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Full-genome sequencing of a Hungarian canine G3P[3] *Rotavirus A* strain reveals high genetic relatedness with a historic Italian human strain

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Abstract A canine *Rotavirus A* strain was identified in the fecal specimen of a young dog during 2012 in Hungary. The strain RVA/Dog-wt/HUN/135/2012/G3P[3] shared complete genotype constellation (G3-P[3]-I3-R3-C3-M3-A15-N2-T3-E3-H6) and high genome sequence similarity (nt, 98.8 %) with a historic human strain, RVA/Human-tc/ITA/PA260-97/1997/G3P[3]. This study provides evidence for the canine origin of the unusual NSP1 genotype, A15, and reinforces the hypothesis of direct interspecies transmission of canine rotaviruses to humans.

Keywords Zoonosis · Semiconductor sequencing · Phylogenetic analysis · Genotype constellation

Introduction

Group A rotaviruses (*Rotavirus A*, RVA) are a major cause of diarrhea in humans and animals. The rotavirus virion is non-enveloped, triple-layered, and contains 11 segments of double-stranded RNA. The segmented genome encodes 6 structural (VP1-4,6,7) and 5 or 6 non-structural (NSP1-6) proteins [1]. Neutralization antigens of the outer capsid layer, VP7 and VP4, serve as the basis for the commonly used dual classification system defining G and P types, respectively. So far, at least 27 G types and 37 P types have been described for RVAs [2, 3]. Recently, a novel classification scheme has been developed for RVAs, which encompasses all 11 segments and defines genotypes for each gene [4]. Universal adoption of the new classification system has helped describe better the impact of interspecies transmission and of reassortment among homologous and heterologous strains on the evolution of human RVAs.

RVA is not considered a major cause of diarrhea in dogs, although in vivo experiments have demonstrated firmly that RVA can induce diarrhea in young dogs [5–9]. All canine RVAs detected thus far and some feline RVAs have been characterized as G3P[3] [9, 10]. Interestingly, canine-like G3P[3] RVAs have also been identified sporadically, but repeatedly, from human patients hospitalized with gastroenteritis worldwide [11–15], suggesting the zoonotic potential and the pathogenic phenotype of canine RVAs in a heterologous host.

Whole-genome sequencing studies clearly indicate a genetic relatedness among canine, feline, and human G3P[3] RVAs [14, 16, 17], although all canine G3P[3] strains were isolated in and preceding the 1990s. A better understanding of common canine RVA genomic configurations, notably of more recent RVA strains, is expected to help track the origin of rare RVA strains causing disease in humans.

Results and discussion

In this study, we determined the full-length genome of a RVA strain detected in the stool of a clinically asymptomatic

