Population, Health & Wellness, Faculty and postdoctoral

Antigenic and genetic characterization of identified rotavirus strains in Qatar in response to Rotarix vaccine usage

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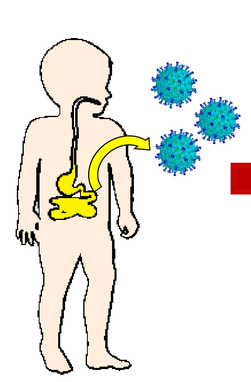
BACKGROUND

Rotavirus (RV) is the most common cause of severe childhood diarrhea worldwide. Despite the introduction of RV vaccines, RV is still contributing to the burden of diarrhea. Concerns exist about the selective pressure that RV vaccines exert on the antigenic and genetic sites among the circulating RV genotypes.

OBJECTIVE

- To genotype and characterize the genetic diversity of RV in hospitalized children
- To study the correlation between circulating genotypes and the efficacy of vaccination
- To identify genetic and antigenic variation in RV strains in response to vaccine usage

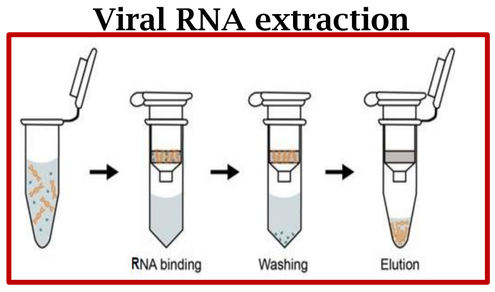
METHODOLOGY



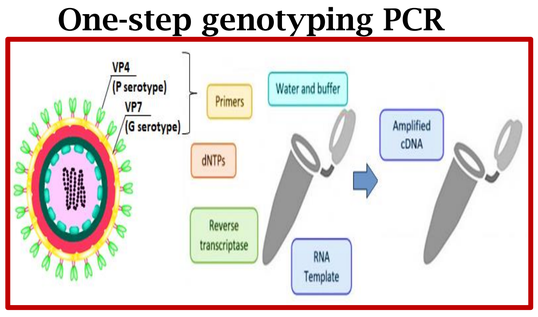
AGE infected children

Film array gastroenteric kit used to detect RV positive samples

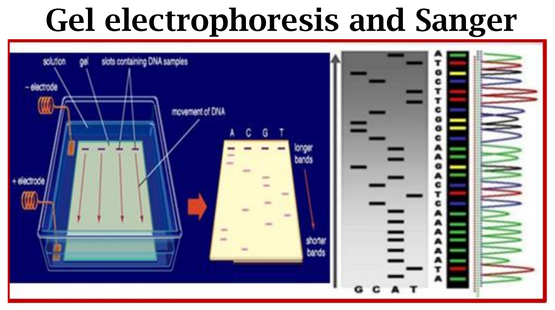
Viral RNA extraction



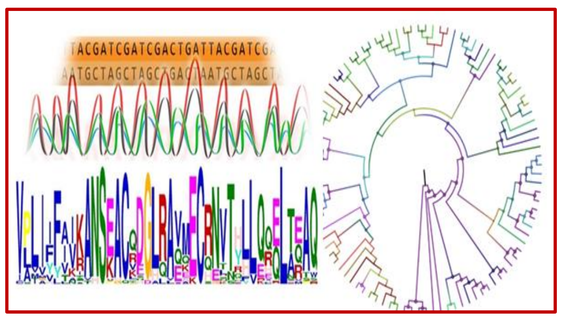
One-step genotyping PCR



