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BRIEF REPORT

Rotavirus infection in children of Nizhny Novgorod, Russia: the gradual change of the virus allele from P[8]-1 to P[8]-3 in the period 1984–2010

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Abstract A collection of rotavirus samples collected over a 26-year period was examined to study the dynamics of change in RV strains of genotype P[8] in a geographically defined population (Nizhny Novgorod, Russia; children under 6 years) with no vaccine pressure. Phylogenetic analysis of gene VP4 (subunit VP8*) showed the presence of two lines of genotype P[8]: P[8]-1 and P[8]-3. Since 1997, the dominant population of rotavirus has been occupied by strains carrying the allele P[8]-3, which is associated with G1, G3 and G4. The complete replacement of the allele P[8]-1 to P[8]-3 took 19 epidemic years.

Human rotaviruses (RVs) (genus *Rotavirus,* family *Reoviridae*) were discovered in 1972 by Bishop and co-workers [4]. Group A RVs are the major etiologic agent of acute gastroenteritis in children during the first years of life throughout the world [26].

Morphologically, RVs are icosahedral particles, containing 11 segments of double-stranded RNA (dsRNA). The outer layer is formed by two capsid proteins: surface protein VP7 (G, glycoprotein) and spike protein VP4 (P, protease-sensitive protein). Based on antigenic and genetic differences of these proteins, RVs are classified into G/P serotypes and G/P genotypes. Currently, 27 G and 35 P genotypes are known to exist in a variety of combinations [20].

Particularly noteworthy are five G/P combinations, G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8], as they are most common in the world among RVs that infect humans [12, 24]. Two external virion proteins, VP7 and VP4, are of particular interest for vaccine development because they are potential targets for neutralising antibodies [15]. The RV G1P[8] type was predominant in Russia up to 2000 [23].

The fourth segment of the genome of RV group A encodes a protease-sensitive VP4, a subunit of which VP8*, is a major determinant of the P serotype (correlated with the P genotype) and an important region, carrying a number of sites for neutralising antibodies [9]. The most widely distributed serotype/genotype in the world, VP4 P1A[8], is included in the developed RV vaccines [6, 8]. This determines the need for monitoring strains of RV, as vaccination and natural immunisation of the population cannot influence the distribution of the same genotype over a long period of time

By the time we started this study, different authors had already found that there are four lineages of P[8] among group A RVs, determined by sequencing the VP8* portion of the VP4 gene, using primers Con2 and Con3 as designed by Gentsch et al. [11]. Allele P[8]-1 was widespread in Bangladesh, Italy, Paraguay, Great Britain, Hungary, Finland, the USA, Taiwan, and Japan [2, 10, 16, 22]. The lineage P[8]-2, which is phylogenetically similar to the reference strains KU and F45, was identified in Paraguay, the USA, Brazil and Korea [10, 18]. The allele P[8]-3 gradually began to spread in the late 1990 s and now is the most common worldwide [2, 10, 16, 18, 22]. The allele P[8]-4 is the least represented and was identified in Finland and Malawi [5].

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This paper presents information about the change of alleles P[8] of rotaviruses circulating in the area of Nizhny Novgorod, Russia, over 26 years.

In the years 1984 to 2010, 16,971 faecal samples were studied in children under 6 years of age who were hospitalised in Nizhny Novgorod with acute gastroenteritis. RVs were found in 5,404 (31.8 %) samples by RNA-PAGE and RT-PCR, and of these, the G/P type was identified in 3663 cases.

RV nucleic acid from a 10 % faecal suspension was extracted according to Herring et al. [14]. RNA-PAGE was performed for 18-20 hours in a thin layer of 10 % separating and 3 % PAGE concentrating in Laemmli buffer solution [17]. The resulting gel was stained with silver nitrate by an established method [14].

The dsRNA of RVs was extracted from 10 % suspension of faecal samples by the standard method, using a reagent kit supplied by InterLabService. The VP8* region of the VP4 gene, 877 bp in size, was obtained by RT-PCR using primers Con2 and Con3mod as described previously [11, 21]. The P-genotypes of rotaviruses were identified using primers suggested by Gentsch et al. [11]. The RV G-genotype was determined using RT-PCR based on a set of differentiating primers: GF, G1R, G2R, G4R, G9R, and G3R [7, 13].

