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Equine G3P[3] rotavirus strain E3198 related to simian RRV and feline/canine-like rotaviruses based on complete genome analyses

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ABSTRACT

Equine group A rotavirus (RVA) strains are the most important cause of gastroenteritis in equine neonates and foals worldwide, and G3P[12] and G14P[12] are epidemiologically the most important genotypes. The genotype constellation of an unusual Argentinean G3P[3] RVA strain (RVA/Horse-wt/E3198/2008/G3P[3]) detected in fecal samples of a diarrheic foal in 2008 was shown to be G3–P[3]–I3–R3–C3–M3–A9–N3–T3–E3–H6. Each of these genotypes has been found typically in feline and canine RVA strains, and the genotype constellation is reminiscent to those of Cat97-like RVA strains. However, the phylogenetic analyses revealed only a distant relationship between E3198 and known feline, canine and feline/canine-like human RVA strains. Surprisingly, a rather close relationship was found between E3198 and simian RVA strains RVA/Simian-tc/USA/RRV/1975/G3P[3] for at least 5 gene segments. RRV is believed to be a reassortant between a bovine-like RVA strain and a RVA strains distantly related to feline/canine RVA strains. These analyses indicate that E3198 is unlikely to be of equine origin, and most likely represents a RVA interspecies transmitted virus, possibly in combination with one or more reassortments, from a feline, canine or related host species to a horse. Further studies are in progress to evaluate if this strain was a single interspecies transmission event, or if this strain started to circulate in the equine population.

1. Introduction

The genus *rotavirus* belongs to the family of *Reoviridae* (Ball, 2005), and is further divided into 8 groups (A–H) based on serological characterization of the inner capsid protein VP6 and recently also based on amino acid sequence comparisons of VP6 (Matthijnssens et al., 2012). The infectious group A rotavirus (RVA) virion is a non-enveloped, triple layered particle with an eleven-segmented, double-stranded RNA genome. Cumulatively, the viral genome encodes five or six nonstructural proteins (NSP1–NSP5, and sometimes, NSP6) and six structural proteins (VP1–VP4, VP6 and VP7) (Estes and Kapikian, 2007). The two outer capsid proteins, VP7 and VP4 are the basis for a widely used classification system defining G-types (glycosylated) and P-types (protease sensitive), respectively. To date, at least 27 G-genotypes and 35 P-genotypes have been described in the literature (Matthijnssens et al., 2011a). Currently, a uniform nomenclature and an extended classification system based on

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nucleotide sequence identity for each of the 11 genome segments is used worldwide, and the acronym Gx–P[x]–Ix–Rx–Cx–Mx–Ax–Nx–Tx–Ex–Hx defines the genotypes of the VP7–VP4–VP6–VP1–VP2–VP3–NSP1–NSP2–NSP3–NSP4–NSP5/6 encoding gene segments (Matthijnssens et al., 2008a,b, 2011a). This new classification system has been proven very useful to study evolution, interspecies transmission and reassortments among a wide range of human and animal RVA strains (Bányai et al., 2009, 2010; Ciarlet et al., 2008; Martella et al., 2010, 2011; Matthijnssens et al., 2006, 2009, 2010a; McDonald et al., 2009; Mukherjee et al., 2011; Park et al., 2011).

Diarrhea is one of the most common health problems of newborn foals, and equine RVA strains are the most common cause of foal enteritis in major horse breeding farms. Both single cases of RVA diarrhea and severe diarrhea outbreaks affecting all the susceptible foals in the farm occur. However, with the use of a commercially available vaccine, farm management practices, and hygiene measures, this disease can be controlled (Barrandeguy et al., 1998; Dwyer, 2007; Estes and Kapikian, 2007; Powell et al., 1997). Worldwide surveys of equine RVA field samples have revealed that the equine RVA population consists mainly of G3 and G14 genotypes associated with genotype P[12] (Ciarlet et al., 1994; Collins et al., 2008; Elschner et al., 2005; Garaicoechea et al., 2011; Monini et al., 2011; Nemoto et al., 2011; Tsunemitsu et al., 2001; van der Heide et al., 2005). We recently described for the first time the complete genomes of 6 equine RVA strains with these prevalent G3P[12] and G14P[12] genotype combinations in addition to the complete genome of the uncommon equine G13P[18] RVA strains L338 (Matthijnssens et al., 2011c). A limited number of RVA strains with unusual G/P combinations have been detected in horses on single occasions, such as RVA/Horse-tc/GBR/H1/1975/G5P[7] shown to be of porcine origin (Ghosh et al., 2012), and RVA/Horse-tc/JPN/R-22/1984/G10P[11] and RVA/Horse-tc/GBR/26-94/199X/G8P[1] believed to be of bovine origin (Imagawa et al., 1994; Iša et al., 1996).

