JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2006, p. 2997–3000 Vol. 44, No. 8
0095-1137/06/$08.00+0 doi:10.1128/JCM.00065-06

Asymptomatic Norovirus Infection in Mexican Children

Coralith García,[1,2,3] Herbert L. DuPont,[1,2,4] Kurt Z. Long,[5] Jose I. Santos,[6] and GwangPyo Ko[7]*

Center of Infectious Diseases, The University of Texas, School of Public Health, Houston, Texas[1]; Baylor College of Medicine, Houston, Texas[2]; Instituto de Medicina Tropical "Alexander von Humboldt," Universidad Peruana Cayetano Heredia, Lima, Peru[3]; St. Luke's Episcopal Hospital, Houston, Texas[4]; Harvard School of Public Health, Boston, Massachusetts[5]; Hospital Infantil de Mexico "Federico Gomez," Mexico City, Mexico[6]; and Institute of Health and Environment, Department of Environmental Health, School of Public Health, Seoul National University, Seoul, Korea[7]

Received 11 January 2006/Returned for modification 17 February 2006/Accepted 22 May 2006

Sixty-three children in periurban Mexico City were examined for the occurrence of asymptomatic norovirus (NoV) infection from June to August 1998. NoV was detected in 48 of 161 stool specimens (29.8%), with 31 children (49.2%) having at least one positive stool. Asymptomatic NoV infection occurred commonly during summertime in a Mexican pediatric population.

Noroviruses (NoVs), belonging to the *Caliciviridae* family, are recognized worldwide as the most common cause of acute nonbacterial gastroenteritis. In the United States, this group of enteric viruses causes an estimated 23 million cases of acute gastroenteritis each year and more than 90% of the outbreaks of nonbacterial gastroenteritis (4). A number of studies in both developed and developing countries have shown that NoV is the major enteric pathogen causing acute diarrhea in children and that such infections can sometimes be fatal (20, 23).

NoV is resistant to various environmental stresses and is commonly transmitted through ingestion of contaminated food and water, direct contact, or aerosols (24). Several studies have reported that not all infections with NoVs result in clinical symptoms (3, 7, 10). It is plausible that asymptomatic individuals act as reservoirs, facilitating the transmission of the NoV. However, the rate of asymptomatic NoV excretion and the genetic diversity of NoV among children in poor communities in developing countries are largely unknown. The objectives of the present study were (i) to determine the rate of asymptomatic NoV excretion in a pediatric population living in a periurban Mexican community and (ii) to identify the genetic diversity of NoV circulating in the community.

