JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1993, p. 955–962
0095-1137/93/040955-08$02.00/0

Vol. 31, No. 4

Characterization and Seroepidemiology of a Type 5 Astrovirus Associated with an Outbreak of Gastroenteritis in Marin County, California

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Received 21 August 1992/Accepted 30 December 1992

The Marin County strain of type 5 astrovirus was associated with two separate outbreaks of nonbacterial gastroenteritis in California in 1978. A safety-tested, bacterium-free filtrate prepared from a stool specimen of an individual who was ill during the original outbreak was given orally to 19 adult volunteers. One volunteer developed a gastrointestinal illness, and nine had serologic responses. Several diarrheal stool specimens from the ill volunteer contained a large number of 27-nm particles. By using immune electron microscopy with acute- and convalescent-phase sera from the original outbreak, these 27-nm particles were shown to be identical to the viral inoculum. The Marin County virus, purified from the stool of the ill volunteer, was shown by immunoprecipitation and polyacrylamide gel electrophoresis to contain a single structural protein with a molecular weight of 30,000. The buoyant density of the virion was 1.39 g/cm^3 in cesium chloride. By electron microscopy, approximately 5% of the particles had the characteristic stellate configuration of astrovirus, and serologic studies by immunofluorescence technique confirmed previous classification of the Marin County virus as a type 5 astrovirus. Radioimmunoassay and biotin-avidin immunoassay were used to detect antibody to the Marin County virus in paired acute- and convalescent-phase sera from 32 outbreaks of nonbacterial gastroenteritis, but none of these outbreaks could be attributed to this virus. Prevalence of antibody to this strain of astrovirus was approximately 13% in children 6 months to 3 years of age and increased to 41% in older children and young adults.

The etiologic agents of a large number of outbreaks of acute nonbacterial gastroenteritis have not been established. The Norwalk agent is clearly an important pathogen and may account for 30 to 40% of outbreaks of acute nonbacterial gastroenteritis (12, 27). Other 27-nm particles, including the Hawaii, Ditchling, and Snow Mountain agents as well as human caliciviruses and astroviruses, have been implicated in a limited number of such outbreaks, but the relative epidemiologic importance of each agent has not been determined (1, 5, 7, 8, 10, 11, 15, 22, 29, 43–45, 50). This report describes the biophysical properties, pathogenicity, and seroepidemiology of the Marin County strain of type 5 astrovirus that was associated with two separate outbreaks of nonbacterial gastroenteritis in California in 1978 (18, 19, 45).

MATERIALS AND METHODS

Volunteer study. A stool specimen from an individual who was ill with gastroenteritis during the original outbreak of the Marin County strain was made into a 0.1% suspension in veal infusion broth with 0.5% bovine serum albumin (BSA) and filtered through a 450-nm-pore-size membrane (45). The filtrate was designated Be,F-T8-0957 and was safety tested at Flow Laboratories, Inc. It was administered orally to adult volunteers who had given written informed consent at the Center for Vaccine Development at the University of Maryland between 1980 and 1982. The studies were approved by the Clinical Research Subpanel of the National Institute of Allergy and Infectious Diseases and the Human Volunteer Research Committee of the University of Maryland Hospital. Of 19 volunteers, 17 received a 1-ml inoculum, and 2 received a 20-ml inoculum. In the week thereafter, volunteers were evaluated daily, and all stool specimens were collected. Serum specimens were obtained before and 4 weeks after inoculation. Loose stools were examined for viral shedding by immune electron microscopy (IEM), and paired sera were tested for antibody to the viral inoculum.

Virus purification. Virus was purified from the diarrheal stools of an ill volunteer by fluorocarbon (Genetron 113) extraction followed by isopycnic and rate zonal centrifugation by methods described previously for purification of Norwalk virus (13). The cesium chloride or sucrose gradient fractions with peak viral antigen activity were identified by immune adherence hemagglutination assay (IAHA) or radioimmunoassay (RIA).

Antisera. Hyperimmune guinea pig serum to the Marin County virus was made by immunizing guinea pigs with purified virus at approximately 1-month intervals over a period of 3 to 4 months. The purified virion was mixed with Freund's complete adjuvant for the first immunization and then with incomplete Freund's adjuvant for subsequent immunizations. The titer of serum antibody to the viral inoculum was monitored by IAHA.

