Infection, Genetics and Evolution 35 (2015) 34–37

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Short communication

Identification of a multi-reassortant G12P[9] rotavirus with novel VP1, VP2, VP3 and NSP2 genotypes in a child with acute gastroenteritis

Simona De Grazia [a,*], Giovanni M. Giammanco [a], Renáta Dóró [b], Floriana Bonura [a], Szilvia Marton [b], Antonio Cascio [c], Vito Martella [d], Krisztián Bányai [b]

[a] Department of Health Promotion Sciences and Mother and Child Care "G. D'Alessandro", University of Palermo, Palermo, Italy
[b] Veterinary Medical Research Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary
[c] Department of Human Pathology, University of Messina, Messina, Italy
[d] Department of Veterinary Medicine, University Aldo Moro of Bari, Valenzano, Italy

ARTICLE INFO

Article history:
Received 29 May 2015
Received in revised form 13 July 2015
Accepted 20 July 2015
Available online 20 July 2015

Keywords:
Human rotavirus
G12P[9]
Full genome sequencing
Genotyping
Interspecies transmission
Reassortment

ABSTRACT

The G12 rotavirus genotype is globally emerging to cause severe gastroenteritis in children. Common G12 rotaviruses have either a Wa-like or DS-1-like genome constellation, while some G12 strains may have unusual genome composition. In this study, we determined the full-genome sequence of a G12P[9] strain (ME848/12) detected in a child hospitalized with acute gastroenteritis in Italy in 2012. Strain ME848/12 showed a complex genetic constellation (G12-P[9]-I17-R12-C12-M11-A12-N12-T7-E6-H2), likely derived from multiple reassortment events, with the VP1, VP2, VP3 and NSP2 genes being established as novel genotypes R12, C12, M11 and N12, respectively. Gathering sequence data on human and animal rotaviruses is important to trace the complex evolutionary history of atypical RVAs.

1. Introduction

Group A rotaviruses (RVAs) are important gastroenteric pathogens of human and animals (Estes and Greenberg, 2013). RVA genome is composed of 11 segments of double stranded RNA, enclosed in a triple-layered capsid. The outer capsid proteins, VP7 and VP4, are the main neutralization antigens, and provide the basis for RVA classification (Estes and Greenberg, 2013). Whole genome classification of RVAs has been implemented to provide a reliable and standardized nomenclature and decipher the relationships between human and animal strains (Matthijnssens et al., 2008). Epidemiological studies and genetic characterization of human RVAs have revealed three major genetic groups, Wa-like (genogroup 1), DS-1 like (genogroup 2) and AU-1 like (genogroup 3), that tend to be stably conserved (Matthijnssens et al., 2008).

RVAs of genotype G12 were first reported in humans in 1987 in the Philippines. A decade later G12 strains began to emerge worldwide, being increasingly detected in several countries (Komoto et al., 2014; Rahman et al., 2007; Wakuda et al., 2003). At present, G12 strains are considered among the six most prevalent human VP7 genotypes, being predominantly found in association with either the P[6] or P[8] VP4 genotype and, sporadically, with P[4] and P[9] (Komoto et al., 2014; Rahman et al., 2007; Wakuda et al., 2003). The ability of G12 RVAs to spread across human populations has been hypothesized to derive from a peculiar genomic plasticity, driven by multiple reassortment events (Rahman et al., 2007). Surveillance for RVAs was started in Sicily, Italy, in 1984 and conducted, uninterruptedly, for more than 30 years, providing a unique epidemiological observatory to monitor the evolution of human RVA (De Grazia et al., 2014). Until 2012, G12 strains were never reported as causative agents of gastroenteritis in Sicily. However, in the years 2012–2014, G12 RVAs accounted for 8.72% (34/390) of the detected RVAs. All the G12 RVA strains had a P[8] type, with exception of a unique strain (ME848/12) that had a P[9] VP4. Upon sequence analyses of VP7 gene, strain ME848/12 displayed a VP7 of lineage G12-II, a peculiar lineage that is shared with human strains of unusual genetic make up, all of which seem to have originated by multiple reassortment events accumulating over an AU-1-like genome backbone (Gomez et al., 2014; Wakuda et al., 2003). In order to define the possible origin of strain ME848/12, the full-length genome of this rare virus was determined and analyzed in detail.

