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Astrovirus as a Cause of Gastroenteritis in Japan

ETSUKO T. UTAGAWA,[1]* SHUICHI NISHIZAWA,[2] SEIJI SEKINE,[3] YUKINAO HAYASHI,[3] YUICHI ISHIHARA,[4] ISAO OISHI,[5] AKIRA IWASAKI,[6] IKUTAKA YAMASHITA,[7] KIKUKO MIYAMURA,[1] SHUDO YAMAZAKI,[1] SAKAE INOUYE,[1] AND ROGER I. GLASS[8]

National Institute of Health, Toyama, Shinjuku-ku, Tokyo 162,[1] Nagano Research Institute for Public Health and Pollution, Amori, Nagano-shi, Nagano 380,[2] Tokyo Metropolitan Research Laboratory of Public Health, Hyakunin-cho, Shinjuku-ku, Tokyo 163,[3] Aichi Prefectural Institute of Public Health, Tsujimachi, Kita-ku, Nagoya-shi, Aichi 462,[4] Osaka Prefectural Institute of Public Health, Nakamichi, Higashinari-ku, Osaka 537,[5] Yamaguchi Prefectural Research Institute of Health, Aoi, Yamaguchi-shi, Yamaguchi 753,[6] Ehime Prefectural Institute of Public Health, Sanban-cho, Matsuyama-shi, Ehime 790,[7] Japan, and Viral Gastroenteritis Section, Centers for Disease Control and Prevention, Atlanta, Georgia 30333[8]

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We used an enzyme immunoassay (EIA) to screen for astrovirus in stool specimens from outbreaks and sporadic cases of gastroenteritis collected between 1982 and 1992 in six prefectural public health institutes in Japan. Three outbreaks of gastroenteritis involving schoolchildren and adults were confirmed to be attributable to astrovirus. Astrovirus was detected in 6 to 10% of the specimens from patients with sporadic gastroenteritis from whom no other bacterial or viral agent had been identified. Among the sporadic cases, astrovirus was most frequently detected in infants less than 1 year of age, and the incidence peaked in March and April. Using specimens from recent outbreaks, we found that the EIA was more sensitive than electron microscopy (EM) for the detection of astrovirus, and many EM-negative specimens were positive by EIA. However, some stool specimens previously found to have astrovirus-like particles by EM were negative by EIA, perhaps because of inadequate storage conditions, such as long-term storage and repeated freezings and thawings. Our results indicate that astrovirus is more commonly associated with childhood gastroenteritis than has been previously appreciated and suggest that further studies to examine the epidemiology and disease burden of this virus are needed.

Rotavirus and enteric adenoviruses constitute the predominant enteric pathogens currently detected among children with acute, nonbacterial gastroenteritis. Besides these agents, small round-structured viruses (SRSVs), such as Norwalk virus and the Snow Mountain, Hawaii, and Taunton agents, have been described in association with outbreaks of gastroenteritis (11) and may cause sporadic cases as well. The epidemiology of gastroenteritis associated with rotavirus and adenoviruses has been well studied because diagnostic reagents for their detection are widely available. However, epidemiologic studies of diverse types of SRSVs have been hampered by difficulties in diagnosis because of their small size, low levels of shedding of SRSVs in feces, failure or difficulty in growing them in cell culture, and the lack of suitable reagents and assays for their detection. Although an etiologic agent cannot be found for a majority of cases of gastroenteritis, we hypothesize that many of these episodes may be attributable to the SRSVs.

Astrovirus is one of the SRSVs. It was first observed by electron microscopy (EM) in 1975 (1, 16) in stool specimens from infants with gastroenteritis. The virus is characterized by a distinct star-like ultrastructure on the surface and a clear margin that forms a distinct rim. Five human serotypes of astrovirus sharing a group-specific antigen have been identified (13). Until recently, astrovirus could be detected only by EM. The virus was subsequently propagated in cell culture by Lee and Kurtz (14) and Willcocks et al. (25), but this method was inefficient for routine use. Consequently, the prevalence of astrovirus-associated gastroenteritis has not been established.