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IAHA. Initially, virus purification was monitored by IAHA with a 1% suspension of human erythrocytes (type O) and a 1:50 dilution of postinfection serum from the ill volunteer. Serial twofold dilutions of each gradient fraction were assayed, and the viral antigen titer was expressed as the reciprocal of the highest dilution positive by IAHA. Antibody to the Marin County virus was measured by IAHA with virus purified from the ill volunteer as test antigen. The methods were adapted from those of previous studies with Norwalk virus (24).

IEM. Detection of antibody to the Marin County virus by IEM was done as previously described (26, 49).

RIA. The RIA used in the purification of the Marin County strain was a modification of the method employed for the Norwalk agent (14). Polyvinyl chloride plates (Dynatech Laboratories; 96 V-bottom wells) were coated with a 1:10,000 dilution of hyperimmune guinea pig serum against the Marin County virus. After overnight incubation at 4°C, the plates were blocked with 1% BSA and then inoculated with 50 μl of test sample per well. After overnight incubation, the plates were inoculated with ^{125}I-labeled immunoglobulin G (IgG) (10^5 cpm per well). The IgG had been purified from hyperimmune guinea pig serum and iodinated by a modification of the Hunter and Greenwood method (46). After incubation at 37°C for 4 h, the plates were washed, and counts for individual wells were determined in a gamma counter. The residual radioactivity (counts per minute) detected in the wells that received test samples was divided by that detected in wells that received phosphate-buffered saline (PBS) (negative-antigen control). A ratio of 2 or greater was considered evidence of the presence of viral antigen.

The RIA was also adapted for use in a blocking test for measurement of antibody to the Marin County virus by methods similar to those employed for the Norwalk agent (14). Plates precoated with hyperimmune guinea pig serum were inoculated with astrovirus-positive stool suspension from the ill volunteer. After overnight incubation at 4°C, the plates were washed and inoculated in duplicate with serial twofold dilutions of paired test sera. The plates were incubated at 4°C overnight and then inoculated with ^{125}I-labeled IgG (10^5 cpm per well). After incubation at 37°C for 4 h, the plates were washed, and residual radioactivity per well was measured. A 50% reduction in residual radioactivity produced by a serum sample compared with that in a buffer control was considered to indicate the presence of antibody. The antibody titer of a given serum specimen was expressed as the reciprocal of the highest dilution that produced such a 50% reduction.

BAI. The biotin-avidin immunoassay (BAI) for the detection of antibody to the Marin County virus was similar to that developed for the Norwalk and Snow Mountain agents (9, 41). IgG from the hyperimmune guinea pig serum against Marin County virus was biotinylated as described previously (16). Polyvinyl chloride plates (Dynatech; 96 U-bottom wells) precoated with a 1:5,000 dilution of hyperimmune guinea pig serum were blocked with PBS containing 0.5 M NaCl, 0.5% gelatin, and 1% fetal calf serum (FCS). Test wells were inoculated with 25 μl of FCS and 25 μl of the ill volunteer's virus-positive stool suspension (diluted in veal infusion broth with 0.5% BSA), and negative-antigen control wells were inoculated with 25 μl of FCS and 25 μl of diluent. After overnight incubation at 4°C, plates were washed and inoculated with twofold dilutions of test serum diluted in 0.5 M NaCl with 0.5% gelatin and 10% FCS. Positive-antigen and negative-antigen control wells received diluting buffer without serum. After incubation at 37°C for 2 h, biotinylated IgG (from hyperimmune guinea pig serum) was added to each well without washing out the serum samples, and the plates were incubated for an additional 2 h at 37°C. After the plates were washed, a 1:4,000 dilution of avidin-horseradish peroxidase (Vector Laboratories) was added to each well. After 30 min at 37°C, the plates were washed, and substrate [0.1 mg of 2,2′-azinobis(3-ethylbenzthiazoline sulfonic acid) per ml of 0.1 M citrate buffer (pH 5.0)–0.012% hydrogen peroxide] was added to each well. The plates were incubated for 30 min at room temperature, and the optical density at 405 nm (OD_{405}) was measured after the enzyme-linked immunosorbent assay reader had been blanked on wells containing substrate alone. The average OD_{405} of the negative-antigen control wells was subtracted from the OD_{405} of the antigen-containing wells. The titer of antibody to the Marin County strain in a given serum was defined as the reciprocal of the highest dilution which reduced the OD_{405} of the wells by 50% or more in comparison with the average OD_{405} of the positive-antigen control wells that had received diluent without test serum.