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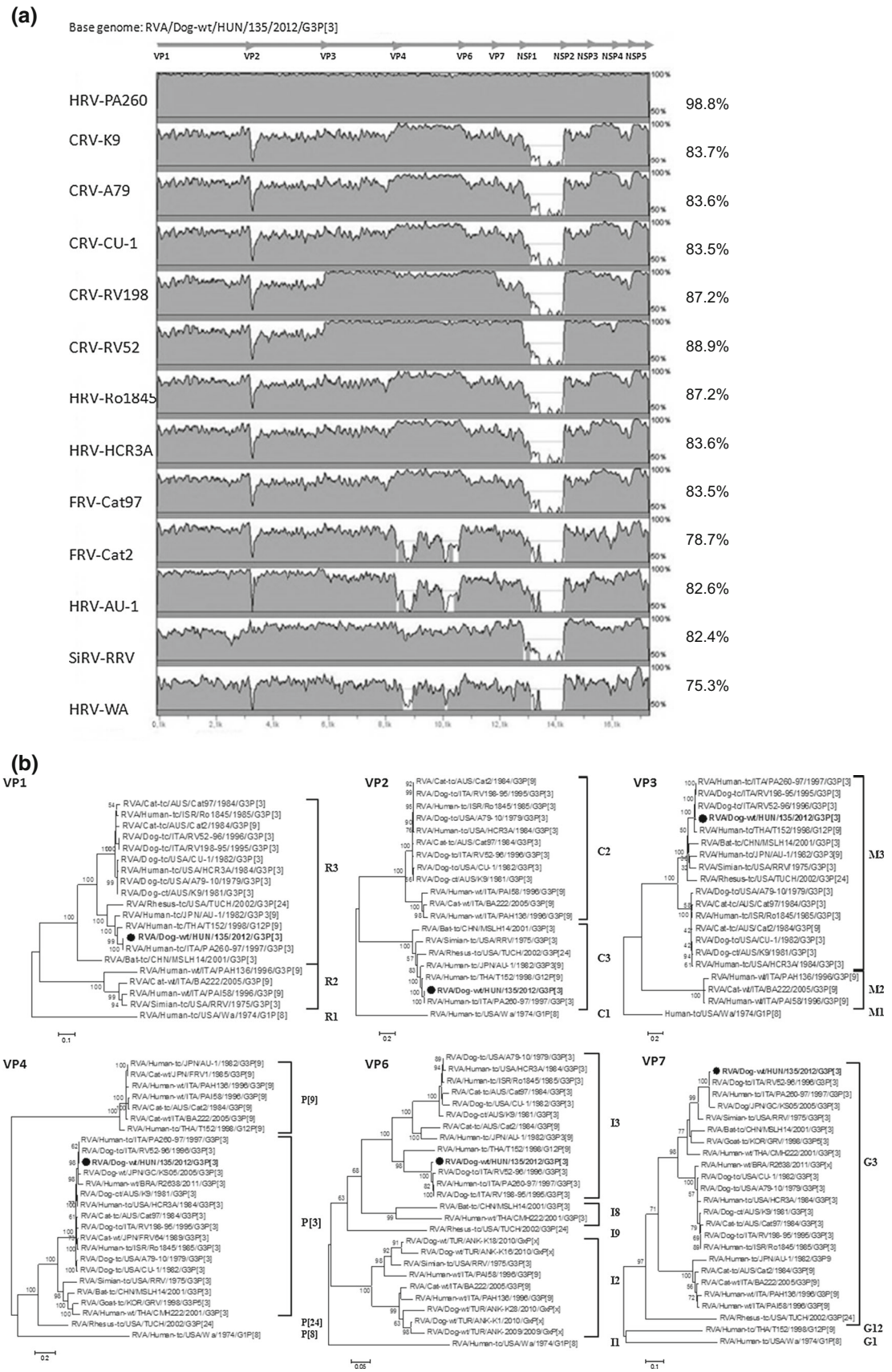
3-week-old dog. The sample was collected during 2012 as part of an ongoing project to detect enteric viruses in sheltered dogs, and it was the sole specimen in which the metagenomic approach identified RVA-specific sequence reads [17]. Briefly, laboratory procedures included the extraction of viral nucleic acid using the Zymo DirectZol kit (Zymo Research) combined with the RiboZol RNA extraction reagent (Amresco). Subsequently, random primed RT-PCR was carried out to produce cDNA for high-throughput sequencing [17]. Library preparation, size selection of library DNA, emulsion PCR, templated Ion Sphere bead enrichment, and semiconductor sequencing were carried out as described in detail elsewhere [18]. The CLC Genomics Workbench (http://www.clcbio.com/) was used to map sequence reads to reference RVA sequences and obtain the consensus genomic sequence for RVA/Dog-wt/HUN/135/2012/G3P[3] (hereafter called 135/2012). Genotypes of individual genes were determined according to the recommendations of the RCWG[4] using the RotaC v2.0 web-based classification tool (http://rotac.regatools.be/). Multiple sequence alignments were constructed and manually adjusted with the GeneDoc software [19]. Phylogenetic analysis was carried out using the MEGA6 package [20]. Best fit substitution models were selected for each data set based on the Bayesian information criterion. Subsequently, maximum likelihood trees were generated and bootstrap analysis was performed with 500 replications. The mVISTA software was used to align and visualize the concatenated whole-genome sequences with reference RVA strains [21].

