Research Article

Characterization of Unusual Human Group A Rotavirus VP4 Genotype Detected in Moroccan Children Fully Vaccinated with Rotarix™

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Abstract | Here we report the molecular characterization, antigenic disparities and the phylogenetic analysis of unusual human rotavirus VP4 genotype detected in Moroccan children fully vaccinated with Rotarix™. After RNA virus extraction, the rotavirus VP7 and VP4 genes were amplified. The DNA was purified, sequenced and genotypes were determined using the RotaC online classification tool. A phylogenetic tree was constructed using the Maximum Likelihood method applying the Tamura 3-parameter model in MEGA 6.06 package and statistically supported by bootstrapping with 1000 replicates. The amino acid sequences of the antigenic regions of the outer capsid proteins VP8* were compared with vaccine and field strains. Here we show that the VP4 gene of the P[14] strains detected in this study exhibited close identity with zoonotic Moroccan goat and bovine strains. The amino acid sequences of the antigenic regions inside VP8* proteins showed high conservation between the amino acid sequences of human and animal strains bearing P[14] proteins, and revealed several changes with respect to the RotaTeq™ and Rotarix™ vaccine strains. This report suggests a possible reassortment event between human and domestic animal rotaviruses. Continuous surveillance in the post vaccine era appears to be necessary in order to identify unusual rotavirus strains that might not be fully contrasted by current vaccines.

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Introduction

Group A rotaviruses (RVA) are the leading cause of severe gastroenteritis in children worldwide (Parashar et al., 2006). The RVA genome contains 11 double-stranded RNA segments that encode six structural (VP1–4 and VP6–7) and five or six non-structural (NSP1–5/6) proteins (Estes and Kapikian, 2007). The traditional binomial RVA classification system was based on the two outer capsid proteins, VP7; a glycoprotein, which defines the G serotype/genotype, and VP4; a protease-sensitive, which defines the P serotype and distincts the P genotype. At least 35 G-types and

50 P-types have been identified (Matthijnssens et al., 2011; Trojnar et al., 2013; Sadiq et al., 2018). Epidemiologically, five common G types (G1–G4 and G9), and three common P types (P[4], P[6] and P[8]) were the most important human rotavirus genotypes worldwide (Gentsch et al., 2005; Banyai et al., 2012; Santos and Hoshino, 2005). In Addition, unusual genotypes (Gentsch et al., 2005; Banyai et al., 2012; Santos and Hoshino, 2005; Matthijnssens et al., 2009; Esona et al., 2009; Mullick et al., 2013), untypable in either G or P genes, and mixed infections were also identified in lower cases of human infections. Many of these rare genotypes are supposed to have originated from animal rotaviruses that were introduced into the human populations through gene reassortments and/or interspecies transmission (Estes and Kapikian, 2007; Gentsch et al., 2005; Mullick et al., 2013; Matthijnssens et al., 2008). Genetic reassortment is common due to the segmented nature of the RVA genome and the two events contribute significantly to RVA genetic diversity (Estes and Kapikian, 2007).

A number of studies have been conducted to identify natural infection. Available data indicates that the immunity induced by natural rotavirus infection can efficiently prevent subsequent severe rotavirus illness (Clarke and Desselberger, 2015). Based on this fact, intensive efforts have been made to develop live attenuated rotavirus vaccines. Currently, two live attenuated orally administered RV vaccines, Rotarix® (RV-1) (Rixensart, Belgium) and RotaTeq® (RV-5) (Blue Bell, Pennsylvania), have been licensed since 2006 (Angel et al., 2007). The two vaccines have been demonstrated to be safe and effective in protecting against severe rotavirus diarrhea among children in middle- and high-income settings (Clarke and Desselberger, 2015). However, in some developing nations, with high rotavirus incidences, these vaccines were less effective (Clarke and Desselberger, 2015).

