Molecular characterization of G2P[4] and G9P[8] rotavirus strains isolated from Moroccan children fully vaccinated with Rotarix™ between 2013 and 2014

Hassan Boulahyaoui a,b, Sanaa Alaoui Amine c,d, Marouane Melloul a, Chaïfa Loutfi a, Reda Tagajdid c,d, Hicham El Annaz a,b, Nadia Touil a,b,9, Yashpal Singh Malik a, Elmostafa El Fahime a,c,d and Saad Mrani a,b

a, Centre de Génomique des Pathologies Humaines (GENOPATH), Faculté de Médecine et de Pharmacie, Université Mohamed V, Rabat, Morocco; b Centre de Virologie, Maladies Infectieuses et Tropicales, Hôpital Militaire d’Instruction Mohamed V, Rabat, Morocco; c, d UATRS, Centre Nationale pour la Recherche Scientifique et Technique, Rabat, Morocco; e Laboratoire de Biotechnologie médicale, Faculté de Médecine et de Pharmacie, Université Mohamed V, Rabat, Morocco; f Laboratoire de physiologie, génétique et ethnopharmacologie, Faculté des Sciences, Université Mohammed Premier, Oujda, Morocco; g Département de Virologie, Société de productions Biologiques et Pharmaceutiques Vétérinaires, Rabat, Morocco; h Laboratoire de Recherche et de Biosécurité, Hôpital Militaire d’instruction Med V de Rabat, Morocco; i Indian Veterinary Research Institute, Izatnagar, India

ABSTRACT

Background: Here, we describe the characterization of the VP7 and VP4 genes of rotavirus group A (RVA) strains isolated from Moroccan infants with acute gastroenteritis (AGE) after receiving the Rotarix.

Methods: Stool samples were collected from seven infants suffering with AGE. Vesikari 20-point was used while collecting the AGE information. RT-PCR followed by sequencing was employed to determine partial sequences from VP7 and VP4 encoding genes. RVA genotypes were determined using the RotaC online classification tool. The Neighbor-Joining phylogenetic tree was performed in the MEGA 6.06 software as well as the amino acid (aa) alignments of VP7 and VP8* antigenic epitopes.

Results: Five children had gastroenteritis episodes defined as severe on the Vesikari scale. Analysis of VP7 gene sequences revealed five G2 and two G9 genotypes. These genotypes were associated with P[4] and P[8], respectively. Phylogenetic analysis demonstrated that G2 and G9 sequences grouped together with Italian strain belonging to lineage II and III respectively. Their P[4] sequences showed only 65-73% identity and grouped into two separate sub-clusters. The Moroccan P[8] isolates did not display this correlation and segregated to lineage III. The VP8* aa of P[8] when compared with that of the Rotarix vaccine, has a six aa differences.

Conclusion: Although, this study does not reveal prevalence rates of RVA in infants after the vaccine introduction, it reports the detection and genetic characteristics of the G2P[4] and G9P[8] RVA types in vaccinated children in Morocco. The findings are suggestive of continuous surveillance program on rotavirus evolution in the vaccinated population.

1. Introduction

Group A rotaviruses (RVA) are implicated as the major viral pathogens in the etiology of acute gastroenteritis (AGE) with an estimated number of rotavirus deaths in children less than 5 years of age of 215,000 deaths in 2013 [1,2]. Over 90% of global child deaths occur in low- and middle-income countries, predominantly in Asia, Africa, and parts of Latin Americas [3]. During 2006–2010, the National Rotavirus Surveillance Network from the Ministry of Health of Morocco, estimated annual diarrhoea cases for children under 5 years of age to be 48,871 and RVA has been established as a causal agent of gastroenteritis presenting 40% of morbidity rates with virtually one death among 389 hospitalized children [4]. Available data indicated that G1P[8] RVA strains predominated (55%) between 2006 and 2009 [6]. Statistically significant differences were found in the geographical distribution of genotypes among four study sentinel sites. G9 was most prevalent in Benimellal (47.8% of all isolates) and Oujda (42%), G1 was the most common strain detected in Tanger and Rabat districts (46% and 33%, respectively) [6]. Rotavirus was also the most frequent virus involved in the development of Moroccan cases of diarrhea, and ranking as the specific second cause of diarrhea after Escherichia coli isolates [7]. Therefore, rotavirus disease prevention through vaccination was a public health priority. Thus, Morocco was the first North African country to include the monovalent Rotarix® (RV-1), a human live attenuated rotavirus vaccine containing one rotavirus strain of G1P[8] in its National Immunization Program in October 2010 with two-dose regimen [8]. RV-1 vaccine covered 88% of infants with the complete schedule achieved in 2013. A decline of approximately
Clinical records from fully vaccinated and non-vaccinated infants, and the genotypes of their rotavirus isolates determined by partial sequencing.

