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ORIGINAL PAPER

Full-length genome analysis of the first human G8P[14] rotavirus strain from Morocco suggests evidence of zoonotic transmission

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Abstract

An unusual group A rotavirus (RVA) strain MAR/ma31/2011/G8P[14] was detected for the first time in Morocco in a stool sample from hospitalized child aged 18 months suffering from acute gastroenteritis and fever in 2011. Complete genome sequencing of the ma31 strain was done using the capillary sequencing technology. The analysis revealed the G8-P[14]-I2-R2-C2-M2-A11-N2-T6-E2-H3 constellation and the backbone genes: I2-R2-C2-M2-A11-N2-T6-E2-H3 are commonly found in RVA strains from artiodactyls such as cattle. The constellation was shared with another Italian zoonotic G8P[14] strains (BA01 and BA02), two Hungarian human strains (182-02 and BP1062) and a sheep RVA strain OVR762. Phylogenetic analysis of each genome segment of ma31 revealed a mixed gene configuration originated from animals and human. Comparison of the antigenic regions of VP7 and VP4 amino acid sequences between ma31 strain and selected animal and human strains bearing G8 and or P[14], showed a high level of conservation, while many substitutions was observed in comparison with RotaTeq™ and Rotarix™ vaccine strains. In contrast, alignment analysis of the four antigenic sites of VP6 revealed a high degree of conservation. These findings reveal a typical zoonotic origin of the strain and confirm a high potential for RVA zoonotic transmission between bovine and humans, allowing the generation of novel rotavirus genotypes.

Keywords Group A rotavirus · G8P[14] genotype · Reassortment · Phylogenetic analysis · Morocco

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Introduction

Group A rotavirus (RVA) is a most important etiologic agent of severe acute gastroenteritis among children under 5 years of age and many animal species worldwide [1, 2], causing 215,000 deaths per year and 37% of hospitalizations annually worldwide, more than 50% of these deaths occur in Africa [3, 4].

RVA belongs to the genus *Rotavirus* within the family *Reoviridae* based on the shared structural and genomic features. Known to be a non-enveloped virus, its genome is composed of 11 segments of double-stranded RNA (dsRNA). These RNA segments encode six structural proteins (VP1-4, VP6, and VP7) and six non-structural proteins (NSP1-NSP6) [5].

The traditional binomial RVA classification system was based on the two outer capsid proteins VP7 and VP4 and it is the most widely used scheme in molecular epidemiology and surveillance programs [6]. These antigens made it possible to define G and P types of the viral strains to better know the predominant strains in human diarrhea and compare them with those isolated from animals to identify

a potential zoonotic risk. Until now, at least 35 G-types and 50 P-types have been identified [7, 8].

The fragmented nature of the genome facilitates the reassortment between and among human and animal strains, which constitutes one of the major processes of the genetic evolution of rotaviruses [2]. Therefore, a new classification system has been proposed in 2008 by the Rotavirus classification working group (RCWG) to better understand the evolution of reassortments and viral inter-species transmission [7]. This new classification system is based on extending the classical binomial genotyping to a full genotype constellation including the 11 segments. These RVA genes VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 are determined as Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx, respectively, with x indicates the genotype number.

In RVA research, complete genome sequencing has become largely used because it offers a good tool allowing the understanding of the intra-genomic variations in the rotavirus genes, hence revealing the pattern of unusual and/or new emerging RVA strains. These latter genotypes are suspected to be originated from animal rotaviruses that have been introduced into human populations through inter-species transmission events, which may be accompanied by reassortment and/or adaptation to a new host [2, 9]. The genotypes G6, G8, and G10 are for example the most common RVA G types found in cattle [10, 11], while the P[14] RVA genotype identified in humans sporadically, takes its origin from several distinct animal species such as rabbits, ungulates and ruminants hosts [12, 13].

The diversity of the human genotypes of circulating RVA as well as the appearance of genotypes considered unusual remain more important in developing countries including Africa than developed countries [14]. The common G and P-type combinations found in Africa were G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]; the uncommon G8 and P[6] genotypes have been well documented and associated to a possible zoonotic transmission [15, 16]. The human G8 RVA strains detected in African children were either DS-1-like or Wa-like genotype constellations with a predominance of DS-1-like in abundance and frequency [17–19]. In contrast, elsewhere in the world, the same human G8 genotype mainly carrying the DS-1-like constellation is less common [20, 21].

In Morocco, monitoring the circulation of such unusual strains is not yet performed. In this regard, our research group highlights in the present study, the full genome sequencing of a rare human RVA strain (RVA/Human-tc/MAR/ma31/2011/G8P[14]) isolated in 2011 from an 18-month-old male child with acute diarrhea during a study conducted between April 2011 and May 2013 in the pediatric division of the military teaching hospital Med V in Rabat, Morocco. The amino acid sequence comparison of VP8*, VP7 and VP6 antigenic regions between the strain under study and other world-wide G8 or P[14] strains and vaccine strains was also performed. This investigation was done to gain more insight into G8P[14] origin and evolution compared to other human and animal G8P[14] strains detected around the world.

Materials and methods

Study sample and initial characterization

Fecal specimen ma31 selected for this study was obtained from an 18-month-old boy with acute gastroenteritis (AGE) during a survey performed on viral gastroenteritis conducted between 2011 and 2013 in Morocco [22]. The child, living in rural region near Rabat, was admitted at the military teaching hospital Mohammed V in Rabat in 2011, diagnosed with mild gastroenteritis with a mean clinical vesikari score of 9. The sample was identified as positive for RVA using the rapid test (Duo Rota-Adenovirus-Check-1, VEDA LAB, France) according to the manufacturer's instructions; then 10% of stool suspension was prepared in PBS and the homogenate was centrifuged at 5000×*g* for 15 min. Briefly, viral nucleic acid was extracted, reverse transcribed and amplified using specific primers targeting VP4 and VP7 genes [23, 24]. The G- and P- types were determined by partial sequencing with the same primers used for PCR. Using RotaC online classification tool [25], the genotype of ma31 was determined as G8P[14]. Details about kits, protocols and instruments used to characterize this strain are provided in the following sections. Both partial and full-genome sequencing were performed at the National Center for Scientific and Technical Research.

Full-length genome sequencing of ma31 strain

Rotavirus isolation and nucleic acid extraction

As this genotype was detected for the first time in Morocco, further studies including the complete genome sequencing of this strain was performed; thus its isolation was necessary to avoid losing the sample due to stool exhaustion. The isolation was achieved following one passage on rhesus monkey kidney cells (MA104) as described by Ennima et al. [26]. The QIAamp Viral RNA Mini Kit (Qiagen, Inc., Valencia, CA) was used to extract total viral RNA from 140 µl of infected cell culture fluid according to the manufacturer's protocol.