In Argentina, Garaicoechea and colleagues reported that RVA circulating in horses between 1992 and 2008 were quite homogeneous in their VP7, VP8* and NSP4 encoding gene segments (Garaicoechea et al., 2011). An apparent shift was described from G3 to G14 over the time, showing that from 1992 to 1999 G3 was the predominant genotype, whereas G14 became the predominant genotype in 2008. In the same study, the equine RVA sequences of the viral enterotoxin (NSP4) were classified in the unusual E12 genotype, which has up to date only been described in RVA strains detected in guanaco and cattle from Argentina (Matthijnssens et al., 2009). Furthermore, a single G3P[3] RVA strain (RVA/Horse-wt/ARG/E3198/2008/G3P[3]) was detected in this study. The G3P[3] genotype combination is typically found in feline and canine RVA strain and has sporadically also been detected in other hosts such as a simian, a goat, cattle and humans (Agnello et al., 2006; Ghosh et al., 2007; Grant et al., 2011; Kang et al., 2007; Lee et al., 2003; Matthijnssens et al., 2010b, 2011b; Oka et al., 2001; Tsugawa and Hoshino, 2008). In this paper we describe the full genome characterization of this unusual equine G3P[3] RVA strain E3198 and its phylogenetic relationship with other human and animal RVA strains.

2. Materials and methods

2.1. Virus

Equine RVA strain RVA/Horse-wt/E3198/2008/G3P[3] was detected from feces of a 3 day-old diarrheic foal on August 12th 2008. The foal was born from a vaccinated mare at a thoroughbred breeding farm in Buenos Aires province, Argentina.

2.2. Rotavirus diagnosis

The fecal sample was initially screened for the presence of RVA antigen by a commercial enzyme-linked immunoassay (Pathfinder Rotavirus, Bio-Rad, Marnes-la-Coquette, France) following the manufacturer's instructions.

2.3. RNA extraction

Viral RNA was extracted using the QIAamp viral RNA kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

2.4. Reverse transcription-PCR

The RT-PCRs and used primers were described previously: VP1, VP2 and VP3 (Tsugawa and Hoshino, 2008), VP6, NSP1–NSP5 (Matthijnssens et al., 2006), VP7 (Garaicoechea et al., 2011), and VP4 (GEN_P[3]_13F: 5′-GCTTCGCTCATTTATAGACAATTGC-3′ and GEN_P[3]_2361R: 5′-GTCACATCCTCTAGAAATTGCTTAC-3′) (Matthijnssens et al., 2011b). Briefly, the extracted RNA was denatured at 95 °C for 2 min before the RT-PCR was carried out using the Qiagen OneStep RT-PCR kit (QIAGEN) on a IVEMA T18 Thermocycler.

2.5. Nucleotide sequencing

The PCR products were purified with the MinElute Gel Extraction kit (QIAGEN) and sent for sequencing to the Unidad de Genómica, Instituto de Biotecnología, CICVyA, INTA, Castelar. The sequencing was performed with the same forward and reverse primers used for the RT-PCR. Primer walking sequencing was performed to cover the complete sequence of the respective fragments on both strands. The 5′ and 3′ ends were determined as described previously (Matthijnssens et al., 2006).

2.6. Nucleotide and protein sequence analysis

The chromatogram sequencing files were analyzed using Bioedit 7.0.9.0 Sequence Alignment Editor (Hall, T.A. 1999). Multiple sequence alignments were constructed using Muscle in MEGA5.0 (Tamura et al., 2011).

2.7. Phylogenetic analysis

Phylogenetic and molecular evolutionary analyses were conducted using MEGA5.0 (Tamura et al., 2011). Genetic

distances were calculated using the Kimura-2 correction parameter at the nucleotide level, and the phylogenetic trees were constructed using the neighbor-joining method with 1000 bootstrap replicates.

