

Feline Origin of Rotavirus Strain, Tunisia, 2008

Mouna Ben Hadj Fredj, Elisabeth Heylen,
Mark Zeller, Imene Fodha,
Meriam Benhamida-Rebai, Marc Van Ranst,
Jelle Matthijssens, and Abdelhalim Trabelsi

In Tunisia in 2008, an unusual G6P[9] rotavirus, RVA/human-wt/TUN/17237/2008/G6P[9], rarely found in humans, was detected in a child. To determine the origin of this strain, we conducted phylogenetic analyses and found a unique genotype constellation resembling rotaviruses belonging to the feline BA222-like genotype constellation. The strain probably resulted from direct cat-to-human transmission.

Group A rotaviruses (RVAs) are a leading cause of severe acute gastroenteritis in infants and young children. An infectious RVA virion is a triple-layered icosahedral particle that contains 11 segments of double-stranded RNA (1). The outer protein layer is formed by virus capsid protein (VP) 4 (P antigen) and VP7 (G antigen), each of which is used for binomial nomenclature (1). At least 27 G genotypes and 35 P genotypes have been identified (2). Globally, only 6 G/P-genotype combinations are of epidemiologic relevance to humans: G1P[8], G3P[8], G4P[8], G9P[8], and G12P[8], which are typically found in combination with a Wa-like genotype constellation (I1-R1-C1-M1-A1-N1-T1-E1-H1), and G2P[4], which is found in combination with a DS-1-like genotype constellation (I2-R2-C2-M2-A2-N2-T2-E2-H2) (3).

Certain G genotypes rarely encountered in humans are commonly associated with RVA strains from animals (4). For example, G6 RVA strains are occasionally detected in humans but are a common genotype in cattle (4). Complete genomes have been determined for 11 human G6 RVA strains: 7 G6P[14], 2 G6P[9], 1 human-animal reassortant G6P[6], and 1 unique G6P[11] (5–9).

The P[9] genotype is commonly associated with the G3 or G6 genotype and is believed to be typical for feline and canine RVA strains (4). A few G3P[9] and G3P[3]

RVA strains have been detected in humans, and they are believed to be the result of direct interspecies transmission from cats or dogs to humans, possibly in combination with reassortment (10–13).

Previously, 2 genotype constellations among feline and canine RVA strains, cat97-like and AU-1-like, were described (13). The genotype constellations were G3-P[3]-I3-R3-C2-M3-A9-N2-T3-E3-H6 and G3-P[9]-I3-R3-C3-M3-A3-N3-T3-E3-H3, respectively. Recently, the complete genomes of a feline strain (RVA/cat-wt/ITA/BA222/2005/G3P[9]) and 2 feline-like human RVA strains (RVA/human-wt/ITA/PAI58/1996/G3P[9] and RVA/human-wt/ITA/PAH136/1996/G3P[9]) were shown to possess a distinct genotype constellation, G3-P[9]-I2-R2-C2-M2-A3-N1-N2-T3/T6-E2-H3 (11), representing a tentative third feline genotype constellation (BA222-like). This tentative third feline BA222-like genotype constellation is an intriguing genotype mosaic, sometimes possessing Wa-like nonstructural protein (NSP) 2 or NSP3 gene segments and partially resembling the genotype constellation found in RVA strains from cattle and other artiodactyla (5,7,10,11). Full-genome sequences of unusual human RVA strains are being analyzed to detect interspecies transmission, reassortment, and evolutionary relationships between human and animal RVAs (10,11).

In 2008, during continuous surveillance for human RVA in Tunisia, we identified an unusual G6P[9] strain in an 8-month-old hospitalized child (14). To understand the evolution and origin of this unusual strain, RVA/human-wt/TUN/17237/2008/G6P[9] (hereafter referred to as strain 17237), we conducted phylogenetic analyses.

The Study

The full-length genome sequence of the virus was determined as described (5). Primers used for all 11 segments are shown in online Technical Appendix Table 1 (wwwnc.cdc.gov/EID/article/19/4/12-1383-Techapp1.pdf). Multiple sequence alignments and phylogenetic analyses were conducted by using MEGA version 5.05 (www.megasoftware.net). Sequences were deposited in GenBank (accession nos. JX271001–JX271011).

Strain 17237 possessed the unique genotype constellation G6-P[9]-I2-R2-C2-M2-A3-N1-T6-E2-H3. This constellation was compared with that of the human G6P[9] strain Se584, feline/canine-like human RVA strains (KF17, PAH136, PAI58, and 0537), and several animal strains (Table). Strain 17237 shared the same combination of genotypes with human RVA strain PAH136 (10) except for VP7 (strain 17237 contained G6 instead G3). Overall, strain 17237 shared 8–10 genotypes with RVA strains possessing the BA222-like genotype constellation and 8–9 genotypes with several bovine or bovine-like RVA strains.

