This indicates that, in patients with a low lymphocyte count, the immunoassay could have a low sensitivity and give false-negative results. Indeed, three negative QFT-IT subjects had active TB with a CD4 cell count of 17, 95 and 19 cells/μL, respectively.

Among QFT-IT negative subjects (CD4 cell count >200 cells/μL), three had active TB, their CD4 cell count was >400 cells/μL, and the percentage of lymphocytes in two cases was >15%, whereas, in one patient, it was 10%. Thus, it is very important to assess not only CD4 cell counts, but also their percentage in the total population because a low percentage may lead to false-negative results. In the two subjects with a high count and a high percentage of lymphocytes, the negative QFT-IT response may not be a result of HIV but, instead, active TB; several studies have been shown that, in immunocompetent individuals with active TB, QFT-IT has a low sensitivity [10].

Patients with active TB and negative QFT-IT (both CD4< and >200 cells/μL) were in stage C3 of HIV infection, and this factor may have led to the failure of the immunological assay. As HIV infection progresses, there is a switch from the cellular to the humoural immune response with a reduction in the production of interferon-γ [11].

A limitation of the present study is the limited number of subjects with active TB who were tested, although our findings confirm the previously published data [9]. The results obtained in the present study indicate that the interferon-γ assay was affected by the CD4 cell count and percentage, as well as by the stage of HIV infection. It would be very useful to establish threshold values with regard to the CD4 parametres below which the immunoassay may not be sensitive and give false-negative or indeterminate results.

Transparency Declaration

The authors declare that there is no financial involvement with the producer of QuantiFERON-TB Gold kit used in this study.

References

1. Chin DP, Osmond D, Page-Shafer K *et al.* Reliability of anergy skin testing in persons with HIV infection. The pulmonary Complications of HIV Infection Study Group. *Am J Respir Crit Care Med* 1996; 153: 1982–1984.
2. Pai M, Kalantri S, Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis: part I. Latent tuberculosis. *Expert Rev Mol Diagn* 2006; 6: 413–422.
3. Harada N, Higuchi K, Yoshiyama T *et al.* Comparison of the sensitivity and specifi city of two whole blood interferon-gamma assays for *M. tuberculosis* infection. *J Infect* 2008; 56: 348–353.
4. Katiyar SK, Sampath A, Bihari S, Mamtani M *et al.* Use of the QuantiFERON-TB Gold In-Tube test to monitor treatment efficacy in active pulmonary tuberculosis. *Int J Tuberc Lung Dis* 2008; 2: 1146–1152.
5. Mori T, Sakatani M, Yamagishi F *et al.* Specific detection of tuberculosis infection: an interferon-gamma-based assay using new antigens. *Am J Respir Crit Care Med* 2004; 170: 59–64.
6. Bua A, Molicotti P, Delogu G *et al.* QuantiFERON TB Gold: a new method for latent tuberculosis infection. *New Microbiol* 2007; 30: 477–480.
7. Palazzo R, Spensieri F, Massari M *et al.* Use of whole-blood samples in in-house bulk and singlecell antigen-specific gamma interferon assays for surveillance of *Mycobacterium tuberculosis* infections. *Clin Vaccine Immunol* 2008; 15: 327–337.
8. Mazurek GH, Zajdowicz MJ, Hankinson AL *et al.* Detection of *Mycobacterium tuberculosis* infection in United States Navy recruits using the tuberculin skin test or whole-blood interferon gamma release assays. *Clin Infect Dis* 2007; 45: 826–836.
9. Raby E, Moyo M, Devendra A, Banda J, De HP *et al.* The effects of HIV on the sensitivity of a whole blood IFN-gamma release assay in Zambian adults with active tuberculosis. *PLoS ONE* 2008; 3: e2489.
10. Dewan PK, Grinsdale J, Kawamura LM. Low sensitivity of a whole-blood interferon-gamma release assay for detection of active tuberculosis. *Clin Infect Dis* 2007; 44: 69–73.
11. Clerici M, Shearer GM. A TH1 → TH2 switch is a critical step in the etiology of HIV infection. *Immunol Today* 1993; 14: 107–111. Review.