2.8. Assignment of genotypes

The genotypes of each of the 11 genome segments of the RVA strain under investigation was determined using the RotaC online classification tool (http://rotac.regatools.be/) (Maes et al., 2009).

2.9. Accession numbers

The following GenBank accession numbers were assigned to the VP1–VP4, VP6, VP7, NSP1–NSP5 gene segments of equine RVA strain E3198: JX036365–JX036375.

3. Results

3.1. Genotype constellation

The (near) complete ORF sequences for each of the 11 gene segments of the unusual G3P[3] RVA strain RVA/Horse-wt/ARG/E3198/2008/G3P[3] detected from a horse in Argentina (Garaicoechea et al., 2011) were determined for this study. The genotypes for each of these gene segments was determined to be G3–P[3]–I3–R3–C3–M3–A9–N3–T3–E3–H6 using the online RotaC genotyping tool (Maes et al., 2009). In Table 1, this genotype constellation is compared to a selection of completely sequenced human and animal RVA strains, including several recently published equine RVA strains isolated in the same epidemiological study (Matthijnssens et al., 2011c). In Table 1 it is shown that this genotype constellation is identical for 9 out of 11 gene segments to that of several canine (RVA/Dog-tc/USA/CU-1/1982/G3P[3], RVA/Dog-tc/AUS/K9/1981/G3P[3], RVA/Dog-tc/USA/A79-10/1 9 79/G3P[3], RVA/Dog-tc/ITA/RV198-95/1995/G3P[3], and RVA/Dog-tc/ITA/RV52-96/1996/G3P[3]) and a feline RVA strain (RVA/Cat-tc/AUS/Cat97/1984/G3P[3]). Several human RVA strains believed to be of feline or canine origin (RVA/Human-tc/ISR/Ro1845/1985/G3P[3], RVA/Human-tc/USA/HCR3A/1984/G3P[3], R VA/Human-tc/ITA/PA260-97/19 97/G3P[3], RVA/Human-wt/USA/6212/2002/G3P[3], RVA/Human-tc/JPN/AU-1/1982/G3P3[9] and RVA/Human-tc/TH A/T152/1998/G12P[9]) had 8–9 genotypes in common with E3198 (Table 1). Furthermore E3198 also showed 8 and 9 genotypes in common with two unusual simian RVA strains RVA/Rhesus-tc/USA/TUCH/2002/G3P[24] and RVA/Simian-tc/USA/RRV/1975/G3P[3], respectively. Only 0–3 genotypes were shared between E3198 and other known equine RVA strains.

3.2. Phylogenetic analyses and pairwise comparison

The VP7 gene segment of E3198 showed the highest sequence identities with a goat RVA strain RVA/Goat-tc/KOR/GRV/1998/G3P5[3] (91.6% on the nucleotide level), and the simian RVA strain RRV (89.5%). Furthermore a few bovine, canine and human canine/feline-like G3 RVA strains showed lower similarities ranging from 87.5 to 88.9%. These RVA strains were also found to cluster near E3198 in the VP7 phylogenetic tree (Fig. 1). The equine G3 RVA strains RVA/Horse-wt/ARG/E30/1993/G3P12 and RVA/Horse-wt/IRL/03V04954/2003/G3P12 were found to cluster rather distantly (86.9–87.1%). The phylogenetic

Table 1
RVA genotype constellations of the equine RVA strains E3198 sequenced in this study as compared to selected canine, feline, human and equine RVA strains.

