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Human G9P[8] rotavirus strains circulating in Cameroon, 1999–2000: Genetic relationships with other G9 strains and detection of a new G9 subtype

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Abstract

Group A rotaviruses (RV-A) are the leading cause of viral gastroenteritis in children worldwide and genotype G9P[8] is one of the five most common genotypes detected in humans. In order to gain insight into the degree of genetic variability of G9P[8] strains circulating in Cameroon, stool samples were collected during the 1999–2000 rotavirus season in two different geographic regions in Cameroon (Southwest and Western Regions). By RT-PCR, 15 G9P[8] strains (15/89 = 16.8%)

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2013.06.005.

were identified whose genomic configurations was subsequently determined by complete or partial gene sequencing. In general, all Cameroonian G9 strains clustered into current globally-spread sublineages of the VP7 gene and displayed 86.6–100% nucleotide identity amongst themselves and 81.2–99.5% nucleotide identity with global G9 strains. The full genome classification of all Cameroonian strains was G9-P[8]-I1–R1–C1–M1–A1–N1–T1–E1–H1 but phylogenetic analysis of each gene revealed that the strains were spread across 4 or more distinct lineages. An unusual strain, RVA/Human-wt/CMR/6788/1999/G9P[8], which shared the genomic constellation of other Cameroonian G9P[8] strains, contained a novel G9 subtype which diverged significantly (18.8% nucleotide and 19% amino acid distance) from previously described G9 strains. Nucleotide and amino acid alignments revealed that the 3′ end of this gene is highly divergent from other G9 VP7 genes suggesting that it arose through extensive accumulation of point mutations. The results of this study demonstrate that diverse G9 strains circulated in Cameroon during 1999–2000.

Keywords

Rotavirus A; Genotype P[8]G9; Genomic phylogenetic analysis; Structural proteins; Non-structural proteins

1. Background

Childhood mortality has been declining worldwide as a result of socioeconomic development and implementation of prevention and survival interventions (Claeson et al., 2000). Group A rotaviruses (RV-A) are the main etiologic agent of acute gastroenteritis in infants and young children worldwide (Estes and Kapikian, 2007) and an estimated 453,000 children aged <5 years die from rotavirus diarrhea each year, with >85% of these deaths occurring in low-income countries of Africa and Asia (Parashar et al., 2009; Tate et al., 2011). Rotaviruses belong to the family *Reoviridae*, and the rotavirus genome consists of 11 double-stranded RNA gene segments that encode six structural (VP) and six non-structural proteins (NSP). Based on the two genes that encode the outer capsid proteins, VP4 (P-type) and VP7 (G-type), a widely used binary classification system was established for RV-A (Estes and Kapikian, 2007). This system has been recently standardized and extended to all 11 genes (Matthijnssens et al., 2008b). To date, at least 27 G, 35 P, 16 I, 9 R, 9 C, 8 M, 16 A, 9 N, 12 T, 14 E and 11 H genotypes have been identified based on the eleven rotavirus A genes (Esona et al., 2010b; Matthijnssens et al., 2011). In humans, at least five RV-A G types (G1–G4 and G9), and two common P types (P[8] and P[4]) circulate worldwide (Banyai et al., 2012; Gentsch et al., 2005; Santos and Hoshino, 2005). G9 strains emerged in 1990s, and there has been a global description of the appearance and dominance of this genotype (Gentsch et al., 2005; Laird et al., 2003; Matthijnssens et al., 2009; Santos and Hoshino, 2005). Genotype G9 strains with a Wa-like or a DS-1-like genomic configuration or a mixture thereof have been detected sporadically in localized outbreaks (Page et al., 2010). In Cameroon, the first molecular identification of genotype G9 in human samples was reported in a study conducted by Steele and colleagues in 2003 (Steele and Ivanoff, 2003).

At least seven major phylogenetic lineages and eleven minor lineages within G9 VP7 genes have been described (Phan et al., 2007; Wu et al., 2011). A molecular evolutionary analysis study utilizing Bayesian inference supported the idea that one single sub-lineage introduced in the 1980s was responsible for all the worldwide spread of G9 in the 1990s (Matthijnssens et al., 2010).

In order to gain insight into the degree of genetic variability of G9P[8] strains circulating in Cameroon, Central Africa, sequence determination and phylogenetic analysis of all eleven genome segments from G9P[8] RV-A strains detected in two different geographic regions of Cameroon (Southwest and Western Regions) was performed in order to infer the genetic relationship of Cameroonian strains with G9P[8] worldwide. The results of these studies revealed a new G9 genetic variant circulating in Cameroon during the 1999–2000 rotavirus seasons.

2. Material and methods

2.1. Fecal samples, strains and nomenclature

Fifteen diarrheic stool specimens collected from children <5 years of age, genotyped as G9P[8] (Esona et al., 2010a), were obtained during the 1999–2000 rotavirus season in two different geographic regions in Cameroon (Southwest and Western Regions). The strains and nomenclature are shown in Table 1.

2.2. Viral RNA extraction, amplification, and sequencing

Viral RNA from each of the 15 specimens was extracted from a 10% stool suspension made from 0.1 g or 100 μl stool in 2 ml of a 1:1 Vertrel/Water solution using either a commercial RNA extraction kit (NucliSens automated extractor, BIOMERIEUX, Durham, NC) according to the protocol specified by the manufacturer or a silica binding method described previously (Boom et al., 1990).

Previously published forward and reverse primers (Das et al., 1994; Gentsch et al., 1992; Iturriza-Gomara et al., 2001, 2002; Kerin et al., 2007; Matthijnssens et al., 2006; Mijatovic-Rustempasic et al., 2011) were used for the amplification of the different gene segments. The extracted dsRNA of each strain was denatured at 97 °C for 5 min and RT-PCR was carried out using a one step RT-PCR kit (Qiagen, Inc., Valencia, CA) according to manufacturer's instructions. Reverse transcription (RT) of each gene from each sample was carried out for 30 min at 42 °C, followed by 15 min at 95 °C to inactivate the reverse transcriptase and activate the Taq polymerase. The cDNA was then subjected to 35 cycles of PCR in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Inc., Foster City, CA) using the following conditions: 30 s at 94 °C; 30 s at 42 °C; 45–90 s at 72 °C (depending upon the expected size of the amplified gene fragment), followed by a 7 min final extension at 72 °C. Amplicons were analyzed by gel electrophoresis in 1% SeaKem agarose gels (Thermo Fisher Scientific, Inc., Waltham, MA) then excised and purified with the QIAquick Gel Extraction kit (Qiagen, Inc., Valencia, CA) following the manufacturer's instructions.