In Morocco, reported G and P RVA genotypes among children were G1-G4, G9, P[4], P[6], P[8] and P[9] (Benhafid et al., 2015; Benmessaoud et al., 2015; Boulahyaoui et al., 2019). Previously, we reported the detection and genetic characteristics of the G2P[4] and G9P[8] RVA strains isolated from children fully vaccinated with Rotarix™ (Boulahyaoui et al., 2019). In this study, we describe the molecular characterization, antigenic disparities and the phylogenetic analysis of P[14] rotavirus strains isolated from vaccinated children admitted to hospital with acute gastroenteritis (AGE) between 2013-2014. To our knowledge, this study is the first one to report the circulation of the animal-like RVA strain with P[14] genotype in a vaccinated Moroccan pediatric populations. These investigations will provide ongoing strain diversity information important for the evaluation of vaccine effectiveness.

Materials and Methods

Ethical approval

Consent to conduct this study was sought from the Biomedical Research Ethics Committee (Comité d'Ethique pour la Recherche Biomédicale) of the Faculté de Médecine et de Pharmacie, Mohamed V University of Rabat, Morocco, following the guidelines set by the Declaration of Helsinki. All parents or legal guardians were informed on the study details and their consents were obtained and documented in the questionnaire form before stool specimen collection.

Clinical samples

Seven stool specimens were collected from children less than one year old during a survey performed on infants hospitalized with AGE in two different cities (Oujda and Rabat) between March 2013 and June 2014. All samples were processed for rapid diagnosis by RotaCheck (VEDA LAB, France). RVA positive samples were conserved at the Centre de Virologie, des Maladies Infectieuses and Tropicales, Military Teaching Hospital Mohamed V at Rabat, Morocco. Vaccination status and analysis of the clinical records were conducted for all infants as summarized in Table 1.

Nucleic acid extraction

Total viral RNA of positive samples was extracted from 100 µl of 10% fecal suspension in phosphate buffered saline by using EZ1 DSP Virus Kit (Qiagen Hilden, Germany) on an automated EZ1 Advanced XL extraction system (Qiagen Hilden, Germany) according to the manufacturer's instructions.

Sequencing and genotype assignment

After viral RNA extraction, the cDNA was synthesized using the reverse transcriptase enzyme and random hexamer primers from Tetro cDNA Synthesis kit (Bioline, France) according to the kit protocol in the presence of 1 µg of total purified RNA in a final volume of 20 µl.

Table 1: *Clinical data from infants included in this study, their VP4 genotypes determined by partial sequencing and their accession numbers.*

Sample number	Birth date	Sex	Location	Age (month)	Vaccine status	Rota check	VP4 genotype	Accession numbers
MA166	03/03/2013	M	Oujda	9.5	Yes (Rotarix)	Positive	P[14]	KX 517802
MA167	09/06/2013	M	Oujda	6.5	Yes (Rotarix)	Positive	P[14]	KX 517803
MA176	29/03/2013	F	Rabat	11.5	Yes (Rotarix)	Positive	P[14]	KX 517804
MA178	03/09/2013	M	Rabat	7	Yes (Rotarix)	Positive	P[14]	KX 517805
MA197	01/04/2013	F	Oujda	9.5	Yes (Rotarix)	Positive	P[14]	KX 517806
MA198	15/06/2013	M	Oujda	7	Yes (Rotarix)	Positive	P[14]	KX 517807
MA235	30/09/2013	M	Rabat	10.5	Yes (Rotarix)	Positive	P[14]	KX 517808

PCR reactions were performed with synthesized cDNA (100 ng) using the MyFiTaq Master Mix 2X (Bioline, France), 500 nM of VP7F/VP7R (Iturriza-Gómara et al., 2004) or VP4F/VP4R (Gentsch et al., 1992) primers in a 25 µl reaction volume under the following conditions: preheating at 95°C for 2 min, then 35 cycles of (95°C, 1 min; 50°C, 1 min; 72°C, 1 min), followed by final extension of 10 min at 72°C employing a verity thermal cycler (Applied Biosystems).

