Hospital-Based Surveillance of Rotavirus and Other Viral Agents of Diarrhea in Children and Adults in Russia, 2005–2007

A. T. Podkolzin,[1] E. B. Fenske,[1] N. Yu Abramycheva,[1] G. A. Shipulin,[1] O. I. Sagalova,[2] V. N. Mazepa,[3] G. N. Ivanova,[4] A. V. Semena,[5] Z. G. Tagirova,[6] M. N. Alekseeva,[7] V. P. Molochny,[8] U. D. Parashar,[9] J. Vinjé,[9] V. V. Maleev,[1] R. I. Glass,[9,10] and V. I. Pokrovsky[1]

[1]Central Research Institute of Epidemiology, Moscow, [2]Chelyabinsk State Medical Academy, Chelyabinsk, [3]Institute of Epidemiology and Microbiology, Nizhni Novgorod, [4]Regional Infectious Clinical Hospital, Tyumen, [5]Russian Army Medical Academy, St. Petersburg, [6]Daghestan State Medical Academy, Makhachkala, [7]Yakut State University, Yakutsk, and [8]Far Eastern Medical University, Khabarovsk, Russia; [9]Centers for Disease Control and Prevention, Atlanta, Georgia; and [10]Fogarty International Center, Bethesda, Maryland

During a 2-year period in 2005–2007, we conducted surveillance of group A rotaviruses and other enteric agents among patients hospitalized with acute gastroenteritis in 8 different cities of the Russian Federation. Fecal specimens were gathered from 3208 children (including 2848 children aged <5 years) and 1354 adults who were admitted to hospitals in Moscow, St. Petersburg, Chelyabinsk, Nizhnii Novgorod, Tyumen, Khabarovsk, Makhachkala, and Yakutsk. Polymerase chain reaction was performed to detect rotaviruses of groups A and C, noroviruses of genogroups I and II, astrovirus, sapovirus, and enteric adenoviruses (group F). Group A rotavirus was the most common viral pathogen detected among children aged <5 years (43.6%), followed by norovirus (12.5%), whereas norovirus was the pathogen most commonly detected in adults (11.9%). P and G genotypes were determined for 515 rotavirus specimens, and the most prevalent genotypes were G1P[8] (44.9%), G4P[8] (40.0%), G2P[4] (8.5%), and G3P[8] (6.6%). This study is the first multicenter study of rotaviruses in the Russian Federation and documents the important burden of disease caused by this pathogen, which soon may be preventable by vaccination.

Although rotavirus infection is believed to be the most common cause of acute infantile gastroenteritis worldwide [1–3], critical data on the local prevalence of disease that are needed for decision making are lacking for many countries. Rotavirus infections are universal, affecting most children during the first few years of life, but the burden of severe rotavirus disease is a particular problem in low- and middle-income countries, where access to medical care may be limited and where enteric infections can be fatal [4, 5]. The development of new vaccines to prevent rotavirus makes it imperative to assess the local burden of rotavirus disease, so that each country can consider the usefulness of the vaccines in its own settings. To assist such studies, the World Health Organization (WHO) has published a generic protocol to survey the prevalence of rotavirus among children with severe diarrhea who are admitted to sentinel hospitals, to provide a window on the burden of severe disease [6].

In 2005, the Central Research Institute of Epidemiology (CRIE) in Moscow began an investigation into the epidemiology of rotavirus and other viral agents of gastroenteritis among patients hospitalized in 8 differ-

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Reprints or correspondence: A. T. Podkolzin, Central Research Institute of Epidemiology, Novogireevskaya, 3a, Moscow, 111123, Russia (apodkolzin@pcr.ru).