* Corresponding author at: Department of Health Promotion Sciences and Mother and Child Care "G. D'Alessandro", University of Palermo, via del Vespro 133, 90127, Italy.
E-mail address: simona.degrazia@unipa.it (S. De Grazia).

http://dx.doi.org/10.1016/j.meegid.2015.07.023
1567-1348/

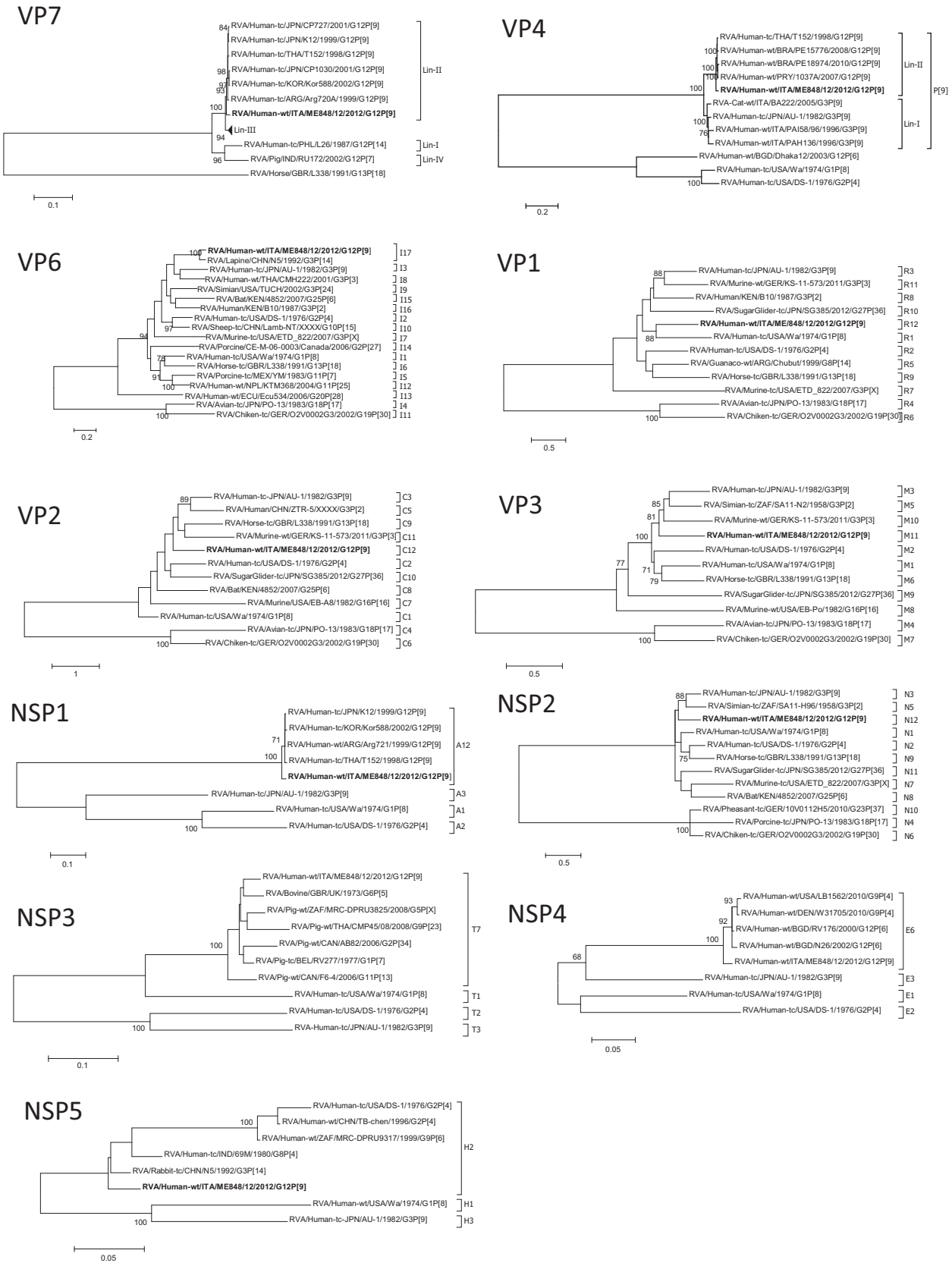

Fig. 1. Phylogenetic trees of the full-length genome of the strain ME848/12. Phylogenetic trees were constructed with the maximum likelihood method using the best fit substitution models for each gene (VP1 and VP2, GTR + G; VP3, VP4, and VP6, GTR + G + I; VP7 and NSP4, HKY + I; NSP1, HKY + G; NSP2, NSP3, and NSP5, T92 + G). Bootstrap values (500 replicates) above 70% are shown. Reference human strains Wa, DS-1 and AU-1, representative of genogroups 1–3, are systematically included in order to provide outgroup strains. In the four RNA segments (VP1, VP2, VP3 and NSP2) where new genotypes were attributed to ME848/12 prototype sequences of all existing genotypes are included. The ME848/12 strain sequences are shown in bold.

Table 1
Genotype constellation and genetic relatedness (in gray) of Italian G12 strain (ME848/12) to all available G12P[9] strains and other selected RVA strains. The new genotypes are indicated in bold.

