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Distribution of Serotypes of Human Rotavirus in Different Populations

PATRICIA A. WOODS,[1]* JON GENTSCH,[1] VERA GOUVEA,[1] LEONARDO MATA,[2] ALBERTO SIMHON,[2] MATHURAM SANTOSHAM,[3] ZHI-SHENG BAI,[4] SHOZO URASAWA,[5] AND ROGER I. GLASS[1]

Viral Gastroenteritis Unit, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333[1]; Instituto de Investigaciones en Salud, Universidad de Costa Rica, Ciudad Universitaria Rodrigo Facio, San José, Costa Rica[2]; Department of International Health, Johns Hopkins School of Hygiene and Health, Baltimore, Maryland 21205[3]; Lanzhou Institute of Biomedical Products, Lanzhou, China[4]; and Department of Hygiene and Epidemiology, Sapporo Medical College, Sapporo 060, Japan[5]

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Serotyping is a useful tool to study the epidemiologic characteristics of rotaviruses in large populations and to assess the need for a vaccine to protect against all strains. By using an enzyme immunoassay with serotype-specific monoclonal antibodies to the four most common rotavirus serotypes, we analyzed 1,183 rotavirus-positive specimens from 16 stool collections in eight countries on four continents that were obtained from 1978 to 1989. Of the 926 strains (78%) that could be serotyped, 48% were serotype 1, 8% were serotype 2, 15% were serotype 3, and 7% were serotype 4. Twenty-two percent had insufficient numbers of double-shelled virus particles to react with the monoclonal antibody of the VP4 rotavirus protein and therefore could not be serotyped. Our results indicate that vaccines being developed must provide the greatest coverage against serotype 1 and that the serotype distribution cannot be predicted currently by the geographic area or prevalence in the preceding year.

Group A rotaviruses are the most important cause of hospitalizations and deaths due to diarrhea in children under 2 years of age in both developed and developing countries (7, 9, 21, 22). Virtually all children are infected by 5 years of age. While the detection of rotaviruses by enzyme immunoassays (EIAs) has become a simple, routine procedure, the serotyping of individual strains can provide additional insights into the epidemiologic features of rotavirus diarrhea. From recent studies of rotavirus surveillance, geographic and temporal patterns of disease in the United States and throughout the world have been identified; these might be clarified if more detailed knowledge of the distribution of the strains were available (26). Similarly, while early vaccine trials with the bovine rotavirus vaccine (RIT 4237, serotype 6) demonstrated good heterotypic protection against rotaviruses of serotypes 1 and 2 (41), subsequent studies with the monovalent rhesus rotavirus vaccine (serotype 3) suggested that homotypic protection was probably important as well (10). Consequently, the distribution of serotypes in a population could alter the effectiveness of vaccines being tested.

Four major and two minor serotypes of human rotavirus have been identified on the basis of the major neutralization glycoprotein, VP7, which is present in the outer capsid of the virus. In the past, the serotyping of rotavirus has been dependent on the ability to grow different viral strains in cell culture and neutralize them with type-specific antiserum (43). The recent development of monoclonal antibodies (MAbs) that specifically and selectively bind to the VP7 glycoprotein of each of the four most-common rotavirus serotypes (serotypes 1 to 4) has made it possible to serotype large collections of specimens by EIA (35).

To examine the global distribution of rotavirus serotypes in different geographic locations and at different times, we have used an EIA with serotype-specific MAbs reactive to rotavirus specimens from 16 separate studies conducted in eight different countries on four continents. These results are compared with those reported in 28 other studies from 23 different countries reported in the literature. Together, these findings suggest that vaccines being developed must provide particularly good coverage for serotype 1, the most common serotype, and that year-to-year variations in the distribution of serotypes may provide clues to the reservoirs and transmission of rotaviruses.

MATERIALS AND METHODS

Specimens. Stool specimens for the serotyping of rotaviruses were received frozen and were stored at −70°C until used. An approximately 20% stool suspension was made in Tris-buffered saline, pH 7.2, (Vogts TBS) and clarified by low-speed centrifugation. Samples from seven collections were obtained from children hospitalized with diarrhea in the following locations: Beer-Sheva, Israel (1986–1987) (8); Seoul, Korea (1987–1988) (23); Puriscal, Costa Rica (1983–1987) (34a); Mexico City, Mexico (1986–1987); Lanzhou, China (1982–1986); Seattle, Wash. (1986–1987); and the White River Indian Reservation, Ariz. (1981–1985). Other collections came from children with diarrhea in day-care centers in Phoenix, Ariz. (1981–1983) (2), from specimens submitted to a diagnostic laboratory that serves the southeastern United States (1987–1988), and from newborns with both symptomatic and asymptomatic infections in South Africa (1978) (29). The five remaining collections from the United States were serotyped as part of an investigation to examine the distribution of rotaviruses in the United States for the period from 1987 to 1989 (19). For 3 of the 16 collections (Costa Rica, China, and White River, Ariz.),

* Corresponding author.