Molecular epidemiology and genetic diversity of human astrovirus in South Korea from 2002 to 2007

A. Y. Jeong[1], H. S. Jeong[1], M. Y. Jo[1], S. Y. Jung[1], M. S. Lee[1], J. S. Lee[1]*, Y. M. Jee[1], J.-H. Kim[2] and D.-S. Cheon[1]

1) *Division of Enteric and Hepatitis Viruses, Center for Infectious Diseases, National Institute of Health, Korea Centers for Disease Control and Prevention, Seoul* and 2) *Department of Pediatrics, College of Medicine, The Catholic University of Korea, St Vincent Hospital, Suwon, South Korea*

Abstract

The present study was conducted to survey the prevalence and genotypic distribution of human astrovirus (HAstV) circulating in South Korea. Of 160,027 patients with acute gastroenteritis, 2,057 (1.3%) were positive for HAstV antigen. We determined the genotypes of 187 HAstV strains collected from laboratories across the country. Genetic analysis revealed genotype 1 to be the most prevalent, accounting for 72.19% of the strains, followed by genotypes 8 (9.63%), 6 (6.95%), 4 (6.42%), 2 (3.21%) and 3 (1.60%). Our findings indicate that HAstV is less common but, even so, a potentially important viral agent of gastroenteritis

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TABLE 1. Yearly distribution of human astrovirus (HAstV) genotypes detected in cases of acute gastroenteritis in Korea, from 2002 to 2007

	Number (%) of HAstV strains of each genotype according to year						
Genotype	2002 (*n* = 12)	2003 (*n* = 67)	2004 (*n* = 55)	2005 (*n* = 20)	2006 (*n* = 15)	2007 (*n* = 18)	Total (*n* = 187)
HastV 1	11 (91.67)	51 (76.12)	38 (69.09)	12 (60.00)	13 (86.67)	10 (55.55)	135 (72.19)
1a[a]	**11 (91.67)**	**48 (71.64)**	**38 (69.09)**	**12 (60.00)**	**8 (53.33)**	**6 (33.33)**	123 (65.78)
1b	0 (0)	2 (2.99)	0 (0)	0 (0)	1 (6.67)	2 (11.11)	5 (2.67)
1d	0 (0)	1 (1.49)	0 (0)	0 (0)	4 (26.67)	2 (11.11)	7 (3.74)
HAstV 2 (2a)	1 (8.33)	3 (4.48)	0 (0)	0 (0)	0 (0)	2 (11.11)	6 (3.21)
HAstV 3	0 (0)	0 (0)	0 (0)	0 (0)	1 (6.67)	2 (11.11)	3 (1.60)
HAstV 4 (4a)	0 (0)	3 (4.48)	8 (14.55)	1 (5.00)	0 (0)	0 (0)	12 (6.42)
HAstV 6	0 (0)	2 (2.99)	4 (7.27)	5 (25.00)	0 (0)	2 (11.11)	13 (6.95)
HAstV 8	0 (0)	8 (11.94)	5 (9.09)	2 (10.00)	1 (6.67)	2 (11.11)	18 (9.63)

[a]Statistically significant (p <0.01) changes in the genotype distribution from year to year are shown in bold.

in South Korea, with significant genetic diversity among circulating HAstV strains.

Keywords: Astrovirus, epidemiology, gastroenteritis, genotype

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Corresponding author: D.-S. Cheon, Division of Enteric and Hepatitis Viruses, Center for Infectious Diseases, National Institute of Health, 5 Nokbun-dong, Eunpyung-gu, Seoul 122-701, Korea

E-mail: cheonds@hanmail.net

*Present address: Department of Microbiology, National Institute of Food and Drug Safety Evaluation, Korea Food and Drug Administration, Seoul, Korea.

Human astroviruses (HAstV) are recognized as a common cause of infantile acute gastroenteritis worldwide [1]. HAstV, along with rotaviruses and caliciviruses, are considered important viral agents of acute gastroenteritis [2]. HAstV are small, single-stranded RNA viruses of 28–34 nm in length, belonging to the recently described family *Astroviridae* [3]. Their genome is 6.8–7.3 kb in length and contains three overlapping open reading frames (ORFs) [4,5]. ORF1a and ORF1b encode the nonstructural serine protease and RNA polymerase proteins, respectively [6]. ORF2 encodes the capsid precursor. A 348-bp segment located between nucleotides 258 and 606 of full-length ORF2 has commonly been used for genotyping HAstV in previous studies, and eight serotypes of HAstV have been identified. Typing surveys indicate that genotype 1 (HAstV-1) is the most prevalent, genotypes 2–4 are common, and genotypes 5–7 are relatively uncommon; genotype 8 has only recently been identified [1,7–12]. The present study summarizes the results of a 6-year period of nationwide surveillance in South Korea (hereafter referred to as 'Korea') conducted to determine the epidemiological prevalence of HAstV gastroenteritis and characterize the HAstV genotypes circulating across the country.