RVA strains	Host	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
RVA/Horse-wt/ARG/E3198/2008/G3P[3]	**Equine**	**G3**	**P[3]**	**I3**	**R3**	**C3**	**M3**	**A9**	**N3**	**T3**	**E3**	**H6**
RVA/Dog-tc/USA/CU-1/1982/G3P[3]	Canine	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Dog-tc/AUS/K9/1981/G3P[3]	Canine	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Dog-tc/USA/A79-10/1979/G3P[3]	Canine	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Dog-tc/ITA/RV198-95/1995/G3P[3]	Canine	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Dog-tc/ITA/RV52-96/1996/G3P[3]	Canine	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Cat-tc/AUS/Cat97/1984/G3P[3]	Feline	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Human-tc/ISR/Ro1845/1985/G3P[3]	Human	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Human-tc/USA/HCR3A/1984/G3P[3]	Human	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Human-tc/ITA/PA260-97/1997/G3P[3]	Human	G3	P[3]	I3	R3	C3	M3	A15	N2	T3	E3	H6
RVA/Human-wt/USA/6212/2002/G3P[3]	Human	G3	*P[3]*	I3	*R1*	*C2*	*M3*	A9	N2	T3	E3	H6
RVA/Simian-tc/USA/RRV/1975/G3P[3]	Simian	G3	P[3]	I2	R2	C3	M3	A9	N2	T3	E3	H6
RVA/Rhesus-tc/USA/TUCH/2002/G3P[24]	Simian	G3	P[24]	I9	R3	C3	M3	A9	N1	T3	E3	H6
RVA/Human-tc/JPN/AU-1/1982/G3P3[9]	Human	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3
RVA/Human-tc/THA/T152/1998/G12P[9]	Human	G12	P[9]	I3	R3	C3	M3	A12	N3	T3	E3	H6
RVA/Cat-tc/AUS/Cat2/1984/G3P[9]	Feline	G3	P[9]	I3	R3	C2	M3	A3	N1	T6	E3	H3
RVA/Cat-wt/ITA/BA222/2005/G3P[9]	Feline	G3	P[9]	I2	R2	C2	M2	A3	N1	T3	E2	H3
RVA/Horse-wt/ARG/E30/1993/G3P[12]	Equine	G3	P[12]	I6	R2	C2	M3	A10	N2	T3	E12	H7
RVA/Horse-wt/IRL/03V04954/2003/G3P[12]	Equine	G3	P[12]	I6	R2	C2	M3	A10	N2	T3	E2	H7
RVA/Horse-wt/ZAF/EqRV-SA1/2006/G14P[12]	Equine	G14	P[12]	I2	R2	C2	M3	A10	N2	T3	E2	H7
RVA/Horse-wt/ARG/E403/2006/G14P[12]	Equine	G14	P[12]	I2	R2	C2	M3	A10	N2	T3	E12	H7
RVA/Horse-wt/IRL/04V2024/2004/G14P[12]	Equine	G14	P[12]	I2	R2	C2	M3	A10	N2	T3	E2	H7
RVA/Horse-wt/ARG/E4040/2008/G14P[12]	Equine	G14	P[12]	I2	R2	C2	M3	A10	N2	T3	E12	H7
RVA/Horse-tc/GBR/L338/1991/G13P[18]	Equine	G13	P[18]	I6	R9	C9	M6	A6	N9	T12	E14	H11

Genotypes identical to those of E3198 are shaded in light gray. The genetic diversity inside the G3 genotype is very large, therefore G3 RVA strains which are only distantly related to E3198 are indicated in dark gray.
Genotypes in italic are based on partial ORF sequences.