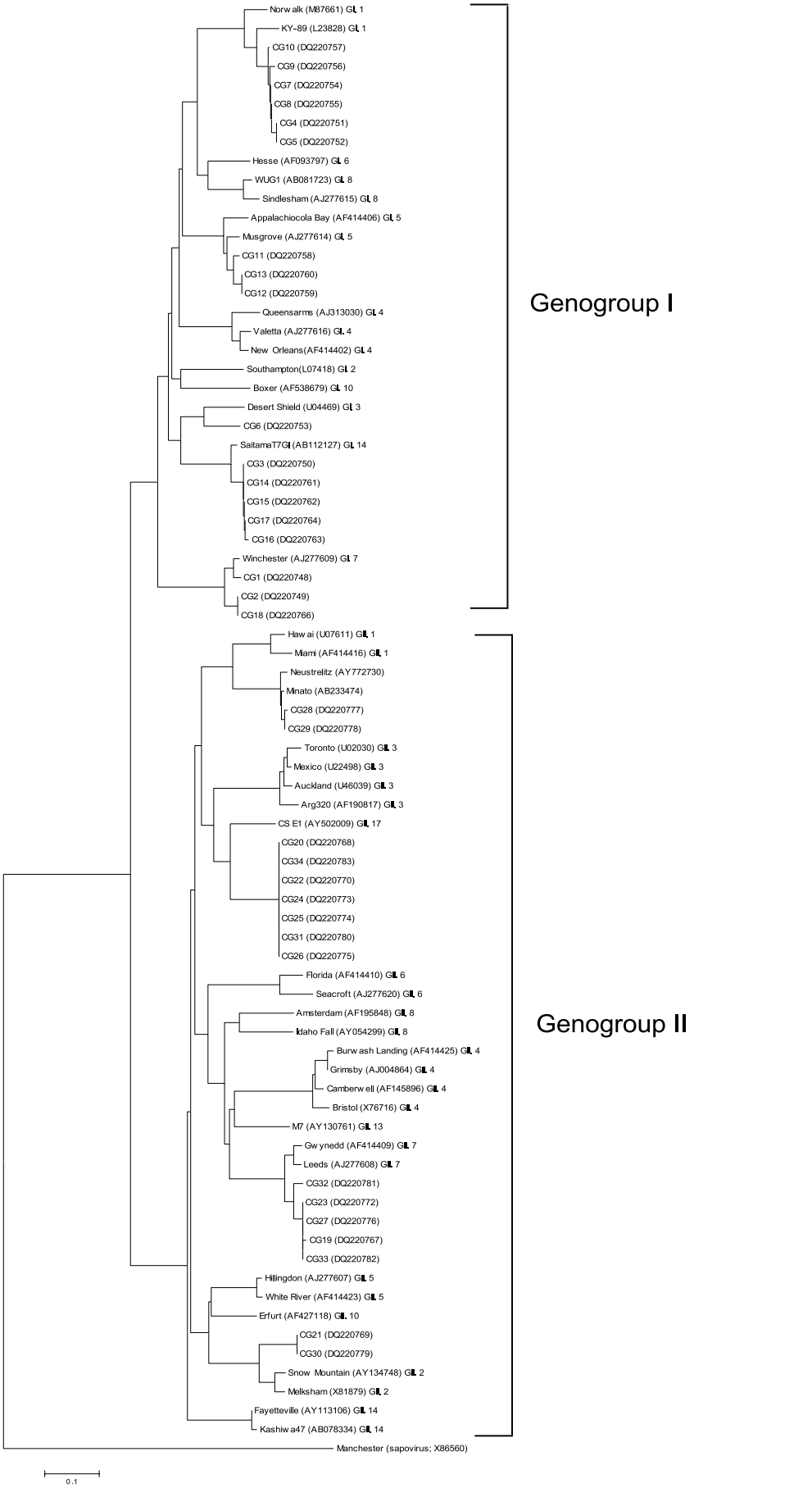
Subjects and fecal specimens. The present study was carried out using clinical information and stool specimens collected during a previous placebo-controlled clinical trial designed to evaluate the impact of vitamin A on diarrheal infections (16). Participants were residents living in the low socioeconomic periurban community of La Magdalena Atlicpac, located along the eastern perimeter of Mexico City. Two hundred children less than 2 years of age were included in this trial. Children were excluded if they were severely malnourished (weight-for-age $<$ 60% of the National Health Statistics median). Trial participants were contacted by field workers. Informed consent for study participation was obtained from parents, and the study was approved by the CONAVA (the Concejo Nacional de Vacunación, Mexico). We included 63 children who were in the placebo arm of the trial and were followed up at least for 1 month between June and August of 1998. Each child's household was visited twice each week to collect the clinical information. Stool specimens were collected twice a month as well. A total of 161 stool specimens were collected for the present study. Asymptomatic infection was defined as NoV detection in stools with no clinical symptoms (diarrhea, vomiting, or fever, etc.) for at least 8 days prior to and at least 2 days after the day of stool collection.

Identification and phylogenetic analysis of NoV in stools. NoV was detected by reverse transcriptase PCR (RT-PCR) as presented in a previous study (13). All RT-PCR products positive for NoV were purified by using the QIAquick PCR purification kit (QIAGEN, Valencia, CA) according to the manufacturer's protocol and then sequenced by using an ABI model 3730XL by a commercial company (SeqWright, Inc., Houston, TX). The nucleic acid sequence was deposited in the GenBank sequence database under accession numbers DQ22048 to DQ22083. The phylogenetic analysis was conducted by using the MegAlign program from the LASERGENE software package (version 6.0; DNAstar, Madison, WI) and MEGA version 3.0 software. A multiple alignment was created by using CLUSTAL W, and the neighbor-joining method was used for the construction of the phylogenetic tree.

Statistical methods. Frequencies and measures of the central tendency were used. Categorical and continuous variables were analyzed with a χ^2 test or the Fisher exact test (two tailed) and an unpaired *t* test, respectively. A *t* test was used to compare the mean age between the of NoV-positive and NoV-negative groups, and a χ^2 test was used to compare the sex between the NoV-positive and -negative groups. The monthly incidence was calculated by using the number of asymptomatic infections as the numerator and the total number of children followed up that month as the denominator. The statistic software used was STATA version 8.