| Sample Number | Birth date  | Sex | Date of consultation (mm/dd/yyyy) | Date of fecal collection (dd/mm/yyyy) | Age (month) | Vesikari score | Vaccine status | Genotypes |
|---------------|-------------|-----|----------------------------------|--------------------------------------|-------------|----------------|---------------|-----------|
| MA001         | 06/07/2009  | M   | 18/04/2011                       | 20/04/2011                           | 21.5        | 11             | No            | G2P [4]*   |
| MA096         | 29/12/2009  | F   | 13/01/2012                       | 16/01/2012                           | 24.5        | 15             | No            | G1P [5]*   |
| MA161         | 15/08/2012  | F   | 30/03/2013                       | 30/03/2013                           | 7.5         | 17             | Yes (RV-1)   | G2P [4]    |
| MA163         | 20/12/2012  | F   | 15/06/2013                       | 16/06/2013                           | 6           | 16             | Yes (RV-1)   | G9P [5]    |
| MA165         | 01/12/2012  | M   | 18/12/2013                       | 21/12/2013                           | 12.5        | 13             | Yes (RV-1)   | G9P [5]    |
| MA170         | 15/04/2013  | F   | 19/02/2014                       | 22/02/2014                           | 10          | 14             | Yes (RV-1)   | G2P [4]    |
| MA175         | 11/06/2012  | F   | 03/03/2014                       | 05/03/2014                           | 21          | 18             | Yes (RV-1)   | G2P [4]    |
| MA190         | 15/06/2013  | M   | 11/04/2014                       | 12/04/2014                           | 10          | 11             | Yes (RV-1)   | G2P [4]    |
| MA201         | 06/07/2013  | M   | 18/04/2014                       | 20/04/2014                           | 9.5         | 16             | Yes (RV-1)   | G2P [4]    |

*Results obtained from the CNR, Dijon, France

41.5% hospitalizations and diarrheal occurrence was observed [8].

Despite, the implementation of RV-1 rotavirus vaccine, data on circulating RVA genotypes in vaccinated cases are not available and specific RVA genotype determination after the use of rotavirus vaccines has not been possible. This is mainly due to limited capacities in the clinical laboratories to establish and maintain molecular biology and sequencing facilities. Here, we describe the molecular characterization of the VP7 and VP4 genes of RVA strains detected in infants suffering with AGE and having history of received monovalent vaccine.

2. Methods

2.1. 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 informed on the study details and their consents were obtained and documented in the questionnaire form before stool specimen collection.

2.2. Clinical Samples

RVA positive human stool samples (Infants) (MA161, MA163, MA165, MA170, MA175, MA190, MA201) were retrieved from a collection bank at the CVMIT Med V at Rabat between March 2013 and June 2014. The RVA infection was laboratory-diagnosed by RotaCheck (VEDALAB, France). All children had received a two-dose regimen of RV-1 (Table 1).

Strains of G2P [4] and G1P [5] genotypes (MA001 and MA96) obtained from symptomatic not vaccinated infants were also included for comparisons (Doblali T, et al. 6th ERBM, Dijon, France, 2015). These infants had mild gastroenteritis with a mean clinical score of 13. Strains had been typed in the CNR of Dijon, (France) as described by Ouédraogo and co-workers [5].

2.3. RVA genotyping

Rotavirus dsRNA was extracted from fecal samples using a RNA extraction kit (QIaAmp® Viral RNA, Germany).

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 [9], 10 mM dNTP mix and H2O in a final volume of 20 µl. After that, cDNA was synthesized using Tetro cDNA Synthesis kit (Bioline, France) by adding the reverse transcription reaction mix which contains 10 U/µl Rnase inhibitor and 200 U/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.