Reverse transcription and whole-genome amplification (RT-PCR)

The extracted RNA was denatured at 97 °C for 5 min and quickly chilled on ice for at least 2 min in the presence of Random Hexamer primers mix, 0,5 mM dNTP mix and H_2O

in a final volume of 20 µl. cDNA was synthetized using Tetro cDNA Synthesis kit (Bioline, London, UK) by adding the reverse transcription reaction mix which contains 10U/µl RNase inhibitor and 20U/µl reverse transcriptase to make up a final volume of 26 µl under the following conditions: 25 °C for 10 min, 45 °C for 60 min and 85 °C for 5 min.

Each rotavirus gene segment (11 segments) was amplified by PCR with newly designed primers in addition to specific primers (Supplementary Table 1) using MyFi Mix (Bioline, London, UK) according to manufacturer's instruction. Briefly, 2,5 µl of cDNA, 0,8 µM of specific primers for each gene and 8 µl of MyFi Mix were added to MiliQ water to make up a final volume of 25 µl. The PCR consisted on an activation step at 95 °C for 2 min and 35 cycles of amplification in a Veriti Thermal Cycler (Life technologies, Inc. Foster City, CA) using the following conditions: 1 min at 95 °C, 1 min at 60 °C and 1 min at 72 °C, with a final extension at 72 °C for 8 min. All PCR products were separated on 1.5% agarose gels containing ethidium bromide (10 mg/ml) and visualized under UV transilluminator.

Nucleotide sequencing

PCR products were purified using ExoSAP-IT purification kit (Thermo Fisher Scientific, Waltham, MA) according to the manufacture's protocol. Sequencing was carried out using Big Dye Terminator Cycle Sequencing kit v3.1 (Life technologies, Inc. Foster City, CA) in both forward and reverse directions with the same primers used for PCR, in addition to primers used only for sequencing to recover the entire fragment. For some genes (VP1, VP3 and NSP1), design of new primers was necessary to complete the sequencing of the whole genome (Supplementary Table 2). The sequence data were collected from an ABI Prism 3130XL Genetic analyzer (Life technologies, Inc. Foster City, CA). The sequences were edited and assembled using DNA Dragon Sequence Assembler version 1.6.0 (Sequentix-Digital DNA Processing, Germany).

The genotypes of each of the 11 gene segments were determined using the RotaC v2.0 automated genotyping tool for group A rotaviruses (http://rotac.regatools.be).

Phylogenetic analysis

Multiple sequence alignments were carried out using the MUSCLE program within the MEGA 6 software [27]. Maximum likelihood phylogenetic trees were constructed using models of nucleotide substitutions based on the lowest Bayesian Information Criterion (BIC) scores [28]: GTR + G+I (NSP1, VP1, VP2, VP3 and VP4), T92 + G (NSP2, NSP3, NSP4 and VP7) and T92 + G+I (NSP5 and VP6) in the MEGA 6 software. The trees were constructed using bootstrap resampling analysis at 1000 replicates. Amino acid alignments for VP7, VP4, and VP6 genes were performed using MEGA 6 software.

The nucleotide and deduced amino acid sequences of all genome segments of RVA/Human-tc/MAR/ma31/2011/G8P[14] strain were deposited in the National Centre of Biotechnology Information (NCBI) database, GenBank, under the accession numbers from MG214332 (NSP1) to MG214342 (VP7).

Results

This RVA isolate was detected in a stool sample collected from an unvaccinated 18-month- old boy. Partial genotyping recovered the RVA isolate as G8P[14]. The full-genome nucleotide sequence obtained was submitted to RotaC database, it results in assignment of G8-P[14]-I2-R2-C2-M2-A11-N2-T6-E2-H3 constellation. Four of the structural genes (VP1, VP2, VP3 and VP6) and two of the non-structural genes (NSP2, NSP4) were DS-1- like, NSP5 was AU-1- like, NSP1 was the A11 genotype and NSP3 the T6 genotype.

Constellation comparison

The human ma31 strain presents the same genotype constellation with the bovine-like human strains from Italy (BA01, BA02) and Hungary (BP1062, 182-02). It shares also the same constellation with human G6 RVAs such as MG6 (Australia), EGY3399 (Egypt), N-1 (India), Hun5 and BP1879 (Hungary) and with G10 rotaviruses from Italy and Australia (V585 et PR457, respectively) (Table 1).

The constellation of the ovine strain OVR 762 from Spain is completely identical to the human ma31 constellation strain with eleven shared genotypes while some strains from animal origin share ten genotypes with ma31 such as the antelope strain RC 18 from South Africa. Other animal strains such as the caprine strains (GO34, 0040), the bovine strain 1604, and the macaque strain PTRV present only nine shared genotypes. Two animal strains from Argentina with the G8P[14] genotype have the minimum number of shared genotypes with the human strain ma31, eight genotypes for the strain Chubut and seven genotypes for the strain 75 (Table 1). According to the comparison of the constellation of ma31 with animal strains having the same constellation, the full-length sequence has bovine backbone indicating that it is probably of ovine origin (Table 1).

Phylogenetic analysis

In total, 11 phylogenetic trees were built using the full-length sequence of the 11 genes of ma31 (Fig. 1a–k). Nucleotide

Table 1 Comparison of complete genomic constellations between the human strain RVA/Human-tc/MAR/ma31/2011/G8P[14], selected animal RVAs and human bovine-like RVAs