DNA cycle sequencing of each amplicon was performed with the same consensus primers used for RT-PCR, using a Big Dye Terminator cycle sequencing Ready kit v1.1 (Applied Biosystems, Inc., Foster City, CA). Previously published primers homologous to internal regions of each gene segment were also used (Mijatovic-Rustempasic et al., 2011). Cycle sequencing products were purified using Centri-sep spin columns (Princeton Separations, Inc., Adelphia, NJ), dried in a DNA speed Vac^{R} (Savant Instruments, Inc., Holbrook, NY) and reconstituted in 15 ml Hi-Di formamide. Automated separation and base-calling of cycle sequencing products was performed using an ABI 3130xl sequencer (Applied Biosystems, Foster City, CA). Overlapping sequence fragments were assembled and edited using Sequencher 4.8 (Gene Codes Corporation, Inc., Ann Arbor, MI).

2.3. Computational analysis

Sequences were aligned using the MUSCLE program within MEGA version 5 (Tamura et al., 2011). Once aligned, the JModel-Test 2 program (Posada, 2008) was used to identify the optimal evolutionary model that best fitted the sequence datasets. Using corrected Akaike Information Criterion (AICc) the following models; TPM3uf + I + G (NSP1), TIM2 + I + G (NSP2), GTR + I + G (NSP3, VP1, VP2, VP6), HKY + G (NSP4), TVM + G (NSP5), TIM3 + I + G (VP3), TPM1uf + G (VP4), and TPM3uf + G (VP7) were found to best fit the sequence data for the different genes. Using these models, maximum likelihood trees were constructed using PhyML 3.0 along with approximate likelihood-ratio test (aLRT) statistics for branch support (Guindon et al., 2010). Nucleotide and amino acid distance matrixes were prepared using the *p*-distance algorithm of MEGA version 5 software (Tamura et al., 2011).

Using the crystal structure of the RRV VP7 protein (PDB accession number 3fmg; (Aoki et al., 2009), amino acid substitutions found in Cameroonian strain RVA/Human-wt/CMR/6788/1999/G9P[8] were mapped spatially onto the 3D protein structure using the PyMOL Molecular Graphics System, version 1.5.0.1 (Schrodinger, 2010).

3. Results

3.1. The genotype configuration of Cameroonian G9P[8] strains

The names and characteristics of the Cameroonian strains analyzed in this study, and lengths of each gene are presented in Table 1. The accession numbers of each gene of the Cameroonian G9 strains and those from the GenBank are in the appendix. Accession numbers in bold face characters represent the gene sequences of these Cameroon G9 strains. Nucleotide sequences analysis based on VP7–VP4–VP6–VP1–VP2–VP3–NSP1–NSP2–NSP3–NSP4–NSP5 genes from all Cameroonian samples analyzed revealed a consensus genotype constellation of G9-P[8]-I1–R1–C1–M1–A1–N1–T1–E1–H1, respectively, according to the classification proposed by Matthijnssens and colleagues (Matthijnssens et al., 2008b).

3.2. Analysis of VP7 nucleotide sequences

Phylogenetic analyses of the eleven genes determined the genetic relationships of the Cameroonian strains with a global collection of rotavirus genotypes. Phylogenetic analysis based on VP7 nucleotide sequences showed that all G9 strains detected in Cameroon during

1999–2000 clustered together in three distinct sub-clusters with lineage III G9 strains isolated worldwide (Fig. 1A). Nucleotide and amino acid identities among Cameroonian strains ranged between 88.6–100% and 84.6–100%, respectively. The strain RVA/Human-wt/CMR/6788/1999/G9P[8] showed a maximum nucleotide identity of 90% with Cameroonian strains RVA/Human-wt/CMR/6806/1999/G9P[8], RVA/Human-wt/CMR/6805/2000/G9P[8] and RVA/Human-wt/CMR/6796/2000/G9P[8], while its nucleotide identity with global G9 strains ranges from 81% to 89% (data not shown). Comparison of the amino acid sequence of strain RVA/Human-wt/CMR/6788/1999/G9P[8] to reference and contemporary human G9 genotypes from the GenBank revealed a low identity in the range of 81–85% as well as one to numerous substitutions in the nine major VP7 variable regions VR-1–VR-9 (Green et al., 1989) described for this protein (Fig. 2); VR-1 (I16L), VR-3 (A43 V), VR-4 (A68T), VR-5/antigenic epitope A (S90A, Q92E, G94A, and D100E), VR-8/antigenic epitope C (I208L, T210P, T212P, A213P, T214P, E217 K, and S221L), and VR-9/antigenic epitope F (D238E, T241P, T243P, and T245P) (Green et al., 1989; Kirkwood et al., 2003). The VR-2, VR-6 and VR-7 were highly conserved amongst both contemporary and older G9 strains. Substitutions in the three major variable regions; VR-5/antigenic epitope A, VR-8/antigenic epitope C and VR-9/antigenic epitope F were mapped to the VP7 crystal structure of the RRV strain (G3P[3]) available in the Protein Data Bank (Fig. 3). Out of the 15 substitutions identified in these three regions, 11 were radical in nature. Radical changes are associated with changes in size, charge and polarity (Zhang, 2000). Out of the 11 radical changes 9 were associated with changes in polarity with the strain 6788 being non-polar when compared to the consensus G9 strains. In VR-5/antigenic epitope A mutation in site 94 is associated with neutralization escape mutants (Aoki et al., 2009). Similar sites associated with neutralization escape mutants were observed in VR-8/antigenic epitope C (positions 213, 217, 221) and in VR-9/antigenic epitope F (position 238). Comparative analysis of this strain with representative strains belonging to the major G9 VP7 lineages revealed a high similarity in the 5′ end of the gene and a lower similarity downstream of the central part of the gene (data not shown).

3.3. Analysis of VP4 nucleotide sequences

Evolutionary analysis of VP4 nucleotide sequences from Cameroonian strains and representatives of genotype P[8] from the Gen-Bank database demonstrated that strains from both regions of Cameroon grouped in four different sub-clusters of lineage P[8]-III together with other rotaviruses from around the world (Fig. 1B). Nucleotide (amino acid) identities between Cameroonian strains ranged between 95.4% and 100% (95.7–100%). Strain RVA/Human-wt/CMR/6735/1999/G9P[8] showed a close genetic relationship with strains isolated in Acre-Brazil during 2005–2006 rotavirus season. Strain RVA/Human-wt/CMR/6778/1999/G9P[8] grouped in a separate cluster together with a previously detected African P[8] strain. Two Cameroonian strains, RVA/Human-wt/CMR/6779/1999/G9P[8] and RVA/Human-wt/CMR/6788/1999/G9P[8], showed complete identity with P[8] strains detected in Tunisia (North Africa) in 2002 and Cote D'Ivoire (West Africa) in 2004 (data not shown).