Strain	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
RVA/human-tc/JPN/AU-1/1982/G3P[9]	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3
RVA/human-tc/THA/T152/1998/G12P[9]	G12	P[9]	I3	R3	C3	M3	A12	N3	T3	E3	H6
RVA/human-wt/ARG/Arg720/1999/G12P[9]	G12	P[9]	–	–	–	–	A12	–	–	–	–
RVA/human-wt/ARG/Arg721/1999/G12P[9]	G12	P[9]	–	–	–	–	A12	–	–	–	–
RVA/human-tc/JPN/K12/1999/G12P[9]	G12	P[9]	–	–	–	–	A12	–	–	–	–
RVA/human-wt/BRA/PE15776/2008/G12P[9]	G12	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H6
RVA/human-wt/BRA/PE18974/2010/G12P[9]	G12	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H6
RVA/human-wt/PRY/1037/2007/G12P[9]	G12	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H6
RVA/human-wt/ITA/ME848/12/2012/G12P[9]	G12	P[9]	I17	**R12**	**C12**	**M11**	A12	**N12**	T7	E6	H2
RVA/human-wt/BGD/RV176/2000/G12P[6]	G12	P[6]	I2	R2	C2	M2	A2	N2	T2	E6	H2
RVA/cow-tc/GBR/UK/1973/G6P[5]	G6	P[5]	I2	R2	C2	M2	A3	N2	T7	E2	H3
RVA/rabbit-tc/CHN/N5/1992/G3P[14]	G3	P[14]	I17	R3	C3	M3	A9	N1	T1	E3	H2

Table 2
Nucleotide identity of the 11 genome segments of the Italian G12P[9] strain ME848/12 against prototype RVA strains and closest matches recovered from GenBank. The new genotypes are indicated in bold.