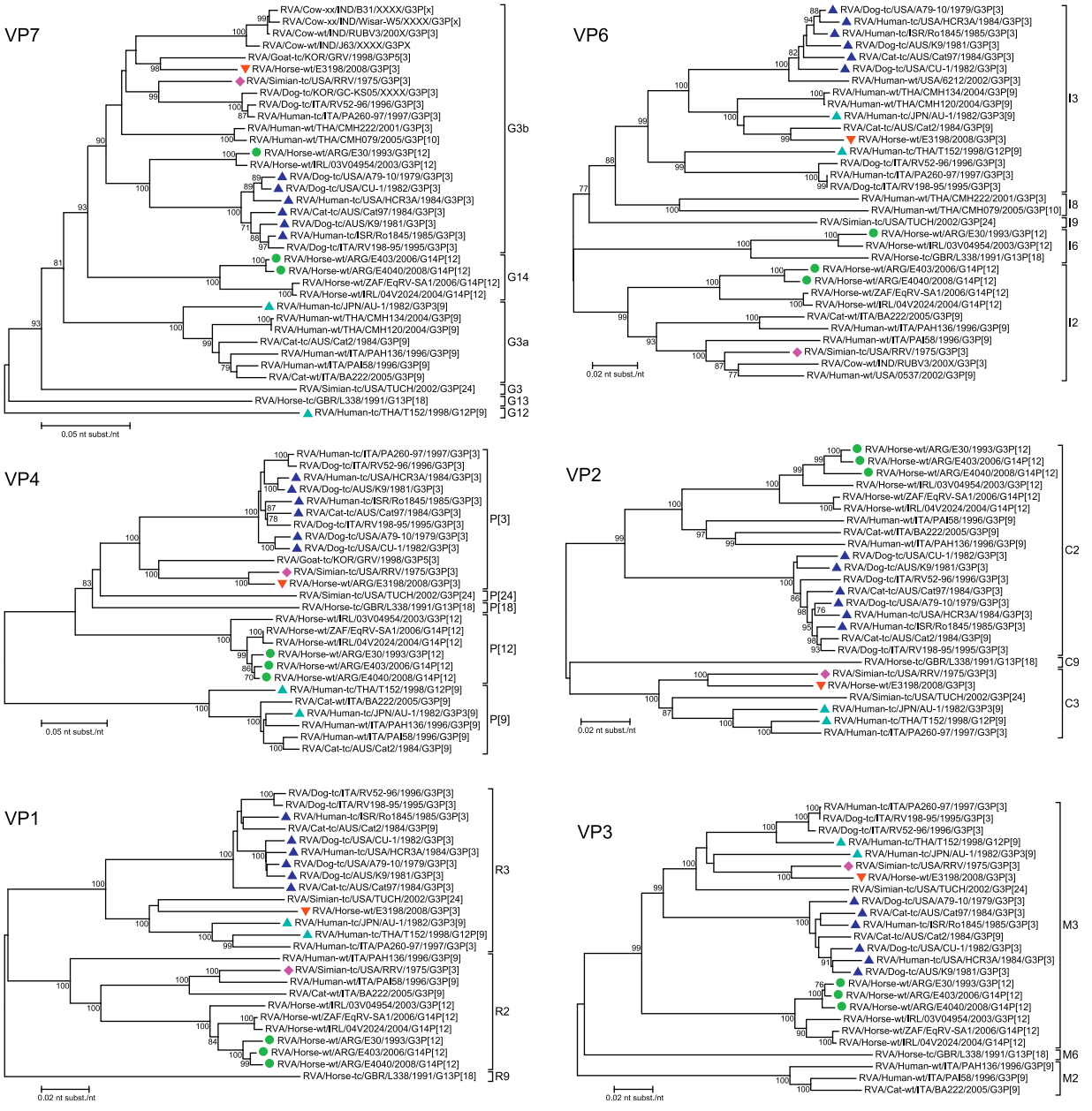

Fig. 1. Phylogenetic trees based on the near full-length ORF nucleotide sequences of selected RVA VP1–4, VP6 and VP7 gene segments. Bootstrap values (1000 replicates) above 70 are shown. Equine G3P[3] RVA strain E3198 is marked with a red inverted triangle, other Argentinean equine RVA strains (E30, E403 and E4040) with a green circle, simian RVA strains RRV with a purple diamond, Cat97-like canine, feline and human RVA strains (Cat97, K9, A79-10, CU-1, Ro1845 and HCR3a) with a dark blue triangle and AU-1-like RVA strains (AU-1 and T152) with a light blue triangle. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

tree of VP4, showed a close relationship between E3198 and RRV (95.9% similar on the nt level) in the P[3] genotype (Fig. 1). More distantly related was the goat RVA strain GRV (only 83.9%), and a large cluster of P[3] RVA strains with a feline/canine origin was found to be even more distantly related (78.1–79.9%). The VP6 gene segment of E3198 belongs to the I3 genotype, which contains G3P[3], G3P[9] and a single G12P[9] RVA strain, from feline, canine and human origin (Fig. 1). E3198 was found to cluster most closely with the feline G3P[9] RVA strains RVA/Cat-tc/AUS/Cat2/1984/G3P[9] (95.6%), in a cluster containing some unusual human G3P[9] RVA strains: AU-1, RVA/Human-wt/THA/CMH120/2004/G3P[9] and RVA/Human-wt/THA/CMH134/2004/G3P[9] (92.6–92.7%). The VP1 gene segment of E3198 is only distantly related to other RVA strains belonging to the R3 genotype (Fig. 1). TUCH, AU-1, T152 and PA260-97 were found to cluster most closely to E3198, although the similarities only ranged from 89.2 to 90.1%. The VP2 gene segment of E3198 belonged to the rather uncommon C3 genotype, distinct from the C2 genotype