Fig. 1 **a** mVISTA whole-genome nucleotide alignment comparing the RVA/Dog-wt/HUN/135/2012/G3P[3] strain with homotypic and partially or fully heterotypic reference RVA strains. Strain names are shown on the *left*. The *y*-axis indicates sequence identity. *Gray shading* indicates the level of conservation. Percentile values on the *right* indicate the whole-genome sequence-based similarity between 135/2012 and the respective reference strains. **b** Maximum likelihood trees of all 11 genes. Bootstrap values greater than 60 % are shown. A heterotypic human RVA strain, Wa, served as outgroup in each analysis. The Hungarian canine RVA strain is labeled with a *dot*. The *scale bars* are proportional to the genetic distance

The genome of strain 135/2012 was 18,524 bp in length (GenBank Accession No. KJ875791-KJ875801). The lengths of the sequenced genomic segments, their respective genotypes, and average sequencing coverage obtained for 135/2012 were as follows: VP1, 3302 bp, R3, 31X; VP2, 2708 bp, C3, 39X; VP3, 2591 bp, M3, 22X; VP4, 2362 bp, P[3], 26X; VP6, 1356 bp, I3, 23X; VP7, 1062 bp, G3, 25X; NSP1, 1599 bp, A15, 26X; NSP2, 1059 bp, N2, 18X; NSP3, 1068 bp, T3, 40X; NSP4, 750 bp, E3, 25X; NSP5, 667 bp, H3, 28X. The whole genotype configuration of the identified canine strain was identical to a rare human strain, RVA/Human-tc/ITA/PA260-97/1997/G3P[3], detected in Sicily in 1997 (Table 1).

Table 1 Complete genotype constellations of G3P[3] and some heterotypic RVA strains

Strain name	Genotypes										
	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
RVA/Dog-wt/HUN/135/2012/G3P[3]	G3	P[3]	I3	R3	C3	M3	A15	N2	T3	E3	H6
RVA/Human-tc/ITA/PA260-97/1997/G3P[3]	G3	P[3]	I3	R3	C3	M3	A15	N2	T3	E3	H6
RVA/Dog-tc/ITA/RV198-95/1995/G3P[3]	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Dog-tc/ITA/RV52-96/1996/G3P[3]	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Dog-tc/USA/CU-1/1982/G3P[3]	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Dog-ct/AUS/K9/1981/G3P[3]	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Dog-tc/USA/A79-10/1979/G3P[3]	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Cat-tc/AUS/Cat97/1984/G3P[3]	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Cat-tc/AUS/Cat2/1984/G3P[9]	G3	P[9]	I3	R3	C2	M3	A3	N1	T6	E3	H3
RVA/Cat-wt/ITA/BA222/2005/G3P[9]	G3	P[9]	I2	R2	C2	M2	A3	N1	T3	E2	H3
RVA/Human-wt/ITA/PAH136/1996/G3P[9]	G3	P[9]	I2	R2	C2	M2	A3	N1	T6	E2	H3
RVA/Human-wt/ITA/PAI58/1996/G3P[9]	G3	P[9]	I2	R2	C2	M2	A3	N2	T6	E2	H3
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-tc/ISR/Ro1845/1985/G3P[3]	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Human-tc/USA/HCR3A/1984/G3P[3]	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Simian-tc/USA/RRV/1975/G3P[3]	G3	P[3]	I2	R2	C3	M3	A9	N2	T3	E3	H6
RVA/Bat-tc/MSLH14/2012/G3P[3]	G3	P[3]	I8	R3	C3	M3	A9	N3	T3	E3	H6
RVA/Horse-wt/E3198/2008/G3P[3]	G3	P[3]	I3	R3	C3	M3	A9	N3	T3	E3	H6
RVA/Rhesus-tc/USA/TUCH/2002/G3P[24]	G3	P[24]	I9	R3	C3	M3	A9	N1	T1	E1	H1