Gene encoding	Cut-off% value for genotype assignation	Nucleotide identity% to		Genotype assigned
		Closest prototype strain (strain name/host origin)	Closest GenBank match (strain name/host origin)	
VP7	80	91 (L26/human)	98.6 (Arg720/human)	G12
VP4	80	88.9 (AU-1/human)	97.7 (T152/human)	P[9]
VP6	85	92 (N5/rabbit)	92 (N5/rabbit)	I17
VP1	83	None above cut-off	None above cut-off	**R12**
VP2	84	None above cut-off	None above cut-off	**C12**
VP3	81	None above cut-off	None above cut-off	**M11**
NSP1	79	97.3 (T152/human)	97.7 (Arg721/human)	A12
NSP2	85	None above cut-off	None above cut-off	**N12**
NSP3	85	95.6 (UK/bovine)	95.6 (RV277/pig)	T7
NSP4	85	97.7 (N26/human)	98.5 (RV176/human)	E6
NSP5	91	88.7 (DS-1/human)	96.5 (N5/rabbit)	H2

2. Materials and methods

2.1. Patient

In August 2012, a 2-year old child was hospitalized with severe acute gastroenteritis at the "G. Martino" University Hospital of Messina, Messina, Italy. On admission, the child had fever (37.5–38 °C), vomiting and diarrhea. Intravenous infusion of rehydrating solution was administered. Liquid stools lasted 6 days with an average of 4–5 stools/day. The child was not vaccinated for rotavirus.

2.2. Rotavirus detection and full-genome analyses

A rotavirus strain (ME848/12) was identified in this sample using enzyme immunoassay (RIDASCREEN Rotavirus, R-Biopharm AG, Darmstadt, Germany) and genotyped by RT-PCR as a G12P[9] (Gentsch et al., 1992; Gouvea et al., 1990; Iturriza-Gomara et al., 2004). In order to understand the evolutionary origin of strain RV A/human-wt/ITA/ME848/12/2012/G12P[9], the complete genome of 18,772 nucleotides (nt) in length was determined as previously described (Doro et al., 2014). Phylogenetic analyses were performed by MEGA 6 software (www.megasoftware.net) on the 11 dsRNA segments. Best fit substitution models were selected for each data set based on the Bayesian information criterion as implemented in MEGA 6. Subsequently, maximum likelihood trees were generated and bootstrap analysis was performed with 500 replications. The nucleotide sequences of the full-length genome of ME848/12 were deposited in GenBank under accession numbers KR632620–KR632630.

3. Results and discussion

Full-length genome analysis revealed that the ME848/12 strain possessed a complex genetic constellation possibly due to multiple reassortment events between human and animal rotaviruses. Strain ME848/12 possessed VP7, VP4 and NSP1 genes closely related to those of human G12P[9] strains. The NSP4 gene was similar to human G12P[6] RVAs and the VP6 and NSP5 genes were similar to the rabbit strain N5. The NSP3 was similar to that of the bovine strain UK and porcine strain RV277, while the VP1–3 and NSP2 genes were genetically distant from all established genotypes (Fig. 1). In detail, the VP1, VP2, VP3 and NSP2 genes of strain ME848/12 showed 65.5–78%, 59.4–78.7%, 50.3–75% and 48.2–78.3% nt identity to established reference strains of genotypes R, C, M and N respectively. Therefore, the VP1–3 and NSP2 genes were classified as novel genotypes R12, C12, M11 and N12, respectively, by the Rotavirus Classification Working Group (RCWG), allowing the classification of strain ME848/12 as G12-P[9]-I17-R12-C12-M11-A12-N12-T7-E6-H2, according to the current classification system (Matthijnssens et al., 2008). The nt identity values of the other genes of strain ME848/12 compared to cognate sequences recovered from GenBank are shown in Table 1.

Whole genome analyses of currently circulating G12 strains from different parts of the world have revealed that the G12 VP7 is mainly associated with any of the three human RVA genogroups, Wa, DS-1 and AU-1-like. Usually, G12P[8] strains possess a Wa-like backbone, while G12P[4]/P[6] and G12P[9] RVAs are generally DS-1-like and AU-1-like, respectively (Rahman et al., 2007). All of these strains are considered to be the result of the introduction of a G12 VP7 into a stable genetic constellation of human RVA genes. However, reassortant G12 strains showing complex gene

combinations have been sporadically reported. Such strains have been considered to be the product of multiple reassortment steps involving both human and animal strains (Wakuda et al., 2003).