The PCR amplicons were electrophoresed on a 1.5% Tris-acetate-ethylene diamine tetra acetic acid-agarose gel stained with 1% ethidium bromide.

Genotyping of RVA strains was performed by sequencing using the ABI 3130xl (Applied biosystems). Amplicons from RVA strains were purified using ExoSAP-IT (Affymetrix) prior to sequencing with BigDye Terminator v3.1 (Applied Biosystems) using the same set of primers as for amplification.

Phylogenetic analysis

The RVA genotypes were determined using the RotaC online classification tool. For phylogenetic analysis, nucleotide sequences of related strains for VP4 gene were retrieved from GenBank and aligned using the MUSCLE program within the MEGA version 6.06 software. Once aligned, a Maximum Likelihood tree was constructed using the MEGA version 6.06 software with the Tamura 3-parameter model (suggested as the best Model by MEGA software) and Gamma distribution. The statistical significance of the nodes was assessed by bootstrap resampling analysis (1000 replicates). Deduced amino acid sequences of antigenic region from VP4 protein of P[14] strains identified in this study were compared with the vaccines and P[14] field strains retrieved from GenBank database using the BioEdit software.

Sequences obtained in the current study were deposited in the GenBank database under the following accession numbers: KX 517802- KX517808.

Results and Discussion

To begin our investigation, we collected samples from children hospitalized with acute gastroenteritis. The samples were subjected to a rapid diagnosis using the RotaCheck test (VEDA LAB, France). Positive samples were identified (Table 1) and used for molecular characterization. The amplification of the VP4 gene was successful in all positive samples analyzed. An amplicon of 663 bp as expected was detected by gel electrophoresis and samples were named as MA166, MA167, MA176, MA178, MA197, MA198 and MA235, whereas no amplification was detected for the VP7 gene (data not shown). To assess the genotypes of the VP4 amplicons, PCR products were sequenced and identified using the RotaC online classification tool. VP4 gene contained in MA166, MA167, MA176, MA178, MA197, MA198 and MA235 samples were defined as RVA strains with P[14] genotype (Table 1). The genotyping of the VP7 was not successful, thus these strains were characterized as G untypable.

To determine the genetic relatedness between P[14] rotaviruses identified in this study and other animal and human P[14] rotaviruses retrieved from the GenBank database, a phylogenetic analysis was performed. The VP4 gene of our P[14] strains clustered in lineage I together with *Artiodactyl* and human P[14] strains (Figure 1). They regrouped with human RVA strains isolated in Italy (BA01/G8P[14], and PA169/G6P[14]), in Belgium (B10925/G6P[14]), in Hungary (hun5/G6P[14]), and in Australia (MG6/G6P[14]). They also grouped together with bovine

strains; Bas1:2S/G10P[14], Maz:6S/G10P[14] and Bas2:6S/G10P[14] (Morocco), 79/G8P[14] and 86/G8P[14] (India), whit goat strains; Ch_S31/G6P[14], Ch_S12/G6P[14], Ch_S61/G6P[14], Ch_S111/G6P[14], and Ch_S11/G6P[14] (Morocco), and guanaco strain detected in 1999 in Argentina (Chubut/G8P[14]) (Figure 1). The human strains included in this tree showed mostly G6, G8 and G10 VP7 genotypes (Figure 1).