3.4. Analysis of VP1, VP2, VP3 and VP6 nucleotide sequences

Phylogenetic analysis based on VP1, VP2, VP3 and VP6 nucleotide sequences demonstrated that each gene of Cameroonian strains detected during the 1999–2000 rotavirus season

grouped in several separate clusters together with strains isolated worldwide (Fig. 1C–F). The Cameroonian strains showed a close genetic relationship with cognate gene sequences of previously reported G1P[8], G3P[8], and G4P[8] strains detected in the USA, Bangladesh and Belgium (McDonald et al., 2009; Mijatovic-Rustempasic et al., 2011; Rahman et al., 2007). Nucleotide (amino acid) identity values among Cameroonian strains ranged from 91.8–100% (94–100%), 90.7–100% (86.8–100%), 89.6–100% (93.9–100%), and 96–100% (99.2–100%) for VP1, VP2, VP3 and VP6, respectively. Complete nucleotide and amino acid similarity (100%) was shared between strains RVA/Human-wt/CMR/6735/1999/G9P[8] and RVA/Human-wt/CMR/6795/1999/G9P[8] (VP1 gene); RVA/Human-wt/CMR/6735/1999/G9P[8] and RVA/Human-wt/CMR/6779/1999/G9P[8], RVA/Human-wt/CMR/6805/1999/G9P[8] and RVA/Human-wt/CMR/6806/1999/G9P[8], RVA/Human-wt/CMR/6790/1999/G9P[8] and RVA/Human-wt/CMR/6792/1999/G9P[8] (VP2 gene); RVA/Human-wt/CMR/6788/1999/G9P[8] and RVA/Human-wt/CMR/6805/1999/G9P[8] (VP3 gene); and RVA/Human-wt/CMR/6806/1999/G9P[8] and RVA/Human-wt/CMR/6807/1999/G9P[8] (VP6 gene). However, when the nucleotide and amino acid homologies of the VP1–VP3 and VP6 gene sequences of the Cameroonian strains were compared with cognate gene sequences of strains belonging to previously identified VP1–VP3 and VP6 genotypes, all of them were more closely related to strains in the R1, C1, M1 and I1 genotypes, respectively. Further comparison showed that within each genotype, the Cameroonian strains had maximum nucleotide (amino acid) identities of 84.8–98.6% (93.1–99.4%) for VP1, 89.8–98.8% (89.8–99.8%) for VP2, 88.3–99.2% (91.4–99.5%) for VP3 and 89.4–99.5% (97.5–100%) for VP6.

3.5. Analysis of NSP4 nucleotide sequences

Phylogenetic analysis based on NSP4 nucleotide sequences showed that the Cameroonian strains grouped into four different sub-clusters together with strains belonging to genotype E1 (Fig. 1G). The nucleotide (amino acid) identity among Cameroonian strains ranged between 90.9%-100% (94.3%-100%). Analysis of deduced amino acid sequences of Cameroonian strains and rotaviruses detected worldwide demonstrated that seven of the fifteen strains detected in Cameroon showed an amino acid substitution within the enterotoxin domain (aa 114–135) (Ball et al., 2005). Three strains exhibited changes at position H131Y and the other four at position N133S. Amino acid substitutions were observed within previously described antigenic sites (Ball et al., 2005; Borgan et al., 2003): ASIV (aa 1–24) at position L7 V; ASIII (aa 112–133) at positions H131Y and N133S; ASII (aa136–150) at positions I136A, P138S, V139A, V141I and I142V and ASI (151–169 aa) at positions E160N, S161N, and L169I when aligned with other E1 genotype strains (Fig. 4).

3.6. Analysis of NSP1- NSP3 and NSP5 nucleotide sequences

Phylogenetic analysis of NSP1- NSP3 and NSP5 nucleotide sequences, demonstrated that for each gene, the Cameroonian strains grouped in small separate sub-clusters of genotypes A1, N1, T1, and H1, respectively, together with other strains from around the world (Fig. 1H–K). Nucleotide (amino acid) identity values among Cameroonian strains ranged from 87.8–99.8% (82.9–99.7%), 86.8–100% (88–100%), 92.8–100% (93.1–100%) and 97.6–100% (98.5–100%) for NSP1, NSP2, NSP3 and NSP5, respectively. Complete nucleotide and amino acid identity was shared between strains RVA/Human-wt/CMR/6777/1999/

G9P[8] and RVA/Human-wt/CMR/6790/1999/G9P[8] (NSP2, NSP3 and NSP5 genes) and RVA/Human-wt/CMR/6806/1999/G9P[8] and RVA/Human-wt/CMR/6807/1999/G9P[8] (NSP3 and NSP5 genes), while only strains RVA/Human-wt/CMR/6779/1999/G9P[8] and RVA/Human-wt/CMR/6791/1999/G9P[8] were completely identical in their NSP5 gene sequences. However, when the nucleotide and amino acid homologies of the NSP1–NSP3 and NSP5 gene sequences of the Cameroonian strains were compared with similar gene sequences of strains belonging to already identified NSP1–NSP3 and NSP5 genotypes, all of them were more closely related to strains in the A1, N1, T1, and H1 genotypes, respectively. Within each of these genotypes, the Cameroonian strains shared maximum nucleotide (amino acid) identity of 75.4–99.6% (68.7–98.8%), 81.2–99.5% (84.5–100%), 85.9–99.6% (87.9–100%), and 94.6–99.8% (93.9–100%), respectively.