TABLE 1. Analysis of rotavirus serotypes from different geographic locations and different years

Place	Yr	No. of samples tested	% (*n*) belonging to serotype:				
			1	2	3	4	NS[a]
United States							
Denver, Colo.	1987–1989	60	75 (45)	2 (1)	23 (14)	0 (0)	0 (0)
Seattle, Wash.	1986–1989	40	93 (37)	0 (0)	0 (0)	0 (0)	7 (3)
Phoenix, Ariz.	1981–1983	29	55 (16)	7 (2)	21 (6)	0 (0)	17 (5)
San Diego, Calif.	1988–1989	35	63 (22)	3 (1)	34 (12)	0 (0)	0 (0)
Houston, Tex.	1987–1988	23	26 (6)	0 (0)	74 (17)	0 (0)	0 (0)
White River, Ariz.	1981–1985	39	36 (14)	15 (6)	28 (11)	8 (3)	13 (5)
Philadelphia, Pa.	1987–1989	30	77 (23)	3 (1)	17 (5)	0 (0)	3 (1)
Buffalo, N.Y.	1987–1989	26	84 (22)	4 (1)	8 (2)	0 (0)	4 (1)
Southeast region	1987–1988	196	83 (163)	1 (1)	8 (16)	0 (0)	8 (16)
Mexico	1986–1989	166	24 (40)	16 (26)	31 (51)	6 (11)	23 (38)
Costa Rica	1983–1987	184	8 (15)	7 (12)	6 (11)	28 (52)	51 (94)
Peru	1986	10	50 (5)	20 (2)	0 (0)	30 (3)	0 (0)
South Africa	1978	31	0 (0)	16 (5)	52 (16)	0 (0)	32 (10)
China	1982–1986	80	47 (38)	29 (23)	14 (11)	0 (0)	10 (8)
Korea	1988	138	52 (71)	4 (6)	1 (1)	0 (0)	43 (60)
Israel	1987	97	55 (52)	10 (10)	0 (0)	19 (19)	16 (16)
Total		1,183	48 (569)	8 (97)	15 (173)	7 (87)	22 (257)

[a] Positive for VP4 but not for serotypes 1 to 4.

specimens were available for a 5-year period and were analyzed both together and for individual years to evaluate changes in the distribution of serotypes in the same area over time.

Several points should be noted about these collections: (i) the specimens were collected from several different groups of children with diarrhea including newborns, children in day-care centers, and ambulatory and hospitalized patients; (ii) some collections span a limited time period; (iii) some collections are small, such as those from South Africa and Peru; and (iv) some of the collections which span several years have limited numbers of samples. They are not meant to be representative of patterns in a specific place or time but to demonstrate the diversity of strains seen in a large reference laboratory.

EIA for serotyping. Stool specimens were serotyped with an EIA by using MAbs KU-4, S2-2G10, YO-1E2, and ST-2G7, which recognize specific serotype 1, 2, 3, and 4 neutralization epitopes, respectively, in the outer capsid protein VP7, and YO-2C2, a MAb which recognizes the VP4 outer capsid protein of all four serotypes. The EIA was performed as described previously with some modifications (35). Microtiter plates were coated with a 1:10,000 dilution of ascitic fluid in phosphate-buffered saline (PBS), pH 7.2. After overnight incubation, the plates were washed three times with PBS-T (PBS containing 0.05% Tween) and incubated with 1% BSA–PBS (2 h at 37°C). The 1% BSA–PBS was removed, and 50 μl of a 20% stool suspension diluted 1:3 in 1% BSA–PBS was added in duplicate to the wells; the plates were then incubated overnight at 4°C. After the plates were washed three times with PBS-T, pooled rabbit serum made against the four rotavirus serotypes was added at a 1:50,000 dilution in 1% BSA–PBS. After three additional washes in PBS-T, 50 μl of horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) was added at a dilution of 1:12,000 in 1% BSA–PBS. The plates were incubated for 1 h at 37°C and washed three times with PBS-T, and 100 μl of the chromogen tetramethylbenzidine was added. After a 10-min incubation at room temperature, the reaction was stopped with 25 μl of 2 M H_2SO_4, and the optical density at 450 nm (OD_{450}) was determined with a microtiter plate reader (Dynatech MR600). A serotype was assigned when the OD value for the reaction with the MAb corresponding to that serotype was higher than 0.2 and the OD value for the reaction corresponding to that serotype was at least twice the mean OD of the other three MAbs reactive to the same sample.