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Table 1. Sequences of Primers and Probes Used for Detection and Typing of Rotavirus Strains

Primer or probe	Sequence,[a] 5′→3′	P or G type	Location (GenBank accession number)
4P385	GAG AAT AAG CAG TTT AAC GTA GA	P[4]	379–401 (M32559)
2T1	CTA TTG TTA GAG GTT AGA GTC	P[4]	494–474 (M32559)
P4z	R6G-CAC TCT GAC TAC TAC CTT TAA ACA GAG CG-BHQ1	P[4]	456–428 (M32559)
8P214	CCT TAT CAR CCT ACT ACA TTT AC	P[8]	210–232 (M21014)
1-T1m	GTA TCT ACT GGA TTA ACG TGC	P[8]	336–356 (M21014)
P8z	FAM-GGA CTG CAG TCR TTG CTG TTG AAC AGT CC-BHQ1	P[8]	314–342 (M21014)
1G86	GAT ATC AAT CAT TCT ACT CAA C	G1	87–108 (M21843)
1G310	GAT TTG AGT ACT TGC TTC AGT	G1	327–307 (M21843)
G1z	R6G-CTG AGC TTT AGT YAA GGC AAA TAA TGC TCA G-BHQ1	G1	201–171 (M21843)
9T4	GGG TCG ATG GAA AAT TCT	G4	423–440 (D86284)
4G319	CCA ACT CAA ATT AGT GAC AC	G4	707–726 (D86284)
G4z	FAM-CTG AAC YTG TCG GCC ATC CTT TGG TTC A-BHQ1	G4	395–368 (D86284)
9T2	GTT AGA AAT GAT TCT CCA CT	G2	281–262 (U73947)
2G170	CTC TGA TAT CAC CAT TTG TG	G2	173–192 (U73947)
G2z	FAM-CAG CGT CTA GTG ATC CCG TTA TTG GCG CTG-BHQ1	G2	244–213 (U73947)
9T3P	GTC CAG TTG CAG TGT AGC	G3	501–484 (AF161823)
3G276	CCT AAC TTC GAC TTT ATG TT	G3	276–295 (AF161823)
G3z	R6G-TAC CCA ACT GAA GCA GCA ACA GGG TA-BHQ1	G3	300–325 (AF161823)

NOTE. Primer labels: BHQ1, black hole quencher 1; FAM; carboxyfluorescein; R6G; rhodamine 6G.

[a] Nucleotide abbreviations are given according to the International Union of Pure and Applied Chemistry system of nomenclature—that is, R represents A or G, and Y represents C or T.

ent cities of the Russian Federation. Because we had access to a platform of molecular diagnostics developed at the CRIE, we expanded on the WHO-recommended algorithms for rotavirus surveillance, included patients of all ages, and tested their fecal specimens for rotavirus and 5 additional viral pathogens. This article summarizes data on the detection of the various viral pathogens and examines in detail the specific role played by rotavirus in gastroenteritis leading to hospitalization among both children and adults.

METHODS

Study design. We enrolled patients with acute gastroenteritis, defined by ⩾3 looser-than-normal stools and/or vomiting during the 24 h before hospitalization, who were admitted to infectious disease hospitals in 8 different cities of the Russian Federation: Moscow, St. Petersburg, Chelyabinsk, Nizhnii Novgorod, Tyumen, Khabarovsk, Makhachkala, and Yakutsk. Uniform epidemiological and clinical information was gathered for all patients. Fecal samples were collected on the day of admission, were diluted in phosphate-buffered saline containing 20% glycerol, and were stored at −15°C to −20°C for no more than 30–40 days. Subsequently, frozen specimens were transported frozen to the CRIE laboratory in Moscow and were tested for a range of enteric viral pathogens.