The resulting RT-PCR products—fragments of the VP4 gene—were sequenced. Identification of the primary structure of cDNA was performed on two circuits using specific primers, the reagent kit ABI PRISM BigDye Terminator v.3.1, and an ABI Prism 3100 Genetic Analyzer in automatic mode as recommended by the manufacturer.

In this study, we identified 21 nucleotide sequences for the fragment of the VP4 gene and deposited them in the GenBank database under accession numbers GU226766-GU226781 and JX089973-JX089977. The numbers of other sequences in the GenBank database that were used for phylogenetic analysis are listed in the names of isolates presented on the phylogenetic tree. The phylogenetic tree was constructed using the neighbor-joining method and the Kimura two-parameter model in the programme MEGA5 [25].

In the analysis of eletropherotypes (e-types) of RNA isolates of RV genotype P[8], it was established that within its range there were genetic variants that differed radically in the electrophoretic mobility of the fourth segment of the genome. Variants of the virus were detected with slow-migrating (3rd, 4th, 86th e-type RNA) and fast-migrating (42nd, 46th, 52nd, 61st, 72nd, 85th, 88th e-type RNA) RNA segments encoding VP4 (Fig. 1), indicating the existence of at least two alleles that determine the P[8] genotype in the RV strains investigated by us. A strong correlation was established between the electrophoretic mobility of the fourth genomic segment and allele P[8],

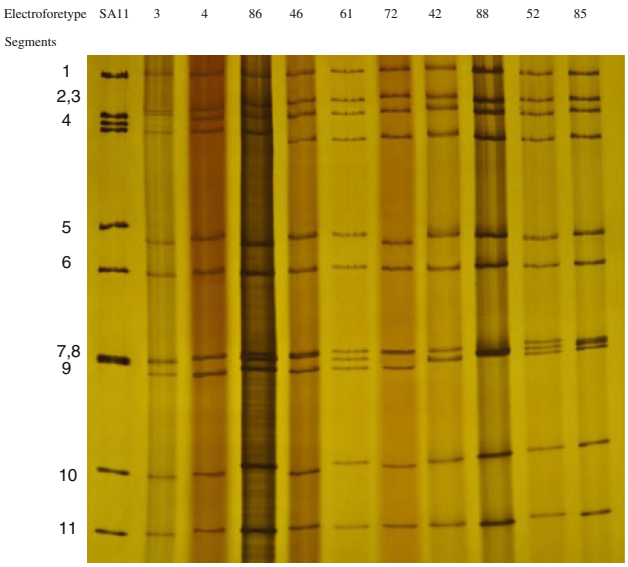

Fig. 1 Profiles of migration of dsRNA segments of rotavirus in PAGE. Numerals indicate the numbers of e-type RNAs that were assigned in the course of identification of e-type RNA types in our laboratory. SA11 is the monkey group A rotavirus

which confirmed the results of Maunula and von Bonsdorff, obtained in 1998 [21].

According to Matthijnssens and co-workers [19], when comparing different strains of RV, more differences are observed in the nucleotide sequences than in the amino acid sequences, and it is therefore more reliable to conduct phylogenetic analysis at the nucleotide level.

We aligned the established partial nucleotide sequences of VP4 with the corresponding nucleotide sequences of the RV strains in the GenBank database using the MEGA5 software (Fig. 2). The figure clearly shows the clustering of strains, revealing at least four clusters within the P[8] genotype, P[8]-1, P[8]-2, P[8]-3, and P[8]-4. As previously reported elsewhere [2, 10], our data show that there is a diversity of VP4 P[8] genotype gene sequences with a high level of variability in the nucleotide sequence encoding the VP8* subunit, which is considered a hypervariable region. In general, the variation in nucleotide sequences of the VP8 P[8] genotype region occurring in these clusters does not exceed 20 % (9.7-12.9 %), which allows them to be assigned to different alleles of the P[8] genotype. Figure 2 shows that RVs detected in Nizhny Novgorod are grouped into clusters, P[8]-1 and P[8]-3. The level of variation in the nucleotide sequences within these clusters reaches 10.2 %.