Strain names	Host	Country	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5	Shared genotypes
RVA/Human-tc/MAR/ma31/2011/G8P[14]	Human	Morocco	G8	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	
RVA/Human-wt/ITA/BA01/2012/G8P[14]	Human	Italy	G8	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	11
RVA/Human-wt/ITA/BA02/2012/G8P[14]	Human	Italy	G8	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	11
RVA/Human-wt/HUN/BP1062/2004/G8P[14]	Human	Hungary	G8	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	11
RVA/Human-wt/HUN/182-02/2001/G8P[14]	Human	Hungary	G8	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	11
RVA/Sheep-tc/ESP/OVR762/2002/G8P[14]	Sheep	Spain	G8	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	11
RVA/Human-wt/ITA/PR1300/2004/G8P[14]	Human	Italy	G8	P[14]	I2	R2	C2	M2	A3	N2	T6	E2	H3	10
RVA/Human-wt/ITA/PR1973/2009/G8P[14]	Human	Italy	G8	P[14]	I2	R2	C2	M2	A3	N2	T6	E2	H3	10
RVA/Human-wt/GTM/2009726790/2009/G8P[14]	Human	Guatemala	G8	P[14]	I2	R2	C2	M2	A13	N2	T6	E2	H3	10
RVA/Human-tc/AUS/MG6/1993/G6P[14]	Human	Australia	G6	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	10
RVA/Human-wt/IND/N-1/2009/G6P[14]	Human	India	G6	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	10
RVA/Human-wt/EGY/EGY3399/2004/G6P[14]	Human	Egypt	G6	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	10
RVA/Human-wt/HUN/Hun5/1997/G6P[14]	Human	Hungary	G6	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	10
RVA/Human-wt/HUN/BP1879/2003/G6P[14]	Human	Hungary	G6	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	10
RVA/Antelope-wt/ZAF/RC-18/2008/G6P[14]	Antelope	South Africa	G6	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	10
RVA/Human-wt/AUS/V585/2011/G10P[14]	Human	Australia	G10	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	10
RVA/Human-wt/ITA/PR457/2009/G10P[14]	Human	Italy	G10	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	10
RVA/Human-wt/USA/2012841174/2012/G8P[14]	Human	United States	G8	P[14]	I2	R3	C2	M2	A3	N2	T6	E2	H3	9
RVA/Human-tc/KEN/B12/1987/G8P[1]	Human	Kenya	G8	P[1]	I2	R2	C2	M2	A3	N2	T6	E2	H3	9
RVA/Cow-wt/ZAF/1604/2007	Bovine	South Africa	G8	P[1]	I2	R2	C2	M2	A3	N2	T6	E2	H3	9
RVA/Macaque-tc/USA/PTRV/1990/G8P[1]	Macaque	United States	G8	P[1]	I2	R2	C2	M2	A3	N2	T6	E2	H3	9
RVA/Human-tc/ITA/PA169/1988/G6P[14]	Human	Italy	G6	P[14]	I2	R2	C2	M2	A3	N2	T6	E2	H3	9
RVA/Human-wt/BEL/B10925/1997/G6P[14]	Human	Belgium	G6	P[14]	I2	R2	C2	M2	A3	N2	T6	E2	H3	9
RVA/Human-wt/THA/SKT-27/2012/G6P[14]	Human	Thailand	G6	P[14]	I2	R2	C2	M2	A3	N2	T6	E2	H3	9
RVA/Human-wt/ITA/111-05-27/2005/G6P[14]	Human	Italy	G6	P[14]	I2	R2	C2	M2	A3	N2	T6	E2	H3	9
RVA/Goat-tc/BGD/GO34/1999/G6P[1]	Goat	Bangladesh	G6	P[1]	I2	R2	C2	M2	A11	N2	T6	E2	H3	9
RVA/Human-tc/GBR/A64/1987/G10P[14]	Human	England	G10	P[14]	I2	R2	C2	M1	A3	N2	T6	E2	H3	9
RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14]	Guanaco	Argentina	G8	P[14]	I2	R5	C2	M2	A3	N2	T6	E12	H3	8
RVA/Vicugna-wt/ARG/75/2010/G8P[14]	Vicugna	Argentina	G8	P[14]	I2	R5	C2	M2	[a]AX	N2	T6	E12	[a]HX	7
RVA/Goat-wt/ARG/0040/2011/G8P[1]	Goat	Argentina	G8	P[1]	I2	R5	C2	M2	A3	N2	T6	E12	H3	6

[a]Undetermined genotypes are indicated with an "X"

identities of the genes compared to the closest related strains present in GenBank were determined (Table 2).

Phylogenetic analysis of ma31 strain showed that VP7, NSP2, NSP3 and NSP4 genes clustered with animal RVA strains while the rest of the genes were closely related to human strains (Table 2 and Fig. 1a–k).

The analysis of the VP7 tree (Fig. 1a) revealed a very close clustering (99% nucleotide identity) of the ma31 strain with the sheep G8P[14] strain detected in Spain in 2002 (sheep-tc/ESP/OVR762/2002/G8P[14]), within a cluster (lineage II) which includes human, goat, roe deer and guanaco strains.

The VP4 tree showed that ma31 was related to P[14] sequences detected in humans, guanaco, antelope and sheep between 1988 and 2012. Human strains included in the tree had mostly G6, G8 and G10 VP7 genotypes (Fig. 1b).

For the ma31 core protein genes, VP1 clustered with human P[14] strains such as ITA/PAI11, ITA/PR1973, EGY/AS970 and HUN/Hun5 (91.3–94.4%) (Fig. 1c), while the VP2 gene phylogeny showed intermediate evolutionary relationships with human, antelope and cow P[14] strains (Human-wt/HUN/182-02, Human-wt/ITA/PAH136, Antelope-wt/ZAF/RC-18-08 and Cow-wt/ZAF/1603) ranging between 94.3–96.1% (Fig. 1d). The VP3 gene phylogeny revealed the same phenomenon as VP2 with an intermediate evolutionary relationships (84.6% to 95.9% at the nucleotide level) with humans, sheep and alpaca P[14] strains (Human-wt/ITA/PR1973, Human-wt/BEL/B10925, Sheep-tc/ESP/OVR762 and alpaca-wt/PER/562) (Fig. 1e). The last gene VP6 clustered closely with the human strain ZAF/2371WC detected in South Africa in 2008 (98.5% nt identity) (Fig. 1f) which was described to be of artiodactyl origin [29].

Concerning non-structural genes, NSP1 gene clustered with human P[14] strains such as HUN/BP1879/2003/G6P[14], Italy/BA02/2012/G8P[14] and Italy/BA01/2012/G8P[14] with 92.5%, 84.1%, 83.9% of nucleotide identity, respectively (Fig. 1g). NSP2 tree showed a close relationship between ma31 strain and the dog 88977 strain detected in Germany in 2013 (98.5% nt identity) (Fig. 1h). For NSP3 and NSP4 genes, their sequences clustered very well with the same sheep strain (ESP/OVR762/2002/G8P[14]) presenting, respectively, 99% and 97.6% of nucleotide identity (Fig. 1i–j). For the latest tree of NSP5 gene, the ma31 strain matched closely with the human strain HUN/182-02/2002/G8P[14] (99.1% nt identity) (Fig. 1k).