Comparison with published results. To examine global trends in the distribution of rotavirus serotypes, we identified studies of rotavirus diarrhea from a Medline search of papers published in English from 1983 to 1991, by using the keywords "rotavirus" and "serotypes." Studies that had used any serotyping method and had reported serotyping results for more than 25 strains were reviewed. Vaccine studies were not included because of a possible serotype-specific protection induced by the vaccine.

RESULTS

A total of 1,183 rotavirus-positive specimens from the 16 collections were assayed for serotype. Of these, 926 (78%) had sufficient double-shelled virus particles to react with the VP4 MAb so that a serotype could be assigned (Table 1). No mixed-serotype samples were observed in this study. Overall, serotype 1 was the most prevalent serotype (48%) and

TABLE 2. Changes in rotavirus serotypes during 5 years in three countries

Place and yr	% (n) belonging to serotype:					No. of samples tested
	1	2	3	4	NS[a]	
Costa Rica						
1983	0 (0)	0 (0)	0 (0)	77 (31)	22 (9)	40
1984	0 (0)	0 (0)	0 (0)	62 (18)	38 (11)	29
1985	0 (0)	14 (5)	14 (5)	3 (1)	68 (24)	35
1986	0 (0)	19 (7)	17 (6)	5 (2)	58 (21)	36
1987	34 (15)	0 (0)	0 (0)	0 (0)	66 (29)	44
Total	8 (15)	7 (12)	6 (11)	28 (52)	51 (94)	184
Lanzhou, China						
1982	76 (16)	19 (4)	0 (0)	0 (0)	5 (1)	21
1983	4 (1)	69 (16)	4 (1)	0 (0)	22 (5)	23
1984	62 (10)	0 (0)	31 (5)	0 (0)	6 (1)	16
1985	56 (9)	12 (2)	31 (5)	0 (0)	0 (0)	16
1986	50 (2)	25 (1)	0 (0)	0 (0)	25 (1)	4
Total	47 (38)	29 (23)	14 (11)	0 (0)	10 (8)	80
Ariz.						
1981	0 (0)	0 (0)	89 (8)	0 (0)	11 (1)	9
1982	33 (2)	0 (0)	50 (3)	0 (0)	17 (1)	6
1983	80 (8)	0 (0)	0 (0)	0 (0)	20 (2)	10
1984	9 (1)	54 (6)	0 (0)	27 (3)	9 (1)	11
1985	100 (3)	0 (0)	0 (0)	0 (0)	0 (0)	3
Total	36 (14)	15 (6)	28 (11)	8 (3)	13 (5)	39

[a] Positive for VP4 but not for serotypes 1 to 4.

the predominant serotype in 12 of the 16 collections. The South African collection, which came from an outbreak of rotavirus in a newborn unit, contained samples with both serotype 2 and 3 specificities and no strains of serotype 1. Serotype 4 was the least common serotype (7%), present in specimens from children in Latin America, Israel, and the White River Indian Reservation, Ariz., but not those from Asia, Africa, and the rest of the United States.

Three of the collections represent samples obtained every year for 5 years and provide some insight into the variability of rotavirus strains in a single location over time (Table 2). In Costa Rica, serotype 4 was the only strain identified for 2 years, after which it was displaced during the next 2 years by serotypes 2 and 3, which gave way the last year to serotype 1. A large number of these strains (51%) could not be serotyped, and this inability to serotype was not related to the age of the specimens in this collection. In Lanzhou, China, serotype 1 was the predominant serotype for 4 of the 5 years, serotype 2 was predominant for a single year, serotype 3 strains were prevalent in a third of cases for two consecutive years, and serotype 4 was absent. In Arizona, with the smallest number of strains, serotypes 1, 2, and 3 shared predominance from year to year.

Data from 28 studies of rotavirus serotypes published previously were identified in the Medline search and reviewed (Table 3). A total of 5,419 specimens were tested, and 74% were serotyped. Serotypes were determined in 26 studies by EIAs with MAbs, in 1 study (Italy) by solid-phase immunoelectron microscopy, and in 1 study (Kenya) by neutralization. Overall, serotype 1 was the most prevalent serotype (61%), and it was the predominant serotype in North America, Africa, Australia, and Europe in 19 of the 28 studies analyzed. Serotypes 2, 3, and 4 were evenly distributed, but serotype 4 was absent in studies from Africa.