Iodination of virion preparation. Five microliters of ^{125}I (carrier-free NaI; 100 mCi/ml; Amersham) and 15 μl of chloramine T (3.5 mg/ml in 0.25 M phosphate buffer, pH 7.4) were added to 20 μl of purified virion in phosphate buffer. After 2 min, 20 μl of metabisulfite (4.8 mg/ml in phosphate buffer) and 20 μl of a solution containing 2 mg of potassium iodide per ml, 22.5% (wt/vol) sucrose, and 0.025% phenol red were added. The free iodine was separated from the labeled protein by gel filtration (Sephadex G-50 medium) with PBS. The labeled protein was made 1% with FCS and kept at 4°C until it was immunoprecipitated.

Immunoprecipitation. Iodinated virion preparations (approximately 10^6 cpm) were mixed with an equal volume of test serum (100 μl of a 1:50 dilution in PBS). After overnight incubation at 4°C, Sepharose-protein A (25 μl) was added, and the mixture was incubated at 37°C for 1 h. The mixture was then centrifuged (10,000 × *g* for 2 min), and the supernatant was discarded. The pellet was washed twice with 1 ml of PBS, and the final pellet was suspended in sample buffer (0.062 M Tris [pH 6.8], 5% mercaptoethanol, 2% sodium dodecyl sulfate, 10% glycerol, 0.0175% bromphenol blue) and boiled for 5 min before electrophoresis on a polyacrylamide gel. Electrophoresis was performed at 25 to 30 mA on 12% polyacrylamide mass gels by the method of Laemmli (36). Molecular markers from Amersham (200,000 to 14,300 Da) were used to estimate the sizes of the virion proteins. Gels were dried and autoradiographed.

Immunofluorescence test (IFT) for astrovirus. Titers of rabbit antisera against human astrovirus strains (serotypes 1 through 5) were determined in the United Kingdom against strains representing each of the five types and the Marin County strain derived from the stool of the ill volunteer (32). Human embryo kidney or LLCMK2 cells were infected with the prototype astrovirus or the Marin County strain and fixed with acetone (37, 38). The titer of the serum was the reciprocal of the highest dilution that gave distinct cytoplasmic fluorescence. Paired sera from the ill volunteer and hyperimmune guinea pig serum against Marin County strain were also tested in this assay.

Human serum specimens. Acute- and convalescent-phase sera from the original Marin County outbreak and from the volunteer study were tested for antibody to the Marin County virus by RIA, BAI, or both. Also, paired sera from 32 outbreaks of nonbacterial gastroenteritis investigated by the Centers for Disease Control (n = 21) (provided by G. W. Gary) or local health departments (n = 11) (provided by