(a)
Base genome: RVA/Dog-wt/HUN/135/2012/G3P[3]
VP1
VP2
VP3
VP4
VP6
VP7
NSP1
NSP2
NSP3
NSP4
NSP5
HRV-PA260
98.8%
CRV-K9
83.7%
CRV-A79
83.6%
CRV-CU-1
83.5%
CRV-RV198
87.2%
CRV-RV52
88.9%
HRV-Ro1845
87.2%
HRV-HCR3A
83.6%
FRV-Cat97
83.5%
FRV-Cat2
78.7%
HRV-AU-1
82.6%
SiRV-RRV
82.4%
HRV-WA
75.3%
(b)
VP1
R3
R2
R1
VP2
C2
C3
C1
VP3
M3
M2
M1
VP4
P[9]
P[3]
P[24]
P[8]
VP6
I3
I8
I9
I2
I1
VP7
G3
G12
G1

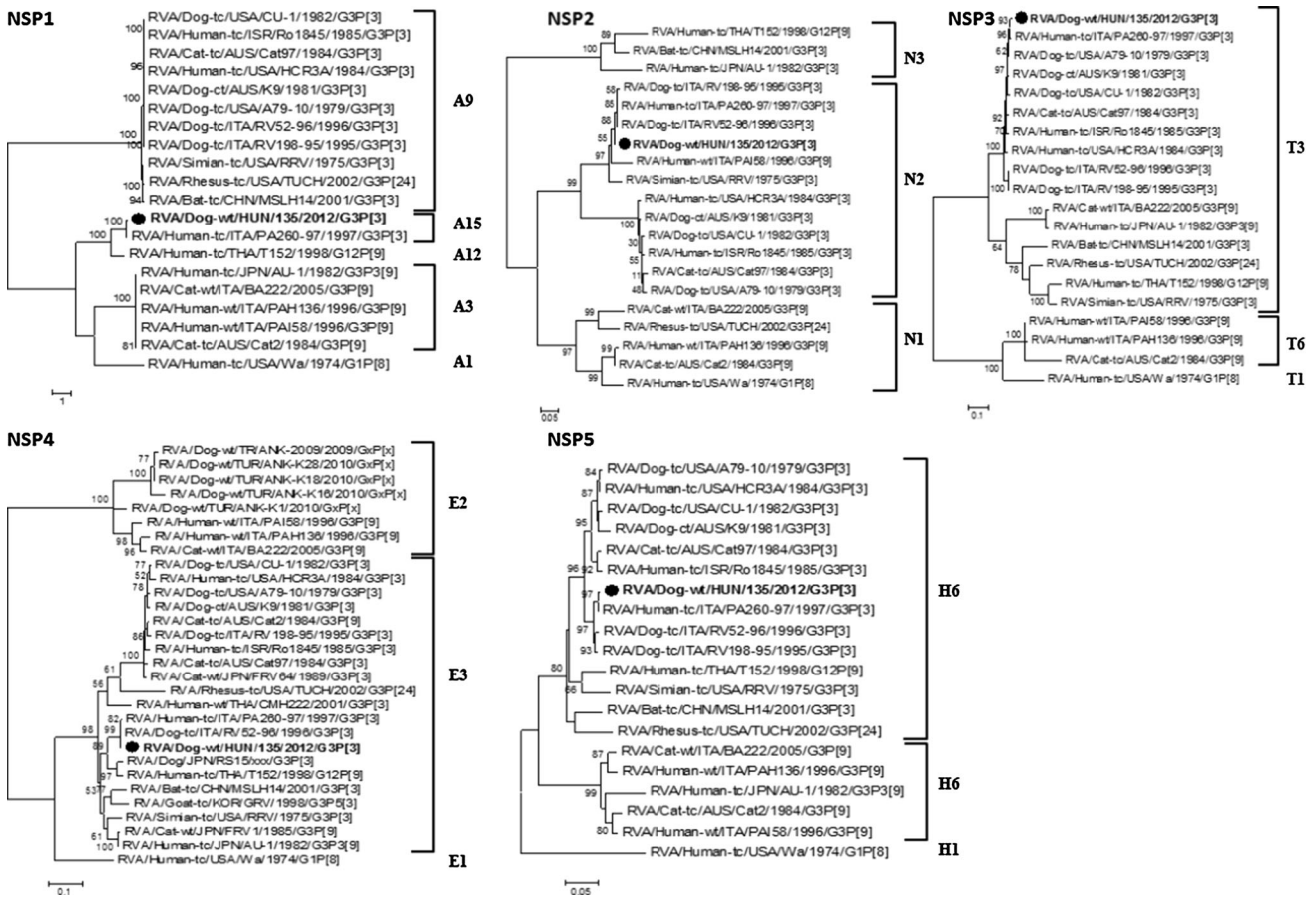


Fig. 1 continued

Whole-genome comparison in the mVISTA module [21] with nine other G3P[3] strains of canine (K9, CU-1, RV198, and RV52), human (PA260-97, Ro1845, and HCR3A), and feline (Cat2 and Cat97) origin, and three additional fully or partially heterotypic reference strains (the human AU-1, the simian RRV, and the human Wa) is depicted in Fig. 1. Overall, the Hungarian canine strain 135/2012 displayed the greatest genomic conservation (98.8 % nt) with the Italian human RVA strain, PA260-97; these two strains shared very high sequence identity in all the 11 genes (range 98.3–99.2 % nt; 98.1–100 % aa) (data not shown). Lower, but still significant genome sequence similarity was found with other canine, feline, and human G3P[3] strains; all these strains were heterotypic to the 135/2012 strain in the genes encoding VP2 and NSP1 (Fig. 1, data not shown).

