornaviruses (Aichi viruses, parechoviruses, and cardioviruses) and established enteric pathogens (e.g., noroviruses and rotaviruses) (8–12). Notably, the aforementioned study on cardioviruses included the same specimens from Brazil, which indicates that poor sample quality was not a factor.

These low concentrations would be compatible with absence of replication in the enteric tract and passive virus ingestion, e.g., from nutritional sources, drinking water, or the respiratory tract. However, nutritional patterns of the tropical countries in which cosavirus have been detected certainly differ. Furthermore, in Brazil, adults are unlikely to have a completely different diet from infants and children. Moreover, the unprecedented detection of cosavirus in a respiratory tract specimen makes ingestion of viruses from nutritional sources alone unlikely, although a link to fluid droplets from drinking water in the respiratory tract is hypothetically possible.

Another explanation for low cosavirus RNA levels in fecal samples is that a cosavirus infection occurred early in the person’s life and produced partial mucosal immunity and limited subsequent cosavirus replication in the gut. This is exemplified for viruses transmitted by the fecal–oral...
route by up to 100-fold higher fecal shedding of vaccine rotavirus and poliovirus among seronegative persons than among seropositive or previously vaccinated persons (13, 14). However, this explanation would be incompatible with the high prevalence of cosavirus in many control children, who were generally older than patients.

Prolonged low concentrations of picornavirus shedding has been demonstrated, e.g., by detectable hepatitis A virus RNA up to 3 months after acute infection (15). Nonetheless, this circumstance is unlikely to explain the low cosavirus concentrations, given the overall high number of persons with positive results.

Although our study extends the known geographic occurrence of cosavirus, whether it is a human pathogen at all remains to be determined. Future studies would be enhanced by serologic analyses and investigations focusing on nutrition and drinking water in tropical countries.

Acknowledgments

We thank Tobias Bleicker, Sebastian Brünink, Monika Eschbach-Bludau, Célia Pedroso, Carlos Brites, Vanusa dos Santos Estrela, Maria Gereh Barberino, Ana-Rute Santos Oliveira, and Milena Carvalho Bastos for outstanding support and Alexander N. Lukashev for helpful suggestions. We are also grateful to the child-care center staff, children, and parents involved in this study.

The study was funded by the Foundation for Research Support of the State of Bahia (Fundação de Amparo à Pesquisa do Estado da Bahia), project codes APR 125/2006/ethics committee protocol 120/2005 and SUS0004/2007/ethics committee protocol 06/2007, and the European Union FP7 project European Management Platform for Emerging and Re-emerging Infectious Disease Entities (grant agreement no. 223498).

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References


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Technical Appendix

Technical Appendix Figure. Cosavirus (CosV) partial 5’ untranslated region phylogeny. The neighbor-joining phylogeny was generated by using MEGA5 (www.megasoftware.net), including a complete deletion option and a nucleotide percentage distance substitution model drawn to scale. Values at node points indicate support from 1,000 bootstrap reiterations (only values >70 are shown). The final dataset comprised 389 t corresponding to positions 512–909 in CosV A1 (GenBank accession no. FJ438902). All available CosV sequences covering the complete genomic region obtained in this study were included in the analysis and are given with strain name (when available), GenBank accession number and geographic origin. CosV 5’ untranslated region types (3) are indicated to the right of taxon names. Viruses from this study are color coded by clinical cohort: red, children with gastroenteritis; green, HIV-infected adults with gastroenteritis; blue, healthy control children from child-care centers. Scale bar indicates nucleotide substitutions per site.