The mean age of children studied was 14 months (range, 6 to 22 months); 56% were females. The average number of analyzed stool specimens per child was 2.5 (total of 161 stool specimens collected from 63 children). The overall rate of NoV detection in stools was 48 of 161 (29.8%). No significant dif-

* Corresponding author. Mailing address: Department of Environmental Health, School of Public Health, Seoul National University, 28 Yunkeon-Dong, Chonro-Ku, Seoul 110-799, Korea. Phone: 82-2-3668-7882. Fax: 82-2-745-9104. E-mail: gko@snu.ac.kr.


Norwalk (M87661) GI, 1
KY-89 (L23828) GI, 1
CG10 (DQ220757)
CG9 (DQ220756)
CG7 (DQ220754)
CG8 (DQ220755)
CG4 (DQ220751)
CG5 (DQ220752)
Hesse (AF093797) GI, 6
WUG1 (AB081723) GI, 8
Sindlesham (AJ277615) GI, 8
Appalachiocola Bay (AF414406) GI, 5
Musgrove (AJ277614) GI, 5
CG11 (DQ220758)
CG13 (DQ220760)
CG12 (DQ220759)
Queensarms (AJ313030) GI, 4
Valetta (AJ277616) GI, 4
New Orleans(AF414402) GI, 4
Southampton(L07418) GI, 2
Boxer (AF538679) GI, 10
Desert Shield (U04469) GI, 3
CG6 (DQ220753)
SaitamaT7GI (AB112127) GI, 14
CG3 (DQ220750)
CG14 (DQ220761)
CG15 (DQ220762)
CG17 (DQ220764)
CG16 (DQ220763)
Winchester (AJ277609) GI, 7
CG1 (DQ220748)
CG2 (DQ220749)
CG18 (DQ220766)
Genogroup I
Hawai (U07611) GII, 1
Miami (AF414416) GII, 1
Neustrelitz (AY772730)
Minato (AB233474)
CG28 (DQ220777)
CG29 (DQ220778)
Toronto (U02030) GII, 3
Mexico (U22498) GII, 3
Auckland (U46039) GII, 3
Arg320 (AF190817) GII, 3
CS E1 (AY502009) GII, 17
CG20 (DQ220768)
CG34 (DQ220783)
CG22 (DQ220770)
CG24 (DQ220773)
CG25 (DQ220774)
CG31 (DQ220780)
CG26 (DQ220775)
Florida (AF414410) GII, 6
Seacroft (AJ277620) GII, 6
Amsterdam (AF195848) GII, 8
Idaho Fall (AY054299) GII, 8
Burwash Landing (AF414425) GII, 4
Grimsby (AJ004864) GII, 4
Camberwell (AF145896) GII, 4
Bristol (X76716) GII, 4
M7 (AY130761) GII, 13
Gwynedd (AF414409) GII, 7
Leeds (AJ277608) GII, 7
CG32 (DQ220781)
CG23 (DQ220772)
CG27 (DQ220776)
CG19 (DQ220767)
CG33 (DQ220782)
Hillingdon (AJ277607) GII, 5
White River (AF414423) GII, 5
Erfurt (AF427118) GII, 10
CG21 (DQ220769)
CG30 (DQ220779)
Snow Mountain (AY134748) GII, 2
Melksham (X81879) GII, 2
Fayetteville (AY113106) GII, 14
Kashiwa47 (AB078334) GII, 14
Genogroup II
Manchester (sapovirus; X86560)
0.1

ferences in the percentage of NoV-positive stools and age ($P = 0.85$) or sex ($P = 0.78$) of the children were found.

The incidences of asymptomatic NoV infection were 0.36, 0.39, and 0.25 episodes per child in June, July, and August, respectively. A total of 31 children of 63 (49.2%) had at least one NoV stool that was positive during the study period. Eight of the thirty-one (25.8%) had two or more different strains. One child had four consecutive positive stool samples with the same strain during July and August, and this was considered as one infection when the incidence was calculated.