Laboratory diagnosis of rotavirus infection. The CRIE laboratory has developed a platform of polymerase chain reaction (PCR)–based assays to detect a variety of enteric viruses that has been commercialized in the Russian Federation and is sold as kits under the brand name AmpliSens. The AmpliSens Rotavirus-290 kit was used to detect rotavirus, and previous comparisons of this kit with the Premier Rotaclone (Meridian Diagnostics) demonstrated very high concordance of both sensitivity and specificity (>96%) (data not shown) [7]. Samples gathered in Moscow and Chelyabinsk during 2005–2006 were tested by conventional PCR for group A rotavirus, norovirus genogroups I and II, astrovirus, and adenovirus. Samples obtained in other cities during this interval and all specimens gathered since 2007 were investigated using the AmpliSens OKI screen PCR kit (CRIE) for multiplex detection of group A rotavirus, norovirus genogroup II, astrovirus, and group F adenovirus by use of fluorescent detection in an end-point format with TaqMan beacon-specific probes. Negative samples were tested for group C rotavirus and sapoviruses [8, 9]. All samples were also tested for the presence of bacterial pathogens (*Shigella* species, enteroinvasive *Escherichia coli, Salmonella* species, and thermophilic Campylobacter species) by use of AmpliSens test systems (data not shown).

Group A rotaviruses were characterized to determine the most prevalent P genotypes (P[4] and P[8]) and G genotypes (G1, G2, G3, and G4) by using PCR with multiplex fluorescent end-point detection. For PCR, we used a collection of reverse primers: 2T1, 1-T1, 9T4, 9T2, and 9T3P, described elsewhere [10, 11] (Table 1). Forward primers and specific TaqMan probes were designed with Sarani software (Strand Genomics), with

Table 2. Rates of Detection of Viral Pathogens Isolated from Children and Adults Hospitalized for Gastroenteritis in the Russian Federation, 2005–2007

Pathogen	Percentage of children aged <5 years (n = 2848)	Percentage of children aged 5–14 years (n = 360)	Percentage of adults (n = 1354)
Rotavirus group A[a]	43.6	20.4	8.2
Norovirus 2	12.0	12.9	10.3
Norovirus 1	0.5	1.4	1.6
Astrovirus	1.1	2.0	2.2
Adenovirus	3.5	5.3	1.8
Rotavirus group C[b]	0.1	1.0	0.7
Sapovirus[b]	0.5	1.4	0.6

[a] Rotavirus group A was found together with another pathogen in 10.3% of children aged <5 years, 9.5% of children aged <14 years, and 1.6% of adults.
[b] Only samples negative for other pathogens were tested for these pathogens.

some probes containing modified nucleotides for improvement of their analytical characteristics. Regions of VP4 and VP7 genome segments of different P and G rotavirus types were cloned into MS2 phages, and these clones were used as positive controls for rotavirus typing.

RESULTS

During 2005–2007, fecal samples from 3208 children aged <14 years (including 2848 children aged <5 years) and 1354 adults who were hospitalized for gastroenteritis were investigated (Table 2). Among children aged <5 years and all children aged <14 years, rotavirus was the most common pathogen, detected in 43.6% and 41.0%, respectively. Of the rotavirus-positive children aged <5 years and those aged <14 years, 33.3% and 30.5%, respectively, had rotavirus identified as the single pathogen, and 10.3% and 9.5%, respectively, had rotavirus detected together with 1 of the other pathogens that can be detected by the test systems used in this study. Rotavirus was also detected in 8.2% of adults with acute diarrhea, including 6.6% with rotavirus as the single pathogen and 1.6% with rotavirus mixed with other pathogens. Norovirus was the second most common viral pathogen in children aged <5 years (12.5%) and was the most common viral pathogen in adults (11.9%).

Of the children aged <5 years with rotavirus diarrhea, 12% were aged <6 months, 43% were aged <1 year, and 74% were aged <2 years (Table 3). The rate of detection of rotavirus was greatest in children aged 7 months to 2 years, but younger infants and older children were also likely to have rotavirus as the detectable pathogen. The peak seasons of hospitalization of patients with rotavirus infection were winter and spring, from December through May (Figure 1). In this study, no significant difference was observed in the seasonality of rotavirus infection between different cities (Figure 2).