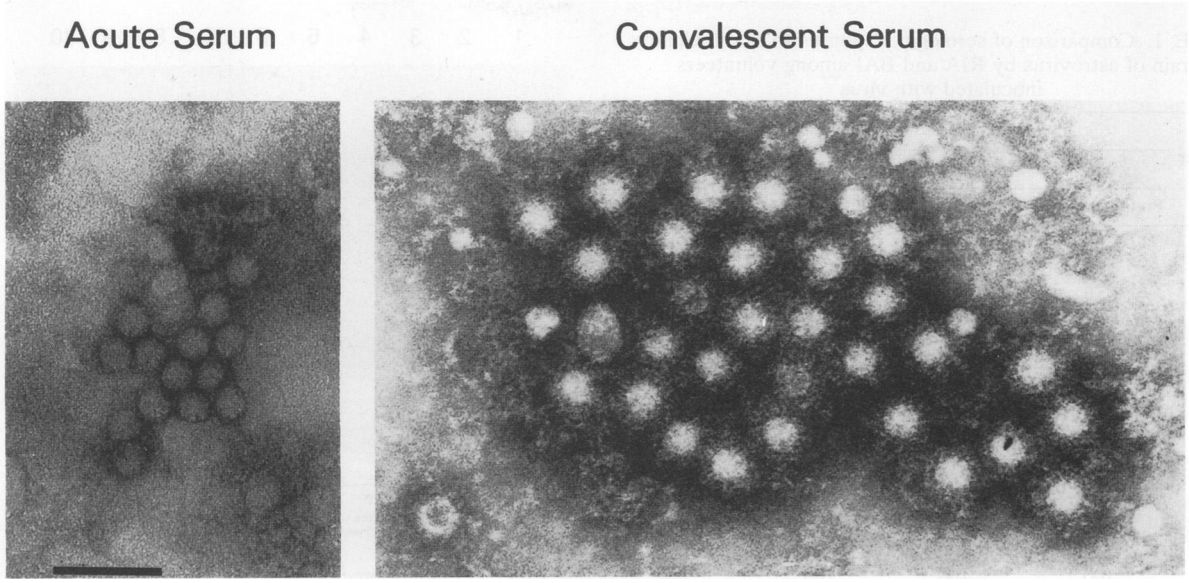

FIG. 1. Immune electron micrographs of 27-nm particles in fecal specimen from volunteer who became ill after inoculation with the Marin County strain of astrovirus. A 10% stool suspension (0.1 ml) was incubated with acute- or convalescent-phase serum (0.1 ml of a 1:5 dilution) from an individual who was ill during the original Marin County outbreak. A significant rise in antibody to the particle is demonstrated (acute phase, 1+; convalescent phase, 3-3-4). Bar = 100 nm.

F.-Y. C. Lin) between 1976 and 1987 were tested for antibody to the Marin County virus. Paired sera from 23 of these outbreaks had been tested previously by RIA for antibody to the Norwalk agent (27). Twenty outbreaks were not associated with the Norwalk agent, since none of the paired sera showed a seroresponse (defined as a ≥4-fold rise in titer of antibody to the Norwalk agent). In the three remaining outbreaks, fewer than 50% of the serum pairs showed a seroresponse to the Norwalk agent, and these outbreaks were categorized as "possibly caused by the Norwalk virus" in an earlier publication (27). Finally, sera from children with respiratory tract disease or other nongastrointestinal illnesses admitted to Children's Hospital National Medical Center in Washington, D.C., between 1965 and 1976; sera obtained from healthy infants and young children at or about the time of their admission to Junior Village, a welfare facility in Washington, D.C., between 1958 and 1969; and sera from students with nongastrointestinal illnesses at the University of Maryland between 1958 and 1959 and between 1964 and 1965 had been gathered as parts of previous studies and were tested for prevalence of antibody to the Marin County virus (3, 14, 23–25).

RESULTS

Volunteer study. Nineteen adult volunteers were orally administered a filtrate prepared from a 0.1% suspension of stool from one of the ill individuals in the original Marin County outbreak. None of 17 volunteers who received a 1-ml inoculum became ill. Because of this, the amount of inoculum was increased to 20 ml. Of two volunteers who received the larger inoculum, one developed a gastrointestinal illness characterized by nausea, vomiting, diarrhea, and malaise. The illness started 5 days after challenge and lasted for 36 to 48 h. IEM examination of several diarrheal stool specimens from this volunteer demonstrated a large number of 27-nm particles. Particles from this volunteer were designated Marin County virus, since IEM examination of paired acute- and convalescent-phase serum specimens from the individual in the original Marin County outbreak from whom the stool filtrate used in the challenge was derived demonstrated a significant rise in antibody to the particle shed by the ill volunteer (Fig. 1). IEM studies with paired sera from two of three additional symptomatic individuals from the original Marin County outbreak also showed significant rises in antibody in response to the volunteer's particles. The individual whose paired sera failed to show a significant rise in antibody had also failed to respond significantly to the particles isolated from the original outbreak. The pre- and postinoculation sera from the 19 volunteers were tested by RIA and BAI for seroresponses to the Marin County virus (Table 1). Although only 1 of 19 volunteers became ill after inoculation, 9 volunteers (47%) developed a fourfold or greater rise in antibody titer by RIA, BAI, or both. Volunteers who lacked prechallenge serum antibody by BAI (titer, <1:50) developed a response more often than those with antibody titers of ≥1:50 by BAI (7 of 10 versus 1 of 9; $P = 0.02$; Fisher's exact test, two-tailed).

Biophysical characteristics of purified virus. The virus purified from the stool of the ill volunteer had a buoyant density in cesium chloride of approximately 1.39 g/cm^3, which corresponds to the fraction with peak viral antigen measured by either IAHA or RIA (Fig. 2). The highly purified virion preparations were examined by negative-stain direct electron microscopy prior to iodination. In some instances, approximately 5% of the particles had the starlike configuration that is characteristic of astrovirus; in most instances, no definite substructure was noted.