Of 48 (56%) NoV-positive stool specimens, 27 were positive for NoV GII and two children were positive for both strains GI and GII in the same specimen. In all, nine different genotypes were observed. Identified genotypes include five GI genotypes (GI 1, GI 3, GI 5, GI 7, and GI 14) and four GII genotypes (GII 1, GII 2, GII 7, and GII 17) (12). One of the NoV GII strains was detected during the entire 3-month period and also was the most frequently identified in seven stool samples. The remaining NoV strains were detected during 1 or 2 months. The phylogenetic tree based on the partial capsid sequences (250 bp) of NoV isolated and the reference NoV strains shows a high genetic diversity, including strains from the GI and GII genotypes (Fig. 1).

The study showed a high rate of asymptomatic NoV infection in children living in a poor periurban Mexican community. Only a few past studies focused on asymptomatic NoV infection. In a community-based study in The Netherlands, NoV was detected in fecal samples from control subjects at a rate of up to 5% (3). A recent study in Japan reported that 6% of fecal specimens collected from asymptomatic infants were infected with NoV (1). Approximately 8% of human calicivirus was detected in nondiarrhea stool specimens collected from a birth cohort of Mexican children (5). Based on enzyme-linked immunoassay and immunosorbent electron microscopy, 11 of 14 children (78.6%) infected with human calicivirus were found to be asymptomatic in a day care center study carried out in the United States (18).

In the present study, almost 30% of the stool specimens collected from asymptomatic children were infected with NoV. Almost half (49.2%) of asymptomatic children had at least one stool positive for NoV during the summer. This rate was much higher than found in other previously reported studies. The children in the present study were residents of a low-class community of the periurban area of Mexico City. The living conditions and poor hygienic measures could facilitate the transmission of enteric infections. Higher rates could be caused by other factors, such as the season or year of the study, or be related to the sensitive RT-PCR methods used.

Ideally, NoV shedding from asymptomatic children should be divided into viral shedding from a latest symptomatic NoV gastroenteritis versus primary asymptomatic NoV infection. In our study setting, children in a poor community in Mexico commonly suffered from diarrhea throughout the year. In a previous challenge study, infected adult were found to excrete NoV in their stool specimens for up to 22 days (21). In the present study, asymptomatic stool specimens were collected from children without clinical symptoms (diarrhea, vomiting, or fever, etc.) for at least 8 days prior to and at least 2 days after the sample was collected. Information as to whether the patients were symptomatic with NoV gastroenteritis prior to the summer study is not available. We cannot therefore be certain in our study whether NoV was shed from a recent symptomatic NoV gastroenteritis or whether the subjects were experiencing primary asymptomatic infections.

High rate of asymptomatic infection in the children described here is surprising since the summer is typically not the season one expects to see heavy rates of NoV infection (19). Recent studies have identified summer peaks of NoV illness (2, 17). Our finding suggests that young children could be an important reservoir for summer transmission of NoV. A recent study found high rates of asymptomatic excretion among adult patients and staff in hospital settings in England, but asymptomatic excreted NoV was not responsible for nosocomial outbreaks (6).

Previous volunteer studies indicated that a significant proportion of infected persons after NoV challenge remained asymptomatic (10). It is not understood what factors would determine the development of clinical symptoms. Recent studies suggest that human histo-blood group antigens, such as secretor factor or blood type, could determine the human susceptibility to NoV (15, 22). In addition, host immunity response, the genotypes and virulence of NoV strain, or a combination of both are likely related to host susceptibility and asymptomatic infection of NoV (9, 14, 15).

Our results are supported by a previous seroepidemiologic study that showed a high percentage of anti-norovirus antibodies in Mexican infants (85%) in 2-year-old infants, indicating widespread early life exposure to NoV in Mexico (11). Prior exposure could result in the development of protective immunity against symptomatic disease later in life. However, in that earlier study a significant association between serum antibodies and resistance to NoV infection was not found, suggesting the role for innate immunity and antigenic diversity of NoV influencing patterns of infection (15).

Our study also demonstrated a high number of NoV strains circulating in this specific community of children living in the outskirts of Mexico City. This result coincides with other stud-