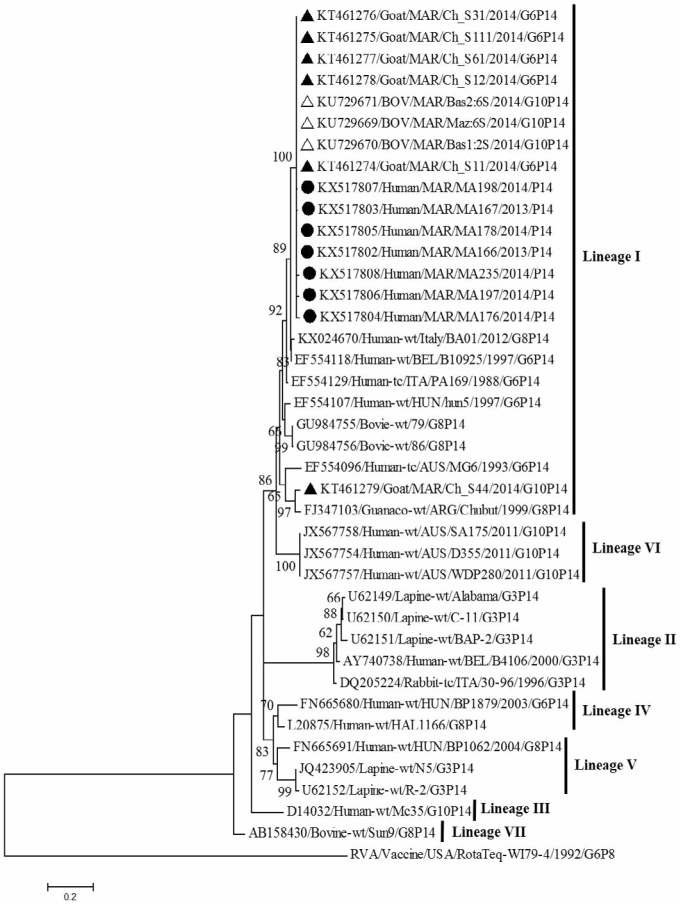

Figure 1: *Phylogenetic analysis based on the VP4 gene nucleotide sequences of Moroccan P[14] strains, and sequences from the Genbank database. Moroccan strains obtained from vaccinated children are marked with a filled circle. Moroccan strains isolated from goat and bovine are marked with a filled and an empty triangle respectively. The lineages appeared at the right. Numbers at the nodes indicate bootstrap values (values <60% are not shown).*

In order to evaluate the possible reduced efficacy of current vaccines to protect children against novel unusual and new evolving virus strains, the deduced VP8* amino acid sequences of our P[14] strains and selected field strains included in the VP4 phylogenic tree were compared with the VP8* sequences of Rotarix™ and RotaTeq™ vaccines strains. The VP8* amino acid comparison was performed for four documented antigenic regions; 8-1, 8-2, 8-3 and 8-4 (Figure 2).

The analyzed amino acid of the VP8* proteins from MA166, MA167, MA178, MA197, MA198 and MA235 showed one amino acid change at position L146V (8-1) compared to MA176 strain (Figure 2) and at residue N133D within the 8-3 antigenic region compared to the human strains detected in Italy (PR457/G10P[14]), in Egypt (AS970/G8P[14]), in Australia (MG6/G6P[14]; V582/G10P[14]) and the sheep strain detected in Spain in 2002 (OVR762/G8P[14]) (Figure 2). The alignment revealed two amino acid substitutions between our strains and the human strain isolated in India in 2009 (N-1/G6P[14]) at residues N133D and N194H (8-3 and 8-1 respectively) (Figure 2).

The alignment of the deduced amino acid sequences inside antigenic epitopes of VP8* proteins from analyzed strains also showed four amino acid differences compared to the human strain detected in Hungary in 2004 (BP1062/G8P14) (at position I89T, S113Q, N133E and N196D), the bovine strain isolated in Japan in 2004 (Sun9/G8P14) (at position I89A, S113Q, N133D and T180I) and the guanaco strain identified in Argentina (Chubut/G8P14) (at position I89V, S113P, N114S and N133D) (Figure 2). Five changes were noted between these strains and a rabbit strain detected in Italy in 1996 (30-96/G3P14) (at position I89T, S113Q, N114S, Q116R and N133D) (Figure 2).

Conversely, no amino acid change was shown in the four compared antigenic regions of the VP8* protein between the MA166, MA167, MA178, MA197, MA198 and MA235 strains and the P[14] strain isolated from goat (Ch_S12/G6P[14]) or bovine (Bas1:2S/G10P[14]) strains detected in Morocco in 2014 (Figure 2).