The Korean Center for Disease Control and Prevention, in collaboration with 16 laboratories of local Public Health Institutes and more than 100 sentinel hospital participants, initiated a viral agent surveillance system for acute gastroenteritis in 1999. Faecal samples submitted for investigation of suspected acute infectious gastroenteritis and tested positive for HAstV antigen were collected for genotyping. From January 2002 to December 2007, faecal samples from 160 027 patients with acute gastroenteritis were investigated. A total of 2057 samples (1.3%) (detection rate per year, 0.6–2.4%) were identified as positive for HAstV by ELISA (IDEIA HAstV, Dako Ltd, Ely, UK).

By performing RT-PCR, we determined the genotypes of 187 HAstV strains received from multiple laboratories around the country. The faecal samples were diluted 1:10 in phosphate-buffered saline (pH 7.2), vortexed, and cleared by centrifugation. Supernatants were collected and stored at −70°C until use. Total RNA was extracted from 25 μL of supernatant samples by using TRIZOL reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. For the detection of HAstV, an RT-PCR assay was carried out using primers Mon 269 and Mon 270 directed against the ORF2 region, as described by Noel *et al.*

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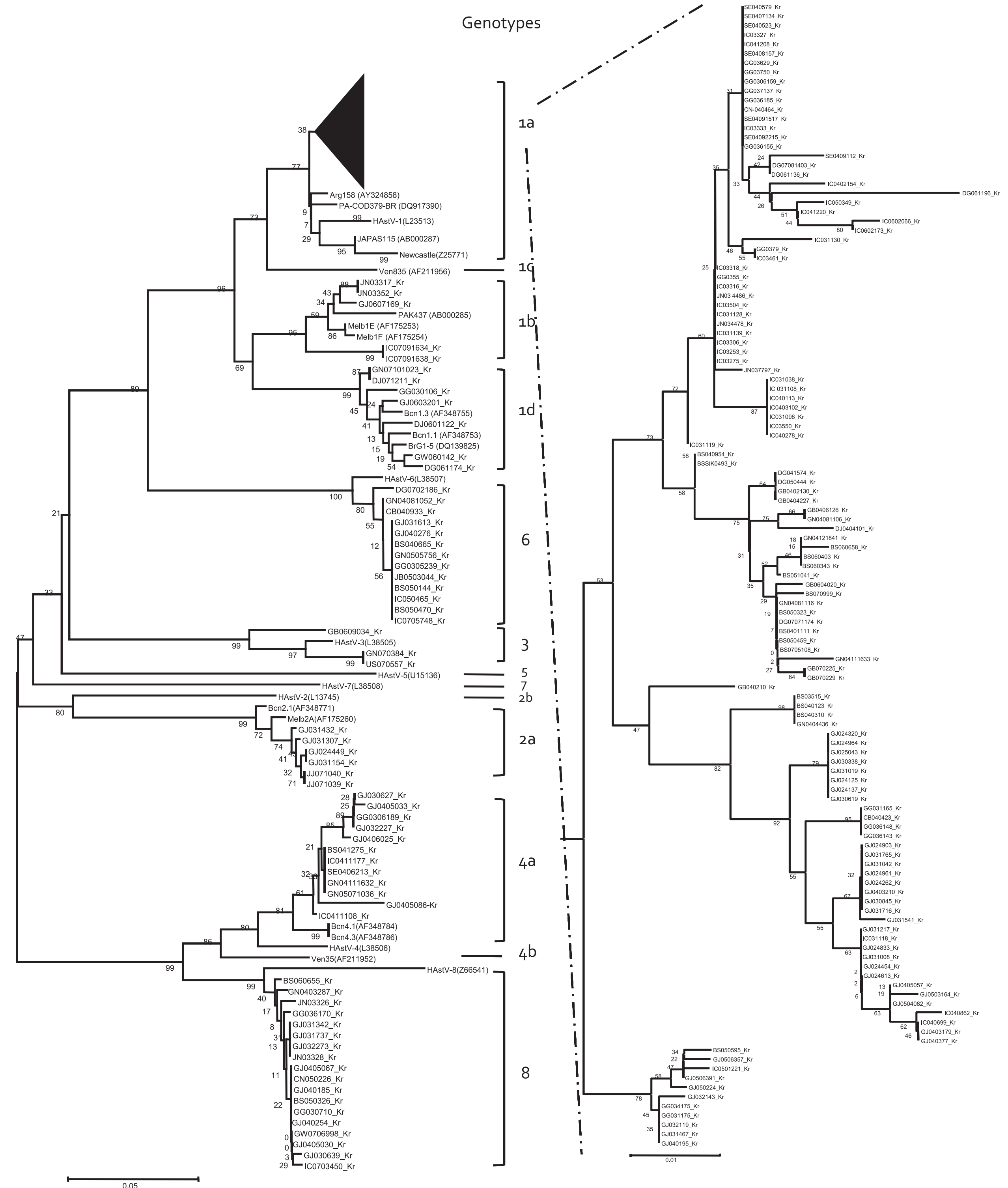