During 2005–2007, G1P[8] was the most common strain type detected (44.9%), followed by G4P[8] (40.0%), G3P[8] (6.6%), and G2P[4] (8.5%). There was considerable variation among strain types between cities and between years (Table 4).

DISCUSSION

This study provides data about the role of group A rotavirus in both children and adults with severe gastroenteritis who required hospitalization at 8 sites in the Russian Federation.

Table 3. Age Distribution of Group A Rotavirus Infections in Children Aged <5 Years Who Were Hospitalized for Gastroenteritis in the Russian Federation, 2005–2007

Age, months	No. of patients with diarrhea of any etiology	No. of patients with rotavirus diarrhea (% of all patients with diarrhea)	Patients with rotavirus diarrhea (n = 1234)	
			Percentage in age group	Cumulative percentage
<6	473	153 (32.3)	12.4	12.4
7–12	834	376 (45.1)	30.5	42.9
13–24	800	387 (48.4)	31.4	74.2
25–36	405	184 (45.4)	14.9	89.1
37–60	335	134 (40.0)	10.9	100
Total	2847	1234 (43.3)	100	…

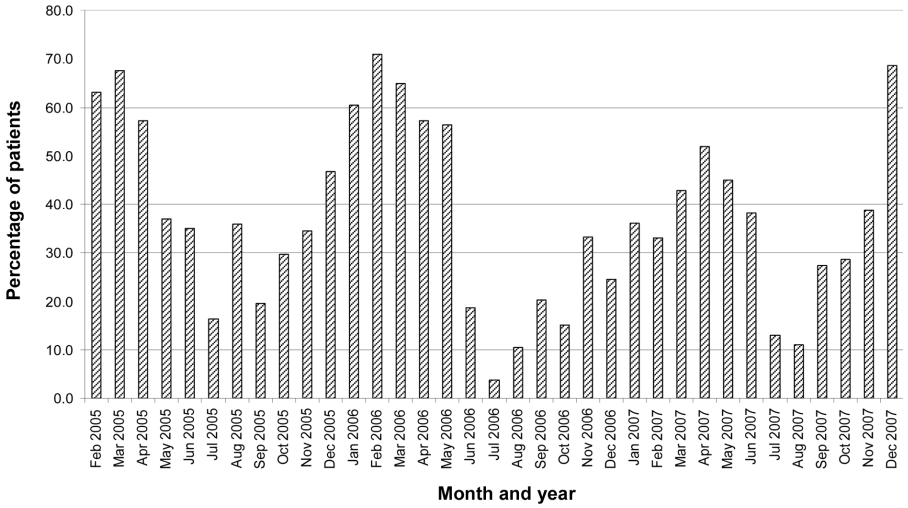

Figure 1. Seasonality of rotavirus infections among children hospitalized in 8 different cities of the Russian Federation, 2005–2007.

The overall detection rate of group A rotavirus among children aged <5 years was 43.6%, a rate comparable to those found in surveys conducted in many other countries in Europe, the Americas, and Asia [3]. However, because this study also looked at different viral enteric pathogens, approximately one-quarter of all children aged <5 years with rotavirus infection were coinfected with another pathogen. This raises the interesting questions of whether rotavirus was truly the etiological agent of disease in these patients and what was the contribution of the other pathogens. Although other surveillance studies that examine only rotavirus could be overreporting rotavirus as the putative cause of diarrhea, vaccine studies have found repeatedly that rotavirus vaccine reduces the rate of diarrhea in children by an amount more than can be attributed to rotavirus alone [12]. The use of this diagnostic platform for detection of multiple viral agents on the basis of PCR could shed new light on the question of when a pathogen is the true agent of disease.