Author affiliations: Sahloul University Hospital, Sousse, Tunisia (M. Ben Hadj Fredj, I. Fodha, M. Benhamida-Rebai, A. Trabelsi); University of Monastir, Monastir, Tunisia (M. Ben Hadj Fredj, I. Fodha, M. Benhamida-Rebai, A. Trabelsi); and University of Leuven, Leuven, Belgium (E. Heylen, M. Zeller, M. Van Ranst, J. Matthijssens)

DOI: <http://dx.doi.org/10.3201/eid1904.121383>

Table. Comparison of genomic constellation of group A rotavirus strain RVA/human-wt/TUN/17237/2008/G6P[9] from Tunisia with reference strains*

Strain	Genotype constellation	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
RVA/human-wt/TUN/17237/2008/G6P[9]	BA222-like	G6	P[9]	I2	R2	C2	M2	A3	N1	T6	E2	H3
RVA/human-wt/ITA/PAH136/1996/G3P[9]	BA222-like	G3	P[9]	I2	R2	C2	M2	A3	N1	T6	E2	H3
RVA/cat-wt/ITA/BA222/2005/G3P[9]	BA222-like	G3	P[9]	I2	R2	C2	M2	A3	N1	T3	E2	H3
RVA/human-wt/ITA/PAI58/1996/G3P[9]	BA222-like	G3	P[9]	I2	R2	C2	M2	A3	N2	T6	E2	H3
RVA/human-tc/USA/Se584/1998/G6P[9]	BA222-like	G6	P[9]	I2	R2	C2	M2	A3	N2	T1	E2	H3
RVA/human-wt/JAP/KF17/2009/G6P[9]	BA222-like	G6	P[9]	I2	R2	C2	M2	A3	N2	T3	E3	H3
RVA/human-wt/USA/0537/2002/G3P[9]	BA222-like	G3	P[9]	I2	R2	C2	M2	A3	N2	T1	E2	H3
RVA/cat-tc/AUS/Cat2/1984/G3P[9]	BA222-like	G3	P[9]	I3	R3	C2	M3	A3	N1	T6	E3	H3
RVA/human-tc/ITA/PA169/1988/G6P[14]	Bovine-like	G6	P[14]	I2	R2	C2	M2	A3	N2	T6	E2	H3
RVA/human-wt/BEL/B10925/1997/G6P[14]	Bovine-like	G6	P[14]	I2	R2	C2	M2	A3	N2	T6	E2	H3
RVA/human-wt/ITA/111-05-27/2005/G6P[14]	Bovine-like	G6	P[14]	I2	R2	C2	M2	A3	N2	T6	E2	H3
RVA/cow-tc/FRA/RF/1982/G6P[1]	Bovine	G6	P[1]	I2	R2	C2	M2	A3	N2	T6	E2	H3
RVA/cow-tc/VEN/BRV033/1990/G6P6[1]	Bovine	G6	P[1]	I2	R2	C2	M2	A3	N2	T6	E2	H3
RVA/cow-tc/USA/WC3/1981/G6P[5]	Bovine	G6	P[5]	I2	R2	C2	M2	A3	N2	T6	E2	H3
RVA/cow-tc/KOR/KJ19-2/2004/G6P[7]	Bovine	G6	P[7]	I2	R2	C2	M2	A3	N2	T6	E2	H3
RVA/rhesus-tc/USA/PTRV/1990/G8P[1]	Bovine-like	G8	P[1]	I2	R2	C2	M2	A3	N2	T6	E2	H3
RVA/human-tc/KEN/B12/1987/G8P[1]	Bovine-like	G8	P[1]	I2	R2	C2	M2	A3	N2	T6	E2	H3
RVA/human-tc/USA/DS-1/1976/G2P[4]	DS-1-like	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/human-tc/JPN/AU-1/1982/G3P3[9]	AU-1-like	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3
RVA/human-tc/USA/Wa/1974/G1P1A[8]	Wa-like	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1

***Boldface** indicates genotypes that are identical to group A rotavirus RVA/human-wt/TUN/17237/2008/G6P[9]. VP, virus capsid protein; NSP, nonstructural protein.

Phylogenetic analyses showed that all 11 genome segments of strain 17237 were most closely related to strains of either feline-like human or feline origin (Figures 1, 2). Strain 17237 clustered most closely with RVA/human-wt/ITA/PA43/2003/G6P[9], RVA/human-wt/JAP/KF17/2009/G6P[9], and RVA/human-wt/BEL/B1711/2002/G6P[6] strains, all of which are suspected to have at least a partial animal (bovine-like or feline-like) origin (6,7). The P[9] genome segment was most closely related to RVA strains RVA/human-wt/RUS/Nov10-N507/2010/G3P[9], BA222, and KF17. The VP1, VP6, NSP2, and NSP4 genome segments of strain 17237 were closely related to BA222, clustering in the R2, I2, N1, and E2 genotypes, respectively. This G3P[9] feline RVA strain BA222 is believed to have a common origin with animal RVA strains and RVA strains that are zoonotically transmissible to humans (11). The NSP2 gene segment

of strain 17237 clustered in the N1 genotype and was distantly related to typical human Wa-like RVA strains. The VP2, NSP3, and NSP5 gene segments were closely related to RVA/human-wt/ITA/PAI58/1996/G3P[9]. The VP3, NSP1, and NSP3 genome segments clustered closely with RVA/human-wt/ITA/PAH136/1996/G3P[9]. NSP1 and NSP5 clustered closely with RVA/human-wt/USA/0537/2002/G3P[9]. These 3 human strains (PAI58-96, PAH136-96, and 0537) are believed to be of feline origin and possess a BA222-like genotype constellation.

Conclusions

The genome constellation of strain 17237 is similar to that of strains belonging to the tentative feline BA222-like genotype constellation (Table). It has been speculated that several of these BA222-like RVA strains resulted from multiple reassortment events among RVA strains

originating from different hosts (cattle, other ruminants, humans, cats, dogs) (10,11). However, a recent article speculates that this genotype constellation, although reminiscent to bovine-like RVA strains, might represent a true feline genotype constellation (12).