FIG. 1. Phylogenetic tree of human astrovirus (HAstV) nucleotide sequences. Neighbour-joining phylogenetic tree based on nucleotide sequences of the capsid region of the HAstV genome. The numbers in the branches indicate the bootstrap values. Reference strains of HAstV selected from GenBank are indicated by accession numbers. The scale indicates nucleotide substitutions per position.

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[13]. All PCR products were sequenced using an ABI Prism 3100 automated sequencer and Big Dye Terminator Cycle Sequencing Mix (Perkin Elmer, Wellesley, MA). The 348-bp HAstV RT-PCR product was sequenced and compared to reference strains by using BLAST software (National Center for Biotechnology Information, Bethesda, MD, USA) to assign a genotype. Multiple sequence alignments were calculated using CLUSTAL X software [14], and, on the basis of different HAstV sequences available from GenBank, a phylogenetic analysis was conducted with MEGA, version 3.1 [15], using the Neighbour-joining method.

We detected six genotypes of HAstV: 1, 2, 3, 4, 6 and 8. As shown in Table 1, HAstV-1 was the dominant genotype in every year investigated. The overall distribution of the genotypes of the 187 HAstV strains detected in clinical specimens and characterized was as: HAstV-1, 72.19% (n = 135); HAstV-8, 9.63% (n = 18); HAstV-6, 6.95% (n = 13); HAstV-4, 6.42% (n = 12); HAstV-2, 3.21% (n = 6); and HAstV-3, 1.60% (n = 3). Previous studies have reported that HAstV-1 is also the predominant HAstV genotype in Egypt, Italy, France, China and Spain [8,11,16–18]. However, our findings regarding the relative prevalence of other genotypes differ from some of these previous investigations [8,11]. In Mexico, the prevalence of HAstV-1 was low (10%) compared to genotypes 2 (42%), 4 (23%), 3 (13%), 5 (6%) and 7 (6%) [19], and a recent study in Madagascar reported a high prevalence of unusual type 8 strains [20].

Phylogenetic analysis indicated that HAstV-1 strains could be classified into four lineages (1a–1d), with the majority of Korean HAstV strains clustered into lineage 1a (65.78%). In 2002, 91.67% of HAstV-1 strains were type 1a, although this prevalence significantly decreased from year to year, reaching 33% in 2007 ($p < 0.01$). Studies conducted in Spain, Germany, Brazil, Vietnam, Japan and China have indicated that HAstV-1d is the predominant type in these countries [14]. In the present study, HAstV-1d was first detected in 2003 and exhibited a peak prevalence of 26.67% in 2006. Phylogenetic analysis was also used to evaluate the relatedness of HAstV strains detected in the present study to reference strains (Fig. 1). Nucleotide comparison revealed that Korean HAstV strains showed homology of 85.9–100% to reference HAstV-1 isolates, 98.9–100% to HAstV-2 isolates, 93.1–99.7% to HAstV-3 isolates, 90.2–100% to HAstV-4 isolates, 98.6–100% to HAstV-6 isolates and 98.6–100% to HAstV-8 isolates. Homology between the Korean strains and other HAstV strains in lineage 1a was in the range 92.5–100%.

This is the first report on a large-scale nationwide surveillance of the prevalence and molecular characterization of HAstV strains circulating in Korea. The findings of the present study clearly indicate that HAstV is less common but, even so, a potentially important viral aetiological agent with a significant role in acute gastroenteritis in Korea. The nucleotide analysis data obtained in the present study regarding the characteristics of HAstV strains may be valuable in the development of a HAstV vaccine.