containing the majority of the feline/canine-like and equine RVA strains (Fig. 1). E3198 clustered most closely with RRV (91.9%), and more distantly with TUCH, AU-1, T152 and 260-97 (87.6–89%). For the VP3 gene segment a similar clustering pattern was observed with E3198 clustering most closely (94.5%) with RRV and more distantly with AU-1, T152, TUCH, 260-97 in addition to RV198-95 and RV52-96 (86.0–88.0%) in the M3 genotype (Fig. 1). The cluster of equine RVA strains with the M3 genotype was only distantly (81.7–82.3%) related to E3198 (Fig. 1). The NSP1 gene segment of E3198 clusters in the A9 genotype with RRV (90.5% similar) and TUCH (84.4%), and more distantly (82.0–83.3%) with typical feline/canine like RVA strains (Fig. 2). E3198 possesses an NSP2 gene segment belonging to the rare N3 genotype, being only distantly related to T152 (89.2%) and AU-1 (85.6%) (Fig. 2). RRV possessed the NSP3 gene segment clustering most closely with E3198 (94.8%), followed by T152 (90.1%) in the T3 genotype. These three RVA strains clustered more closely with simian RVA strain TUCH and Argentinean equine RVA strains E30, E403 and E4040 (87.9–88.5%), than to typical feline/canine like RVA strains (84.5–85.5%) (Fig. 2). The NSP4 gene segment of E3198 is again most closely related to RRV (94.1%), in addition to three Japanese

