Human caliciviruses (HuCVs), especially noroviruses, are a major cause of food- and waterborne outbreaks in industrialized countries. Their role as a cause of gastroenteritis outbreaks in economically developing areas is unclear because little information is available (1–3). Five norovirus genogroups have been described, with serogroup II (GII) prevailing in outbreaks worldwide since 1990 (3–6). Strains differing significantly from GI and GII prototypes are being increasingly reported since detection methods have improved (5,6).

Chile is a rapidly developing country. Studies have shown seroprevalence for HuCVs of >70% for children 5 years of age and incidence of 8% in acute sporadic cases of diarrhea in children (7–9). A small number of norovirus-associated outbreaks have been reported but information is scarce because no surveillance system for gastroenteritis exists (8). The capital city of Chile, Santiago, with ≈6.1 million persons, contains 48% of the country’s population. Ninety-six public hospitals, private clinics, and emergency outpatient clinics distributed within 6 healthcare services centers are responsible for notifying the Health Ministry when infectious diseases that are on the National Mandatory Notification List are identified.

The Study

In 1994, the Metropolitan Area Environmental Health Service (health service) began a gastroenteritis outbreak surveillance program in the centers. This program was improved in 2000 by using a standard protocol for pathogen detection. This study was to determine the role of HuCVs as a cause of gastroenteritis outbreaks from June 1, 2000, to January 30, 2003, in Santiago, Chile, by using recently improved antigen and genome detection assays, and to characterize genetically the circulating strains.

Sentinel sites were instructed to report gastroenteritis outbreaks ≤48 hours after detecting the sentinel case. A health service epidemiologist would initiate an investigation and make home visits to identify all persons possibly involved in the outbreak. Specific attack rates for implicated food products were calculated.

Stools samples for pathogen detection were collected during home visits from affected persons and were cultured for Salmonella, Shigella, Campylobacter, and Vibrio spp., according to standard techniques using selective media (10). Enteropathogenic Escherichia coli, enterotoxigenic E. coli, and enterohemorrhagic E. coli were studied by multiplex polymerase chain reaction (11) and enzyme-linked immunosorbent assay (ELISA). Rotavirus and enteric adenoviruses were detected by ELISA or by commercial kits (SAS Rota Test, SA Scientific Inc., San Antonio, TX, USA; Premier Adenoclone, Meridian Diagnostics Inc., Cincinnati, OH, USA; 40/41 AdenoStrip, Coris Bioconcept, Gembloux, Belgium) and parasites were detected by Burrows technique.

All samples were tested for HuCV by a novel ELISA specific for noroviruses based on pools of sera obtained from rabbits and guinea pigs hyperimmunized with a total of 9 different norovirus capsids (12) and by reverse transcription–polymerase chain reaction (RT-PCR) targeting conserved sequences in the polymerase region of HuCVs (9). Primers used for RT-PCR were 289 (RT)/290 (PCR) or a pool of degenerate primers of last generation, 289hijk for PCR, that detect norovirus and sapovirus (13,14). RT-PCR products were cloned by using pGEM-T Easy vector system (Promega, Madison, WI, USA). The 327-base nucleotide sequences that encode for the polymerase dependent RNA were aligned by using OMIGA 2.0 (Oxford Molecular, Madison, WI, USA) software and compared with 21 prototype sequences retrieved using BLAST searches from the GenBank database. Phylogenetic distances were calculated by Kimura 2-parameter method and a phylogenetic tree was plotted by the neighbor-joining method using MEGA, version 2.1 (15). Bootstrap values were based on 1,000 generated trees.

Conclusions

During the 30-month study, a total of 82 outbreaks affecting ≤100 persons in the Santiago metropolitan area were reported properly to the health service and investigated. In each outbreak, a rectal swab from ≥1 person was
collected for microbial studies. In each of 55 outbreaks, 
≥1 stool sample was collected for virus studies, and in 
each of 31 outbreaks, ≥1 stool sample was collected for 
parasite studies. Enteric microbial pathogens were isolat-
ed in samples from ≥1 person in 32% of the 82 outbreaks, 
and potentially pathogenic parasites were isolated in 6 
(19%) of 31 outbreaks (Table 1). A total of 175 samples 
from 55 outbreaks were obtained for viral detection, of 
which 47 (27%) from 25 (45%) outbreaks were positive 
for HuCV by using ≥1 method. HuCV outbreaks affected 
≥99 persons with a median of 5 persons (Table 1). In 16 
outbreaks, ≥2 persons were positive by using ELISA or 
RT-PCR; in 9 outbreaks, 1 person was positive by ≥1 
method. Overall, 20% of the outbreaks were detected only 
by ELISA, 24% only by RT-PCR, and 56% by both 
techniques.

Most HuCV outbreaks occurred in the home, with out-
breaks in childcare centers and schools occurring next 
most frequently; only a small fraction occurred in restaur-
ants. The most commonly implicated food products were 
seafood, including raw oysters and clams (Table 2). Among a total of 1,137 persons exposed in the 25 HuCV 
outbreaks, 283 (25%) had typical acute gastroenteritis 
symptoms. Thirty-nine percent of the cases occurred in 
children <5 years of age, 28% occurred in children 5–14 
years of age, 27% occurred in adolescents and adults 
15–60 years of age, and 4% occurred in adults >60 years 
of age. Most commonly reported symptoms were diarrhea 
(86%), vomiting (36%), and fever (16%).