Unlike other G12P[9] RVAs, strain ME848/12 did not possess an AU-1-like genetic make up (Table 2). With exception of the G12 VP7 of lineage-II, of the P[9] VP4 gene, and of the A12 NSP1, that are usually associated with G12P[9] RVAs (Gomez et al., 2014; Wakuda et al., 2003), the virus exhibited a complex constellation of genome segments. The NSP3 gene (genotype T7) of strain ME848/12 was closely related to that of porcine RVAs, of the bovine prototype UK (Martel-Paradis et al., 2013) and of unusual human reassortant viruses (Papp et al., 2013). The NSP4 gene of ME848/12 clustered in the E6 lineage together with other human G12P[6] strains. This was not unexpected, as the E6-genotype NSP4 has been found in several AU-1-like strains (Rahman et al., 2007; Sharma et al., 2009). In the NSP5 and VP6 genes, strain ME848/12 was more closely related to the H2-type NSP5 gene and I17-type VP6 gene of the multi-reassortant G3P[14] lapine strain N5. This rare NSP5–VP6 combination may be a serendipitous event or may suggest a common point during the evolution of these two distinct strains.

The mechanisms giving rise to human RVAs with novel or atypical genotypes are interspecies transmission, genetic reassortment events, point mutations and genome recombination. Comparison of the ME848/12 genotype constellation with other completely sequenced RVA genomes revealed that this unusual G12P[9] strain shared several genome segments, respectively, with either mono- or multi- reassortant human (T152 and RV176), and animal (lapine N5 and bovine UK) RVAs. The G12P[9] strain T152 showed an AU-1-like genetic make up, with the NSP1 and NSP5 genes likely derived from bovine viruses, while the G12P[6] strain RV176-00 showed a DS-1 genetic backbone with a distantly related NSP4 gene (Rahman et al., 2007). The rabbit G3P[14] strain N5 is supposed to have originated after multiple reassortments involving canine, feline and human rotaviruses (Guo et al., 2012). The bovine G6P[5] strain, UK, is a mono-reassortant with a porcine-derived NSP3 gene and a bovine genetic backbone (Martel-Paradis et al., 2013).

4. Conclusions

Overall, it is impossible to establish the temporal order of the reassortment events leading to the unusual genomic constellation of this novel G12P[9] strain. It is unclear whether this RVA strain was generated in a human host by reassortment with a homologous virus, or it was transmitted directly from an animal host that acted as a mixing vessel, or, more likely, it was the result of consecutive interspecies transmissions intermingled with reassortment events in various hosts. Whole genome analysis of strain ME848/12 revealed an impressive genetic diversity and allowed to identify novel VP1, VP2, VP3 and NSP2 genotypes. In order to allowed identification of novel alleles, and whether the complex gene constellation observed in strain ME848/12 is unique or it is retained in other RVAs, it will be important to gather sequence data on other human and animal RVA strains, extending RVA surveillance also to non-domesticated animals.

Acknowledgments

The study was supported, in part, by the Hungarian Scientific Research Fund (OTKA, T100727) and the Momentum program.

References

De Grazia, S., Bonura, F., Colomba, C., Cascio, A., Di Bernardo, F., Collura, A., Terranova, D.M., Martella, V., Giammanco, G.M., 2014. Data mining from a 27-years rotavirus surveillance in Palermo, Italy. Infect. Genet. Evol. 28, 377–384.

Doro, R., Mihalov-Kovacs, E., Marton, S., Laszlo, B., Deak, J., Jakab, F., Juhasz, A., Kisfali, P., Martella, V., Melegh, B., Molnar, P., Santha, I., Schneider, F., Banyai, K., 2014. Large-scale whole genome sequencing identifies country-wide spread of an emerging G9P[8] rotavirus strain in Hungary, 2012. Infect. Genet. Evol. 28, 495–512.