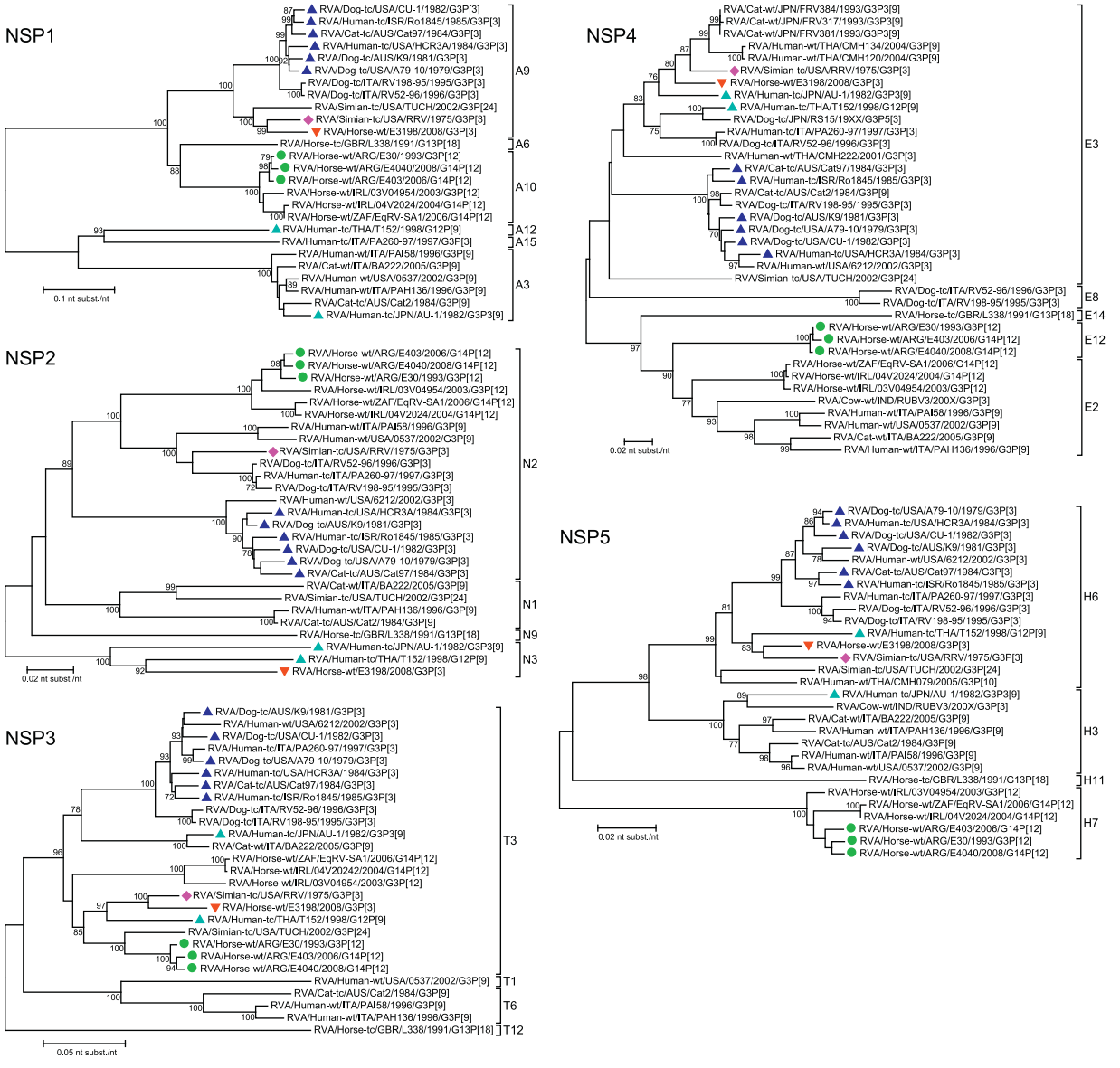

Fig. 2. Phylogenetic trees based on the near full-length ORF nucleotide sequences of selected RVA NSP1-5 gene segments. Bootstrap values (1000 replicates) above 70 are shown. Equine G3P[3] RVA strain E3198 is marked with a red inverted triangle, other Argentinean equine RVA strains (E30, E403 and E4040) with a green circle, simian RVA strains RRV with a purple diamond, Cat97-like canine, feline and human RVA strains (Cat97, K9, A79-10, CU-1, Ro1845 and HCR3a) with a dark blue triangle and AU-1-like RVA strains (AU-1 and TI52) with a light blue triangle. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

feline RVA strains (RVA/Cat-wt/JPN/FRV384/1993/G3P3[9], RVA/Cat-wt/JPN/FRV317/1993/G3P3[9] and RVA/Cat-wt/JPN/FRV381/1993/G3P3[9]; 93.5–93.7%), two human RVA strains with feline characteristics (CMH134 and CMH120; 92.6%), and AU-1 (91.6%), which is reflected in the phylogenetic tree (Fig. 2). NSP5 of E3198 clustered most closely with RRV (96.3%) and T152 (94.6%). Similarities of 93.6–94.6% were found between E3198 and a large cluster containing feline, canine and feline/canine-like human RVA strains (Fig. 2).

4. Discussion

RVA surveillance in horses has been conducted for more than 20 years in Argentina, and is still ongoing (Garaicoechea et al., 2011). In this period 878 diarrheic samples have been tested for RVA. From these samples approximately 24% (213/878) were RVA positive. Up to now only the G3 and G14 genotypes have been detected in this surveillance. Both G3 and G14 equine RVA strains are usually found in combination with P[12] worldwide (Ciarlet et al., 1994; Collins et al., 2008; Elschner et al., 2005; Garaicoechea et al., 2011; Monini et al., 2011; Nemoto et al., 2011; Tsunemitsu et al., 2001; van der Heide et al., 2005). However, in 2008 an unusual G3 RVA strain (RVA/Horse-wt/E3198/2008/G3P[3]) was detected in Argentina in combination with the P[3] genotype (Garaicoechea et al., 2011).

The complete genome of several equine RVA strains from Argentina, Ireland, South-Africa and the UK were recently described, showing the presence of a single rather conserved genotype constellation (G3/14–P[12]–I6/2–R2–C2–M3–A10–N2–T3–E12–H7) in equine RVA strains from several continents and one unique genotype constellation (G13–P[18]–I6–R9–C9–M6–A6–N9–T12–E14–H11) for RVA strain L338 (Matthijnssens et al., 2011c). The complete genome sequence of E3198 was determined to study the origin and relationships among these and other RVA strains.

