Detection and Characterization of Human Caliciviruses Associated with Sporadic Acute Diarrhea in Adults in Djibouti (Horn of Africa)

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Abstract. Recent advances in molecular diagnostics have allowed us to recognize Human caliciviruses (HuCVs) as important agents of acute diarrhea in industrialized countries. Their prevalence and genetic diversity in developing countries remains unknown. We report on the characterization of HuCVs among adults presenting acute diarrheas in Djibouti; 108 stool samples collected were screened by EIA, RTPCR, or cell cultures for the group A Rotaviruses, Adenoviruses, Astroviruses, and HuCVs, which were further characterized by genotyping. Among stool samples screened for HuCVs, 25.3% were positive. The other enteric viruses were less prevalent. The 11 HuCV strains sequenced revealed a large diversity (3 sapoviruses and 8 noroviruses). GII strains noroviruses were predominant, five were newly described genotypes, and two were recombinant with a pol gene related to GGIIB strains with the particularity to associate a unique pol gene to different capsid genes. These results could help to the knowledge of HuCV infections in Tropical Africa.

INTRODUCTION

Acute infectious diarrhea represents a public health concern throughout the world. In industrialized countries, the role and the diversity of the 4 main viruses causing gastroenteritis, group A rotaviruses (RV), human caliciviruses (HuCVs), astroviruses (AsV), and enteric adenoviruses (AdV) have now been well documented. HuCVs have notably been identified as the second leading cause of acute gastroenteritis in young children1 and as a major cause of non-bacterial gastroenteritis in sporadic and outbreak cases in all age groups.2–4 HuCVs are a very diverse group of single-stranded RNA viruses of the Caliciviridae family. They are divided into 2 genera, Norovirus and Sapovirus; human noroviruses are divided into 3 distinct genogroups, GI, GII, and GIV, each including genotypes. The classification is constantly evolving with the discovery of new strains. Recently Kageyama and others6 proposed a classification with 31 genotypes, and two were recombinant with a pol gene related to GGIIB strains with the particularity to associate a unique pol gene to different capsid genes. These results could help to the knowledge of HuCV infections in Tropical Africa.

Patients and samples. Djibouti, situated in the Horn of Africa, is surrounded by Eritrea, Ethiopia, and Somalia; it is an important regional crossroads for trade and exchange. The total population of Djibouti is estimated about 600,000, two-thirds of which live in the city of Djibouti. The French Military Hospital Bouffard (Djibouti city) is open to expatriates (French, American, and other European soldiers are the majority), Djiboutians (especially soldiers and their families), and more rarely patients from other countries of the Horn of Africa living in Djibouti.

Patients were enrolled in the study from September 2002 to February 2004. They were all over 15 years old, largely urban, seen for acute diarrhea (more than 3 stools per day for a duration of less than 7 days) at Bouffard Hospital. For each patient, the physician filled out an epidemiologic and clinical questionnaire, and a stool sample was immediately sent to the laboratory for bacteriological and parasitological analysis. Only negative samples were further tested for viruses.

Virus detection. After shipment of frozen samples (stored at −80°C in dry ice) to the Teaching Military Hospital Val de Grâce in Paris, France, specimens were screened by enzyme immunoassay (EIA) for the presence of group A RV (IDEIA™ Rotavirus, Dako Ltd.), AdV (IDEIA™ Adenovirus, Dako Ltd.), and AstV (Amplified IDEIA™ Astrovirus, Dako Ltd.). Cell cultures (Vero, MRC-5) were inoculated for AdV detection by immunofluorescence with monoclonal antibodies (11-020, Argene, France). HuCVs and also AsV were detected by a commercial reverse transcription polymerase chain reaction test (RT-PCR) (Calici/Astrovirus Consensus™, Argene, France).13

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**HucVs characterization.** HuCVs were further characterized by genotyping by the National Reference Center for enteric viruses in Dijon, France. Strains were sequenced in the polymerase gene using the following primers: JV12-JV13 for noroviruses and Sr80/p110 for sapoviruses. Some strains were sequenced in the capsid gene using primers previously reported. Briefly, a RT-PCR was performed with primers JV12 and G1SKR or NI and Mon383 depending on genogroup I or II, and the PCR products were cloned into the pGEM-T Easy Vector System (Promega Corporation, Madison, WI). The cycling conditions were as follows: one cycle of reverse transcription at 42°C for 15 min; PCR: denaturation for 2 min at 94°C; 40 amplification cycles with denaturation for 30 sec at 94°C, annealing for 1 min at 50°C, and extension for 1 min at 72°C; and a final cycle of incubation at 72°C for 15 min. Genotyping was realized by direct sequencing of the PCR products or plasmids using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit in an automated sequencer (model 3100 DNA Sequencing System), both from Applera Corporation, Foster City, California, United States. Sequence alignments with the GenBank library were carried out by using Fasta Version 3.3t06. For the RNA polymerase, alignments were also performed with reference strains available in the database of the European Foodborne Viruses network.

For the phylogenetic analyses, sequences were first aligned using CLUSTAL-W program in the PHYLIP format. The replicates represented by bootstrap resampling and were analyzed by the neighbor-joining method.

**Nucleotide sequence accession numbers.** The nucleotide sequences determined in this study have been deposited in GenBank under accession numbers EF190918, EF190919, and EF190920 for samples VDG5, VDG50, and VDG66, respectively.