In the P[8]-1 cluster, two subclusters, P[8]-1a and P[8]-1b, are seen, with a variation in the nucleotide sequences of 4.8 %. In the subcluster P[8]-1a, there are Wa-like strains that circulated mainly in the 80-90 s, including in the area of Nizhny Novgorod. In the subcluster P[8]-1b, there are more recent strains that were identified in 2000. In the

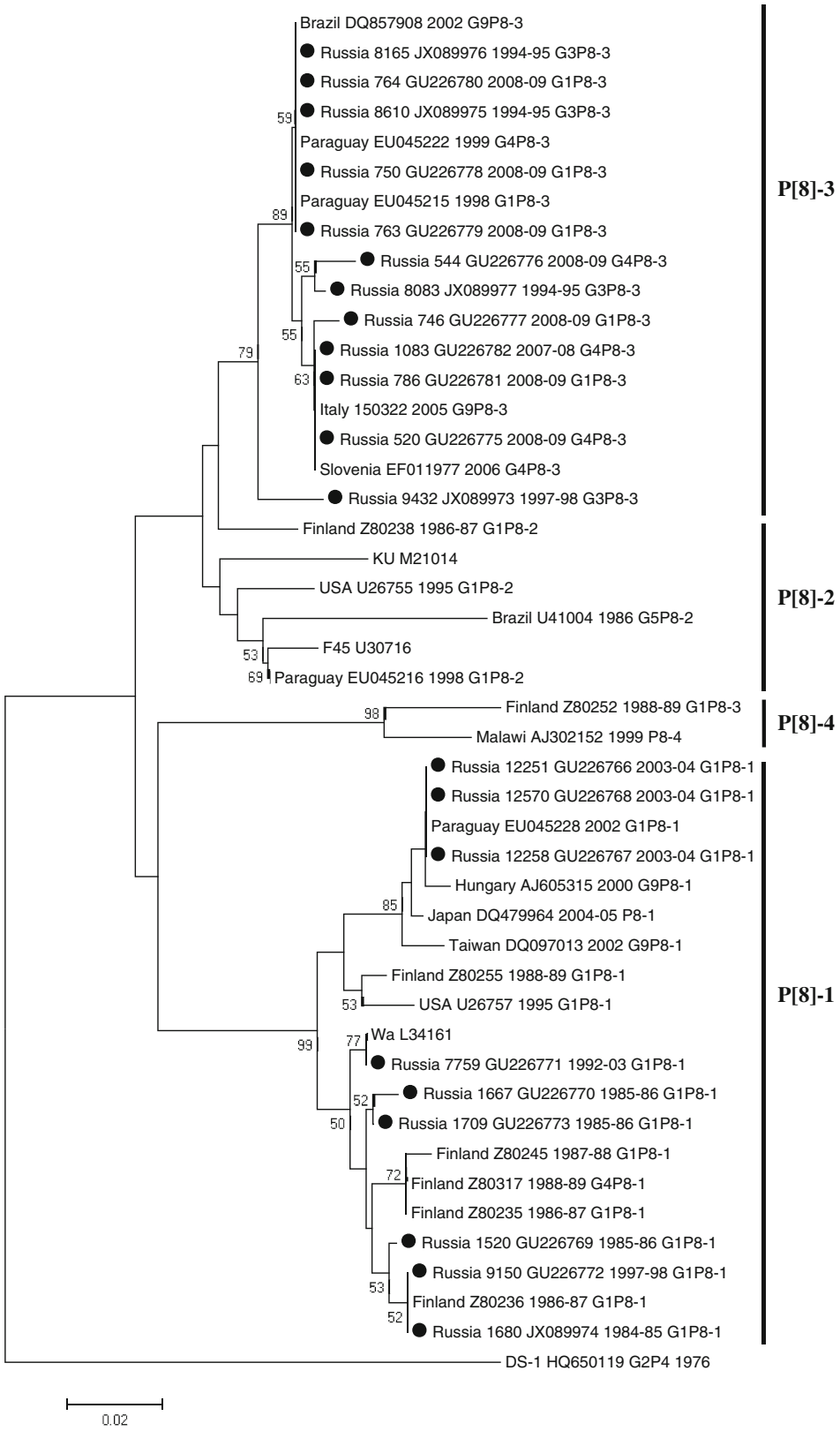

Fig. 2 Phylogenetic trees based on partial nucleotide sequences of the VP4 gene (region VP8*) of rotavirus strains P[8] isolated worldwide. The sequences obtained by us are marked with circles. Numbers of sequences obtained from the GenBank/EMBL/DDBJ database are indicated in the names of the isolates. The phylogenetic tree was made by the neighbour-joining method and the Kimura two-parameter model. Bootstrap values above 50 % are shown at branch nodes

population of Nizhny Novgorod, only one genetic variant of RV was identified, which was included in this subcluster: RV with 86th e-type RNA, which dominated in the 2003-2004 epidemic season and then disappeared.