Partial amplifications of VP7 (881 bp) and VP4 (663 bp) genes were carried out using 2.5 µl of cDNA, 10 mM specific primers for each gene (VP7F: ATGTATGGTATTTGAAATACAC; nucleotide position 51–71), VP7R: AAATTTGGCACCATTTTTCC (nt 914–932) and VP4F: TATGCTCCAGTNAATTTGG (nt 132–149), VP4R: ATTGCATTTCTTTCCATAATG (nt 775–795)) as previously published [10,11] and MyFi mix (Bioline, France) according to the manufacturer’s protocol. PCR was carried out with an activation step for 2 min at 95°C followed by 35 cycles of amplification in a Verity thermal cycler (Applied Biosystems, France) using the following conditions: 1 min at 95°C, 1 min at 58°C (for VP7) and 62°C (for VP4), and 1 min at 72°C, a final extension was done 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.

2.4. Nucleotide sequencing and phylogenetic analysis

PCR products were purified using ExoSAP-IT purification system (ThermoFisher Scientific, France)
according to the manufacturer’s protocol. Sequencing was carried out using Bigdye Terminator cycle sequencing Kit V3.1 (Applied Biosystems, France) in both forward and reverse directions with the same primers used for PCR. The sequence data were collected from an ABI Prism 3130XL Genetic analyzer (Applied Biosystems, France).

The RVA genotypes were determined using the RotaC online classification tool [12]. Then, the neighbor-joining phylogenetic tree was performed using the MEGA 6.06 software package as well as the amino acid (aa) alignments of VP7 and VP8* antigenic epitopes [13]. The evolutionary distances were computed at the nucleotide level, with bootstrapping of 1000 replicates [14,15].

The partial VP7 and VP4 sequences characterized in this study were deposited into GenBank library data under accession numbers (KX517809 to KX517827) (supplementary data; Table S1).

3. Results

The children identified in the present study were found to be moderately to severely affected with RVGE (Vesikari score > 11–18) (Table 1).

VP4 and VP7 amplicons were identified on the basis of their size (663 and 881 bp). An analysis of each of these nucleotide sequences shows that detected genotypes were different from that contained in RV-1 vaccine. Strains were of G2P [4] and G9P [5] genotypes (Table 1). They were not vaccine-derived.

Phylogenetic analysis demonstrated that the Moroccan human G2 and P [4] isolates grouped together with a human RVA strain detected in fecal

---

**Figure 1.** Nucleotide based phylogenetic analysis of Moroccan human rotavirus G2 type strains with other human and porcine G2 sequences retrieved from the GenBank database.

Moroccan strains obtained from vaccinated and non-vaccinated children are marked with a black filled and an empty triangle, respectively. The lineages, I to IV, are depicted at the right. Numbers at the nodes indicate bootstrap values (values <65% are not shown). Scale bar at bottom indicates nucleotide substitutions per site.
sample from the non-vaccinated Moroccan child (MA001/2011). All Moroccan G2 RVA strains grouped together with the Italian strain (ITA/BERG08/2012) belonging to the lineages II. Their G2 clustered in different groups from those from Latin America during 2009–2011 seasons (Figure 1). Their VP4 sequences showed however only 65–73% identity; they clearly grouped into two separate sub-clusters within the tree (Figure 2). MAR/MA175/2014 showed the highest VP4 gene identity with the strain ITA/BERG08/2012 (68% of similarity).

The analysis of the Moroccan G9 isolates demonstrates that these genotypes are identical. They grouped into a one separate sub-cluster within the tree (Figure 3) with the Italian BA29 isolated in 2012 and clustered inside lineage G9-III.

However, when the Moroccan P [5] isolates were compared to their counterparts published in NCBI, they did not display this correlation and segregate to a different separate cluster while grouped within lineage III with those from Canada, Belgium, and Brazil (95% of homology was detected). Interestingly, their P [5] have only 66% homology at the nucleic acid level with the Moroccan field MA096 isolate. The latter was almost identical and grouped together with the Italian AST123/2007 and Hungarian isolates (100% homology) (Figure 4).