VP8*, VP7 and VP6 hypervariable regions

The deduced VP4 (VP8*), VP7 and VP6 amino acid sequences of ma31 strain were compared with selected relevant strains present in the corresponding trees and with sequences of Rotarix™ and RotaTeq™ vaccine strains.

The comparison of VP4 amino acid sequences was carried out for antigenic regions 8–1, 8-2, 8-3, and 8-4 (Fig. 2).

Analysis of the VP8* alignment showed no amino acid substitution in the antigenic region 8-1 (Fig. 2) in contrast five amino acid substitutions were found between ma31 strain and Guatemalan strain 2009726790/2009 (G8P[14]) at residues T180I (8-2), S113Q, T125A and N133D (8-3) and I89A (8-4). One amino acid change differentiated the ma31 strain from all related strains included in the comparison at residue N133D (8-3) with the exception of Belgian B10925/1997/G6P[14] and Australian WAG8.1/2002/G8P[14] strains. In addition, one amino acid substitution was found at residue N183D (8-2) with the Belgium strain B10925/1997/G6P[14] (Fig. 2).

Comparison of the Moroccan G8P[14] strain with the VP8* antigenic regions (8-1, 8-2, 8-3 and 8-4) of the P[8] vaccine strains Rotarix™ and RotaTeq™ showed 20 and 22 amino acid substitutions, respectively (Fig. 2).

The amino acid sequences in the VP7 antigenic regions (7-1a, 7-1b and 7-2) of the ma31 G8P[14] strain showed a single change compared to animal and human strains Vicuna-wt/ARG/C75/2010/G8P[14], Cow-wt/JPN/Sun9/2000/G8P[14], Goat-wt/ARG/0040/2011/G8P[1] and Human-tc/KEN/B12/1987/G8P[1] at residue T87 V (7-1a). Additionally, two amino acid substitutions were found in comparison with Guanaco-wt/ARG/Chubut/1999/G8P[14] strain at residues T87 V (7-1a), N238S (7-1b), with Dog-wt/GER/88977/2013/G8P[1] strain at T87 V, T91 K (7-1a) and with human strain GTM/2009726790/2009/G8P[14] at T87 V (7-1a), N147S (7-2). Another two amino acid substitutions were observed between ma31 strain and animal strain roe deer-wt/SLO/D110-05/2015/G8P[14] at residues T87I and N147S (7-1a and 7-2, respectively) (Fig. 3).

Comparison with the VP7 antigenic regions of vaccine strains showed that 14 residues from 29 were conserved between the ma31 strain and the G1 Rotarix™ strain, and from 10 to 18 residues compared with the five VP7 RotaTeq™ strains (Fig. 3).

The analysis of the four antigenic sites of VP6 (I-IV) demonstrated a high degree of conservation between ma31 strain and most of the human and animal strains analyzed. Only two amino acid substitutions were observed in antigenic site I; at residue V56I from ZAF/RC-18-08/G6P[14] to GTM/2009726790/G8P[14] and at residue D62A with SEN/MRC-DPRU2053/G8P[6]. In the antigenic site III, one amino acid substitution was reported at residue I211 V with two strains ZAF/MRC-DPRU3010 and GTM/2009726790 (Fig. 4).

The comparison of the VP6 antigenic regions with the vaccine strains showed four amino acid substitutions between ma31 strain and the Rotarix™ strain (I and IV), and only one substitution with the RoTateq™ strains at residue I211 V (III) (Fig. 4).

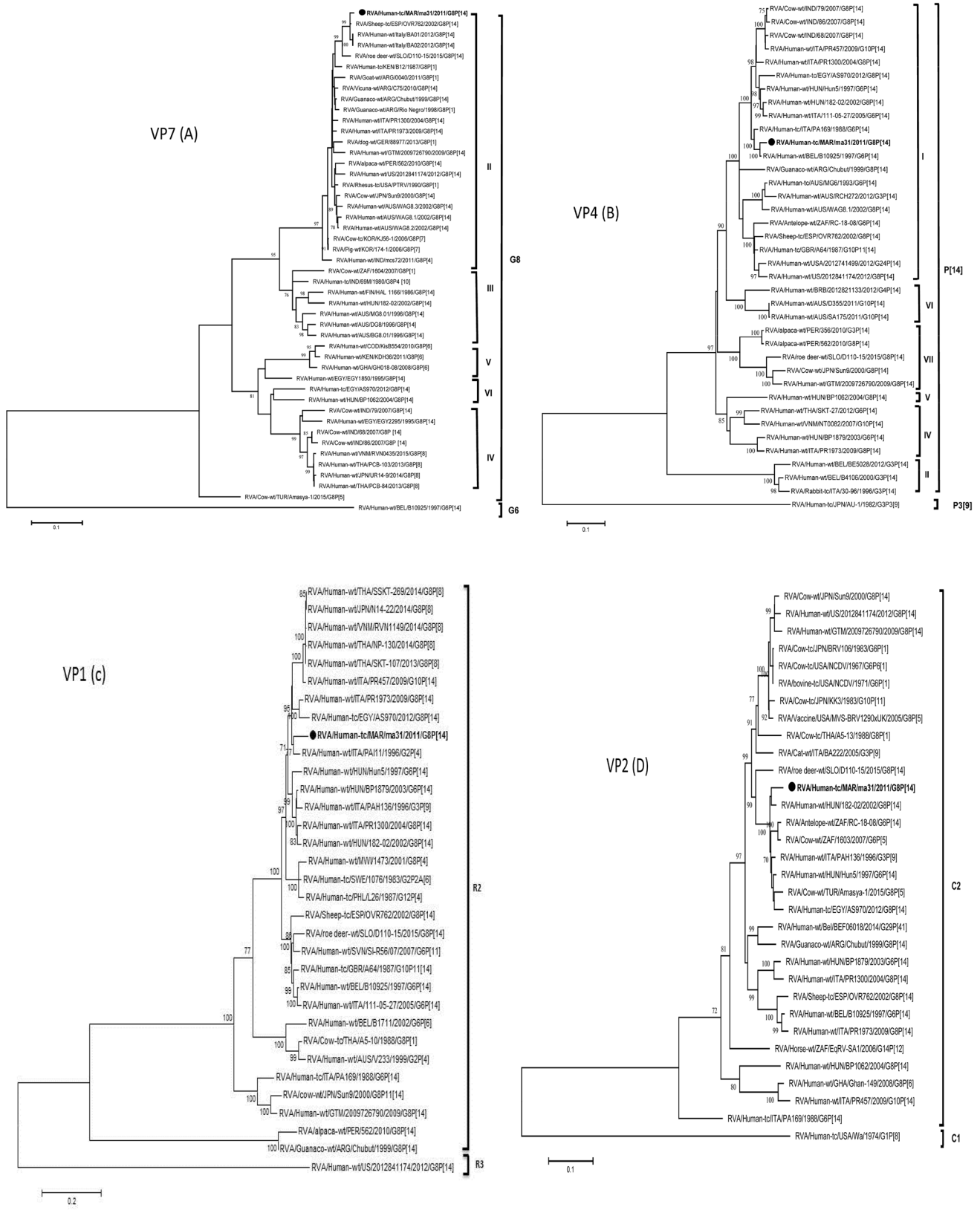

Fig. 1 Phylogenetic trees based on full nucleotide sequences of all 11 RVA gene segments. ma31 strain is indicated in bold. Maximum likelihood method was used, with bootstrap at 1000 repetitions. Scale bars represent the number of nucleotide substitution per site