4. Discussion

The genetic variability of RV-A strains is the result of accumulation of single nucleotide mutations (genetic drift) and sudden changes in the RV-A genome (genetic shift), primarily by reassortment and recombination events (Estes and Kapikian, 2007; Matthijnssens et al., 2008c; McDonald et al., 2009; Ramig, 1997). Since the proposal that RV-A classification should be based on all 11 RV-A gene segments (Matthijnssens et al., 2008b), the number of studies reporting RV-A full genome sequences has increased (Banyai et al., 2011; Esona et al., 2010b, 2011; Matthijnssens et al., 2008a; McDonald et al., 2009, 2011; Mijatovic-Rustempasic et al., 2011). Previous studies have shown that the predominance of a specific G type is related to the emergence of atypical VP7 lineages (Banyai et al., 2009; Parra, 2009; Parra et al., 2005). The results obtained in this study revealed multiple amino acids changes in 6 of the 9 variable regions (Green et al., 1989; Kirkwood et al., 1993) when comparing Cameroon G9 strain RVA/Human-wt/CMR/6788/1999/G9P[8] to both contemporary and older G9 strains from the GenBank database. This suggests that this Cameroon G9 strain might represent a new genetic variant of VP7 gene G9 genotype. The relatively low overall amino acid homology with other G9 strains together with numerous changes in important antigenic regions raises questions on whether this strain may be antigenically distinct from typicalG9 strains. Also, the previously described conserved N-glycosylation site found within VR-4 at amino acid residues 69–71 (Green et al., 1989) was found to be conserved in all G9 strains used in this analyses. Definitive conclusions on the possible origin of this variant could not be made by bioinformatics analysis of the VP7 gene sequence. However, in this case it remains unclear what selective pressures on this gene fragment could have driven this strong diversification, given that other gene segments of this strain retained their identity with related G9P[8] strains identified in the same region and time period. In this context, it is difficult to determine if a single sub-lineage of G9 can be responsible for the worldwide spread of G9 rotavirus as proposed recently (Matthijnssens et al., 2010).

At least six different neutralization epitopes (A through F) have been identified in the RVA VP7 protein, with A–C and F described as the most important (Kirkwood et al., 2003). The strain RVA/Human-wt/CMR/6788/1999/G9P[8] shows distinct changes in its antigenic regions when compared to G9 strains circulating in the same region and also globally (Fig. 2). A strong shift in polarity, with strain RVA/Human-wt/CMR/6788/1999/G9P[8] being

strongly non-polar as compared to other G9 strains suggest possible inaccessibility of epitopes on the VP7 protein of strain RVA/Human-wt/CMR/6788/1999/G9P[8] as the region becomes more hydrophobic in nature. Multiple sites previously identified as important in producing neutralization escape mutants show substitutions in strain RVA/Human-wt/CMR/6788/1999/G9P[8] when compared with global G9 strains. If strain RVA/Human-wt/CMR/6788/1999/G9P[8] is also a neutralization escape mutant it could be due in part to changes in polarity at the antigenic epitopes. The amino acid sequences at positions 87–101 and 208–211 (epitope region A and C) is said to be conserved within serotypes (Green et al., 1988). However, we observe substitutions within these regions in an alignment of a global collection of G9 strains.

Seven of the fifteen strains detected in Cameroon showed an amino acid substitution in the enterotoxin domain (114–135 aa) of NSP4. These changes occur at amino acid positions 131 and 133, which are in the region (amino acid 131–140) reported to be responsible for altered pathogenesis mediated by the NSP4 protein (Zhang et al., 1998). Also observed are amino acid substitutions in the four previously described NSP4 antigenic sites (Ball et al., 2005). Antigenic sites AS IV, AS III, AS II and AS I had 1, 1, 5, 3 amino acids substitutions, respectively, when compared with strains in the E1 genotype. NSP4 is a trans-membrane glycoprotein known to be involved in virus assembly and is capable of inducing diarrhea in infant mice (Ball et al., 1996; Tian et al., 1996). It is possible these changes may affect the conformation or activity of NSP4 and also alter ability of host responses to neutralize enterotoxic function of the NSP4 gene segment (Ball et al., 1996; Tian et al., 1995).

Recent studies on origin and spread of the G9 rotavirus have revealed a high degree of genetic diversity within this genotype worldwide (Martinez-Laso et al., 2009; Phan et al., 2007; Wu et al., 2011). These studies have also shown close similarity between human and porcine G9 strains and zoonotic transmission and convergent evolution were proposed as the possible evolutionary mechanisms. Studies in Brazil have implicated porcine G9 strains in many outbreaks (Leite et al., 2008). Also, reassortment events among animal and human strains have continued to be an important mechanism for rotavirus evolution and emergence in developing countries (Banyai et al., 2010; Esona et al., 2010b). This present report confirms the circulation of a diverse G9 genotype among children in two regions of Cameroon. However, all of the G9P[8] strains reported here showed close similarity in all gene segments except VP7 with human rotavirus strains of different G genotypes including G1P[8], G3P[8] and G4P[8]. Nonetheless, due to the increased close contact between human and animals in most developing countries, the full genome sequence data obtained from any future G9 study from Cameroon maybe entirely different.

Two live oral vaccines from Merck (RotaTeq®) and Glaxo-SmithKline (Rotarix®) have been licensed in more than 100 countries and are being introduced into routine immunization programs in several countries worldwide (Glass et al., 2006; Vesikari et al., 2007), but has not been introduced in Cameroon. Therefore, it will be important to monitor the circulation of G9s in Cameroon before the introduction of these vaccines and, once introduced, to monitor the abilities of the vaccines to provide heterotypic protection against divergent G9 strains.