Our results support this hypothesis in 2 ways. The first source of support comes from the fact that BA222-like RVA strains have been detected on several continents:

Europe (Italy), North America (United States), Asia (Japan), and now Africa (Tunisia) (7,10). RVA strains with this BA222-like genotype constellation are much more likely to circulate in a certain host species rather than result from distinct multiple reassortment events in each of the above-mentioned countries. The second source of support comes from the fact that our phylogenetic analyses confirmed that each of the 11 gene segments

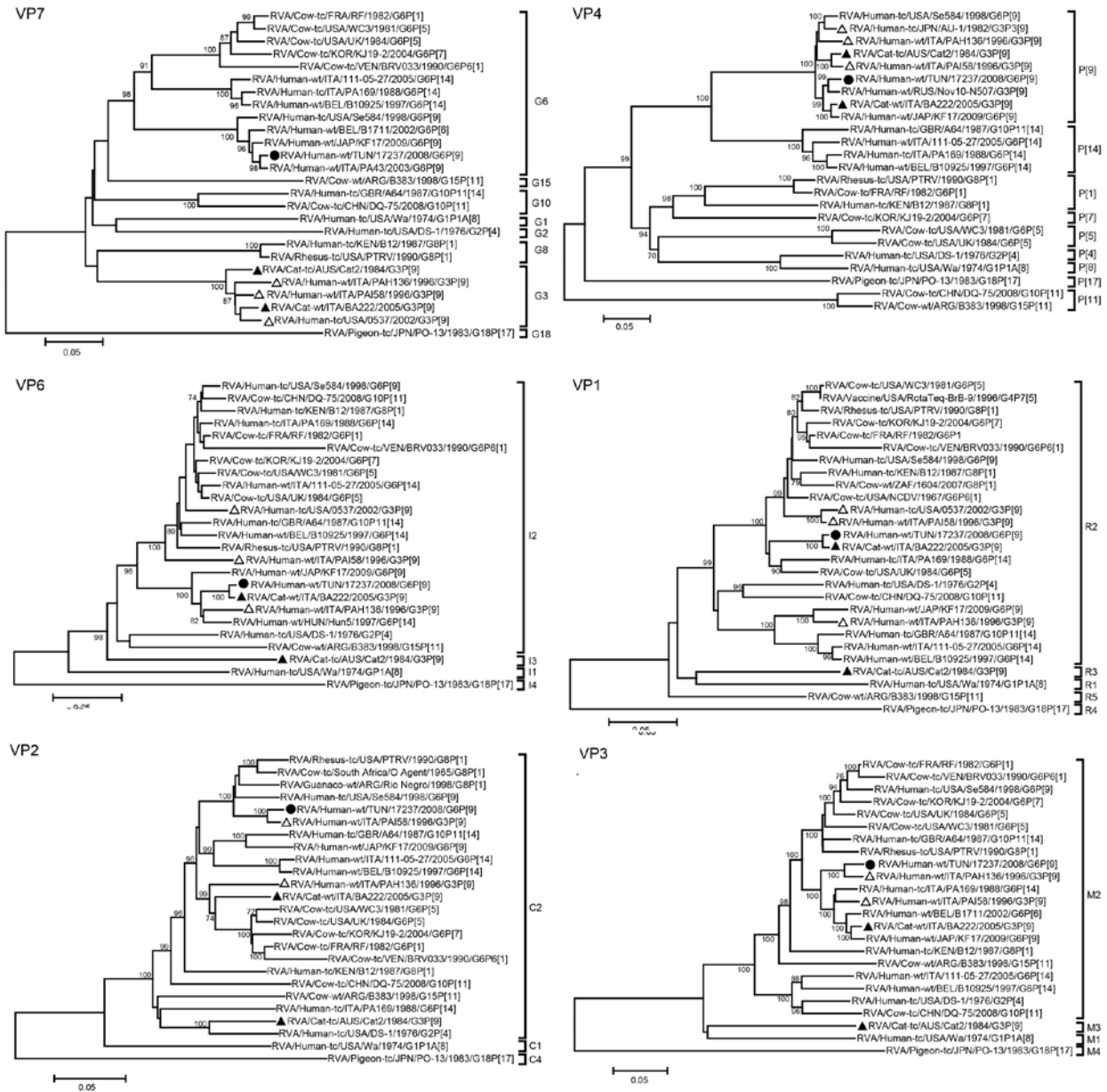


Figure 1. Phylogenetic trees of the full-length nucleotide sequences of the group A rotavirus (RVA) virus capsid protein (VP) 7, VP4, VP6, VP1, VP2, and VP3 genes. Phylogenetic trees were constructed by using the neighbor-joining method with the Kimura 2-parameter method. Bootstrap values (1,000 replicates) >70% are shown. Filled circles indicate strain RVA/human-wt/TUN/17237/2008/G6P[9] from Tunisia; filled triangles indicate feline RVA strains; and open triangles indicate feline/canine-like human RVA strains. GenBank accession numbers of the sequences of reference strains are shown in online Technical Appendix Table 2 (wwwnc.cdc.gov/EID/article/19/4/12-1383-Techapp1.pdf). Scale bars indicate nucleotide substitutions per site.

of strain 17237 was more closely related to BA222-like RVA strains than to bovine or bovine-like RVA strains. This finding strengthens the hypothesis that each of the BA222-like RVA strains did not result from individual multiple reassortment events but rather that this genotype constellation now circulates (most likely in cats) around the world and might have resulted from >1 reassortment events in the more distant past.