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Transparency Declaration

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References

1. Glass RI, Noel J, Mitchell D *et al.* The changing epidemiology of astrovirus-associated gastroenteritis: a review. *Arch Virol Suppl* 1996; 12: 287–300.
2. Walter JE, Mitchell DK. Astrovirus infection in children. *Curr Opin Infect Dis* 2003; 16: 247–253.
3. Monroe SS, Jiang B, Stine SE, Koopmans M, Glass RI. Subgenomic RNA sequence of human astrovirus supports classification of *Astroviridae* as a new family of RNA viruses. *J Virol* 1993; 67: 3611–3614.
4. Jiang B, Monroe SS, Koonin EV, Stine SE, Glass RI. RNA sequence of astrovirus: distinctive genomic organization and a putative retrovirus-like ribosomal frameshifting signal that directs the viral replicase synthesis. *Proc Natl Acad Sci USA* 1993; 15: 10539–10543.
5. Monceyron C, Grinde B, Jonassen TO. Molecular characterisation of the 3′-end of the astrovirus genome. *Arch Virol* 1997; 142: 699–706.
6. Guix S, Bosch A, Pinto RM. Human astrovirus diagnosis and typing: current and future prospects. *Lett Appl Microbiol* 2005; 41: 103–105.
7. Gaggero A, O'Ryan M, Noel JS *et al.* Prevalence of astrovirus infection among Chilean children with acute gastroenteritis. *J Clin Microbiol* 1998; 36: 3691–3693.
8. Guix S, Caballero S, Villena C *et al.* Molecular epidemiology of astrovirus infection in Barcelona, Spain. *J Clin Microbiol* 2002; 40: 133–139.
9. Mendez-Toss M, Romero-Guido P, Munguia ME, Mendez E, Arias CF. Molecular analysis of a serotype 8 human astrovirus genome. *J Gen Virol* 2000; 81: 2891–2897.
10. Mustafa H, Palombo EA, Bishop RF. Epidemiology of astrovirus infection in young children hospitalized with acute gastroenteritis in Melbourne, Australia, over a period of four consecutive years, 1995 to 1998. *J Clin Microbiol* 2000; 38: 1058–1062.
11. Naficy AB, Rao MR, Holmes JL *et al.* Astrovirus diarrhea in Egyptian children. *J Infect Dis* 2000; 182: 685–690.
12. Sakamoto T, Negishi H, Wang QH *et al.* Molecular epidemiology of astroviruses in Japan from 1995 to 1998 by reverse transcription-

©2010 University of Sassari

polymerase chain reaction with serotype-specific primers (1 to 8). *J Med Virol* 2000; 61: 326–331.

13. Noel JS, Lee TW, Kurtz JB, Glass RI, Monroe SS. Typing of human astroviruses from clinical isolates by enzyme immunoassay and nucleotide sequencing. *J Clin Microbiol* 1995; 33: 797–801.
14. Nguyen TA, Hoang L, Pham le D *et al.* Identification of human astrovirus infections among children with acute gastroenteritis in the Southern Part of Vietnam during 2005–2006. 2008; 50: 298–305.
15. Kumar S, Tamura K, Nei M. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* 2004; 5: 150–163.
16. Galdiero E, Marinelli A, Pisciotta MG, Pagliara I, Di Monteforte ES, Liguori G. Reverse transcriptase-PCR for the detection of Astrovirus in children with nosocomial diarrhea in Naples, Italy. *Med Mal Infect* 2005; 35: 213–217.
17. Liu MQ, Yang BF, Peng JS *et al.* Molecular epidemiology of astrovirus infection in infants in Wuhan, China. *J Clin Microbiol* 2007; 45: 1308–1309.
18. Medina SM, Gutierrez MF, Liprandi F, Ludert JE. Identification and type distribution of astroviruses among children with gastroenteritis in Colombia and Venezuela. *J Clin Microbiol* 2000; 38: 3481–3483.
19. Walter JE, Mitchell DK, Guerrero ML *et al.* Molecular epidemiology of human astrovirus diarrhea among children from a periurban community of Mexico City. *J Infect Dis* 2001; 183: 681–686.
20. Papaventsis DC, Dove W, Cunliffe NA *et al.* Human astrovirus gastroenteritis in children, Madagascar, 2004–2005. *Emerg Infect Dis* 2008; 14: 844–846.

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