FIG. 1. Phylogenetic tree construct based on partial sequences of the capsid gene of norovirus isolated in asymptomatic Mexican children. The accession numbers (DQ220748 to DQ220783) of our strains (CG1 to CG34) are indicated. The reference strains and their GenBank accession numbers were as follows: Norwalk (M87661), KY89 (L23828), WUG (AB081723), Valetta (AJ277616), Queensarms (AJ313030), Southampton (L07418), Desert Shield (U04469), Boxer (AF538679), New Orleans (AF414402), Sindleham (AJ277615), Musgrove (AJ277614), Appalachicola Bay (AF414406), Hesse (AF093797), Hawaii (U07611), Miami (AF414416), Winchester (AJ277609), CS E1 (AY502009), Saitama T7G1 (AB112127), Fayetteville (AY113106), Kashiwa47 (AB078334), White River (AF414423), Erfurt (AF427118), Snow Mountain (AY134748), Mexico (U22498), Auckland (U46039), Arg320 (AF190817), Seacroft (AJ277620), Melksham (X81879), Burwash Landing (AF414425), Grimsby (AJ004864), Camberwell (AF145896), Toronto (U02030), Bristol (X76716), M7 (AY130761), Hillingdon (AJ277607), Florida (AF414410), Leeds (AJ277608), Gwynedd (AF414409), Amsterdam (AF195848), Idaho Falls (AY054299), Minato (AB233474), Neustrelitz (AY772730), and Manchester (X86560).

ies based on NoV associated with sporadic cases of acute gastroenteritis in children (8, 20). Future studies are needed to understand the importance of asymptomatic NoV infection in children as it relates to the transmission of infection and gastroenteritis.

This study was supported by an unrestricted grant from The University of Texas–Health Science Center in Houston and Public Health Service (grant DK56338), which funds the Texas Gulf Coast Digestive Diseases Center. C.G. was supported by the Baylor-Cayetano Heredia Training Program in Global Infectious Diseases (D43TW006569).