Interestingly, we further analyzed the deduced aa sequences of the G2 VP7 [16] and VP8* [17] of P [5] epitopes from strains circulating in Moroccan and Brazilian non- and RV-1-vaccinated children.

G2 antigenic epitopes of the MA001, MA161, MA170, MA175, MA190, and MA201 strains share 100% homologies with RVA Brazilian (RJ17745/2010, BA17990/2010 and MA19557/2011 strains) antigenic epitopes.

On the other hand, when the VP8* aa of the P [5] of the MA163 and MA165 strains were compared with that of the RV-1 vaccine, six aa substitutions are detected. They are S125N, S131R, N135D, S146G, S1791 and G207A.
S190N, and N196G. Only one aa substitution was found when VP8* of the P [5] of the MA163 and MA165 strains compared with the Moroccan MA096 strain recovered from the fecal sample from the unvaccinated child.

### 4. Discussion

Although we detected RVA in infants suffering with AGE, we are still uncertain on the role of RVAs as etiological agents of diarrhea because none of their fecal samples were screened for any other enteric pathogens. Thus, no direct association between the obtained severe Vesikari scores and the RAGE may be extrapolated from the present data.

As far as we know, only one study reported the etiology, demographic and clinical characteristics of RAGE in children adequately vaccinated with RV-1 under 5 years of age [7]. This study highlights the presence of G1P [5] and unusual G8P [5] and G3P [9] genotypes detected in five patients. Our investigation is based on the molecular analyses of VP7 and VP4 genes of RVA strains collected from only fully RV-1 vaccinated infants. We demonstrated that the sequencing data analysis of VP7 and VP4 genes are different from those contained in the RV-1 vaccine.

The highest similarity of G2P [4] Moroccan genotypes from vaccinated children with the Italian RVA/Human-wt/ITA/BERG08/2012/G2P [4] detected in a 14-year old boy, gives interesting information on the circulation of strains in these particular Mediterranean countries during 2012–2014. This genotype is documented to be the major human genotype persisting in >5 years Italian adults [18], while, it has been the major serotype circulating in the post-vaccination era in Morocco (24.4% vs. 15%) or in other Eastern Mediterranean Region [8,19]. The high conservation of VP7 and VP4 genes between either circulating G2P [4] strains in the pre- and post-vaccination periods involve their stability over time. We may speculate that these strains are

---

**Figure 3.** Nucleotide based phylogenetic analysis of Moroccan G9 human rotavirus strains with other human G9 strain sequences retrieved from the GenBank database. Moroccan strains obtained from the vaccinated children are marked with filled black triangle. The lineages, I to V, are depicted at the right. Numbers at the nodes indicate bootstrap values (values <65% are not shown). Scale bar at bottom indicates nucleotide substitutions per site.
not vaccine-derived while it would have been interesting to study their whole genomes to better characterize them.

G9P [5] genotype was also found to emerge as the second most prevalent strain after 3 years of immunization in Morocco (15% vs. 7.8% 11.3%) [8]. Both G9 genotypes were found close to the Italian ITA/BA29/2012 and ITA/JES11/2010; a G9P [5] rotavirus strains circulating in Italy between 2010 and 2011 [20,21]; showing high similarities between clinical and environmental strains. However, they presented the most genetic divergence, with a separate clustering pattern for encoding VP4 genes. Their P [5] segregate into a separate sub-cluster, allowing to hypothesize a possible intra-genotype reassortment event at the base of the formation of the modern Moroccan G9P

Figure 4. Nucleotide based phylogenetic analysis of Moroccan P [5] human rotavirus strains with other human P [5] strain sequences retrieved from the GenBank database. Moroccan strains obtained from vaccinated children are marked with a black filled triangle. The lineages, I to IV, are depicted at the right. Numbers at the nodes indicate bootstrap values; only values above 65% are shown. Scale bar at bottom indicates nucleotide substitutions per site.
strains. This hypothesis is supported by the substitutions found at the level of their aa. Whether this is the reason why RV-1 vaccine could not provide any protection against the partly heterotypic rotavirus strains remains to be clarified.