Phylogenetic analysis of the 11 genes was in agreement with similarity data (Fig. 1). With regard to the virion components, the VP7, VP4, and VP6 genes of 135/2012 clustered together with the Italian human strain PA260-97 and two Italian canine strains, RV198 and RV52. In addition, in the VP1, VP2, and VP3 phylogenies 135/2012 segregated with the Italian human G3P[3] strain, the reference human AU-1 strain and several simian strains, including TUCH (VP1-VP3) and RRV (VP2 and VP3) [22]. Concerning the non-structural genes, again, the phylogenetic analysis revealed very close genetic relatedness between 135/2012 and PA260-97. The NSP1 was an interesting example of this close relationship between 135/2012 and PA260-97, given that only these two strains have been described so far to carry the unusual A15 NSP1 genotype. In the NSP2, NSP3, NSP4, and NSP5 phylogenies, the Hungarian canine strain clustered with several Italian canine, feline, and human strains as well as with simian (RRV; NSP2 gene), caprine (GRV; NSP4 gene), and bat (MSLH14; NSP4 gene) G3P[3] RVAs.

Genome sequencing studies revealed that human, feline, and canine origin G3P[3] and G3P[9] RVAs can be classified into three distinct groups, one each retaining a relatively stable constellation of genotypes. The Cat97-like group includes feline, canine, and human G3P[3] strains, while feline and human G3P[9] RVAs form two separate groups based on whole-genome configurations, AU-1-like