Most RV strains circulating in the area of Nizhny Novgorod since the mid-1990 s to the present are characterized by fast-migrating fourth segments of RNA in PAGE. These strains were grouped on the phylogenetic tree in the cluster P[8]-3. Clustering did not exhibit time dependence. RV allele P[8]-3 VP4 was specific to VP7 G1, G3 and G4. On the phylogenetic tree, the cluster P[8]-3 is divided into two groups of sequences with a low level of variation (0.5 %) but a high index of support (89). It is probable that within lineage P[8]-3 VP4 there are at least two geographically independent phylogenetic lines. RV strains in Nizhny Novgorod circulating since 2005 are genetically related to the VP4 gene in strains isolated in European countries (Fig. 2). In the area of Nizhny Novgorod, the alleles P[8]-2 and P[8]-4 of gene VP4 were not detected.

We analyzed the long-term dynamics of circulating genetic variants of RV P[8] with the dominant e-type RNA, which was set with allele P[8]. Figure 3 shows that during 26 years of RV circulation with the VP4 P[8] genotype, a gradual replacement of RV strains P[8]-1 with strains of allele P[8]-3 has occurred. Our research began in 1984. At that time, and until the mid-1990 s, strains of RV P[8]-1, which had a specificity for G1 VP7, had a dominant position.

The allele P[8]-3 of the VP4 gene was first detected in the 1985-86 epidemiological season in the RV strains with the 46th e-type RNA, which also showed specificity for G1 VP7. Over time, the RV lineage P[8]-3 increased in circulation, gradually displacing the P[8]-1 strains. In 19 epidemic years, the P[8]-1 allele RV strains were displaced completely (Fig. 3).

Analysis of the evolution of a specific gene is of scientific and practical interest, especially when it comes to genes encoding protective antigens. Of particular importance are studies conducted in one geographical area over a long period of time. Such studies allow the assessment of trends in the changes of genotypes of lineages of viral proteins and their further spread, as well as analysis of the sources of the emergence of new strains. In our study, RVs samples were collected during a 26-year period in a geographically defined population group (Nizhny Novgorod, Russia; children under 6 years) with no vaccine pressure. The first observations carried out in Russia showed that in a population of RV, genotype P[8] VP4 had a dominant position throughout the period of study. We have confirmed that within genotype P[8] VP4, there are several alleles of the gene. In Nizhny Novgorod RV strains, P[8]-1 and P[8]-3 lineages were identified. At the same time, within allele P[8]-1, associated with G1 VP7, the existence of two genetic lines of RV was established: 1a and 1b, which circulated at different periods. Early strains, carrying the allele P[8]-1a associated only with G1, dominated in Nizhny Novgorod until 1995 and now is no longer detected. The RV lineage P[8]-1b in the area of Nizhny Novgorod was detected only in the 2003-04 epidemic season, after which it was also no longer detected. Since 1997 until