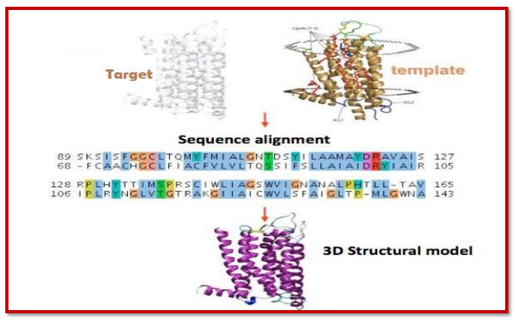
Gel electrophoresis and Sanger



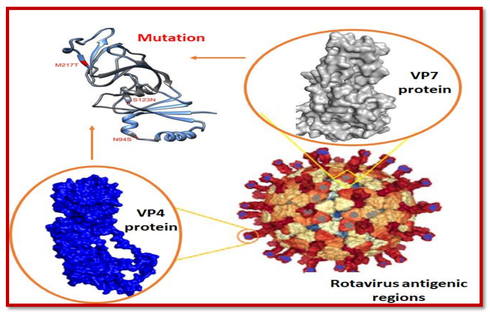
Multiple sequence alignment and Phylogeny



Homology modeling



Identifying mutations in antigenic sites



RESULTS

Distribution of RV strains among infected study subjects

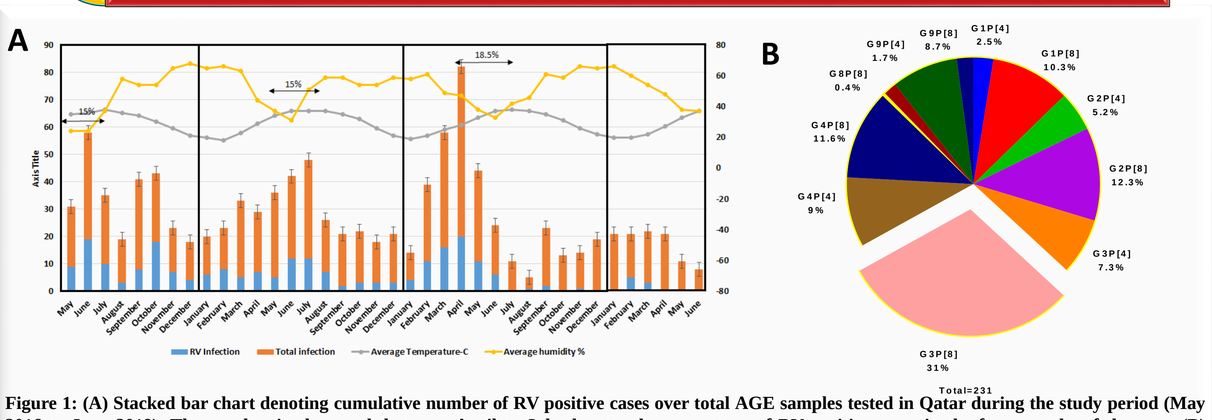


Figure 1: (A) Stacked bar chart denoting cumulative number of RV positive cases over total AGE samples tested in Qatar during the study period (May 2016 to June 2019). The number in the graph between April to July denotes the percentage of RV positive cases in the four months of the year. (B) Percentage of circulating genotypes detected in Qatar between May 2016 to June 2019.

Association of RV vaccination with age group and circulating genotypes

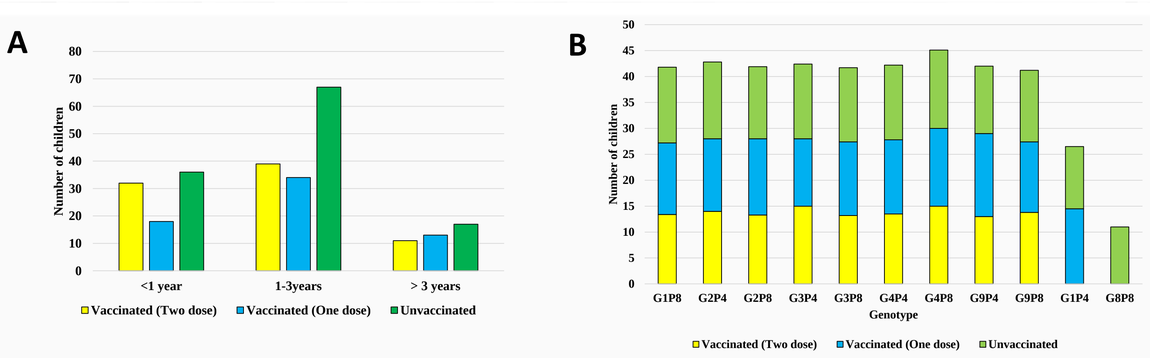


Figure 2: (A) Distribution by age and RV vaccination status of children. Highest vaccine coverage occurs in children under 1- 3 years of age. (B) Association between genotypes and RV vaccination status in infected children. Despite of vaccination, data analysis shows a greater tendency of vaccinated pediatrics contracted RV infection including G1P[8].