The evolution of the G9P [5] and G2P [4] strains detected in this study might be due to two main factors which can explain the failure of the vaccine in our context. The first most important factor is the environmental enteric dysfunction leading to reduced ability to mount an immune response [22–24] and the second factor is the existence of the maternal antibodies which could adversely impact antibody responses to RVA [25,26]. However, both these assumptions have not been evaluated during the current work. In the presence of limited epidemiological-serological data on RV-1 vaccinated children with AGE due to RVA, we cannot predict that the study strains have evaded immunity conferred by the RV-1 vaccine. Our findings provide additional genetic information and phylogenetic relationships of recovered G2P [4] and G9P [5] RVA strains from fully RV-1 vaccinated children. They are different from those contained in the vaccine (G1P [5]).

Author’s contributions

The study presented here was approved by all authors. HB, SM, and NT defined the research theme and protocol. HB wrote the paper under the supervision of NT, RT and HE contributed to the samples collection and analyses, HB and SA carried out the RT-PCR, HB and MM did the sequencing and phylogenetic analyses. HB, CL, and NT discussed, interpreted, and presented the data under the supervision of SM and EE. YSM analyzed the data, updated the paper and helped in revision of the final manuscript. All authors have read and approved the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Notes on contributors

Hassan Boulayaoui is a Master graduated in the field of Immuno-Virology and Applied Microbiology at Hassan II University of Casablanca. He is a PhD Student in field of Molecular Virology at Mohamed V University, Faculty of Medicine and Pharmacy of Rabat.

Sanaa Alaoui Amine is a Master graduated in the field Biology and Health at Mohamed V University of Rabat. She is a PhD Student in field of Medical Biotechnology at Mohamed V University, Faculty of Medicine and Pharmacy of Rabat.

Marouane Melloul is a PhD graduated in the field of Plants Molecular Genetics in Ibn Tofail University. He is Assistant professor in Genetics at Mohamed 1st University, Faculty of science, Oujda, Morocco. He is specialist in sequencing and bioinformatics.

Chaïfaa Loutfi is DSA graduated in Biology Science at Mohamed V University of Rabat. She is the Head of Laboratory of Virology, responsible for vaccine production at Biopharma Company of Rabat, Morocco

Reda Tagajdid is a Biologist pharmacist and Assistant Professor in Microbiology at Sidi Mohamed Ben Abdellah University, Faculty of Medicine of Fes, Morocco

Hicham El Annaz is a PhD graduated in the field of Molecular Virology at Mohamed V University. He is an Assistant Professor in Microbiology at Mohamed V University, Faculty of Medicine and Pharmacy of Rabat, Morocco.

Nadia Touil is a Professor of Virology in Mohamad V University of Rabat. She is currently works at the Virology field, Mohammed V University. Nadia does research in Virology, Cryobiology and Animal Communications.

Yashpal Singh Malik is an Editorial Board/Reviewer of 10 Int. journals. He is a Principal Scientist at the Indian Veterinary Research Institute, Izatnagar, India. Working on Diagnosis and Epidemiology of principal enteric viral infections in animals, Development of Molecular Techniques and their application to viral diagnosis, Evolutionary analysis of viral pathogens.

Elmostafa El Fahime is the head of Genomics Platform at National Center for Scientific and Technical Research (CNRST) (Rabat, Morocco). He is a Professor of Higher Education in Genetics and Biotechnology at Mohamed V University of Rabat, Morocco.

Saad Mrani is a Biologist Doctor. He is a PhD graduated in the field of Molecular Virology at Claude Bernard Lyon 1 University, France. He is Professor of Higher Education in Virology at Mohamed V University, Faculty of Medicine and Pharmacy of Rabat, Morocco.

ORCID

Hassan Boulayaoui http://orcid.org/0000-0001-5668-397X

References

[1] Parashar UD, Gibson CJ, Bresee JS, et al. Rotavirus and severe childhood diarrhea. Emerg Infect Dis. 2006;12:304–306.

[2] Tate JE, Burton AH, Boschì-Brínto C, et al. Global, regional, and national estimates of rotavirus mortality in children <5 years of age, 2000–2013. Clin Infect Dis. 2016;62:S96–S105.

[3] Parashar UD, Burton A, Lanata C, et al. Global mortality associated with rotavirus disease among children in 2004. J Infect Dis. 2009;200:S9–S15.

[4] Benhafid M, Rguig A, Trivedi T, et al. Monitoring of rotavirus vaccination in Morocco: establishing the
baseline burden of rotavirus disease. Vaccine. 2012;30:6515–6520.