To further support or refute this hypothesis, more complete genomes must be determined from RVA strains from cats and dogs. Moreover, because P[9] is believed to be typical for feline/canine RVA strains, it would be intriguing to determine whether this strain could persist in the human population and could become competitive with already established P genotypes in humans. The recently emerged human G9 RVA strain is believed to have

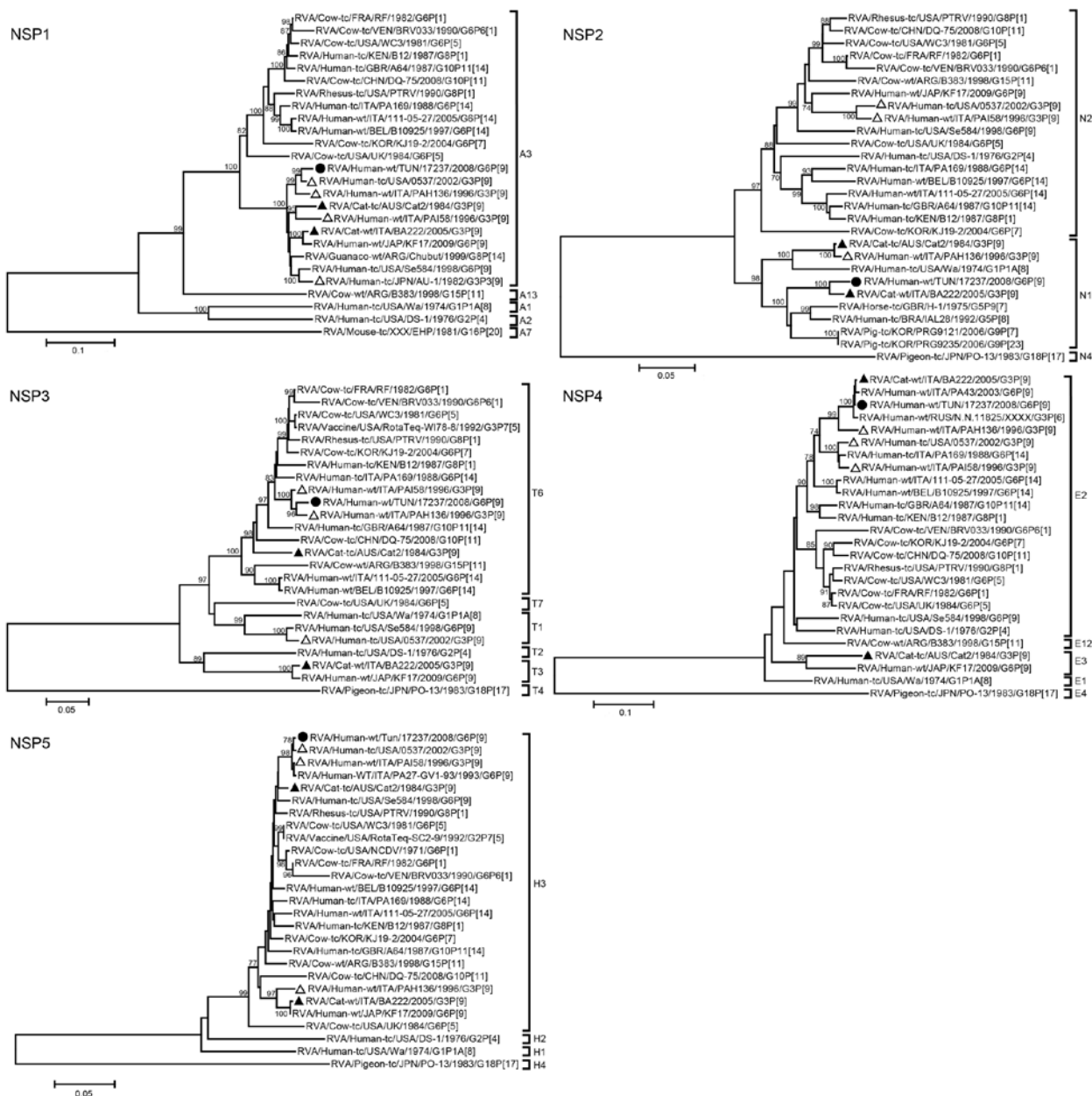


Figure 2. Phylogenetic trees of the full-length nucleotide sequences of the group A rotavirus (RVA) nonstructural protein (NSP) genes. Phylogenetic trees were constructed by using the neighbor-joining method with the Kimura 2-parameter method. Bootstrap values (1,000 replicates) >70% are shown. Filled circle indicates strain RVA/human-wt/TUN/17237/2008/G6P[9] from Tunisia, filled triangles indicate the feline RVA strains, and open triangles indicate the feline/canine-like human RVA strains. GenBank accession numbers of the sequences of reference RVA strains are shown in online Technical Appendix Table 2 (wwwnc.cdc.gov/EID/article/19/4/12-1383-Techapp1.pdf). Scale bars indicate nucleotide substitutions per site.

originated from pigs and to have become established in the human population as the fifth major human RVA genotype, after multiple genome reassortment events with typical human Wa-like RVA strains (15).