Purified virus was iodinated and immunoprecipitated with pre- and postinfection sera from the ill volunteer and acute- and convalescent-phase sera from two individuals who were ill during the original outbreak, each of whom had developed a seroresponse (Fig. 3). The postinfection or convalescent-phase sera precipitated a single major protein with a molecular weight of 30,000 from the virion preparation. A faint band, possibly representing low levels of preexisting anti-

TABLE 1. Comparison of serologic responses to Marin County strain of astrovirus by RIA and BAI among volunteers inoculated with virus

Volunteer no.[a]	Reciprocal antibody titer of serum[b]			
	RIA		BAI	
	Preinoculation	Postinoculation	Preinoculation	Postinoculation
1	**<50**	**3,200**	**<50**	**6,400**
2	**<50**	**1,600**	**<50**	**6,400**
3	**<50**	**200**	**<50**	**1,600**
4	**50**	**800**	**<50**	**1,600**
5	**<50**	**1,600**	**50**	**800**
6	**<50**	**200**	**<50**	**100**
7	**50**	**200**	**<50**	**100**
8	<50	<50	**<50**	**100**
9	**<50**	**100**	<50	<50
10	<50	<50	50	100
11	<50	50	100	200
12	400	200	400	400
13	<50	50	<50	<50
14	<50	50	<50	<50
15	100	100	200	100
16	100	100	50	100
17	<50	<50	50	100
18	100	200	50	50
19	50	50	200	100

[a] Volunteers 1 and 9 received a 20-ml inoculum; other volunteers received a 1-ml inoculum. Only volunteer 1 became ill.

[b] Boldface print indicates a fourfold or greater rise in antibody titer between the pre- and postinoculation serum specimens.

body, was precipitated in the same location by preinfection or acute-phase sera. Postinfection sera from two volunteers who did not become ill but who had seroresponses by antibody blocking RIA also specifically immunoprecipitated a 30,000-molecular-weight protein (data not shown).

Antigenic relationship with established astrovirus serotypes. The antigenic relationship of the Marin County virus with established astrovirus serotypes 1 through 5 was studied by IFT with reference antigens and antisera for each of the serotypes and with the hyperimmune guinea pig serum prepared against the Marin County virus. Table 2 shows that in tests with reference rabbit antisera against five established astrovirus types, the Marin County strain reacted only with astrovirus type 5 serum. In the reciprocal serologic study, the hyperimmune guinea pig serum made against the Marin County strain reacted with the homologous Marin County strain and the prototype strain of astrovirus type 5 to a

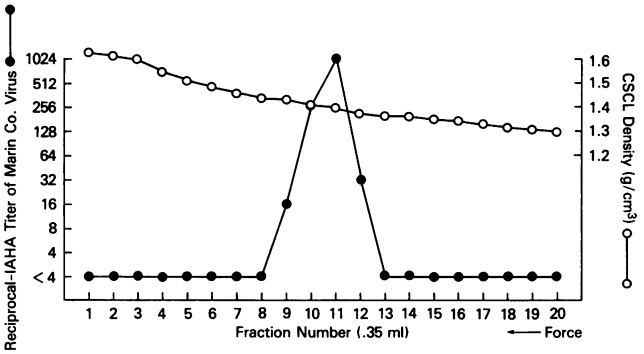

FIG. 2. Cesium chloride buoyant density gradient of Marin County strain of astrovirus.

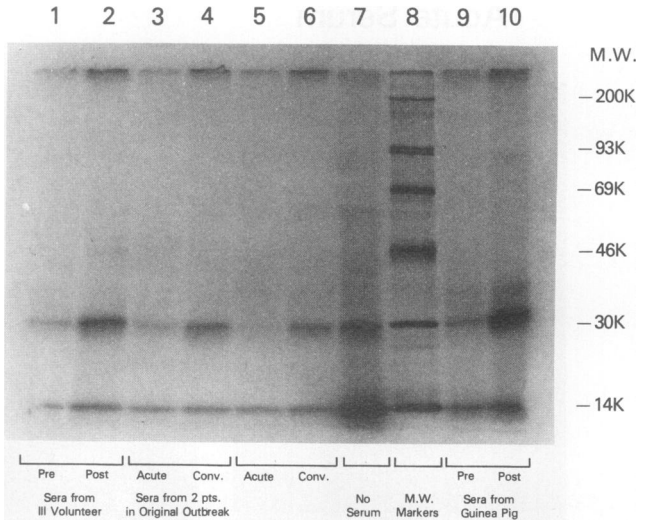

FIG. 3. Immunoprecipitation and polyacrylamide gel electrophoresis of purified Marin County virus. Purified virus was iodinated, immunoprecipitated, run on a polyacrylamide gel, and autoradiographed. Lanes 1 and 2, pre- and postinoculation sera from ill volunteer (RIA antibody titers of <1:50 and 1:3,200, respectively); lanes 3 and 4, acute- and convalescent-phase sera from individual who was ill during original Marin County outbreak (RIA antibody titers of <1:50 and >1:1,600); lanes 5 and 6, acute- and convalescent (conv.)-phase sera from another individual who was ill during original outbreak (RIA antibody titers of <1:50 and 1:800); lane 7, iodinated Marin County virus added directly to gel; lane 8, molecular weight (M.W.) markers (200,000 to 14,300 [200K to 14.3K]); lanes 9 and 10, pre- and hyperimmune guinea pig sera.