Furthermore, monitoring temporal changes in all 11 gene segments may help us to comprehend the nature and pattern of rotavirus evolution. This study revealed the presence of a novel VP7 genetic variant and diverse G9P[8] strains in Cameroon. Surveillance to monitor the strain diversity of circulating RV-A to detect possible strain replacement following the introduction of universal RV-A vaccine is a priority of the World Health Organization. Such studies are important to estimate potential impact of vaccination programs on circulating strains including whether escape mutants of known serotypes or novel strains that evade vaccine immunity will emerge.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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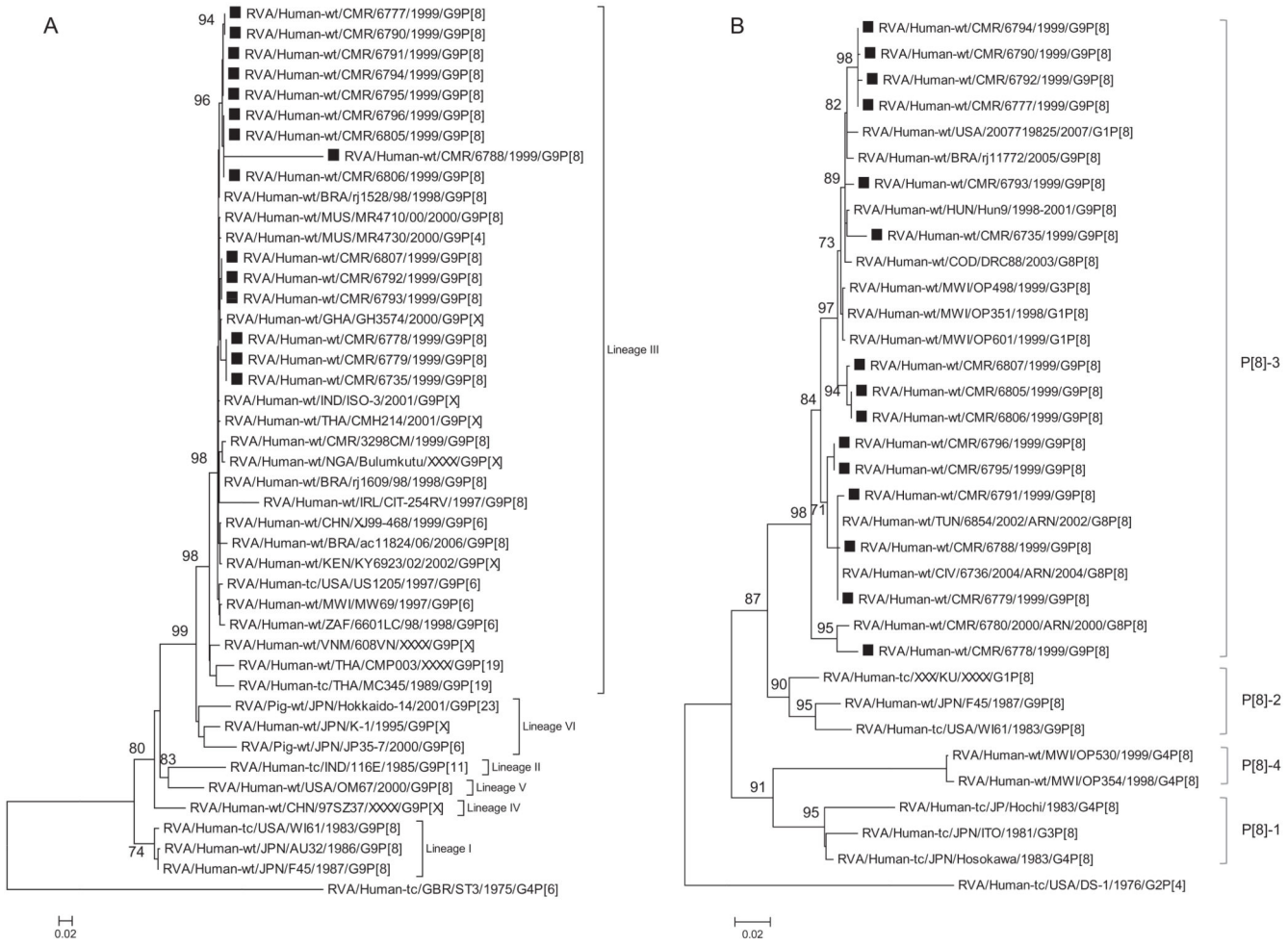