Each of the individual genotypes and the entire encountered genotype constellation of E3198, G3–P[3]–I3–R3–C3–M3–A9–N3–T3–E3–H6, are very reminiscent of feline and canine RVA strains, and to human RVA strains believed to have a feline or canine origin. Currently the complete genomes of 3 feline RVA strains (Cat97, Cat2 and BA222), 5 canine RVA strains (A79-10, K9, CU-1, RV 198-95 and RV52-96) and 10 human RVA strains (6212, RVA/ Human-wt/USA/6235/2002/G3P[3], Ro1845, HCR3A, PA260-97, RVA/Human-wt/ITA/PAH136/1996/G3P[9], RVA/Human-wt/USA/0537/2002/G3P[9], RVA/Human-wt/ITA/PAI58/1996/G3P[9] and AU-1) which are believed to have a (partial) feline/canine origin, have been determined (De Grazia et al., 2010; Grant et al., 2011; Martella et al., 2011; Matthijnssens et al., 2008a, 2011b; Rahman et al., 2007; Tsugawa and Hoshino, 2008). Among these strains, a number of reassortments and at least three distinct genotype constellations have been described, represented by Cat97, AU-1 and BA222 (Matthijnssens et al., 2011b). The genotype constellation of E3198 is very reminiscent (identical genotypes for 9 out of 11 genotypes) to canine, feline and human RVA strains belonging to the Cat97-like genotype constellation (Table 1). E3198 possesses the C3 and the N3 genotype, whereas the Cat97-like RVA strains possess the C2 and N2 genotypes respectively (Table 1). However, the C3 and N3 genotypes are typical for RVA strains possessing an AU-1-like genotype constellation (Table 1). These findings suggest that the equine RVA strains E3198 may be of feline/canine origin, and may be the result of a reassortment among different feline/canine-like RVA strains.

However, phylogenetic analyses revealed that none of the 11 gene segments of E3198 clustered very closely to any of the currently described Cat97-like or AU-1-like RVA strains, with similarities below the 90% sequence identity for the majority of the gene segments of E3198. Only VP7 (~95%) and NSP5 (~94%) of E3198 were relatively closely related with Cat97-like RVA strains (Figs. 1 and 2). Interestingly, 5 gene segments (VP4, VP3, NSP3, NSP4 and NSP5) of E3198 showed high identities (between 94.1 and 96.3%) and clustered closely with the unusual simian RVA strains RRV. RRV was also one of the RVA strains most closely related (~90%) to E3198 for the VP7, VP2 and NSP1 gene segments (Figs. 1 and 2). The VP1, VP6 and NSP2 gene segments of E3198 and RRV were only distantly related (<82%), belonging to different genotypes. The complete genome of the simian RVA strain RRV was analyzed recently, and it was hypothesized that RRV was the result of a reassortment between a bovine-like RVA strain (donating gene segments encoding VP1, VP6 and NSP2), and a RVA strain having a common ancestor with feline/canine RVA strains donating the remaining 8 gene segments (Matthijnssens et al., 2010b). These data indicate that RVA strain E3198 is the most closely related relative of the feline/canine ancestor of RRV, currently known. The NSP2 gene segment of E3198 clusters in the rare N3 genotype only distantly related to AU-1 and T152 (Fig. 2). Also for VP1, E3198 is found to cluster distantly with AU-1 and T152 in addition to the feline-like human RVA strain PA260 and the simian RVA strains TUCH. All these strains are also believed to have a distant common ancestor with feline/canine RVA strains (Matthijnssens et al., 2010b, 2011b). For VP6, E3198 again clusters relatively distant with AU-1 and more closely with Cat2, which is believed to be a reassortant of feline RVA strains belonging to different genotype constellations (Matthijnssens et al., 2011b).

An observation further complicating our attempts to determine the origin of E3198 is the relative close relationship of the G3 VP7 gene segment of E3198 with caprine and bovine RVA strains from South Korea and India respectively (Fig. 1). However, these caprine and bovine RVA strains are also believed to be unusual reassorted or interspecies transmitted RVA viruses (Ghosh et al., 2007; Lee et al., 2003; Varshney et al., 2002). The majority of the genotypes of E3198 are different from those of typical equine RVA strains (Table 1). For VP3, they were found in the same genotype, although they formed highly distinct clusters inside M3 (Fig. 1), also for VP7, E3198 and equine G3 RVA strains E30 and RVA/Horse-wt/IRL/03V04954/2003/G3P[12], were only distantly related. However, surprisingly the NSP3 of E3198 was found to cluster relatively close to 3 Argentinean equine RVA strains (Fig. 2).

All these combined observations do not allow an unambiguous determination of the origin of E3198, but it is unlikely to be of equine origin, suggesting an interspecies transmitted RVA strain. The data suggest that E3198 has a distant common ancestor with currently circulating typical feline/canine RVA strains. In addition, E3198 may be more closely related to the feline/canine RVA strain involved in a reassortment resulting in simian RVA strains RRV. To better understand the complex interaction between RVA strains from different species, more complete genomes of different animal RVA strains will be necessary.

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