Estes, M.K., Greenberg, H.B., 2013. Rotaviruses, 6th ed. Lippincott Williams and Wilkins, Philadelphia.

Gentsch, J.R., Glass, R.I., Woods, P., Gouvea, V., Gorziglia, M., Flores, J., Das, B.K., Bhan, M.K., 1992. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J. Clin. Microbiol. 30, 1365–1373.

Gomez, M.M., Resque, H.R., Volotao Ede, M., Rose, T.L., da Silva, M.F., Heylen, E., Zeller, M., Matthijnssens, J., Leite, J.P., 2014. Distinct evolutionary origins of G12P[8] and G12P[9] group A rotavirus strains circulating in Brazil. Infect. Genet. Evol. 28, 385–388.

Gouvea, V., Glass, R.I., Woods, P., Taniguchi, K., Clark, H.F., Forrester, B., Fang, Z.Y., 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J. Clin. Microbiol. 28, 276–282.

Guo, D., Liu, J., Lu, Y., Sun, Y., Yuan, D., Jiang, Q., Lin, H., Li, C., Si, C., Qu, L., 2012. Full genomic analysis of rabbit rotavirus G3P[14] strain N5 in China: identification of a novel VP6 genotype. Infect. Genet. Evol. 12, 1567–1576.

Iturriza-Gomara, M., Kang, G., Gray, J., 2004. Rotavirus genotyping: keeping up with an evolving population of human rotaviruses. J. Clin. Virol. 31, 259–265.

Komoto, S., Wandera Apondi, E., Shah, M., Odoyo, E., Nyangao, J., Tomita, M., Wakuda, M., Maeno, Y., Shirato, H., Tsuji, T., Ichinose, Y., Taniguchi, K., 2014. Whole genomic analysis of human G12P[6] and G12P[8] rotavirus strains that have emerged in Kenya: identification of porcine-like NSP4 genes. Infect. Genet. Evol. 27, 277–293.

Martel-Paradis, O., Laurin, M.A., Martella, V., Sohal, J.S., L'Homme, Y., 2013. Full-length genome analysis of G2, G9 and G11 porcine group A rotaviruses. Vet. Microbiol. 162, 94–102.

Matthijnssens, J., Ciarlet, M., Rahman, M., Attoui, H., Banyai, K., Estes, M.K., Gentsch, J.R., Iturriza-Gomara, M., Kirkwood, C.D., Martella, V., Mertens, P.P., Nakagomi, O., Patton, J.T., Ruggeri, F.M., Saif, L.J., Santos, N., Steyer, A., Taniguchi, K., Desselberger, U., Van Ranst, M., 2008. Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. Arch. Virol. 153, 1621–1629.

Papp, H., Borzak, R., Farkas, S., Kisfali, P., Lengyel, G., Molnar, P., Melegh, B., Matthijnssens, J., Jakab, F., Martella, V., Banyai, K., 2013. Zoonotic transmission of reassortant porcine G4P[6] rotaviruses in Hungarian pediatric patients identified sporadically over a 15 year period. Infect. Genet. Evol. 19, 71–80.

Rahman, M., Matthijnssens, J., Yang, X., Delbeke, T., Arijs, I., Taniguchi, K., Iturriza-Gomara, M., Iftekharuddin, N., Azim, T., Van Ranst, M., 2007. Evolutionary history and global spread of the emerging g12 human rotaviruses. J. Virol. 81, 2382–2390.

Sharma, S., Paul, V.K., Bhan, M.K., Ray, P., 2009. Genomic characterization of nontypeable rotaviruses and detection of a rare G8 strain in Delhi, India. J. Clin. Microbiol. 47, 3998–4005.

Wakuda, M., Nagashima, S., Kobayashi, N., Pongsuwanna, Y., Taniguchi, K., 2003. Serologic and genomic characterization of a G12 human rotavirus in Thailand. J. Clin. Microbiol. 41, 5764–5769.