and BA222-05-like [16]. Additional heterologous G3P[3] strains with shared genotype constellations have been detected, for example, in horse, monkey, and bat. Phylogenetic analysis studies that had involved these strains often uncovered genetic relationship with canine/feline G3P[3] RVAs raising interesting scenarios concerning the origin and evolution of the particular genotype constellations, occasionally pointing out unexpected interspecies transmission events of RVA strains among these heterologous hosts [22–24]. The human G3P[3] strain, PA260-97, was detected in a child hospitalized with severe gastroenteritis in Palermo, Sicily, 1997 [12]. This virus shared the genomic configuration with Cat97-like strains and was suspected to have originated by spill-over event. Also PA260-97 was found to possess a novel NSP1 genotype, A15, whose host species origin was unknown [16]. The results presented in this study seem to provide evidence that this unusual NSP1 genotype may be of canine origin. However, the occurrence of A15 is known based on merely two RVA strains, therefore the possibility that this genotype had been acquired by reassortment from another host species to become the component of a Cat97-like canine RVA strain cannot be excluded either. It remains to be investigated whether the A15 NSP1 genotype is a stable component of some Cat97-like RVA strains. At last, the identification of RVA strains (i.e., 135/2012 and PA260-97) with a stable genome configuration and minimal genetic variation in two distinct geographical areas over a time span of 15 years might indicate an optimal level of adaptation of this particular RVA genotype configuration to the putative reservoir carnivore host species.

Comprehensive reviews of human RVA genotypes uncovered that G3P[3] strains are very rare in human populations (<0.2 %) in comparison to the prevalence of medically important genotypes, such as G1P[8] (37.7 %), G2P[4] (10.6 %), G3P[8] (8.7 %), G4P[8] (6.3 %), and G9P[8] (11.3 %) [25]. Unfortunately, the epidemiology of canine RVAs is not systematically investigated leaving gaps in our knowledge about their prevalence and true genetic diversity. Thus, although independent reports on the identification of feline/canine-like RVAs in human patients hospitalized with severe gastroenteritis demonstrate that interspecies transmission between humans and pets may occur, this risk is probably underestimated in the absence of structured epidemiologic investigations in pets. Additional epidemiological and molecular data are needed to assess the extent of RVA interspecies transmissions and to evaluate the potential zoonotic risks posed by companion animals.