comparable titer (only a fourfold difference), whereas its titer to astrovirus types 1 through 4 was more than 20 antibody units lower than its homologous titer. Thus, the Marin County virus was shown to be a strain of type 5 astrovirus in reciprocal immunofluorescence assays. Of interest, the pre- and postinfection serum pair from the ill volunteer showed broadly reactive seroresponses, with fourfold antibody rises to astrovirus prototypes 1 through 5 and the Marin County strain.

Comparison of serologic assays for detection of antibody. Eleven pairs of acute- and convalescent-phase sera from the original Marin County outbreak that had been tested previously (45) or in this study by IEM were tested by RIA and IAHA. Six individuals developed a seroresponse to the Marin County strain by both IEM and RIA, two had responses by IEM only, and one had a response by RIA only. Only 5 of 11 pairs were tested by IAHA for rises in titer of antibody because of limited amounts of serum. IAHA failed to detect seroresponses in four pairs that had responses by IEM. IAHA detected a significant rise in titer of antibody in one pair which had been negative by IEM but positive by RIA. These results suggested that IEM and RIA were equally efficient in detecting rises in titer of antibody and more efficient than IAHA. As noted earlier, BAI and RIA were comparably efficient in detecting a serologic response to the Marin County strain of astrovirus type 5 (Table 1).

Role of Marin County strain of astrovirus in outbreaks of acute nonbacterial gastroenteritis. Because the Marin County virus was associated with 2 outbreaks of nonbacterial gastroenteritis, we attempted to determine the role, if any, of this agent in 32 outbreaks from various settings and different

TABLE 2. Antigenic relationships of Marin County strain with astrovirus serotypes 1 through 5 by IFT

Astrovirus strain (serotype)	Antibody titer with[a]:							
	Rabbit antisera to astrovirus serotypes:					Sera from volunteer ill with Marin County strain		Guinea pig serum against Marin County strain[b]
	1	2	3	4	5	Acute phase	Convalescent phase	
MA (1)	1,280	<20	<20	<20	<20	<20	80	160
SP (2)	<20	3,200	<20	<20	<20	<20	80	40
PE (3)	80	<20	320	<20	<20	<20	320	640
BY (4)	<20	<20	<20	1,280	<20	<20	80	640
BE (5)	20	<20	<20	<20	320	<20	160	10,240
Marin County strain[b]	<20	<20	<20	<20	320	<20	320	40,960

[a] Antibody titers are expressed as reciprocals of the highest dilutions of serum which produce clear cytoplasmic fluorescence with acetone-fixed, virus-infected cells (human embryo kidney or LLCMK2 cells). Homologous titers are underlined.
[b] Derived from stool of ill volunteer.

seasons over an 11-year period (1976 through 1987). The majority of individuals involved were adults of all ages, but some children were also affected. Outbreaks 1 through 23, which had been screened previously for seroresponses to the Norwalk virus, were chosen selectively, since none of them could be attributed to the Norwalk virus. In addition, nine outbreaks which occurred in Maryland and which had not been studied for Norwalk virus were included. Acute- and convalescent-phase sera from individuals involved in these outbreaks were tested by RIA or BAI for antibody that reacted with the Marin County virus (Table 3). None of the outbreaks could be associated with the Marin County strain, because seroresponses were not detected in 29 of 32 outbreaks and were found in only one individual in each of the other 3 outbreaks.