Comparison of the VP8* from Moroccan P[14] strains with the four VP8* antigenic regions of the P[8] vaccine strains Rotarix™ (A41CB052A) and RotaTeq™ (WI79-4) showed 20 and 22 amino acid substitutions, respectively (Figure 2).

To our knowledge, one study is available in Morocco, in which the authors reported the circulation of the RVA strains with P[14] genotype among neonatal diarrheic calves in the central region of Morocco (Laarayche, Kenitra, and Moulay Bouslham) (Ennima et al., 2016). In addition, Moroccan P[14] goat RVA strains were presented as an oral communication by

Strain	Antigenic sites VP8*																								
	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
KX 517802/Human/MAR/MA166/2013/P14	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
KX 517803/Human/MAR/MA167/2013/P14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KX 517804/Human/MAR/MA176/2014/P14	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KX 517805/Human/MAR/MA178/2014/P14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KX 517806/Human/MAR/MA197/2014/P14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KX 517807/Human/MAR/MA198/2014/P14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KX 517808/Human/MAR/MA235/2014/P14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KP198626/Human-wt/ITA/PR457/2009/G10P14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.
JX040420/human-wt/IND/N-1/2009/G6P14	.	.	.	.	.	.	.	.	H	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.
KU317469/Human-tc/EGY/AS970/2012/G8P14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.
EF554096/Human-tc/AUS/MG6/1993/G6P14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.
JX567756/Human-wt/AUS/V582/2011/G10P14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.
FN665691/Human-wt/HUN/BP1062/2004/G8P14	.	.	.	.	.	.	.	.	.	.	D	.	.	Q	.	.	.	.	.	.	E	.	.	.	T
KU729670/BOV/MAR/Bas1:2S/2014/P14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KT461278/Goat/MAR/Ch_S12/2014/P14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AB158430/Bovine-wt/Sun9/G8P14	.	.	.	.	.	.	.	.	.	.	.	I	.	Q	.	.	.	.	.	.	D	.	.	.	A
FJ347103/Guanaco-wt/ARG/Chubut/1999/G8P14	.	.	.	.	.	.	.	.	.	.	.	.	.	P	S	.	.	.	.	.	D	.	.	.	V
EF554151/Sheep-tc/ESP/OVR762/2002/G8P14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.
DQ205224/Rabbit-tc/ITA/30-96/1996/G3P14	.	.	.	.	.	.	.	.	.	.	.	.	.	Q	S	.	R	.	.	.	D	.	.	.	T
G1P[8] (A41CB052A) of Rotarix	.	S	S	N	S	S	A	.	L	N	.	E	R	N	P	V	D	S	.	.	D	N	N	T	N
G6P[8] (WI79-4) of Rotateq	.	S	S	N	S	N	A	.	L	N	D	E	R	N	P	V	D	N	R	.	D	D	N	T	N

Figure 2: *Alignment of the deduced amino acid sequences inside antigenic epitopes of VP8* proteins of Moroccan P[14] stains analyzed and compared with vaccines and field P[14] strains retrieved from GenBank database.*

Alaoui et al at the 6th European Rotavirus Biology Meeting (ERBM), held in Dijon, France, from Mai 17 to 20, 2015. Of note, the authors reported the molecular analyses of the Moroccan caprine rotavirus strains detected during a severe outbreak of diarrhea in April 2012 in Bouaarfa, Eastern part of Morocco.

RVA strains with a P[14] genotype have not been reported in Moroccan vaccinated children previously, thus it was of interest to us to characterize a P[14] rotavirus strain detected in our state. Here we report the molecular characterization and VP4 phylogenetic relationships of P[14] RVA strains circulating in infants who had been administered Rotarix vaccine and who had subsequently presented with rotavirus gastro-enteritis during the study period (2013-2014). In addition, the antigenic disparities between these strains and RVA vaccines strains were investigated by comparing the amino acid composition of the antigenic epitopes on the VP8* domain; to evaluate their possible implication on the RVA vaccine effectiveness.