**RESULTS**

**Virological testing.** A total of 108 fecal samples were collected; 103–105 samples were first analyzed with an immunologic test, and 75 were analyzed by RT-PCR (the difference between the total fecal samples collected and the samples analyzed was due to the amount of stool collected). Ninety-five questionnaires were usable. The characteristics of the population are presented in Table 1. In expatriates, the majority of diarrheas occurred during the first 2 months after arriving in Djibouti (Table 2). The average number of stools was 8 per day (predominately watery), and 33 cases needed treatment (30.5%) with anti-secretory agents (19/33) and intestinal coating agents (15/33). Only 6 patients (all with fever) received antibiotics.

Patients presenting nausea were the most often treated (19/44, 43%) in comparison with those without nausea (12/51, 23.5%), \( P = 0.03 \).

The results of virological testing are presented in Table 3. Nineteen samples (25.3%) were positive for HuCVs. The other enteric viruses were less prevalent: AstV: 4.8% and 4% by EIA and RT-PCR, respectively (the AstV were not phylogenetically investigated); AdV: 3.8% and 4.8% by EIA and IFI respectively; RV: 1.9%. No co-infection was detected. The ELISA technique allowed us to identify 5 AstV strains; the PCR only 3. However, the numbers analyzed were different for the 2 techniques (105 and 75 stool samples, respectively). For the AdVs, the difference can be explained by the higher expected sensitivity of the IFI. There was no significant difference (\( P > 0.05 \)) between the patients with HuCV infection and patients with infection caused by another virus with regards to sex, origin, age, status, and number of stools per day (mean = 8/day). The frequency of asthma was higher among patients infected by HuCVs (\( P = 0.03 \)). There was no significant difference regarding the other symptoms (abdominal pain, vomiting, nausea, fever, myalgia), but this may arise from the low number of comparable cases of each virus in each age group.

**HuCV characterization.** Eleven strains of HuCVs could be genotyped in the pol and/or the capsid region. A diversity of strains was observed: among them, 8 (73%) were noroviruses and 3 (27%) were sapoviruses. Two of the sapoviruses were related to Parkville virus (Accession number: U73124, 90.8% and 92% nucleotide identity in a 285 bp region of the pol gene, respectively) and one showed 85.8% nucleotide identity with Mexico/339/1991 virus (Accession number: AY157865). Among the noroviruses (Figure 1), 1 belonged to genogroup I (VDG50) and was close to Saitama-KU8G1/99/JP virus, which has been proposed recently as a new GI genotype (GI-11). 6 belonged to genogroup II and 1, VDG63, to genogroup IV (Alphatron). The 6 GI strains were the following: 2 M7/99/US-like strains, VDG68 and VDG82 (new proposed genotype, GII-14), and 1 CS-E1-like strain, VDG39 (new proposed genotype GII-17) and 2 putative recombinants showing a pol gene that could not be
assigned to any known genotype but was close to GIIb recombinant strains.23 For one of them, a capsid gene belonging to GII-2 (Melksham genotype), the second capsid gene could not be sequenced.

TABLE 3
Results of the Virological testing

<table>
<thead>
<tr>
<th>Stool samples (N)</th>
<th>Virus</th>
<th>Method</th>
<th>Positive samples (N)</th>
<th>%</th>
</tr>
</thead>
<tbody>
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<td>105</td>
<td>Adenovirus</td>
<td>EIA</td>
<td>4</td>
<td>3.8</td>
</tr>
<tr>
<td>105</td>
<td>Astrovirus</td>
<td>EIA</td>
<td>5</td>
<td>4.8</td>
</tr>
<tr>
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<td>Rotavirus</td>
<td>EIA</td>
<td>2</td>
<td>1.9</td>
</tr>
<tr>
<td>105</td>
<td>Adenovirus</td>
<td>IFI</td>
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<td>4.8</td>
</tr>
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<td>75</td>
<td>Calicivirus</td>
<td>RT-PCR</td>
<td>19</td>
<td>25.3</td>
</tr>
<tr>
<td>75</td>
<td>Astrovirus</td>
<td>RT-PCR</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

DISCUSSION

Viral gastroenteritis is now well documented in industrialized countries, in contrast to Africa where epidemiologic data concerning viruses other than RV are still lacking. This may be attributed to the fact that methods for the detection of these viruses, especially HuCVs, are costly and require specialized laboratories. Moreover, local adaptation of surveys remains difficult because of preservation and shipment of samples.

In this survey, which was focused on the detection and characterization of Human Caliciviruses associated to acute diarrhea among adults (outside of the context of an outbreak) the results of virological testing confirm the relative...
frequencies generally observed in industrialized countries for HuCVs (and also RV, AstV, AdV) in this age group, which remains the main agents of gastroenteritis among children. 

Among noroviruses, GII strains were predominant as recently in African countries. Five of the 8 noroviruses were newly described genotypes, GI-11, GI-9, GII-14, and GII-17 and 2 of them were recombinant strains showing a pol gene related to GGIIb strains. These strains, which were detected first in 2000 in France have been predominant in gene related to GGIIb strains. These strains, which were de-

In conclusion, this study shows the occurrence and diversity of HuCVs among adults presenting acute diarrheas in a tropical area (Djibouti, Horn of Africa). Considering a virus as readily transmissible as norovirus, we cannot judge if the strains are really endemic and if the traffic in and out of Djibouti has played a role in the introduction of new strains from elsewhere. The majority of our patients were European soldiers at the beginning of their deployment in Djibouti. Because their deployment in other countries before coming to Djibouti were for months at a time, it is likely that they contracted the diarrhea in Djibouti. Moreover they did not present any gastroenteritis before their arrival. Still, it is difficult to extrapolate our findings to the Djiboutian population in general. Surely, the development of RT-PCR assays among some references laboratories in tropical Africa should be considered to survey those viruses.

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REFERENCES