* Corresponding author. Mailing address: Department of Epidemiology, National Institute of Health, Toyama 1-23-1, Shinjuku-ku, Tokyo 162, Japan. Phone: 81(3)5285-1111. Fax: 81(3)5285-1177.

Cultivation of astrovirus facilitated the development of immunologic reagents, including polyclonal antisera and a group-specific monoclonal antibody (8), that have been combined for use in an indirect double-antibody enzyme immunoassay (EIA) for astrovirus detection (18). Use of this new EIA for epidemiologic surveys has demonstrated that astrovirus is the cause of a substantial percentage of the cases of viral gastroenteritis in institutions with infants and young children (2, 15, 17, 18, 21). The method is more sensitive than EM, is very specific, and is well suited for screening large numbers of stool samples. The recent cloning and sequencing of astrovirus have led to its classification in a new family of single-stranded RNA viruses, the *Astroviridae*, and have provided molecular approaches for the detection of astrovirus by using probes and PCR (7, 10, 18, 20).

In Japan, Konno et al. (12) first described an outbreak of astrovirus infections among the children and staff of a kindergarten. Recently, one remarkable large outbreak occurred in Osaka Prefecture in 1991, in which more than 4,000 schoolchildren, teachers, and cooking staff were affected. In that outbreak, the causative agent was identified as astrovirus by EM, PCR, and EIA (23). Other outbreaks of astrovirus-associated gastroenteritis have been diagnosed by EM examination of stool specimens from patients (Table 1). In addition, sporadic cases of diarrhea attributable to SRSVs have been reported each year from virus laboratories of the prefectural public health institutes (PHIs) participating in the Infectious Agents Surveillance Program in Japan (22). In these surveys, which used EM for virus detection, astroviruses often could not be distinguished from other SRSVs. Because this method is relatively insensitive, the etiologic agents could not be detected from a majority of the stool specimens examined in those laboratories.

TABLE 1. Astrovirus-associated gastroenteritis outbreaks in Japan, 1989 to 1992

Outbreak no.	Date (yr, mo)	Prefecture	Suspected vehicle	Patients			Specimen collection (day)[a]	Tests for astrovirus[b]	
				Group	No. ill	Attack rate (%)		EM	EIA
1	1989, Dec.	Nagano	Meal at restaurant	Dinner guest	22	67	2–4	2/9	3/7[c]
2	1991, Dec.	Yamaguchi	School lunch	Children and staff	1,419	62	4–8	0/18	6/28
3	1992, Feb.	Nagano	Meal at lodging house	High-school students	45	66	4–8	1/6	6/6

[a] Days after the onset of illness.
[b] No. of specimens positive/total no. tested
[c] EM-negative specimens were tested.

In the present study we used the new EIA (18) to investigate the epidemiology of astrovirus diarrhea in Japan and to examine the disease burden of astrovirus compared with that reported in previous studies in which EM was the only detection method available. We report here the results of astrovirus detection by EIA in stool samples from patients with gastroenteritis collected between 1982 and 1992 in five PHIs in Japan.

MATERIALS AND METHODS

Stool specimens. Stool specimens were collected from three groups of patients with gastroenteritis. The first group (Table 1) included a total of 41 specimens from three outbreaks that occurred in two prefectures between 1989 and 1992; two were associated with meals served at a restaurant and a lodging house, and the other one was a large outbreak in a school that was suspected of being caused by contaminated lunches. The second group (Table 2) was obtained from two PHIs and included 165 specimens that were randomly selected from patients with sporadic cases of gastroenteritis between 1982 and 1991 in whom no bacterial or viral pathogen had previously been detected. The third group (Table 3) included 49 specimens from patients with sporadic cases of gastroenteritis who were screened previously in two PHIs and found to have astrovirus by EM. All stool specimens (undiluted) or 10% suspensions of stool specimens in phosphate-buffered saline (PBS) had been stored at −20 or −70°C since the time of collection.