Due to lack of availability of tests for rotaviruses analysis, the RotaCheck (VEDA LAB, France) was used as a routine rotavirus diagnosis in our laboratory while validated for the detection of RVA.

Despite the unsuccessful characterization of the VP7 genotype in our study, Moroccan P[14] bovine strains were identified by Ennima and coworkers as combined with the G10 genotype (Ennima et al., 2016), also the G10P[14] combination was detected from a nomadic goat (KT461288), while G6 genotype was found to predominate in association with P[14] genotype among goats surveyed in Eastern part of Morocco (goat rotavirus sequences were available in GenBank database under accession numbers: KT461280-KT461287), thus it has been suggested by Alaoui et al, that the Bedouin livestock farming systems may favor the introduction of new strains from a heterologous host by interspecies transmission given their nomadic lifestyle [Alaoui et al., 2015, oral presentation, 6th ERBM, France].

A fraction of rotavirus strains cannot be typed for P or G genotype or for both P and G genotypes. Untypeable rotavirus strains may result from an insufficient or degraded RNA, the presence of residual stool inhibitors in the RNA extract, technical problems with the assay itself, the presence of a novel strains, or a genetic variation in common strains. Since the successful identification of the P (VP4) genotype using the same RNA extract, we suggest that the inability to identify the G (VP7) genotype in our study might be result of the presence of a novel rotavirus strains, or a genetic variation in common strains.

Seven lineages have been reported within the P[14] genotype, and all of them include human strains (Tam et al., 2014). The VP4 gene from RVA strains reported in this study belongs to lineage I within the P[14] genotype together with human and *Artiodactyl* P[14] strains detected in Morocco and others countries (Matthijnssens et al., 2008; Ennima et al., 2016; Matthijnssens et al., 2009; Delogu et al., 2016; Chitambar et al., 2011). Phylogenetic analysis of our strains revealed close relatedness (100% bootstrap value) to several zoonotic goat and bovine

strains reported in Morocco in 2014. In addition, all Moroccan P[14] strains (human and animal) were very related to the P[14] strains isolated from an Italian child in 2012 (BA01/G8P[14]) (Delogu et al., 2016) and human B10925/G6P[14] strains identified in Belgiumin 1997 (Matthijnssens et al., 2008).

In the last decades, the unusual RVA strains with P[14] genotype, have been associated with gastroenteritis in humans in several countries (Mullick et al., 2013; Ghosh et al., 2007; Medici et al., 2008; Banyai et al., 2010; El Sherif et al., 2011; Cowley et al., 2013). The P[14] genotype is commonly found in rabbits (always associated with G3) (Ciarlet et al., 1997), but it has also been identified in other ruminants such as cattle, goats, sheep, guanacos, and antelope (in association with G6, G8, or G10), suggesting that the human P[14] strains have common origins with those of the even-toed ungulates belonging to the mammalian order *Artiodactyla* (Matthijnssens et al., 2009).

Based on the data reported above, we can speculate that the P[14] strains isolated in Moroccan children, maybe, represent an example of a direct zoonotic transmission event. This hypothesis might partially reinforced by the fact that the human and animal (goat/bovine) P[14] strains were isolated in the same area in Morocco (Rabat and Kenitraare located in central region of Morocco, and both Oujda and Bouaarfa are located in Eastern part of Morocco). In addition, the transmission of P[14] genotype between goats, bovines and humans was probable due to the close relationship between human and domestic animals in areas where the samples were retrieved during the study period, as has being described for G6 and G10 genotype [Alaoui et al., 2015; oral presentation, 6th ERBM, France].

Even the fact that the segmented nature of the RVA genome facilitates the reassortment between and among human and animal strains (Estes and Kapikian, 2007), the full-genome classification system, as described by Matthijnssens and Coworkers (2011), is important to better understand the evolution of reassortments and viral interspecies transmission. Thus, the identification of the 11 genomic segments of our strains is mandatory to confirm the suggested direct interspecies transmission.