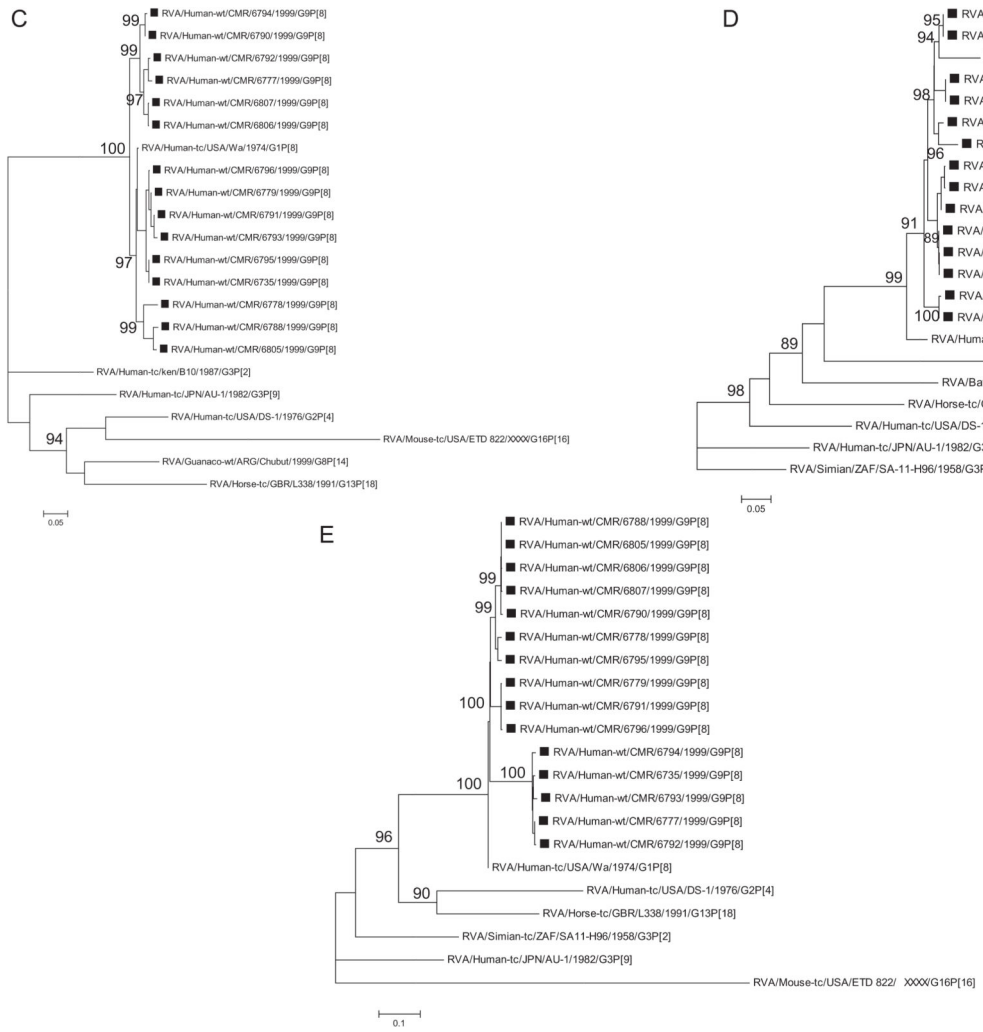

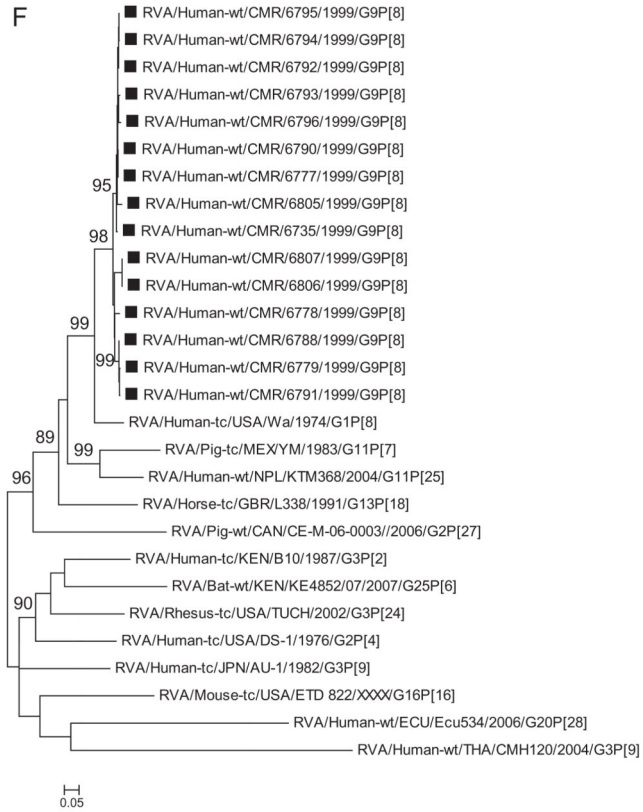

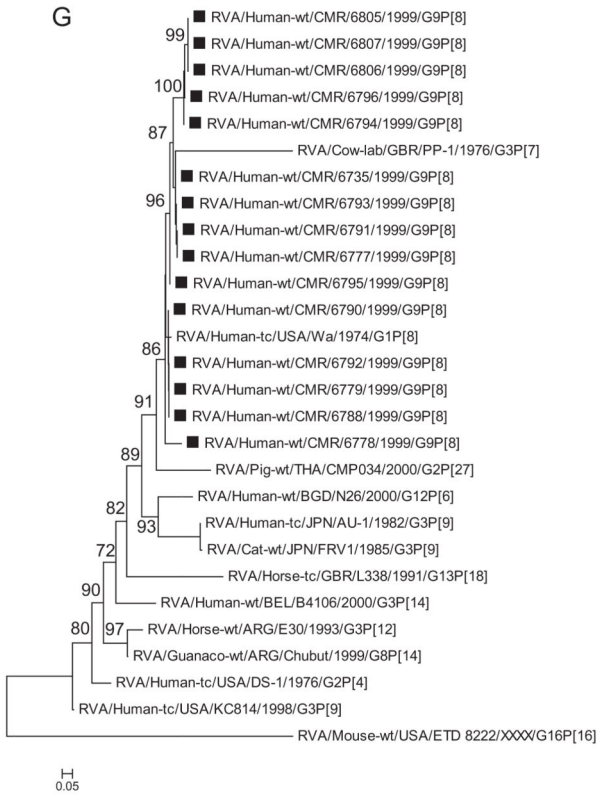

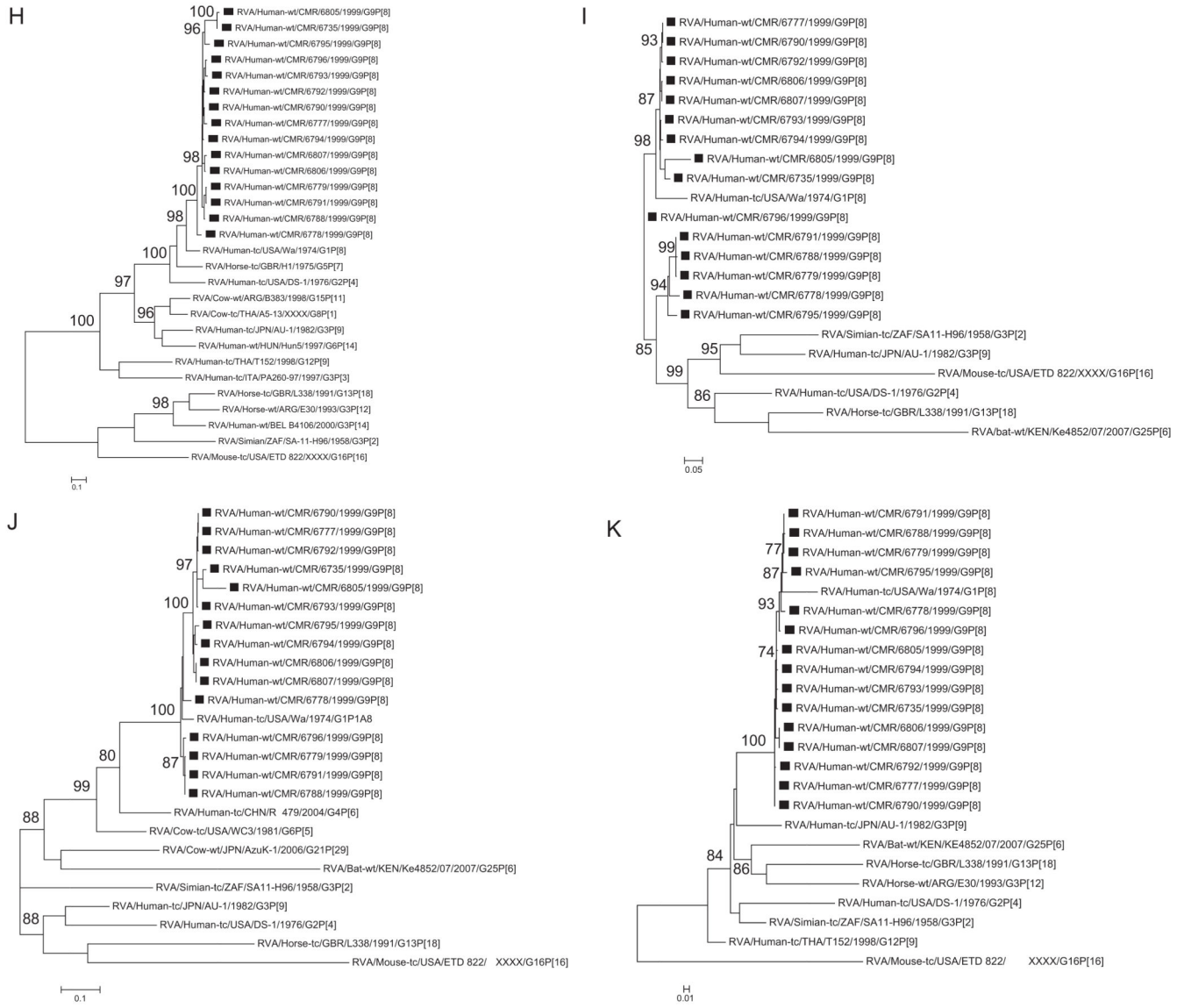

Fig. 1.