EIA. Fecal specimens were tested for astrovirus by using the biotin-avidin EIA prepared at the Centers for Disease Control and Prevention (18). In brief, a group-reactive monoclonal antibody (8E7) (8) was used for capture, and biotinylated immunoglobulin G (IgG) from hyperimmune rabbit antiserum to astrovirus type 2 (CDC 909) was used as the detector. Antiadenovirus hexon monoclonal antibody (AD2/8) was included in the test as a control capture antibody. Microtiter wells were coated in duplicate with the capture antibodies (diluted in carbonate-bicarbonate buffer [pH 9.6]) for 2 h at 37°C before they were washed with PBS–Tween 20 (0.05%). The 10% stool suspension (clarified by low-speed centrifugation) was added to the wells for 2 h at 37°C, and the wells were washed with PBS–Tween 20. Fifty microliters of the biotinylated detector (about 2 μg of protein per ml in PBS with 0.05% gelatin and 0.15% Tween 20 [PBS-GT]) was added to each well, and the wells were incubated for 1.5 h at 37°C. After the wells were washed with PBS–Tween 20, 50 μl of a 1:5,000 dilution of streptavidin-horseradish peroxidase conjugate (Kirkegaard & Perry Laboratories, Gaithersburg, MD) in PBS-GT was added to each well, and the wells were incubated for 30 min at 37°C. ABTS [2,2′-azino-di-(3-ethyl-benzthiazoline sulfonate); Kirkegaard & Perry Laboratories] and hydrogen peroxide were used for color development. After 10 min, the reaction was stopped with 5% sodium dodecyl sulfate in water and the A_{405} of the solution was measured in a plate reader spectrophotometer (Titertek Multiskan Plus; Flow Laboratories, Tokyo, Japan).

Samples were considered positive when $P - N$ was ≥ 0.07 and P/N was ≥ 2, where P is the average absorbance of two test wells (wells coated with 8E7) and N is the average absorbance of the two control wells (wells coated with antiadenovirus

TABLE 2. Astrovirus detection by EIA from sporadic cases of gastroenteritis with nonbacterial, nonrotavirus, nonadenovirus etiology in Japan, 1982 to 1991

Year	Nagano PHI		Aichi PHI	
	No. tested	No. (%) positive	No. tested	No. (%) positive
1982	2	0	10	0
1983	10	0	10	2 (20)
1984	4	0	10	0
1985	32	3 (9)	10	0
1986	7	1 (14)	10	0
1987	3	0	10	3 (30)
1988	3	0	10	4 (40)
1989	4	0	10	0
1990	—[a]	—	10	0
1991	—	—	10	1 (10)
Total	65	4 (6)	100	10 (10)

[a] —, no specimen.

TABLE 3. Astrovirus detection by EIA from EM-positive specimens in Japan, 1982 to 1991

Year	Tokyo PHI		Ehime PHI	
	No. tested	No. (%) positive	No. tested	No. (%) positive
1982	1	1 (100)	—[a]	—
1983	12	7 (58)	—	—
1984	1	1 (100)	—	—
1985	3	2 (67)	1	0 (0)
1986	—	—	2	1 (50)
1987	—	—	4	2 (50)
1988	—	—	6	2 (33)
1989	—	—	7	4 (57)
1990	—	—	—	—
1991	12	9 (75)	—	—
Total	29	20 (69)	20	9 (45)

[a] —, no specimen.

TABLE 4. Age distributions of astrovirus-positive subjects among patients with sporadic cases of gastroenteritis

Prefecture	No. positive among those ages (yr):																Total no. tested
	Total	<1	1	2	3	4	5	6	7	8	9	10–19	20–29	30–39	40–49	50–	UK[a]	
Nagano	4							1					1			1	1	65
Aichi	10	2	1	2		1	1	1				2						100
Tokyo	20			1	1		2	1	1	1	1		1				11	29
Ehime	9	5	1		2	1												20
Total positive	43	7	2	3	3	2	3	3	1	1	1	2	2			1	12	
Total tested		53	26	21	9	8	8	6	6	5	1	12	14	1	1	12	31	214

[a] UK, unknown.

monoclonal antibody). When the paired positive or negative wells did not behave uniformly, the sample was retested.