[5] Ouédraogo N, Kaplon J, Bonkoungou JJ, et al. Prevalence and genetic diversity of enteric viruses in children with diarrhea in Ouagadougou, Burkina Faso. PLoS One. 2016. DOI:10.1371/e0153652.

[6] Benhafid M, Elomari N, Elqazoui M, et al. Diversity of rotavirus strains circulating in children under 5 years of age admitted to hospital for acute gastroenteritis in Morocco, June 2006 to May 2009. J Med Virol. 2013;85:354–362.

[7] Benmessaoud R, Jroundi I, Nezha M, et al. Aetiology, epidemiology and clinical characteristics of acute moderate-to-severe diarrhea in children under 5 years of age hospitalized in a referral pediatric hospital in Rabat, Morocco. J Med Microbiol. 2015 Jan;64(Pt 1):84–92.

[8] Benhafid M, Elomari N, Azzouzi IM, et al. Effect of monovalent rotavirus vaccine on rotavirus disease burden and circulating rotavirus strains among children in Morocco. J Med Virol. 2015;90:1–10.

[9] Iturriza-Gomara M, Green J, Brown DW, et al. Comparison of specific and random priming in the reverse transcriptase polymerase chain reaction for genotyping group A rotaviruses. J Virol Methods. 1999;78:93–103.

[10] Iturriza-Gomara M, Cubitt D, Desselberger U, et al. Amino acid substitution within the VP7 protein of G2 rotavirus strains associated with failure to serotype. J Clin Microbiol. 2001;39:3796–3798.

[11] Simmonds MK, Armah G, Asmah R, et al. New oligonucleotide primers for P-typing of rotavirus strains: strategies for typing previously untypeable strains. J Clin Virol. 2008;42:368–733.

[12] Maes P, Matthijssens J, Rahman M, et al. RotaC: a web-based tool for the complete genome classification of group A rotaviruses. BMC Microbiol. 2009;9:238.

[13] Tamura K, Stecher G, Peterson D, et al. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013;30:2725–2729.

[14] Kimura M. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980;16:111–120.

[15] Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evol. 1985;39:783–791.

[16] Aoki ST, Settembre EC, Trask SD, et al. Structure of rotavirus outer-layer protein VP7 bound with a neutralizing Fab. Science. 2009;324(5933):1444–1447.

[17] Dormitzer PR, Sun ZJ, Wagner G, et al. The rhesus rotavirus VP4 sialic acid binding domain has a galectin fold with a novel carbohydrate binding site. Embo J. 2002;21:885–897.

[18] Ianiro G, Delogu R, Bonomo P, et al. Molecular analysis of group A rotaviruses detected in adults and adolescents with severe acute gastroenteritis in Italy in 2012. J Med Virol. 2014;86(6):1073–1082.

[19] Almalki SSR. Circulating rotavirus G and P strains post rotavirus vaccination in Eastern Mediterranean Region. Saudi Med J. 2018;39(8):755–766.

[20] Ruggeri FM, Bonomo P, Ianiro G, et al. Rotavirus genotypes in sewage treatment plants and in children hospitalized with acute diarrhea in Italy in 2010 and 2011. Appl Environ Microbiol. 2015;81(1):241–249.

[21] Ianiro G, Heylen E, Delogu R, et al. Genetic diversity of G9P[8] rotavirus strains circulating in Italy in 2007 and 2010 as determined by whole genome sequencing. Infect Genet Evol. 2013;16:426–432.

[22] Madhi SA, Cunliffe NA, Steele D, et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. N Engl J Med. 2010;362:289–298.

[23] Armah GE, Sow SO, Breiman RF, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. Lancet. 2010;376:606–614.

[24] Zaman K, Anh DD, Victor JC, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. Lancet. 2010;376:615–623.

[25] Siegrist CA. Mechanisms by which maternal antibodies influence infant vaccine responses: review of hypotheses and definition of main determinants. Vaccine. 2003;21:3406–3412.

[26] Moon SS, Wang Y, Shane AL, et al. Inhibitory effect of breast milk on infectivity of live oral rotavirus vaccines. Pediatr Infect Dis J. 2010;29:919–923.