TABLE 3. Global review of serotyping studies

Place	Yr	Reference	No. of samples	% Belonging to serotype				
				1	2	3	4	NS[a]
N. America								
Canada	1984	3	41	61	10	29	0	7
Mexico	1984–1987	31	132	43	17	15	25	33
United States	1979–1989	28, 30	1,676	59	3	31	7	26
S. America								
Argentina	1983–1986	16	123	41	19	14	26	44
Brazil	1984–1985	27	57	29	21	10	40	9
Peru	1983	3	54	20	62	6	12	7
Venezuela	1981–1983	12	134	49	16	20	15	24
Africa								
Africa	1983–1985	13	152	72	15	13	0	6
Gambia	1982–1984	33	24	41	36	23	0	8
Kenya	1982–1983	40	16	59	33	8	0	25
Asia								
Bangladesh	1988	1	123	42	30	3	25	71
Bangladesh	1985–1986	42	143	31	16	15	38	0
Burma	1986	3	17	25	19	37	19	6
India	1983–1985	6	117	48	9	6	37	73
Indonesia	1978–1979	5	111	2	9	53	36	48
Japan	1984	38	16	63	12	25	0	33
Japan	1986–1988	39	562	51	31	11	7	25
Pakistan	1985	3	12	0	0	0	100	0
Sri Lanka	1987	3	5	0	0	0	100	0
Thailand	1983–1984, 1987–1988	32	88	31	29	0	40	34
Australia								
	1986–1987	37	344	95	4	.5	.5	38
	1973–1986	36	647	67	9	9	15	37
	1973–1989	4	943	75	7	3	15	29
Europe								
England	1983–1988	3	353	55	20	16	9	2
Finland	1984, 1986–1987	3	135	87	5	3	5	4
Italy	1981–1985	14	129	72	14	0	14	3
Sweden	1983	3	32	50	34	16	0	0
Total			5,419	61	10	16	13	26

[a] Percentage of HRV-positive samples whose serotypes could not be determined.

DISCUSSION

Serotyping is a useful tool to study the epidemiologic features of rotaviruses in large populations and to assess the need for a vaccine to protect against all strains. This study indicates that serotype 1 is the most common global serotype in circulation and any vaccine must provide good protection against it. At the same time, from three collections spanning 5 years and from many collections with 1 year of data alone, great variations in the distribution of serotypes occur over time and from place to place. These indicate that, to be highly effective, a vaccine must protect against all of these serotypes at the same time. Furthermore, collections having

a high proportion of nonserotypeable strains (i.e., samples that were VP4-positive by EIA but could not be serotyped) must be examined further for other less-common serotypes (S8, S9, and S12) or new serotypes yet to be described. While 78% of all strains could be serotyped using the four MAbs, the rates of nontypeable isolates varied greatly, from 50% in Costa Rica to none in Peru.

The serotyping technique described herein is easily reproducible and suitable to study large collections of epidemiologic interest. At the same time, it is based on MAbs binding to single VP7 epitopes. Recent studies have demonstrated that subtypes of rotavirus serotypes 1 (4) and 4 (15) exist, on the basis of the binding of their VP7 polypeptides to some neutralizing, serotype-specific MAbs but not others. These subtypes have been designated monotypes by some workers (4). Although insufficient intact virions may account for most nontypeable strains (about 30% in most studies), data that some isolates of rotaviruses do not bind to all MAbs commonly used in serotyping have been presented (42). Due to these epitope variations of isolates, it has been suggested that the range of this method could be enhanced by the use of MAbs specific for more than one VP7 epitope (42). The range of the method may also be enhanced by the addition of MAbs for serotypes 8, 9, and 12, which appear to be uncommon except in certain geographic regions. Other techniques for serotyping nontypeable strains include the polymerase chain reaction (18), nucleotide sequencing (20), hybridization methods (11, 34), and solid-phase immunoelectron microscopy (15). The polymerase chain reaction is also amenable to the identification of new serotypes through the addition of new primers as sequence information becomes available.

Recent studies have delineated the human rotaviruses into three VP4 serotypes and one subtype (17). These results suggest that a more-complete characterization of strains in the future will depend upon the neutralization associated with VP4 as well as VP7 polypeptides. Techniques already developed for VP7 serotyping may also be applicable for VP4. Neutralizing MAbs that can be used to separate human rotaviruses into four overlapping VP4-antigenic groups have already been reported (24). Although these antibodies are not specific enough to distinguish all VP4-antigenic groups in a solid-phase assay, it is plausible that these and other antibodies may be used to develop an EIA-based serotyping method for VP4. Similarly, a hybridization-based method for the discrimination of gene 4 types has been reported (25). When reduced to practice, these methods will allow a more complete understanding of the prevalence of rotavirus genes important for neutralization and help in the design of future vaccine candidates.

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