RESULTS

Detection of astrovirus from patients in three gastroenteritis outbreaks. Forty-one stool specimens collected from patients in three gastroenteritis outbreaks suspected of being associated with astrovirus were tested for astrovirus by EIA (Table 1). Previous testing of these specimens failed to identify a bacterial pathogen, rotavirus, or adenovirus. Clinical and epidemiologic observations suggested a viral cause, and EM examination of some specimens indicated the presence of SRSVs that were thought to be astrovirus.

In the first outbreak, 22 (67%) of 33 people who dined at a restaurant in Nagano Prefecture developed gastroenteritis, with a median incubation interval of 33 h. Symptoms included vomiting (46%), diarrhea (73%), fever (46%), and abdominal pain (36%). The median incubation period was 36 h, and the median duration of illness was 72 h. Two of nine stool specimens from these patients had SRSV-like particles by EM, and these two specimens were used up during the examination. The remaining seven EM-negative specimens were tested by EIA, and three were positive for astrovirus.

The second outbreak occurred in Yamaguchi Prefecture and was probably caused by a contaminated school lunch, similar to the outbreak in Osaka Prefecture (23). In that outbreak (n = 1,419 people), the median incubation period was 44 h and the median duration of illness was 72 h. Predominant clinical features of the patients were vomiting (70%), abdominal pain (65%), fever (64%), and diarrhea (40%). Twenty-eight stool specimens were obtained from 22 kitchen staff and 6 schoolchildren; of these, 6 specimens were positive for astrovirus by EIA, including 2 of 18 specimens that were EM negative and 4 of 10 specimens that were not examined by EM. Only six specimens from children were available, and all were negative by both EM and EIA.

In the third outbreak, 45 of 68 high-school students became ill within 24 h after eating at a ski lodge. The clinical symptoms were similar to those seen in the other outbreaks: vomiting (78%), fever (73%), diarrhea (69%), and abdominal pain (69%). Of six specimens available from the third outbreak, only one was positive for astrovirus by EM but all were positive for astrovirus by EIA, including two samples obtained 8 days after the onset of illness. These two specimens were obtained late after the students returned home from the ski resort.

Detection of astrovirus from sporadic cases of gastroenteritis. Stool specimens from patients with sporadic cases of gastroenteritis in which no bacterial pathogen, rotavirus, or adenovirus was detected were tested for astrovirus by EIA (Table 2). These specimens were collected by two PHIs from 1982 to 1991 and were stored at −20 or −70°C until tested. In the Aichi PHI, a total of 1,720 specimens of this category were collected during this period and 10 specimens from each year were randomly selected for testing. Of the 100 specimens tested by EIA, 10 (10%) were positive for astrovirus. In the Nagano PHI, 65 specimens were examined by EIA, of which 10 had been SRSV-positive by EM, including 1 that was considered to contain an "astrovirus-like" particle. Four specimens (6%) were positive for astrovirus, including two that were EM negative, one in which an SRSV was detected, and one in which an astrovirus-like SRSV was detected.

Results of EIA of EM-positive specimens. Stool specimens from two PHIs previously determined to contain astrovirus by EM were tested by EIA (Table 3). Twenty (69%) of 29 EM-positive specimens in the Tokyo PHI and 9 (45%) of 20 EM-positive specimens in the Ehime PHI were positive by EIA.

Age and seasonal distributions. The age distributions of the patients with sporadic cases of astrovirus-associated diarrhea were examined for the 31 patients for whom ages were available (Table 4). Astrovirus was found primarily in the young; 7 patients (23%) were infants (<1 year old), 10 patients were 1 to 4 years of age, and 9 patients were 5 to 9 years of age. Five cases occurred in older children and adults; the oldest patient was 53 years of age. The seasonal distributions of astrovirus from sporadic cases indicated a peak in March and April (65% of all cases) (Table 5). Sporadic cases occurred from November to May (one to five cases), but no case was detected from June to October.