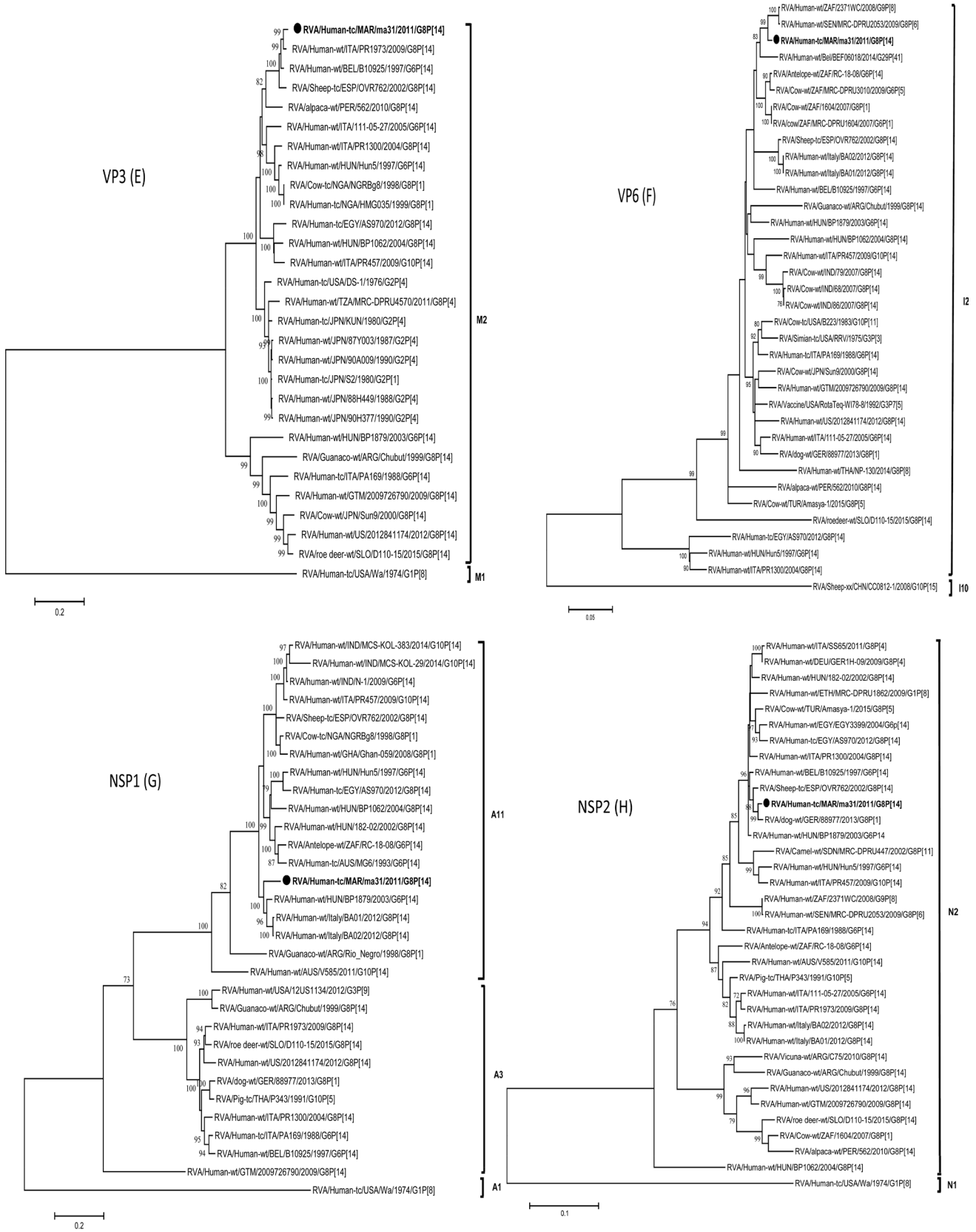

Fig. 1 (continued)