Prevalence of antibody. In a limited survey, sera obtained

TABLE 3. Evaluation of role of Marin County strain of astrovirus type 5 in 32 outbreaks of acute nonbacterial gastroenteritis by serologic assay

Location	Setting	Date (mo/yr)	No. of cases with ≥4-fold rise in titer of serum antibody[a]/no. tested	
			Marin County virus	Norwalk virus
At sea	Cruise ship	09/76	0/4	0/4
New York	Hospital	11/76	0/8	0/19
North Carolina	College	03/77	1/6	0/8
Wyoming	Recreational area	07/77	0/7	1/7
Vermont	Nursing home	01/78	0/3	1/3
Florida	Nursing home	02/78	0/8	0/25
Missouri	Community	05/78	0/6	0/6
At sea	Cruise ship	01/79	0/6	0/10
At sea	Cruise ship	02/79	0/11	0/12
Florida	Hospital	05/79	0/3	0/5
Alaska	Family	06/79	0/12	0/13
Virginia	Swimming pool	07/79	0/7	0/7
Hawaii	Restaurant	01/80	0/11	0/9
Florida[b]	Nursing home	03/80	0/4	0/4
California[b]	Restaurant	05/80	0/8	0/8
Illinois	College	05/80	0/6	1/7
Illinois	Restaurant	05/80	0/4	0/12
Washington	Hospital	05/80	0/7	0/17
Colorado	Nursing home	06/80	0/3	0/6
New York	Camp	07/80	0/11	0/4
Florida	Hospital	07/80	0/10	0/10
New York	Restaurant	04/82	0/7	0/7
Wisconsin	Hospital	06/82	0/7	0/14
Maryland[b]	Day camp	02/86	1/12	NT
Maryland[b]	College	12/86	0/6	NT
Maryland[b]	Nursing home	12/86	0/3	NT
Maryland[b]	Nursing home	12/86	0/9	NT
Maryland[b]	Nursing home	02/87	1/12	NT
Maryland[b]	Nursing home	03/87	0/8	NT
Maryland[b]	Nursing home	03/87	0/10	NT
Maryland[b]	Nursing home	04/87	0/8	NT
Maryland[b]	Nursing home	04/87	0/4	NT

[a] Acute- and convalescent-phase antibody titers were measured by RIA or BAI. NT, not tested.
[b] These outbreaks were investigated by local health departments; others were investigated by the Centers for Disease Control.

TABLE 4. Prevalence of antibody to Marin County strain of astrovirus type 5 in sera from infants and children as determined by BAI

Age	Location	No. tested	No. (%) positive at dilution of 1:50
6–12 mo	Junior Village	47	7 (15)
13–24 mo	Junior Village	6	0 (0)
25–36 mo	Junior Village	7	1 (14)
37–48 mo	Junior Village	12	5 (42)
7–11 yr	Children's Hospital	32	13 (41)
11–20 yr	Children's Hospital	18	12 (67)
17–26 yr	University of Maryland	60	20 (33)

from various age groups at several locations were evaluated for the presence of antibody that reacted with the Marin County strain of astrovirus type 5 by BAI (titer, ≥1:50) (Table 4). Prevalence of astrovirus type 5 antibody was approximately 13% in children 6 months to 3 years of age, rising to 41% in older children and young adults.

DISCUSSION

In this study, the Marin County virus, a type 5 astrovirus associated with two separate outbreaks of nonbacterial gastroenteritis in California in 1978, has been further characterized in terms of its biophysical properties, pathogenicity in a volunteer study, and possible role as an etiologic agent in outbreaks of nonbacterial gastroenteritis. Direct electron microscopy of purified virus revealed that in most preparations, the viral particle lacked definite substructure, but in occasional preparations, approximately 5% of the particles had a stellate configuration characteristic of astrovirus. The buoyant density of the virus in cesium chloride was 1.39 g/cm^3, which is similar to that described for ovine and human astroviruses in previous studies (17, 29). The Marin County strain of astrovirus had one structural protein with a molecular weight of 30,000 by specific immunoprecipitation. In contrast, a human type 1 astrovirus has been described as having four proteins with molecular weights ranging between 32,000 and 36,500 (33), and a human type 2 astrovirus has been described as having three proteins with molecular weights of 20,000, 29,000, and 31,000 (42). In the latter study, the 29,000-molecular-weight protein was predominant, with only faint bands corresponding to the other two proteins. Among animal astroviruses, an ovine strain has been described as having two proteins, each with a molecular weight of 33,000 (17), and a porcine strain has been described as having five proteins with molecular weights ranging between 13,000 and 39,000 (47). The reason for these discrepancies is unclear, and the exact number of astrovirus polypeptides remains to be defined. By IFT that included the reciprocal assay in which hyperimmune serum against the Marin County virus was tested against prototype astroviruses (types 1 through 5), the Marin County virus was shown to be a type 5 astrovirus, a finding confirmatory of previous reports (18–20).