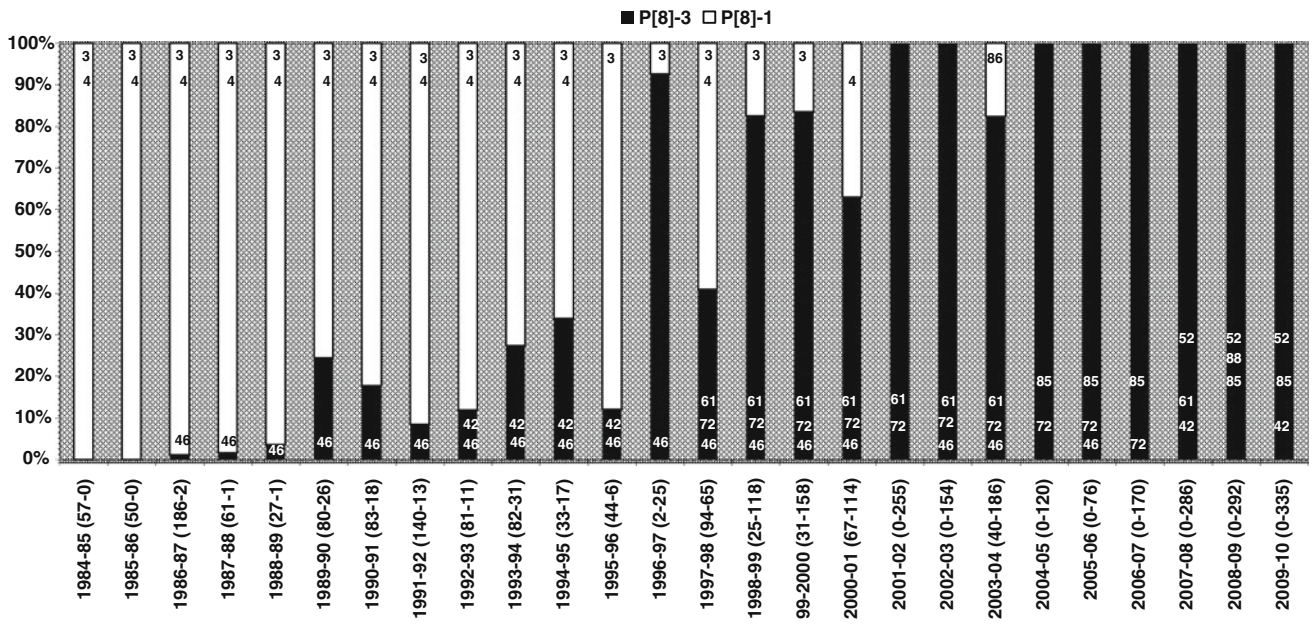

Fig. 3 The change of subgenotypes from P[8]-1 to P[8]-3 during the 26-year-long circulation of group A rotavirus among the children in Nizhny Novgorod. The actual numbers of samples with the genotype P[8] (P[8]-1 – P[8]-3) are shown in brackets after the year. The numbers on the bars indicate the e-types (as in Fig. 1) prevailing for the given epidemiological season

the present time, the dominant position in the RVs in the Nizhny Novgorod population is held by the RV strains carrying the allele P[8]-3, which is associated with G1, G3 and G4. The shift of the dominant RV allele P[8] VP4 in Nizhny Novgorod reflects the trends occurring in the world. Thus, Wa-like allele VP4 (P[8]-1a), found in RV in the 1970 s in the U.S., Asia and Europe are now not detected. Since 1995, strains G4 and G9 circulating in the world have had the allele VP4 P[8]-3 [1–3, 10].

Despite the large variety of variants of the P-genotype, P[8] is evolutionary the most successful and widespread in the world. The present article demonstrates the change of alleles within genotype P[8], which is the mechanism of its evolutionary success. These data may help to predict the emergence of a new allele or a cardinal change of genotypes in the circulation of rotaviruses.