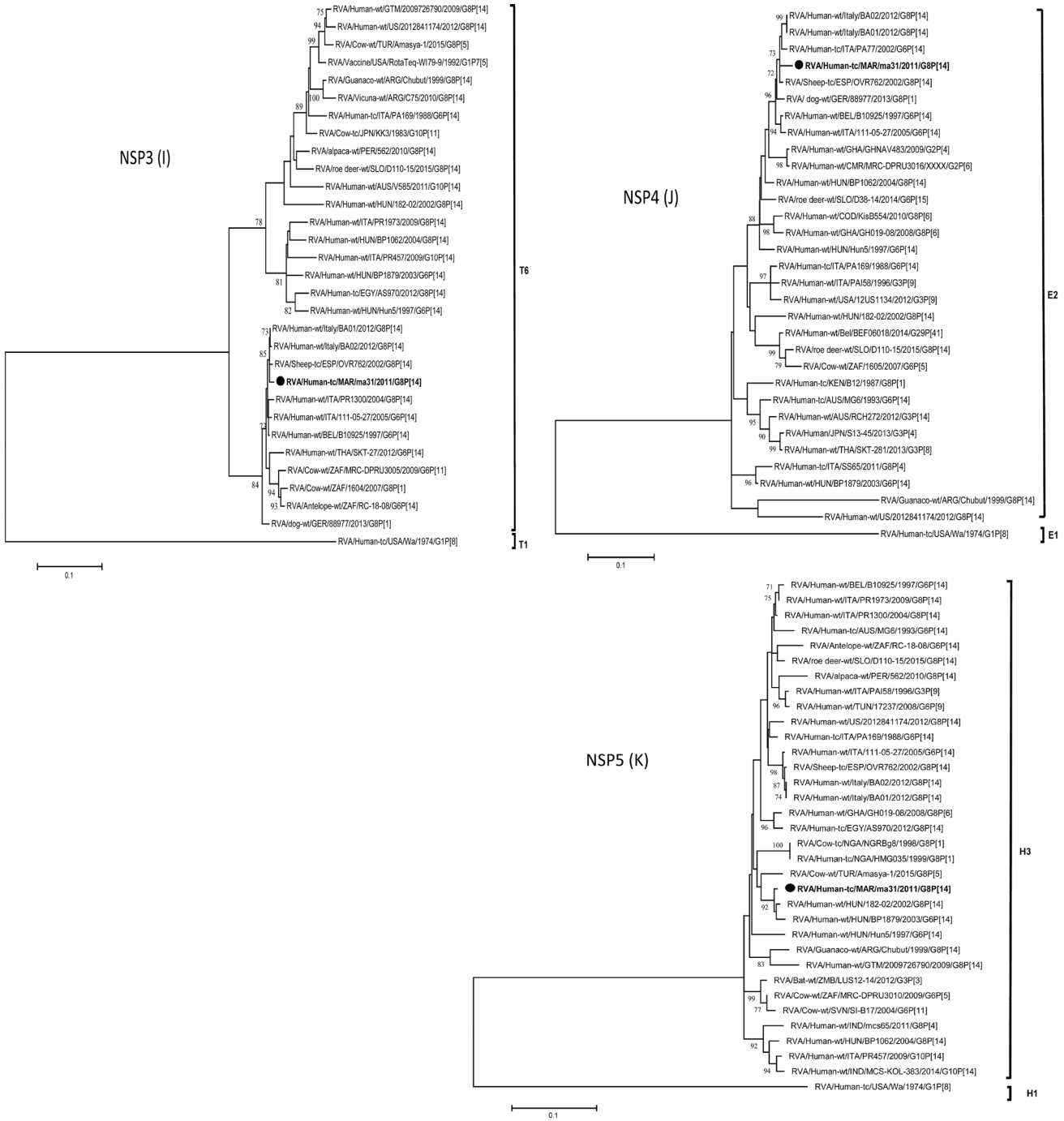

Fig. 1 (continued)

Discussion

The rare genotype G8P[14] is reported for the first time in Morocco and appeared in natural conditions. It represents to our knowledge, the second case reported in Africa after Egypt [30]. Genotypes circulating in Morocco even after a successive introduction of Rotarix™ and RoTateq™ vaccines into the national immunization programs in 2010 and 2014, respectively, are G1P[8], G9P[8], G2P[4], G4P[8] and G3P[8] [30–32]. The limited cases reported worldwide (Italy, Denmark, Finland, Hungary, United states, Guatemala, Taiwan, Australia,) [33, 34] suggest that this rare genotype of rotaviruses is not fully adapted to humans which explains its limited spread. To further investigate the genetic relationship of this strain with other G8P[14] strains, it was necessary to characterize its full genome.

Table 2 Nucleotide sequence identities of RVA/Human-tc/MAR/ma31/2011/G8P[14] strain to closely related strains

Gene	% identity to closest related strain	Closely related strain	GenBank accession no.	RotaC classification
NSP1	92.5	RVA/Human-wt/HUN/BP1879/2003/G6P[14]	FN665681	A11
NSP2	98.5	RVA/Dog-wt/GER/88977/2013/G8P[1]	KJ940158	N2
NSP3	99	RVA/Sheep-tc/ESP/OVR762/2002/G8P[14]	EF554156	T6
NSP4	97.6	RVA/Sheep-tc//ESP/OVR762/2002/G8P[14]	EF554157	E2
NSP5	99.1	RVA/Human-wt/HUN/182-02/2002/G8P[14]	KU508379	H3
VP1	94.4	RVA/Human-wt/ITA/PAI11/1996/G2P[4]	KC178764	R2
VP2	96.1	RVA/Human-wt HUN/182-02/2002/G8P[14]	KU508381	C2
VP3	95.9	RVA/Human-wt/BEL/B10925/1997/G6P[14]	EF554117	M2
VP4	98.1	RVA/Human-wt/BEL/B10925/1997/G6P[14]	EF554118	P[14]
VP6	98.5	RVA/Human-wt/ZAF/2371WC/2008/G9P[8]	JN014002	I2
VP7	99	RVA/Sheep-tc/ESP/OVR762/2002/G8P[14]	EF554153	G8

	8-1											8-2		8-3									8-4		
	100	146	148	150	188	190	192	193	194	195	196	180	183	113	114	115	116	125	131	132	133	135	87	88	89
Human-tc/MAR/ma31/2011/G8P[14]	D	L	K	G	Y	L	I	N	N	D	N	T	N	S	N	T	Q	T	S	N	N	S	T	Q	I
Human-wt/BEL/B10925/1997/G6P[14]	.	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.	.	.	.	.	.	.	.	.
Sheep-tc/ESP/OVR762/2002/G8P]14]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.
Human-wt/HUN/182-02/2002/G8P[14]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.
Human-wt/EGY/AS970/2012/G8P[14]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.
Human-wt/ITA/PR1300/2004/G8P[14]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.
Human-wt/GTM/2009726790/2009/G8P[14]	.	.	.	.	.	.	.	.	.	.	.	I	.	Q	.	.	.	A	.	.	D	.	.	.	A
Human-wt/US/2012841174/2012/G8P[14]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.
Cow-wt/IND/79/2007/ G8P[14]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.
Human-wt/AUS/WAG8.1/2002/ G8P[14]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Rotarix/USA/2009/G1P[8]	.	S	S	N	S	S	A	.	L	N	.	E	R	N	P	V	D	S	.	.	D	N	N	T	N
RotaTeq-WI79-4/USA/1992/G6P1A[8]	.	S	S	N	S	N	A	.	L	N	D	E	R	N	P	V	D	N	R	.	D	D	N	T	N

Fig. 2 Alignment of antigenic residues in the four VP8* (VP4) antigenic regions between the ma31 strain, the vaccine strains Rotarix™ and RotaTeq™, and relevant strains selected from the VP4 tree