The volunteer study demonstrated that the Marin County strain of astrovirus was transmissible but of low pathogenicity for adults. While 9 of 19 volunteers had a serologic response to the inoculum, only 1 volunteer developed a gastrointestinal illness. It should be noted that the ill volunteer was one of two volunteers who received the 20-fold-higher inoculum. It is impossible to know whether the volunteer developed illness because of the higher inoculum or whether other host factors were involved. Nonetheless, our findings are similar to those of a previous volunteer study with an unspecified serotype of astrovirus in which only 1 of 17 volunteers became ill, although 13 of 16 volunteers who were tested had a serologic response by IFT (34). It is of interest that the volunteer who developed illness in our study had a broadly cross-reactive antibody response, developing a rise in titer of antibody to the Marin County strain as well as to all five prototype astrovirus strains (serotypes 1 through 5) by IFT. It had been reported previously that the volunteer developed a rise in titer of antibody by IEM to serotypes 1 and 5; no mention was made of serotypes 2 through 4 (18). Whether such a broadly cross-reactive response is characteristically seen in primary or subsequent astrovirus infections is unknown. Although the preinoculation serum of this individual had no detectable antibody by IFT, RIA, or BAI, it is still possible that this volunteer had been infected previously and was having an anamnestic response. It is also noteworthy that volunteers without preexisting antibodies by BAI were more likely to develop infection, as manifested by a serologic response, than those with detectable antibody (7 of 10 versus 1 of 9, respectively). A similar trend had been noted in the earlier astrovirus volunteer study, in which all eight individuals without preexisting antibody as determined by IFT had a serologic response but only five of eight with detectable antibody had a serologic response (34). The pattern was more pronounced when the serologic responses in that study were measured by IEM. A rise was detected in all six individuals without preexisting antibody, in three of five with equivocal (+/−) antibody, and in one of five with detectable antibody (≥+).

Astroviruses have characteristically been found in the feces of infants and young children with mild gastroenteritis and have been associated with outbreaks of gastroenteritis in a nursery for newborns, a pediatric ward, a kindergarten, and a nursing home (2, 11, 28, 29, 35, 40). Antibody prevalence studies in the United Kingdom have shown that over 70% of children have acquired antibody to astrovirus by the age of 5 years (31). Studies of children hospitalized with gastroenteritis in the United States, Japan, and Ecuador have identified small round viruses in the feces by electron microscopy in less than 2% of cases, unlike rotavirus, which was identified in 35 to 45% of cases, and adenovirus, which was identified in 4 to 5% of cases (4, 30, 48). However, a recent study of children with gastroenteritis who were evaluated at an outpatient clinic in Thailand showed that astrovirus was a significant etiologic agent (21). Astrovirus was found in 8.6% of cases compared with rotavirus in 19% of cases and enteric adenovirus in 2.6% of cases. Likewise, longitudinal, community-based surveillance studies in Guatemala and the United States suggest that astrovirus-caused diarrhea occurs relatively frequently (6, 39). From these studies, it appears that the role of astrovirus in childhood diarrhea has not yet been clearly delineated; its relative absence in hospital-based studies suggests that it is not an important cause of severe childhood diarrhea.

The role of astrovirus as an etiologic agent in outbreaks of gastroenteritis has not been examined systematically. In the present study, the RIA and BAI were developed and shown to be comparable to IEM for detection of antibody to the Marin County strain of astrovirus. These assays were used to screen paired acute- and convalescent-phase sera from 32 outbreaks of acute nonbacterial gastroenteritis that had occurred in different settings and seasons of the year over an 11-year period. The majority of these outbreaks had been

previously screened for the 27-nm Norwalk virus and found to be negative. None of these outbreaks could be associated with the Marin County strain of astrovirus type 5, suggesting that it is not an important cause of outbreaks of nonbacterial gastroenteritis in adults and older children. However, antibody prevalence data showed that the Marin County strain of astrovirus type 5 or related viruses that induced cross-reactive antibody were common in that more than 40% of children had acquired antibody by the age of 4 years. Perhaps the role of astrovirus will be more clearly defined when longitudinal studies of gastroenteritis of any severity in infants and young children are evaluated for etiology.

ACKNOWLEDGMENTS

This work was supported in part by the Burroughs Wellcome Young Investigator Award in Virology of the Infectious Diseases Society of America.

We thank Kenneth Hope for his assistance in manuscript preparation.

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