HuCV amplicons from 13 outbreaks evaluated 
belonged to the norovirus genus, including 8 GI, 2 GI, and 
3 in a potentially novel genogroup. The 3 new strains dif-
fered >40% in nucleotide identity from all prototype 
strains compared (Figure). Bootstrap analysis based upon 
1,000 generated trees yielded a node for the potentially 
novel genogroup in 100% of the trees. Two of the out-
breaks caused by this potentially novel genogroup 
occurred during the same month, while the third occurred 
a year later. The distribution of the 8 genogroup II strains 
fell into 3 genetic clusters. One of the genetic clusters, 
represented by strain 028/10-2001, was closely related with a 
distance of 0.11 to Saitama virus (SaiU1, accession no. 
AB039775), a Japanese strain found in 1998 in a child 
with acute gastroenteritis. The 2 other genetic clusters are 
proposed as novel genetic clusters and include strains (i) 
O55/5-2002, O64/10-2002, O62/9-2002, O71/11-2002, 
O78/11-2002, and (ii) O77/11-2002, O85/1-2003 (Figure). 
Both clusters are also most closely related to SaiU1. The 
first cluster has 2 independent nodes with a distance of 
0.19 to 0.28 from SaiU1, the second cluster is represented 
by 2 strains with a distance of 0.18 and 0.19 from SaiU1, 
respectively.

HuCVs were associated with almost half of 55 fully 
evaluated gastroenteritis outbreaks in Santiago, Chile, and

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**Table 1. Proportion of acute diarrhea outbreaks associated with a bacterial enteropathogen or a human calcivirus (HuCV) and number of persons affected during the HuCV outbreaks**

| Year | Bacteria positive* no. tested | HuCVs (%) | No. affected in HuCV outbreaks | Range (median) |
|------|------------------------------|-----------|-------------------------------|----------------|
| 2000 | 8/13 (61)                    | 4/12 (33) | 3–28 (4)                     |
| 2001 | 11/32 (34)                   | 6/18 (33) | 2–54 (5)                     |
| 2002 | 6/34 (18)                    | 14/22 (64)| 2–99 (5)                     |
| 2003† | 1/3 (33)                     | 1/3 (33)  | 5                             |
| Total| 26/62 (32)                   | 25/55 (45)| 2–99 (5)                     |

*An outbreak was associated with a given pathogen if ≥1 sample was positive.
†Bacteria isolated included: enteropathogenic Escherichia coli (EPEC) (2), enterotoxigenic E. coli (ETEC) (3), enterohemorrhagic E. coli (3), EPEC + ETEC (1), Salmonella sp. (12), Shigella sp. (2), Staphylococcus aureus (3).
‡January 1–10, 2003.
§In 1 outbreak, ETEC and EPEC and in another, Shiga toxin—producing E. coli, were concomitantly isolated with HuCV. In 1 additional outbreak the only pathogens simultaneously detected in 1 patient were rotavirus and adenovirus by enzyme-linked immunosorbent assay.

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**Table 2. Human calcivirus outbreak settings and implicated food products by study years**

|               | 2000–2001 | 2002–2003 | Total (%) |
|---------------|-----------|-----------|-----------|
| No. outbreaks | 10        | 15        | 25        |
| Outbreak settings |         |           |           |
| Home         | 6         | 11        | 17 (66)   |
| Childcare center or school | 2       | 3         | 5 (20)    |
| Restaurant   | 1         | 1         | 2 (8)     |
| Picnic       | 1         | 0         | 1 (4)     |
| Food products implicated |       |           |           |
| Seafood      | 3         | 11        | 14 (56)   |
| Meat products | 2        | 3         | 5 (20)    |
| Prepared cooked food | 2      | 1         | 3 (12)    |
| Other        | 3*        | 0         | 3 (12)    |

*Goat cheese, mayonnaise, celery.
were more common than outbreak-associated enteric bacterial pathogens such as *Salmonella* sp. and diarrheogenic *E. coli*. To our knowledge, this is the first prospective, active surveillance for gastroenteritis outbreaks in Latin America that included a thorough search for HuCVs. Publications from the region have described high sero-prevalence for these viruses (3,16) and have reported isolated outbreaks affecting children and adults (3,8).

HuCV-associated outbreaks mostly affected children that ate seafood in homes; other implicated sources included meat products and vegetables. Estimated attack rates were ≈25%. The reported outbreaks in this study reflect the tip of the iceberg; only 10% of all reported outbreaks could be studied because of capacity and resources for prompt reporting and investigation. This study should stimulate efforts for appropriate outbreak investigation in developing regions where food products safety is important for the health of the population, tourism, and international commerce.

Genogroup II strains dominated, as in other studies (3–6), but only 1 of these strains fell into the same genetic cluster of a previously described strain, Saitama virus; in contrast, most strains grouped into 2 closely related new clusters. In addition, 3 strains, 2 temporally related, likely belong to a new genogroup. The circulation of genetically diverse strains indicates the need for further studies to understand the clinical and epidemiologic importance of such diversity.

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