A–K Maximum likelihood phylogenetic trees built in PhyML with aLRT statistics as support show the genetic relationships of nucleotide sequences of VP7 (A), VP4 (B), VP1(C), VP2 (D), VP3 (E), VP6 (F), NSP4 (G), NSP1 (H), NSP2 (I), NSP3 (J) and NSP5 (K) of human G9P[8] rotaviruses from Cameroon with known human and animal rotavirus strains from GenBank database. The trees were drawn to scale. Only aLRT values of 70% and greater are shown. The strains labeled with filled squares indicate the Cameroon G9P[8] isolates sequenced in this study. The scale bar at the bottom of the trees indicates genetic distance.

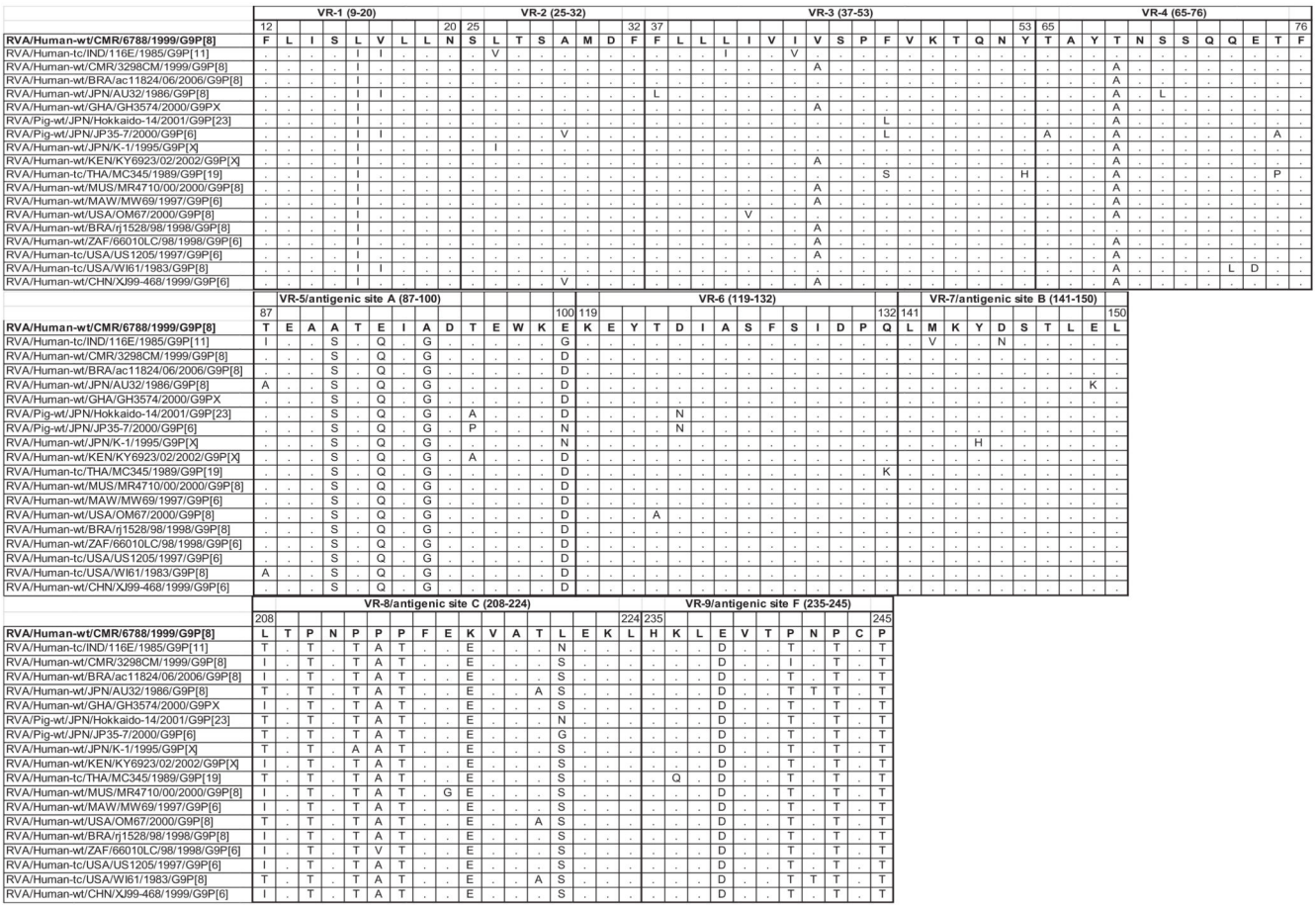

Fig. 2.

Comparison of the deduced amino acid sequence of gene segment 9 of strain RVA/Human-wt/CMR/6788/1999/G9P[8] to a selection of older and contemporary G9 sequences from the GenBank. Only amino acids which differ are shown. Variable regions designated VR-1-VR-9 are shown.