	7-1a														7-1b						7-2								
	87	91	94	96	97	98	99	100	104	123	125	129	130	291	201	211	212	213	238	242	143	145	146	147	148	190	217	221	264
Human-tc/MAR/ma31/2011/G8P[14]	T	T	A	S	S	W	K	D	Q	D	A	I	N	K	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
Sheep-tc/ESP/OVR762/2002/G8P[14]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Human-wt/Italy/BA01/2012/G8P[14]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Human-wt/Italy/BA02/2012/G8P[14]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Roe deer-wt/SLO/D110-15/2015/G8P[14]	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.
Vicuna-wt/ARG/C75/2010/G8P[14]	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Guanaco-wt/ARG/Chubut/1999/G8P[14]	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.	.	.	.	.
Human-wt/GTM/2009726790/2009/G8P[14]	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.
Cow-wt/JPN/Sun9/2000/G8P[14]	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Dog-wt/GER/88977/2013/G8P[1]	V	K	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Human-tc/KEN/B12/1987/G8P[1]	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Goat-wt/ARG/0040/2011/G8P[1]	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Rotarix/USA/2009/G1P[8]	.	.	N	G	E	.	.	.	.	S	V	V	D	.	.	N	V	D	.	.	.	D	Q	.	L	.	M	N	.
RotaTeq-SC2-9/USA/1992/G2P7[5]	A	N	S	D	E	.	E	N	.	.	T	M	.	.	.	.	V	S	.	S	R	D	N	T	.	D	I	S	.
RotaTeq-WI78-8/USA/1992/G3P7[5]	.	.	N	N	.	.	.	.	.	.	.	V	D	.	.	.	A	N	K	D	.	D	.	T	L	.	.	.	.
RotaTeq-BrB-9/USA/1996/G4P7[5]	S	.	S	T	E	.	.	.	N	L	.	D	.	.	.	.	.	A	D	.	R	A	S	G	E	.	T	S	.
RotaTeq-WI79-4/USA/1992/G6P1A[8]	V	N	.	T	E	.	.	.	.	.	.	V	E	.	.	N	P	D	.	A	.	D	S	T	Q	.	T	T	.

Fig. 3 Alignment of antigenic residues in the three VP7 antigenic regions between ma31 strain, the vaccine strains Rotarix™ and RotaTeq™, and relevant strains selected from the VP7 tree

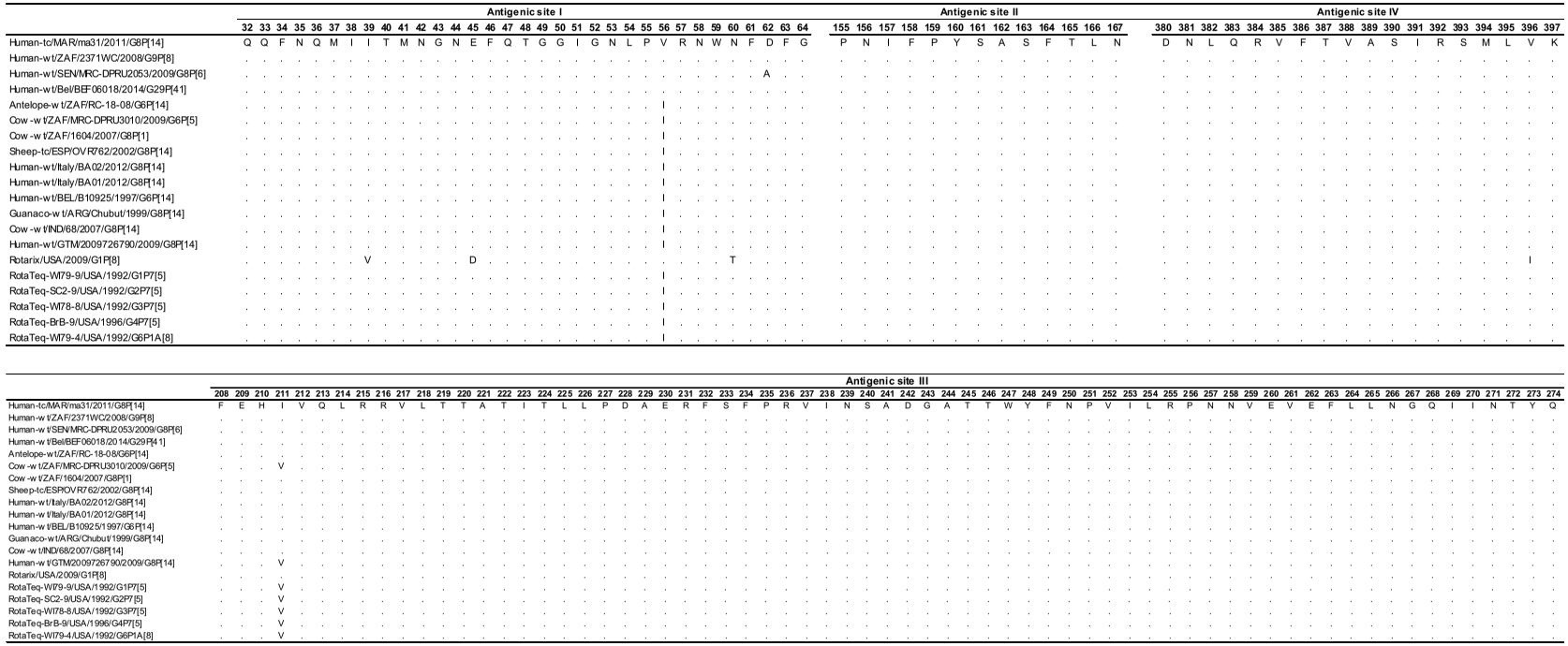

Fig. 4 Alignment of antigenic residues in the four VP6 antigenic regions between ma31 strain, the vaccine strains Rotarix™ and RotaTeq™, and relevant strains selected from the VP6 tree

The constellation results demonstrated that this strain belongs to DS-1-like genogroup 2 for most genes except for H3 genotype which is presented normally among AU-1-like rotaviruses (genogroup 3) and for A11 and T6 genotypes, which are characteristic of artiodactyl bovine-like rotavirus strains [35]. Consequently, the human ma31 strain represent interspecies transmissions from RVA strains belonging to members of the *Artiodactyla* family such as cattle, guanaco and sheep like it was previously reported by Matthijnssens et al. [36]. The full length genome analysis of ma31 strain showed a mixed configuration of genes of possible animal origin and zoonotic human origin which indicates the generation of reassortment between human and animal rotaviruses revealing this unusual human strain (Fig. 1a–k). Four out of eleven genes (VP7, NSP2, NSP3, NSP4) of the strain RVA/Human-tc/MAR/ma31/2011/G8P[14] presented a very close identity with the respective genes of Dog-wt/GER/88977/2013/G8P[1] and Sheep-tc/ESP/OVR762/2002/G8P[14] strains while the remaining genes (VP1, VP2, VP3, VP4, VP6, NSP1, NSP5) were more closer to human rotavirus strains such as HUN/182-02/2002/G8P[14], BEL/B10925/1997/G6P[14] and ZAF/2371WC/2008/G9P[8] detected several years before in Hungary, Belgium and South Africa respectively (Table 2).