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References

1. M.K. Estes, A.Z. Kapikian, in *Fields Virology*, ed. by D.M. Knipe, P.M. Howley, D.E. Griffin, M.A. Martin, B. Roizman, S.E. Straus (Lippincott Williams & Wilkins, Philadelphia, 2007), p. 1917
2. J. Matthijnssens, M. Ciarlet, S.M. McDonald, H. Attoui, K. Bányai, J.R. Brister, J. Buesa, M.D. Esona, M.K. Estes, J.R. Gentsch, M. Iturriza-Gómara, R. Johne, C.D. Kirkwood, V. Martella, P.P. Mertens, O. Nakagomi, V. Parreño, M. Rahman, F.M. Ruggeri, L.J. Saif, N. Santos, A. Steyer, K. Taniguchi, J.T. Patton, U. Desselberger, M. Van Ranst, Arch. Virol. **156**, 1397 (2011)
3. E. Trojnar, J. Sachsenröder, S. Twardziok, J. Reetz, P.H. Otto, R. Johne, J. Gen. Virol. **94**, 136 (2013)
4. J. Matthijnssens, M. Ciarlet, M. Rahman, H. Attoui, K. Bányai, M.K. Estes, J.R. Gentsch, M. Iturriza-Gómara, C.D. Kirkwood, V. Martella, P.P. Mertens, O. Nakagomi, J.T. Patton, F.M. Ruggeri, L.J. Saif, N. Santos, A. Steyer, K. Taniguchi, U. Desselberger, M. Van Ranst, Arch. Virol. **153**, 1621 (2008)
5. C.A. Johnson, R.W. Fulton, W.G. Henk, T.G. Snider, Am. J. Vet. Res. **44**, 1682 (1983)
6. C.A. Johnson, T.G. Snider, R.W. Fulton, D. Cho, Am. J. Vet. Res. **44**, 1687 (1983)
7. C.A. Johnson, T.G. Snider, W.G. Henk, R.W. Fulton, Vet. Pathol. **23**, 443 (1986)
8. B.K. Kang, D.S. Song, K.I. Jung, C.S. Lee, S.J. Park, J.S. Oh, D.J. An, J.S. Yang, H.J. Moon, S.S. Lee, Y.D. Yoon, B.K. Park, J. Vet. Diagn. Invest. **19**, 78 (2007)
9. V. Martella, A. Pratelli, G. Greco, M. Gentile, P. Fiorente, M. Tempesta, C. Buonavoglia, Virus Res. **74**, 17 (2001)
10. Y. Hoshino, R.G. Wyatt, F.W. Scott, M.J. Appel, Arch. Virol. **72**, 113 (1982)
11. P. Khamrin, N. Maneekarn, S. Peerakome, F. Yagyu, S. Okitsu, H. Ushijima, J. Med. Virol. **78**, 986 (2006)
12. S. De Grazia, V. Martella, G.M. Giammanco, M. Iturriza-Gòmara, S. Ramirez, A. Cascio, C. Colomba, S. Arista, Emerg. Infect. Dis. **13**, 1091 (2007)
13. T. Tsugawa, Y. Hoshino, Virology **380**, 344 (2008)
14. L. Grant, M. Esona, J. Gentsch, J. Watt, R. Reid, R. Weatherholtz, M. Santosham, U. Parashar, K. O'Brien, J. Med. Virol. **83**, 1288 (2011)
15. F.T. Wu, K. Bányai, J.S. Lin, H.S. Wu, C.A. Hsiung, Y.C. Huang, K.P. Hwang, B. Jiang, J.R. Gentsch, Vector-Borne Zoonot. Dis. **12**, 170 (2012)
16. J. Matthijnssens, S. De Grazia, J. Piessens, E. Heylen, M. Zeller, G.M. Giammanco, K. Bányai, C. Buonavoglia, M. Ciarlet, V. Martella, M. Van Ranst, Infect. Genet. Evol. **11**, 1396 (2011)
17. E. Mihalov-Kovács, Á. Gellért, S. Marton, S.L. Farkas, E. Fehér, M. Oldal, F. Jakab, V. Martella, K. Bányai, Emerg. Infect. Dis. (2015)
18. K. Bányai, R. Borzák, K. Ihász, E. Fehér, Á. Dán, F. Jakab, T. Papp, U. Hetzel, R.E. Marschang, S.L. Farkas, Arch. Virol. **159**, 153 (2014)
19. K.B. Nicholas, H.B. Nicholas, D.W. Deerfield, Embnet News **4**, 14 (1997)
20. K. Tamura, G. Stecher, D. Peterson, A. Filipski, S. Kumar, Mol. Biol. Evol. **30**, 2725 (2013)

21. K.A. Frazer, L. Pachter, A. Poliakov, E.M. Rubin, I. Dubchak, Nucleic Acids Res. **32**, W273 (2004)
22. J. Matthijnssens, Z.F. Taraporewala, H. Yang, S. Rao, L. Yuan, D. Cao, Y. Hoshino, P.P. Mertens, G.R. Carner, M. McNeal, K. Sestak, M. Van Ranst, J.T. Patton, J. Virol. **84**, 2013 (2010)
23. B. He, F. Yang, W. Yang, Y. Zhang, Y. Feng, J. Zhou, J. Xie, Y. Feng, X. Bao, H. Guo, Y. Li, L. Xia, N. Li, J. Matthijnssens, H. Zhang, C. Tu, J. Virol. **87**, 12357 (2013)
24. S. Miño, J. Matthijnssens, A. Badaracco, L. Garaicoechea, M. Zeller, E. Heylen, M. Van Ranst, M. Barrandeguy, V. Parreño, Vet. Microbiol. **161**, 239 (2013)
25. K. Bányai, B. László, J. Duque, A.D. Steele, E.A. Nelson, J.R. Gentsch, U.D. Parashar, Vaccine. **30**(Suppl 1), A122 (2012)