Several features identified in this study are particularly noteworthy. First, the relatively high rates of rotavirus detection found in 8 cities are similar to the rate found in previous pilot studies in Moscow in 2005–2007 (740 [45.9%] of 1613 children aged <5 years) (A. Mukhina, personal communication). Second, the age distribution of children with rotavirus infection indicated that 42% were aged <1 year, a rate similar to that found in the United States and other industrialized countries [5]. Third, rotavirus continues to have a distinct seasonality in the winter and spring. Finally, most of the rotavirus strains had common serotypes. However, unusual types were identified by other authors; thus, long-term monitoring will be needed to identify novel strains that may arise [13–16]. Special attention to this issue is required in territories with active application of rotavirus vaccines.

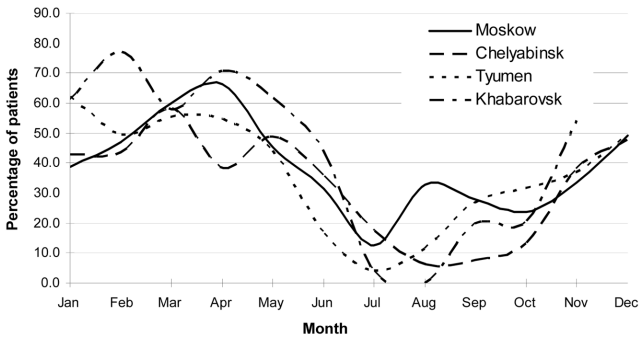

Figure 2. Year-round seasonality of rotavirus infections among children hospitalized in the Russian Federation, by city, in 2005–2007.

This study also examined rotavirus in adults and looked at multiple viral pathogens. It is noteworthy that, despite the general perception that adults have good immunity to rotavirus disease, many adults with diarrhea had rotavirus detected in their fecal specimens. This suggests either that rotavirus is a problem in adults as well as children or that these patients may have coinfections and rotavirus is not contributing to the development of disease. A study in Japan found that rotavirus was shed by 14% of 693 adult patients with gastroenteritis who presented to emergency departments and by 5% of patients without diarrhea [17]. Previous studies have demonstrated the presence of rotavirus in several risk groups of adults [18]—elderly individuals, parents and caretakers of small children, immunocompromised individuals, and travelers—but we did not have enough information to investigate whether any of these risk factors were more common in our study population. We found that norovirus was the second most common viral agent of gastroenteritis among children and the most common pathogen among adults. Efforts to develop methods of pre-

Table 4. Rotavirus Genotype Distribution among Children Aged <5 Years in Several Cities of the Russian Federation, 2005–2007

	No. of patients					
Year(s), genotype	Moskow	Chelyabinsk	Tyumen	Makhachkala	Khabarovsk	Total
2005						
G1P[8]	7	22	0	0	0	29
G4P[8]	42	0	0	0	0	42
G3P[8]	10	0	0	0	0	10
G2P[4]	4	5	0	0	0	9
Total	63	27				90
2006						
G1P[8]	3	17	64	1	9	94
G4P[8]	15	3	5	24	12	59
G3P[8]	1	0	9	0	4	14
G2P[4]	4	0	3	2	17	26
Total	23	20	81	27	42	193
2007						
G1P[8]	38	18	36	4	12	108
G4P[8]	34	8	2	27	34	105
G3P[8]	8	0	1	0	1	10
G2P[4]	6	1	1	1	0	9
Total	86	27	40	32	47	232
2005–2007						
G1P[8]	48	57	100	5	21	231
G4P[8]	91	11	7	51	46	206
G3P[8]	19	0	10	0	5	34
G2P[4]	14	6	4	3	17	44
Total	172	74	121	59	89	515

vention of norovirus infection may need to be given a higher priority, because the importance of this pathogen has always been underestimated as a result of the lack of sensitive diagnostic methods to detect it. This study demonstrates the added value of using a broad panel of diagnostic tests to understand the etiology of enteric infections and to prioritize their importance on a national basis.

In conclusion, this study has laid the groundwork to consider the need for future interventions with rotavirus vaccines, to examine more fully the role of rotavirus in adults, and to search more comprehensively for the full spectrum of causative agents of diarrhea, so that we can develop interventions to target those that are most common and most important.

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