Concerning human P[14] strains, previous studies proposed their common origin with strains affecting ungulates belonging to the order Artiodactyla [36] and within the P[14] genotype, the Moroccan strain occupies lineage I shared with human P[14] strains from Italy, Belgium and Hungary as well as animal RVA P[14] strains (bovine, Antelope and sheep) from India, South Africa and Spain. This genotype was reported in neonate calves from different Moroccan geographical regions in combination with G10 genotype [26] suggesting a possible origin of infection from a bovine species. Moreover, the susceptibility of the child to the heterologous genotype of rotavirus P[14] might be related to the phenotype of the ABO blood group. Indeed, the VP8* fragment of the P[14] VP4 protein for example was demonstrated to interact with type A histo-blood group antigens of humans and only people carrying this kind of antigens are likely to be infected with these genotypes [37, 38].

The animal G8 rotavirus genotype is known to be present in bovine species but circulating with a lower frequency compared to the G6 and G10 genotypes, however this genotype represented the dominant strain identified in Japan [39] and Tunisia [40]. In humans, G8 rotavirus has been detected sporadically throughout the world in combination with different P-types such as P[4], P[6] and P[8] [21, 41]. In contrast, this rare genotype presented a prevalence of 12% in some African countries compared to other genotypes detected in that continent [16] combined with other rotavirus genes from animal origin [18, 41, 42]. In Morocco, human G8 genotype has been reported among circulating strains for the first time in 2015 in Rabat as G8P[9] isolated strain [43]. Within the G8 genotype, the literature described the presence of 6 lineages identified to date [21]. The VP7 ma31 gene belongs to lineage II which contains mostly sheep, goat, porcine, bovine and artiodactyl-like human strains. This lineage was demonstrated to be of animal origin [41].

People living nearby intensive livestock farms especially close to small ruminants are at increased risk exposure to animal rotaviruses which may lead to a possible co-infection and generation of reassortants between human and animal rotaviruses. The infected case reported in the present study lives in a rural area near Rabat in close contact with sheep, goats, bovine and dogs. This may explain in part the ma31 genomic rare constellation as described in other studies [42]. However, the fact that this rare G8P[14] genotype has been detected in a single patient among all the samples collected during the survey, may suggest that this genotype or reassortant strain is not yet well adapted to spread effectively among humans.

Considering that the two most used vaccines Rotarix™ and RotaTeq™ have shown great efficacy against the five most widespread G genotypes (G1, G3, G4, G9, and G12) in the world and a large cross-protection against serotypes not present in the vaccine, the efficacy data against genotypes of non-human origins are still limited [44]. A recent study carried out on G8 strains in sub-Saharan Africa showed that RotaTeq™ has good efficacy against this serotype, this may be due to the composition of the vaccine which carries a bovine genetic background [45].

Comparison of the major antigenic regions (8-1, 8-2, 8-3, 8-4, 7-1a, 7-1b, 7-2) of the VP8* and VP7 amino acid sequences [46] between ma31 strain and some relevant animal and human P[14] and/or G8 strains analyzed in various parts of the word showed several differences in some residues positions. Even if these strains present a genetic variability, they share the common genetic background that appears to be modified by independent interspecies transmission events (Figs. 2, 3 and 4).

Unsurprisingly, the analysis of VP7 antigenic region of ma31 strain revealed 15 differences out of 29 amino acids contained in the major antigenic sites related to the neutralization of the G1 Rotarix™ strain, as well as 11 to 19 differences with the four RotaTeq™ strains (G1–G4) (Fig. 3). Similar results were observed for the antigenic regions of VP8*, where the ma31 strain antigenic sites differed significantly from those of vaccine strains (P[8]) with 20 and 22 different amino acids out of 29 compared to Rotarix™ and RotaTeq™ strains, respectively (Fig. 2). However, these differences noted in the VP7 and VP8* of the ma31 strain do not provide a clear conclusion as to whether or not the effectiveness of the immunity induced by the Rotarix™ and RotaTeq™ vaccines in protection against infections with rotavirus strains of zoonotic origin will be reduced.

In contrast, the analysis of the VP6 amino acid sequences of the four major antigenic sites [47] showed a high degree of conservation between ma31 strain, animal and human strains included in the analysis, while it represented 1 and 4 differences with the RotaTeq™ and Rotarix™ vaccine strains, respectively (Fig. 4).

The mechanisms of protection against rotavirus infection are still unclear until now and it seems that other genes in addition to VP4 and VP7 are involved [48] such as the VP6 gene which might play a role in vaccine-induced immunity [49]. Furthermore, multiple factors may influence the effectiveness of both vaccines and the potential impact of amino acid changes in the antigenic regions cannot be assumed by sequence information alone [50, 51].

In conclusion, and since the VP6 amino acid sequences of the four major antigenic sites in ma31 strain showed a high degree of conservation with RotaTeq™ and Rotarix™ vaccine strains, this would probably initiate an effective immune response against G8P[14] strain. Unfortunately, in Morocco, there are no RVA strain surveillance strategies to predict the possible introduction of new strains resulting from interspecies transmission into humans.

In summary, this study present the first report of G8P[14] RVA strain isolated in Morocco, Africa with a genomic constellation G8-P[14]-I2-R2-C2-M2-A11-N2-T6-E2-H3. This strain belongs to lineage I within the P[14] genotype. The results of this study confirm the importance of interspecies transmission which plays a role in the generation of a large diversity of human rotaviruses through zoonotic reassortements. Further studies are, therefore, warranted to monitor at the full-genome level if such emerging strains will spread globally to ensure the successful use of vaccines and to explain the vaccine failure if it occurs.

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Author contributions NT and EE conceived and designed the study. SA and MM carried out the experiments. SA, MM and ME performed the data analysis. SA wrote the draft. NT, EE, MM and ME reviewed the manuscript. All the authors read the final version of the manuscript and approved it for publication.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Ethical approval for this study was obtained from the Biomedical Research Ethics Committee of the Faculty of Medicine and Pharmacy of Rabat, Mohamed V University, Morocco following the guidelines set by the Declaration of Helsinki.

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