The unusual G6P[9] RVA strain 17237 most likely resulted from direct interspecies transmission from a cat to a human. Interspecies transmission increases potential for spread of unusual and uncommon RVA strains. The findings of this study highlight the need for continuous monitoring of RVA strains and timely recognition of novel or rare genotypes. Continued surveillance of RVA strains in industrialized and developing countries, and in humans and animals, will provide more insights into interspecies transmission processes of RVAs. In turn, this information could help determine how the introduction of novel genes might affect the evolution of the RVA populations that infect humans.

Acknowledgments

We thank all colleagues of the Laboratory of Clinical and Epidemiological Virology, Department of Microbiology and Immunology, Rega Institute for Medical Research, University of Leuven, Belgium, for their help and valuable advice.

This study was supported by a grant from the World Health Organization (GL.GLO.IVD.646.XC.04.2.999.00). M.Z. was supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders. J.M. was supported by a Fonds voor Wetenschappelijk Onderzoek postdoctoral fellowship.

Dr Ben Hadj Fredj is a PhD student at the Faculty of Pharmacy of Monastir, Tunisia, and a member of the research unit UR06SP20, Laboratory of Microbiology, Sahloul University Hospital, Sousse, Tunisia. Her primary research interests focus on virus typing, viral enteric pathogens, and virus epidemiology.

References

- Estes M, Kapikian A. Rotaviruses. In: Knipe M, Howley P, editors. *Fields virology*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 1917–74.
- Matthijnssens J, Ciarlet M, McDonald SM, Attoui H, Banyai K, Brister JR, et al. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). *Arch Virol*. 2011;156:1397–413. <http://dx.doi.org/10.1007/s00705-011-1006-z>
- Matthijnssens J, Van Ranst M. Genotype constellation and evolution of group A rotaviruses infecting humans. *Curr Opin Virol*. 2012;2:426–33. <http://dx.doi.org/10.1016/j.coviro.2012.04.007>
- Martella V, Banyai K, Matthijnssens J, Buonavoglia C, Ciarlet M. Zoonotic aspects of rotaviruses. *Vet Microbiol*. 2010;140:246–55. <http://dx.doi.org/10.1016/j.vetmic.2009.08.028>
- Matthijnssens J, Potgieter CA, Ciarlet M, Parreno V, Martella V, Banyai K, et al. Are human P[14] rotavirus strains the result of interspecies transmissions from sheep or other ungulates that belong to the mammalian order Artiodactyla? *J Virol*. 2009;83:2917–29. <http://dx.doi.org/10.1128/JVI.02246-08>
- Matthijnssens J, Rahman M, Van Ranst M. Two out of the 11 genes of an unusual human G6P[6] rotavirus isolate are of bovine origin. *J Gen Virol*. 2008;89:2630–5. <http://dx.doi.org/10.1099/vir.0.2008/003780-0>
- Yamamoto D, Kawaguchiya M, Ghosh S, Ichikawa M, Numazaki K, Kobayashi N. Detection and full genomic analysis of G6P[9] human rotavirus in Japan. *Virus Genes*. 2011;43:215–23. <http://dx.doi.org/10.1007/s11262-011-0624-6>
- El Sherif M, Esona MD, Wang Y, Gentsch JR, Jiang B, Glass RI, et al. Detection of the first G6P[14] human rotavirus strain from a child with diarrhea in Egypt. *Infect Genet Evol*. 2011;11:1436–42. <http://dx.doi.org/10.1016/j.meegid.2011.05.012>
- Steyer A, Sagadin M, Kolenc M, Poljsak-Prijatelj M. Whole genome sequence analysis of bovine G6P[11] rotavirus strain found in a child with gastroenteritis. *Infect Genet Evol*. 2013;13:89–95. <http://dx.doi.org/10.1016/j.meegid.2012.09.004>
- De Grazia S, Giammanco GM, Potgieter CA, Matthijnssens J, Banyai K, Platia MA, et al. Unusual assortment of segments in 2 rare human rotavirus genomes. *Emerg Infect Dis*. 2010;16:859–62. <http://dx.doi.org/10.3201/eid1605.091826>
- Martella V, Potgieter AC, Lorusso E, De Grazia S, Giammanco GM, Matthijnssens J, et al. A feline rotavirus G3P[9] carries traces of multiple reassortment events and resembles rare human G3P[9] rotaviruses. *J Gen Virol*. 2011;92:1214–21. <http://dx.doi.org/10.1099/vir.0.027425-0>
- Matthijnssens J, De Grazia S, Piessens J, Heylen E, Zeller M, Giammanco GM, et al. Multiple reassortment and interspecies transmission events contribute to the diversity of feline, canine and feline/canine-like human group A rotavirus strains. *Infect Genet Evol*. 2011;11:1396–406. <http://dx.doi.org/10.1016/j.meegid.2011.05.007>
- Tsugawa T, Hoshino Y. Whole genome sequence and phylogenetic analysis reveal human rotavirus G3P[3] strains Ro1845 and HCR3A are examples of direct virion transmission of canine/feline rotaviruses to humans. *Virology*. 2008;380:344–53. <http://dx.doi.org/10.1016/j.virol.2008.07.041>
- Ben Hadj Fredj M, Zeller M, Fodha I, Heylen E, Chouikha A, Van Ranst M, et al. Molecular characterization of the NSP4 gene of human group A rotavirus strains circulating in Tunisia from 2006 to 2008. *Infect Genet Evol*. 2012;12:997–1004. <http://dx.doi.org/10.1016/j.meegid.2012.02.011>
- Matthijnssens J, Heylen E, Zeller M, Rahman M, Lemey P, Van Ranst M. Phylodynamic analyses of rotavirus genotypes G9 and G12 underscore their potential for swift global spread. *Mol Biol Evol*. 2010;27:2431–6. <http://dx.doi.org/10.1093/molbev/msq137>

Address for correspondence: Abdelhalim Trabelsi, Laboratory of Microbiology, Sahloul University Hospital, 4054 Sousse, Tunisia; email: abdelhalim.trabelsi@gmail.com



Sign up to receive email announcements when
a new article is available.