REFERENCES

1. **Akihara, S., T. G. Phan, T. A. Nguyen, G. Hansman, S. Okitsu, and H. Ushijima.** 2005. Existence of multiple outbreaks of viral gastroenteritis among infants in a day care center in Japan. Arch. Virol. **150:**2061–2075.
2. **Boga, J. A., S. Melon, I. Nicieza, I. De Diego, M. Villar, F. Parra, and M. De Ona.** 2004. Etiology of sporadic cases of pediatric acute gastroenteritis in asturias, Spain, and genotyping and characterization of norovirus strains involved. J. Clin. Microbiol. **42:**2668–2674.
3. **de Wit, M. A., M. P. Koopmans, L. M. Kortbeek, W. J. Wannet, J. Vinje, F. van Leusden, A. I. Bartelds, and Y. T. van Duynhoven.** 2001. Sensor, a population-based cohort study on gastroenteritis in The Netherlands: incidence and etiology. Am. J. Epidemiol. **154:**666–674.
4. **Fankhauser, R. L., S. S. Monroe, J. S. Noel, C. D. Humphrey, J. S. Bresee, U. D. Parashar, T. Ando, and R. I. Glass.** 2002. Epidemiologic and molecular trends of "Norwalk-like viruses" associated with outbreaks of gastroenteritis in the United States. J. Infect. Dis. **186:**1–7.
5. **Farkas, T., X. Jiang, M. L. Guerrero, W. Zhong, N. Wilton, T. Berke, D. O. Matson, L. K. Pickering, and G. Ruiz-Palacios.** 2000. Prevalence and genetic diversity of human caliciviruses (HuCVs) in Mexican children. J. Med. Virol. **62:**217–223.
6. **Gallimore, C. I., D. Cubitt, N. du Plessis, and J. J. Gray.** 2004. Asymptomatic and symptomatic excretion of noroviruses during a hospital outbreak of gastroenteritis. J. Clin. Microbiol. **42:**2271–2274.
7. **Graham, D. Y., X. Jiang, T. Tanaka, A. R. Opekun, H. P. Madore, and M. K. Estes.** 1994. Norwalk virus infection of volunteers: new insights based on improved assays. J. Infect. Dis. **170:**34–43.
8. **Hansman, G. S., K. Katayama, N. Maneekarn, S. Peerakome, P. Khamrin, S. Tonusin, S. Okitsu, O. Nishio, N. Takeda, and H. Ushijima.** 2004. Genetic diversity of norovirus and sapovirus in hospitalized infants with sporadic cases of acute gastroenteritis in Chiang Mai, Thailand. J. Clin. Microbiol. **42:**1305–1307.
9. **Hutson, A. M., R. L. Atmar, and M. K. Estes.** 2004. Norovirus disease: changing epidemiology and host susceptibility factors. Trends Microbiol. **12:**279–287.
10. **Hutson, A. M., R. L. Atmar, D. Y. Graham, and M. K. Estes.** 2002. Norwalk virus infection and disease is associated with ABO histo-blood group type. J. Infect. Dis. **185:**1335–1337.
11. **Jiang, X., D. O. Matson, F. R. Velazquez, J. J. Calva, W. M. Zhong, J. Hu, G. M. Ruiz-Palacios, and L. K. Pickering.** 1995. Study of Norwalk-related viruses in Mexican children. J. Med. Virol. **47:**309–316.
12. **Kageyama, T., M. Shinohara, K. Uchida, S. Fukushi, F. B. Hoshino, S. Kojima, R. Takai, T. Oka, N. Takeda, and K. Katayama.** 2004. Coexistence of multiple genotypes, including newly identified genotypes, in outbreaks of gastroenteritis due to norovirus in Japan. J. Clin. Microbiol. **42:**2988–2995.
13. **Ko, G., C. Garcia, Z. D. Jiang, P. C. Okhuysen, J. Belkind-Gerson, R. I. Glass, and H. L. DuPont.** 2005. Noroviruses as a cause of traveler's diarrhea among students from the United States visiting Mexico. J. Clin. Microbiol. **43:**6126–6129.
14. **Lindesmith, L., C. Moe, J. Lependu, J. A. Frelinger, J. Treanor, and R. S. Baric.** 2005. Cellular and humoral immunity following Snow Mountain virus challenge. J. Virol. **79:**2900–2909.
15. **Lindesmith, L., C. Moe, S. Marionneau, N. Ruvoen, X. Jiang, L. Lindblad, P. Stewart, J. LePendu, and R. Baric.** 2003. Human susceptibility and resistance to Norwalk virus infection. Nat. Med. **9:**548–553.
16. **Long, K. Z., and N. Nanthakumar.** 2004. Energetic and nutritional regulation of the adaptive immune response and trade-offs in ecological immunology. Am. J. Hum. Biol. **16:**499–507.
17. **Lopman, B. A., M. Reacher, C. Gallimore, G. K. Adak, J. J. Gray, and D. W. Brown.** 2003. A summertime peak of "winter vomiting disease": surveillance of noroviruses in England and Wales, 1995 to 2002. BMC Public Health **3:**13.
18. **Matson, D. O., M. K. Estes, T. Tanaka, A. V. Bartlett, and L. K. Pickering.** 1990. Asymptomatic human calicivirus infection in a day care center. Pediatr. Infect. Dis. J. **9:**190–196.
19. **Mounts, A. W., T. Ando, M. Koopmans, J. S. Bresee, J. Noel, and R. I. Glass.** 2000. Cold weather seasonality of gastroenteritis associated with Norwalk-like viruses. J. Infect. Dis. **181**(Suppl. 2)**:**S284–S287.
20. **Phan, T. G., M. Okame, T. A. Nguyen, N. Maneekarn, O. Nishio, S. Okitsu, and H. Ushijima.** 2004. Human astrovirus, norovirus (GI, GII), and sapovirus infections in Pakistani children with diarrhea. J. Med. Virol. **73:**256–261.
21. **Rockx, B., M. De Wit, H. Vennema, J. Vinje, E. De Bruin, Y. Van Duynhoven, and M. Koopmans.** 2002. Natural history of human calicivirus infection: a prospective cohort study. Clin. Infect. Dis. **35:**246–253.
22. **Rockx, B. H., H. Vennema, C. J. Hoebe, E. Duizer, and M. P. Koopmans.** 2005. Association of histo-blood group antigens and susceptibility to norovirus infections. J. Infect. Dis. **191:**749–754.
23. **Subekti, D., M. Lesmana, P. Tjaniadi, N. Safari, E. Frazier, C. Simanjuntak, S. Komalarini, J. Taslim, J. R. Campbell, and B. A. Oyofo.** 2002. Incidence of Norwalk-like viruses, rotavirus and adenovirus infection in patients with acute gastroenteritis in Jakarta, Indonesia. FEMS Immunol. Med. Microbiol. **33:**27–33.
24. **Widdowson, M. A., A. Sulka, S. N. Bulens, R. S. Beard, S. S. Chaves, R. Hammond, E. D. Salehi, E. Swanson, J. Totaro, R. Woron, P. S. Mead, J. S. Bresee, S. S. Monroe, and R. I. Glass.** 2005. Norovirus and food-borne disease, United States, 1991–2000. Emerg. Infect. Dis. **11:**95–102.