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References

1. Arista S, Giammanco GM, De Grazia S, Migliore MC, Martella V, Cascio A (2004) Molecular characterization of the genotype G9 human rotavirus strains recovered in Palermo, Italy, during the winter of 1999–2000. Epidemiol Infect 132:343–349
2. Arista S, Giammanco GM, De Grazia S, Colomba C, Martella V (2005) Genetic variability among serotype G4 Italian human rotaviruses. J Clin Microbiol 43:1420–1425
3. Arista S, Giammanco GM, De Grazia S, Ramirez S, Lo Biundo C, Colomba C, Cascio A, Martella V (2006) Heterogeneity and temporal dynamics of evolution of G1 human rotaviruses in a settled population. J Virol 80:10724–10733
4. Bishop RF, Davidson GP, Holmes IH, Ruck BJ (1973) Virus particles in epithelial cells of duodenal mukosa from children with acute non-bakterial gastroenteritis. Lancet 8:1281–1283
5. Cunliffe NA, Gondwe JS, Graham SM, Thindwa BD, Dove W, Broadhead RL, Molyneux ME, Hart CA (2001) Rotavirus strain diversity in blantyre, Malawi, from 1997 to 1999. J Clin Microbiol 39:836–843
6. Clark HF, Offit PA, Ellis RW, Eiden JJ, Krah D, Shaw AR, Pichichero M, Treanor JJ, Borian FE, Bell LM, Plotkin SA (1996) The development of multivalent bovine rotavirus (strain WC3) reassortant vaccine for infants. J Infect Dis 174:73–80
7. Das BK, Gentsch JR, Cicirello HG, Woods PA, Gupta A, Ramachandran M, Kumar R, Bhan MK, Glass RI (1994) Characterization of rotavirus strains from newborns in New Delhi, India. J Clin Microbiol 32:1820–1822
8. Dennehy PH (2008) Rotavirus vaccines: an overview. J Clin Microbiol 21:198–208
9. Estes MK, Cohen J (1989) Rotavirus gene structure and function. Microbiol Rev 53:410–449
10. Espinola EE, Amarilla A, Arbiza J, Parra GI (2008) Sequence and phylogenetic analysis of the VP4 gene of human rotaviruses isolated in Paraguay. Arch Virol 153:1067–1073
11. Gentsch JR, Glass RI, Woods P, Gouvea V, Gorziglia M, Flores J, Das BK, Bhan MK (1992) Identification of group A rotavirus gene 4 types by polymerase chain reaction. J Clin Microbiol 30:1365–1373
12. Gentsch JR, Laird AR, Bielfelt B, Griffin DD, Banyai K, Ramachandran M, Jain V, Cunliffe NA, Nakagomi O, Kirkwood CD, Fischer TK, Parashar UD, Bresee JS, Jiang B, Glass RI (2005) Serotype diversity and reassortment between human and animal rotavirus strains: implications for rotavirus vaccine programs. J Infect Dis 12:146–159
13. Gouvea V, Glass RI, Woods P, Taniguchi K, Clark HF, Forrester B, Fang ZY (1990) Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J Clin Microbiol 28:276–282
14. Herring AJ, Inglis NF, Ojeh CK, Snodgrass DR, Menzies JD (1982) Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. J Clin Microbiol 16:473–477
15. Hoshino Y, Kapikian AZ (2000) Rotavirus serotypes: classification and importance in epidemiology, immunity, and vaccine development. J Health Popul Nutr 18:5–14
16. Iturriza-Gómara M, Green J, Brown DW, Desselberger U, Gray JJ (2000) Diversity within the VP4 gene of rotavirus P[8] Strains: implications for reverse transcription-PCR genotyping. J Clin Microbiol 38:898–901
17. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685
18. Le VP, Chung YC, Kim K, Chung SI, Lim I, Kim W (2010) Genetic variation of prevalent G1P[8] human rotaviruses in South Korea. J Med Virol 82:886–896
19. Matthijnssens J, Ciarlet M, Heiman E, Arijs I, Delbeke T, McDonald SM, Palombo EA, Iturriza-Gómara M, Maes P, Patton JT, Rahman M, Van Ranst M (2008) Full genome-based classification of rotaviruses reveals a common origin between human Wa-like and porcine rotavirus strains and human DS-1-like and bovine rotavirus strains. J Virol 82:3204–3219
20. Matthijnssens J, Ciarlet M, McDonald SM, Attoui H, Bányai K, Brister JR, Buesa J, Esona MD, Estes MK, Gentsch JR, Iturriza-Gómara M, Johne R, Kirkwood CD, Martella V, Mertens PP, Nakagomi O, Parreño V, Rahman M, Ruggeri FM, Saif LJ, Santos N, Steyer A, Taniguchi K, Patton JT, Desselberger U, Van Ranst M (2011) Uniformity of rotavirus strain nomenclature proposed by the rotavirus classification working group (RCWG). Arch Virol 156:1397–1413
21. Maunula L, von Bonsdorff CH (1998) Short sequences define genetic lineages: phylogenetic analysis of group A rotaviruses based on partial sequences of genome segments 4 and 9. J Gen Virol 79:321–332
22. Nagashima S, Kobayashi N, Paul SK, Ghosh S, Chawla-Sarkar M, Hossain MA, Krishnan T (2010) Identification of P[8]b subtype in OP354-like human rotavirus strains by a modified RT-PCR method. Jpn J Infect Dis 63:208–211
23. Novikova NA, Fedorova OF, Epifanova NV, Chuprova AB (2007) G[P] type profiles of group A human rotavirus and their distribution in Nizhni Novgorod and Dzerzhinsk in 1997–2005. Vopr Virusol 52:19–23
24. Santos N, Hoshino Y (2005) Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. Rev Med Virol 15:29–56
25. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599
26. Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD, WHO-coordinated Global Rotavirus Surveillance Network (2012) 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infect Dis 12:136–141