The four VP8* antigenic regions of our P[14] strains shared 100% amino acid similarity with VP8* antigenic regions of P[14] strains identified in goats and bovines in Morocco except the substitution noted within 8-1 region ofMA176 strains (L146V). A high amino acid identity (96%) was noted between our strains and human P[14] strains isolated in Egypt (AS970) [KU317469], in Italy (PR457) (Medici et al., 2015) and in Australia (MG6 and V582) (Matthijnssens et al., 2008; Cowley et al., 2013). They also shared a 96% amino acid similarity with the sheep P[14] strains (OVR762) isolated in Spain in 2002 (Matthijnssens et al., 2008). However, amino acid similarity was lower compared to the P[14] human strains isolated in India (92%) and Hungary (84%) (Mullick et al., 2013; Banyai et al., 2010).

The VP8* region of the P[14] VP4 protein has been shown to interact with type A histo-blood group antigens of humans (Hu et al., 2012; Liu et al., 2012) as well as bovine and porcine mucins, as consequent, it has been suggested by Liu et al., 2012 that this mechanism is thought to play a role in cross-species transmission of P[14] RVAs (Liu et al., 2012).

The results of this study reinforce the potential role of inter-species transmission and reassortment in generating novel and unusual rotavirus strains which infect humans as previously reported in diverse parts of the worlds (Estes and Kapikian, 2007; Gentsch et al., 2005; Mullick et al., 2013; Matthijnssens et al., 2008; El Sherif et al., 2011).

The comparison of the P[14] amino acid sequences inside previous described antigenic epitopes in VP8*, with the P[8] protein from Rotarix and Rotateq strains was made, and revealed several amino acid differences as expected since P[14] is rather different from P[8]. The same substitutions have been previously described by Delogu et al. (2016).

However, it is unclear whether the changes described within the VP8* of P[14] strains isolated from vaccinated children might imply a reduced effectiveness of the immunity produced by Rotarix™ vaccines in protection against infection with reassortants or animal-like rotavirus strains. The possible implication of this amino acid changes in reduction of the vaccine effectiveness against genotypes of non-human origins is still unknown and their evaluation with a broad cross-neutralization studies may be important. However, the efficacy of Rotarix and Rotateq was supported by strong vaccine trials and field studies indicating

that the two vaccines provide a significant protection against severe rotavirus gastroenteritis caused by homotypic, partially heterotypic, and heterotypic circulating rotavirus (Patel et al., 2011; Payne et al., 2013). In addition, and as suggested for G8 strains in preliminary studies conducted in Sub-Saharan Africa (Heylen et al., 2015), the bovine genetic background present in the RotaTeq™ vaccine may provide a good effectiveness against animal-like strains.

Conclusions and Recommendations

In conclusion, our study while restricted to a VP4 gene analysis of a limited number of cases, demonstrated the circulation of animal-like RVA strains with a P[14] genotype in Rotarix vaccinated children in Morocco and provides important findings of antigenic differences between VP4 of these strains, vaccines and P[14] field strains. Our results may be of relevance in explaining recent evolving of non-human RVA strains with a P[14] genotype worldwide (Mullick et al., 2013; Tam et al., 2014; Delogu et al., 2016; Ghosh et al., 2007; Medici et al., 2008; Banyai et al., 2010; El Sherif et al., 2011; Cowley et al., 2013). Surveillance of human rotaviruses, especially identification of rare/new G-types and P-types may be important to estimate the origin of the emerging human rotavirus and their possible effect on decreasing the efficacy of rotavirus vaccines.

Authors Contributions

HB, SM and NT defined the research theme and protocol. HB wrote the paper. FH contributed to the samples collection and analyses. HB extracted the RNA virus. HB and SA carried out the RT-PCR. HB and MM did the sequencing and phylogenetic analyses. HB and NT discussed, interpreted, and presented the data under the supervision of SM and EE. All authors have read and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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