Get an online subscription at www.cdc.gov/ncidod/eid/subscrib.htm

Feline Origin of Rotavirus Strain, Tunisia

Technical Appendix

Table 1. Primers used for amplification and sequencing of the whole genome of group A rotavirus strain RVA/human-wt/TUN/17237/2008/G6P[9] from Tunisia, 2008

Gene	Primer name	Primer sequence	
VP1	Gen_VP1Fb	5'-GGC TAT TAA AGC TRT ACA ATG GGG AAG -3'	
	Gen_VP1Rb	5'-GGT CAC ATC TAA GCG YTC TAA TCT TG -3'	
	MG6_VP1_447F	5'-TGC AGT TAT GTT CTG GTT GG -3'	
	Hosokawa_VP1_2587R	5'-ACG CTG ATA TTT GCG CAC -3'	
	LAP_VP1_1200F	5'-GCT GTC AAT GTC ATC AGC -3'	
	Gen_VP1_2417R	5'-GCT ATY TCA TCA GCT ATT CCY G -3'	
	30-96_VP1_3163F	5'-GGA TCA TGG ATA AGC TTG TTC TG -3'	
	26097_VP1_269R	5'-GCG TTA TAC TTA TCA TAC GAA TAC G -3'	
	VP2	Gen_VP2Fc	5'-GGC TAT TAA AGG YTC AAT GGC GTA CAG -3'
		Gen_VP2Rbc	5'-GTC ATA TCT CCA CAR TGG GGT TGG -3'
26097_VP2_458F		5'-AGT TGC GTA ATA GAT GGT ATT GG -3'	
B1711_VP2_2112R		5'-GCA ATT TTA TCT GAG GCA CG -3'	
NCDV_VP2_1868F		5'-AGG ATT AAT GAT GCA GTG GC -3'	
LAP_VP2_2543F		5'-GAC ATC AAA TCT TAC CTT CAC TG -3'	
260-97_VP2_345R		5'-GAC TCT TTT GGT TCG AAA GTA GG -3'	
FR5_VP2_23F		5'-TAC AGG AAA CGT GGA GCG -3'	
260-97_VP2_744R		5'-GTACTCTTTGTCTCATTTCGC -3'	
Gen_VP2_2739Ra		5'-TAC AAC TCG TTC ATG ATG CG -3'	
VP3	Gen_VP3_24F	5'-TGY GTT TTA CCT CTG ATG GTG-3'	
	Gen_VP3_2584R	5'-TGA CYA GTG TGT TAA GTT TYT AGC-3'	
	NCDV_VP3_2026R	5'-CAT GCG TAA ATC AAC TCT ATC GG -3'	
	MG6_VP3_488F	5'-GCA GCT ACA GAT GATGAT GC -3'	
	B10925_VP3_2416F	5'-ACA ATC GAG AAT GTT CAT CCC -3'	
	TUN1_VP3_167R	5'-TTT CTA CTG CAG CTA TGC CAG-3'	
VP4	LAP_VP4_788F	5'-CCT TGT GGA AAG AAA TGC-3'	
	VP4_2348-2368Re	5'-GGT CAC ATC TTA AAA TAG ACA G -3'	
	TUN1_VP4_2101F	5'-CAG ACT CAC CAG TTT TAT CAG C -3'	
VP6	TUN1_VP4_199R	5'-ACT GGT AAG TTG AGT GAG GTG -3'	
	Gen_VP6F	5'-GGC TTT WAA ACG AAG TCT TC -3'	
	Gen_VP6R-RC	5'-GGT CAC ATC CTC TCA CT -3'	
VP7	E403_VP6_1192F	5'-TGG CTT CCA TTA GAA GCA TGC -3'	
	TUN1_VP6_229R	5'-TGC ATT ACG CGC AGT TTC AAC -3'	
	Beg9-deg	5'-GGC TTT AAA AGM GAG AAT TTC CG -3'	
	End9-deg	5'-GGC TTT AAA AGM GAG AAT TTC CG -3'	
NSP1	PA169_VP7_895F	5'-AGA ATG ATG CGA GTG AAT TGG -3'	
	TUN1_VP7_250R	5'-AAG GGT CGA TGT CAA AAA CGG -3'	
	Gen_NSP1F	5'-GGC TTT TTT TTA TGA AAA GTC TTG-3'	
	Gen_NSP1R-RC	5'-GGT CAC ATT TTA TGC TGC C-3'	
	Chu_NSP1_1463R	5'-CAT TCT TCC TAT TCA GTG CTC G -3'	
NSP2	Chu_NSP1_1310F	5'-ATG GAT GGA AGA GTG CCA AGG -3'	
	Chu_NSP1_265R	5'-GGG TTC TTC ATC TAA GAA ACA CC -3'	
	Gen_NSP2F	5'-GGC TTT TAA AGC GTC TCA G-3'	
	Gen_NSP2R	5'-GGT CAC ATA AGC GCT TTC -3'	
	Au-1_NSP2_951F	5'-CCT GTC AAC TGA TAG AAA GAT GG -3'	
NSP3	POR_NSP2_132R	5'-ACT TTT GCT GTC AAC ATG C -3'	
	D2-25_NSP2_471R	5'-TGA CCA ATA GCT ATC AAC ACA G -3'	
	Gen_NSP3F	5'-GGC TTT TAA TGC TTT TCA GTG-3'	
	Gen_NSP3R	5'-ACA TAA CGC CCC TAT AGC-3'	
NSP4	Au-1_NSP3_519R	5'-ACT TCC ATT TTC TCT TCA ACG A -3'	
	TUN1_NSP3_786F	5'-GCC TGA TGA AAT TAA GAC GGA C -3'	
	NSP4F	5'-GGC TTT TAA AAG TTC TGT TCC GAG-3'	
	NSP4R	5'-GGT CAC ATC AAG ACC ATT CC-3'	
NSP5	PA169_NSP4_553F	5'-TGA CTG CAG CAA TGT GAG -3'	
	TUN1_NSP4_122R	5'-AAA GAC TGT CAG GAC AGA TGC -3'	
	Gen_NSP5F	5'-GGC TTT TAA AGC GCT ACA G-3'	
	Gen_NSP5R	5'-GGT CAC AAA ACG GGA GT -3'	
	MG6_NSP5_503F	5'-CGA TGA TTC TGA TAG TGA TGA TGG -3'	
	LAP_NSP5_314R	5'-CGT GAT TGT GTT GAT GAA TCC -3'	