DISCUSSION

The study described here is the first to examine the epidemiology of astrovirus in outbreaks and sporadic cases of gastroenteritis in Japan. Several characteristic features of astrovirus diarrhea are apparent. First, large outbreaks of gastroenteritis caused by astrovirus have occurred in Japan (Table 1) (23), an observation not reported previously from other countries; this is in contrast to the association of Norwalk-like agents with outbreaks of gastrointestinal illness, which are relatively common (11). Second, we found astrovirus infection to be more common after the rotavirus season in the late winter and spring (March and April), which is consistent with other reports (5, 6, 19). Third, we found that sporadic cases of astrovirus-associated diarrhea were most common in infants and young children, which is consistent with other reports (3, 7, 15, 18, 19), although older children and adults were also affected. By contrast, the community-based outbreaks that were identified involved older schoolchildren and adults. Although outbreaks of astrovirus-associated diarrhea have been reported among elderly patients in institutions (4,

TABLE 5. Seasonal distribution of astrovirus-positive specimens from patients with sporadic cases of gastroenteritis in Japan

Prefecture	No. of positive subjects in the following month:														Total no. tested
	Total	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	UK[a]	
Nagano	4				3									1	65
Aichi	10	1		2	6	1									100
Tokyo	20		3	9	3							1	4		29
Ehime	9	3		4	1								1		20
Total positive	43	4	3	15	13	1						1	5	1	
Total tested		16	26	53	35	23	13	2	8	5	3	5	14	11	214

[a] UK, unknown.

24) and in immunocompromised patients (7), the outbreaks that we studied involved healthy schoolchildren and adults, suggesting further that astrovirus infection may occur in all age groups in Japan. This observation is in contrast to the epidemiology of rotavirus infection, 80% of all cases of which occur among children less than 2 years of age (9). Seroepidemiologic studies are needed to look further at the age distribution of infection.

The EIA appeared to be more sensitive than EM and worked best with fresh specimens. Many EM-negative specimens from outbreaks 1 and 3 were positive by EIA, and antigen was detectable as late as 8 days after the onset of illness in specimens from outbreak 3 (Table 1). While the EIA can detect astroviruses of the five major serotypes, the relative sensitivity is not known, nor are the distributions of these serotypes in Japan. Furthermore, some specimens gave discordant results; EM-positive specimens were not always confirmed by EIA, and vice versa. The volume of samples was insufficient for us to use PCR or cultivation for confirmation. Although some negative results may be due to prolonged storage and repeated freezing and thawing of specimens and loss of antigen, our findings indicate that the EIA is sensitive and identifies more positive specimens when compared with the results of EM.

On the basis of our results from the sporadic cases (Table 2), for 6 to 10% of the samples from patients with nonbacterial gastroenteritis that were negative for rotavirus and adenovirus, the gastroenteritis appeared to be due to astrovirus infection, although we did not test stool specimens from healthy individuals. Moe et al. (18) reported that 2 to 4% of stool specimens from children with diarrhea in the United States, Korea, and Peru were EIA positive for astrovirus; these estimates were based on the percentage of all cases of diarrhea, including those caused by bacteria or other viruses. The higher rate (6 to 10%) estimated in our study is comparable to results from Thailand (8.6%) (9).

Our preliminary studies indicate that astrovirus is a more common cause of both sporadic cases and outbreaks of gastroenteritis in Japan than was previously appreciated. Our ability to arrive at this conclusion was a direct result of improvements in the methods for detecting astrovirus by EIA, a sensitive assay suitable for large-scale screening. Application of the EIA more broadly to epidemiologic studies should help to delineate the natural history, epidemiology, and disease burden of astrovirus gastroenteritis in Japan.

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