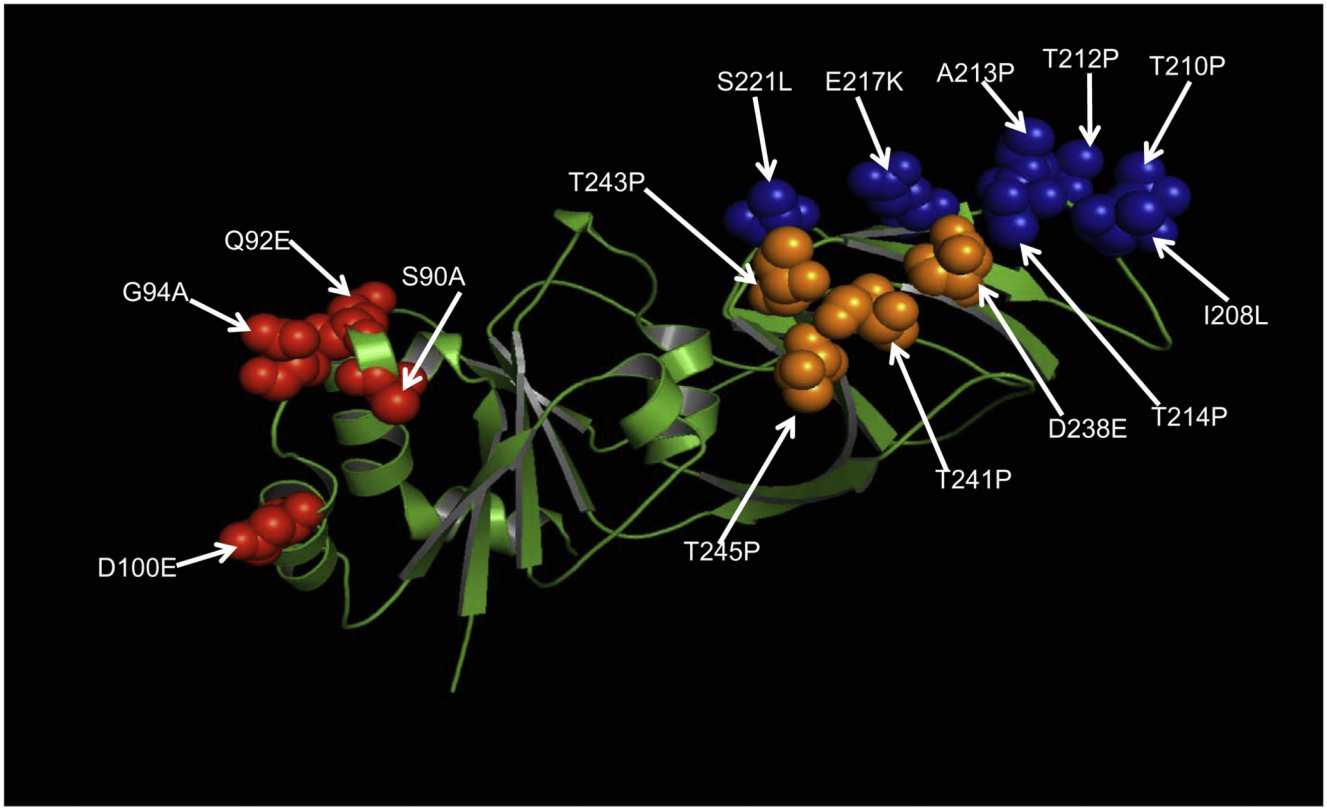

Fig. 3.

Substitutions in strain RVA/Human-wt/CMR/6788/1999/G9P[8] highlighted on the crystal structure of RRV VP7 protein (3fmg). The molecule is colored in green. Residues corresponding to previously describe major antigenic sites A, C and F are indicated in red, blue and orange spheres, respectively. Arrows indicate substitutions at amino acid positions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

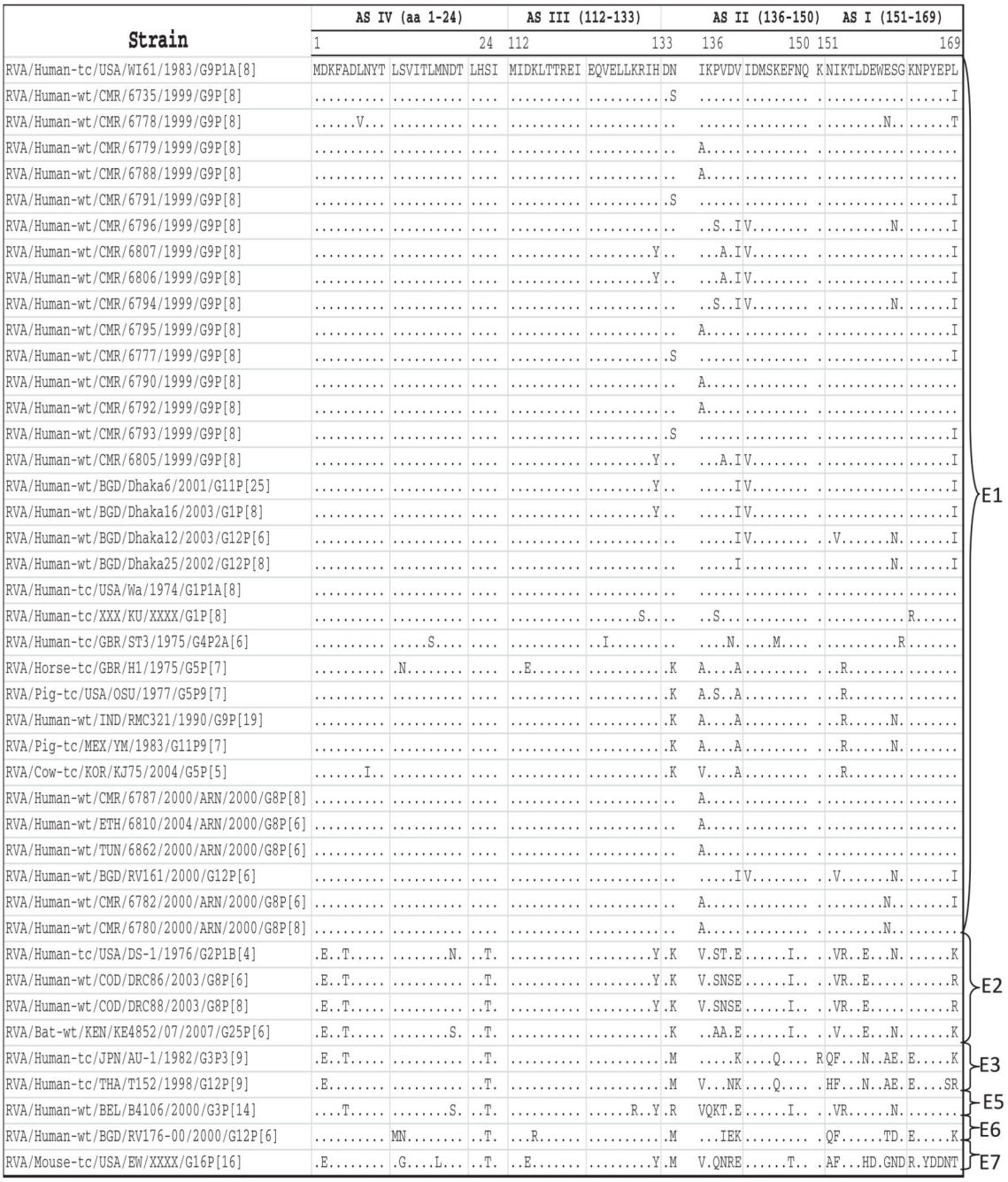

Fig. 4.

Alignment showing amino acid substitutions inside the four NSP4 antigenic sites of the Cameroon G9 strains.

Table 1

Characteristics of the Cameroon G9P[8] strains.

	Genotypes												
	VP7	**Lineage**	**VP4**	**Lineage**	**VP6**	**VP1**	**VP2**	**VP3**	**NSP1**	**NSP2**	**NSP3**	**NSP4**	**NSP5**
Length of gene sequenced (nucleotide)[a]	**840**		**834**		**1191**	**1917**	**1367**	**1187**	**1043**	**948**	**930**	**525**	**591**
Presence of ORF[b]	**P**		**P**		**C**	**P**	**P**	**P**	**P**	**C**	**C**	**C**	**C**
Strain name													
RVA/Human-wt/CMR/6735/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6778/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6779/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6788/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6791/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6796/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6807/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6806/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6795/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6777/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6790/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6792/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6793/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6805/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6794/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1

[a] Length of gene sequenced in nucleotides.

[b] P and C denotes partial or complete ORF, respectively.