Table 2. GenBank accession numbers of the sequences of reference strains used in the phylogenetic analysis

Strain	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
RF	X65940	U65924	K02254	J04346	X14057	AY116592	M22308	Z21640	Z21639	AY116593	AF188126
WC3	AY050272	AY050271	AF411322	EF560615	EF560616	EF560617	EF990699	EF990700	EF990701	AY050273	EF990702
UK	JF693056	JF693051	JF693053	JF693048	JF693049	JF693050	JF693052	JF693054	JF693055	JF693057	JF693058
KJ19-2	FJ206095	HM988969	HM988973	HM988966	HM988961	HM988963	HM988971	FJ206116	FJ206167	FJ206106	FJ206054
BRV033	U62154	<i>U62155</i>	AF317126	EF560612	EF560613	EF560614	EF990703	EF990704	EF990705	AF144804	EF990706
111-05-27	EF554142	EF554140	EF554141	EF554137	EF554138	EF554139	EF554143	EF554144	EF554145	EF554146	EF554147
PA169	EF554131	EF554129	EF554130	EF554126	EF554127	EF554128	EF554132	EF554133	EF554134	EF554135	EF554136
B10925	EF554120	EF554118	EF554119	EF554115	EF554116	EF554117	EF554121	EF554122	EF554123	EF554124	EF554125
Se584	EF672609	EF672605	EF583044	EF583041	EF583042	EF583043	EF672606	EF672608	EF672607	EF672610	EF672611
B1711	AF532202	<i>EF554085</i>	<i>EF554086</i>	<i>EF554082</i>	<i>EF554083</i>	EF554084	<i>EF554088</i>	<i>EF554089</i>	<i>EF554090</i>	<i>EF554091</i>	<i>EF554092</i>
KF17	JF421980	JF421978	JF421979	JF421975	JF421976	JF421977	JF421981	JF421982	JF421983	JF421984	JF421985
PA43	JF793944	<i>JF793939</i>	<i>JF793933</i>	–	–	–	–	–	–	JF793928	<i>JF793922</i>
B383	FJ347116	FJ347114	FJ347115	FJ347111	FJ347112	FJ347113	FJ347117	FJ347118	FJ347119	FJ347120	FJ347121
A64	EF672567	EF672563	EF583020	EF583017	EF583018	EF583019	EF672564	EF672566	EF672565	EF672568	EF672569
DQ-75	GU144587	GU181281	GU384194	GU384192	GU384191	GU384193	GU384195	GU384196	GU384197	GU181282	GU384198
Wa	JX406755	JX406750	JX406752	JX406747	JX406748	JX406749	JX406751	JX406754	JX406753	JX406756	JX406757
DS-1	HQ650124	HQ650119	HQ650121	HQ650116	HQ650117	HQ650118	HQ650120	HQ650123	HQ650122	HQ650125	HQ650126
B12	HM627547	HM627545	HM627546	HM627542	HM627543	HM627544	HM627548	HM627549	HM627550	HM627551	HM627552
PtrV	FJ422138	FJ422134	FJ422136	FJ422131	FJ422132	FJ422133	FJ422135	FJ422139	FJ422137	FJ422140	FJ422141
Cat2	EU708961	EU708959	EU708960	EU708956	EU708957	EU708958	EU708962	EU708963	EU708964	EU708965	EU708966
PAH136	GU296430	GU296426	GU296428	GU296420	GU296422	GU296424	GU296410	GU296412	GU296415	GU296416	GU296418
PAI58	GU296431	GU296427	GU296429	GU296421	GU296423	GU296425	GU296411	GU296413	GU296414	GU296417	GU296419
BA222	GU827411	GU827409	GU827410	GU827406	GU827407	GU827408	GU827412	GU827413	GU827414	GU827415	GU827416
0537	JF805014	<i>JF805012</i>	JF805013	JF805010	<i>JF805011</i>	<i>JF805015</i>	JF805005	JF805006	JF805007	JF805008	JF805009
AU-1	<i>D86271</i>	D10970	<i>DQ490538</i>	<i>DQ490533</i>	<i>DQ490536</i>	<i>DQ490537</i>	D45244	<i>DQ490534</i>	<i>DQ490535</i>	<i>D89873</i>	<i>AB008856</i>
Nov10-N507	<i>JX088008</i>	JQ289055	<i>JQ230092</i>	–	–	–	–	–	–	–	–
Hun5	<i>EF554109</i>	<i>EF554107</i>	EF554108	<i>EF554104</i>	<i>EF554105</i>	<i>EF554106</i>	<i>EF554110</i>	<i>EF554111</i>	<i>EF554112</i>	<i>EF554113</i>	<i>EF554114</i>
1604	<i>JN831225</i>	<i>JN831223</i>	<i>JN831224</i>	JN831220	<i>JN831221</i>	<i>JN831222</i>	<i>JN831215</i>	<i>JN831216</i>	<i>JN831217</i>	<i>JN831218</i>	<i>JN831219</i>
NCDV	<i>JF693034</i>	<i>JF693029</i>	<i>JF693031</i>	JF693026	<i>JF693027</i>	<i>JF693028</i>	<i>JF693030</i>	<i>JF693032</i>	<i>JF693033</i>	<i>JF693035</i>	JF693036
Chubut	<i>FJ347105</i>	<i>FJ347103</i>	<i>FJ347104</i>	<i>FJ347100</i>	<i>FJ347101</i>	<i>FJ347102</i>	<i>FJ347106</i>	<i>FJ347107</i>	<i>FJ347108</i>	<i>FJ347109</i>	<i>FJ347110</i>
O-Agent	<i>JF693045</i>	<i>JF693040</i>	<i>JF693042</i>	<i>JF693037</i>	JF693038	<i>JF693039</i>	<i>JF693041</i>	<i>JF693044</i>	<i>JF693043</i>	<i>JF693046</i>	<i>JF693047</i>
Rio Negro	<i>FJ347127</i>	<i>FJ347125</i>	<i>FJ347126</i>	<i>FJ347122</i>	<i>FJ347123</i>	<i>FJ347124</i>	<i>FJ347128</i>	<i>FJ347129</i>	<i>FJ347130</i>	<i>FJ347131</i>	<i>FJ347132</i>
H-1	<i>AF242393</i>	<i>FJ870377</i>	<i>AF242394</i>	<i>JQ309138</i>	<i>JQ309139</i>	<i>JQ309140</i>	<i>JQ309141</i>	<i>JQ309142</i>	<i>JQ309143</i>	<i>AF144800</i>	<i>JQ309144</i>
IAL28	<i>EF672588</i>	<i>EF672584</i>	<i>EF583032</i>	<i>EF583029</i>	<i>EF583030</i>	<i>EF583031</i>	<i>EF672585</i>	<i>EF672587</i>	<i>EF672586</i>	<i>EF672589</i>	<i>EF672590</i>
PRG9121	<i>JF796739</i>	<i>JF796737</i>	<i>JF796738</i>	<i>JF796734</i>	<i>JF796735</i>	<i>JF796736</i>	<i>JF796729</i>	<i>JF796730</i>	<i>JF796731</i>	<i>JF796732</i>	<i>JF796733</i>
PRG9235	<i>JF796706</i>	<i>JF796704</i>	<i>JF796705</i>	<i>JF796701</i>	<i>JF796702</i>	<i>JF796703</i>	<i>JF796696</i>	<i>JF796697</i>	<i>JF796698</i>	<i>JF796699</i>	<i>JF796700</i>
N.N.11825	–	–	–	–	–	–	–	–	–	DQ270109	–
PA27-GV1-93	<i>JF793942</i>	<i>JF793937</i>	<i>JF793931</i>	–	–	–	–	–	–	<i>JF793926</i>	<i>JF793921</i>
RotaTeq	<i>GU565090</i>	<i>GU565088</i>	<i>GU565089</i>	<i>GU565085</i>	<i>GU565086</i>	<i>GU565087</i>	<i>GU565091</i>	<i>GU565092</i>	<i>GU565093</i>	<i>GU565094</i>	<i>GU565095</i>
BrB-9	–	–	–	–	–	–	–	–	–	–	–
RotaTeq	<i>GU565079</i>	<i>GU565077</i>	<i>GU565078</i>	<i>GU565074</i>	<i>GU565075</i>	<i>GU565076</i>	<i>GU565080</i>	<i>GU565081</i>	<i>GU565082</i>	<i>GU565083</i>	<i>GU565084</i>
WI78-8	–	–	–	–	–	–	–	–	–	–	–
RotaTeq	<i>GU565068</i>	<i>GU565066</i>	<i>GU565067</i>	<i>GU565063</i>	<i>GU565064</i>	<i>GU565065</i>	<i>GU565069</i>	<i>GU565070</i>	<i>GU565071</i>	<i>GU565072</i>	<i>GU565073</i>
SC2-9	–	–	–	–	–	–	–	–	–	–	–
PO-13	D82979	AB009632	D16329	AB009629	AB009630	AB009631	<i>AB009633</i>	AB009625	AB009626	AB009627	AB009628
EHP	<i>U08425</i>	<i>U08424</i>	–	–	–	–	<i>U08423</i>	–	–	<i>U96336</i>	–

*Numbers in italic were not used in this study. – indicates that no sequence data were available in GenBank.