Multiple sequence alignment of neutralizing domains in VP7 and VP4 RV strains

A Alignment of amino acid residues defining the neutralization domains in VP7 protein (7-1a, 7-1-b and 7-2) of RV strains analyzed

RV Strain from Qatar	Antigenic sites / RV Vaccination status	Epitope 7-1a														Epitope 7-1b						Epitope 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
	▲RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A[8]	T	T	N	G	E	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	N	L	S	M	N	G
SG1P[8] (n=5)	Vaccinated (two dose)	*	*	S	*	*	*	*	*	*	N	*	*	*	-	*	*	*	*	*	*	*	*	*	*	*	*	T	*	*
SG1P[8] (n=3)	Vaccinated (one dose)	*	*	S	*	*	*	*	*	*	N	*	*	*	-	*	*	*	*	*	*	*	*	*	*	*	*	T	*	*
SG1P[8] (n=9)	Unvaccinated	*	*	N	*	*	*	*	*	*	S	*	*	*	-	*	*	*	*	*	*	*	*	*	*	*	*	M	*	*
SG1P[4] (n=3)	Vaccinated (two dose)	*	*	S	*	*	*	*	*	*	N	*	*	*	-	*	*	*	*	*	*	*	*	*	*	*	*	T	*	*
SG1P[4] (n=2)	Vaccinated (one dose)	*	*	S	*	*	*	*	*	*	N	*	*	*	-	*	*	*	*	*	*	*	*	*	*	*	*	T	*	*
SG1P[4] (n=1)	Unvaccinated	*	*	N	*	*	*	*	*	*	S	*	*	*	-	*	*	*	*	*	*	*	*	*	*	*	*	M	*	*

B Alignment of amino acid residues defining the neutralization domains in VP4 subunit (8-1, 8-2, 8-3 and 8-4) of VP4 of RV strains analyzed

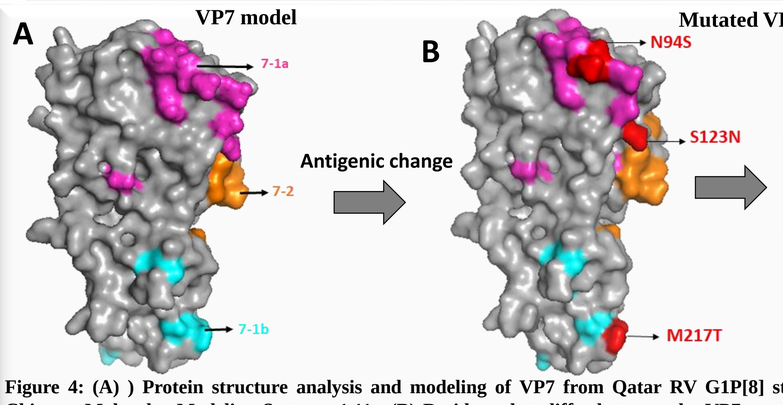
RV Strain from Qatar	Antigenic sites / RV Vaccination status	Epitope 8-1											Epitope 8-2		Epitope 8-3										Epitope 8-4				
		100	146	148	150	188	190	192	193	194	195	196	180	183	113	114	115	116	125	131	132	133	135	86	87	88	89	90
	▲RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A[8]	D	S	S	N	S	S	A	N	L	N	N	E	R	N	P	V	D	S	S	N	D	N	S	N	T	N	G
SG1[P8] (n=5)	Vaccinated (two dose)	*	G	*	*	*	N	*	*	*	*	G	*	*	*	*	*	*	N	R	*	*	D	*	*	*	*	*
SG1[P8] (n=3)	Vaccinated (one dose)	*	G	*	*	*	N	*	*	*	*	G	*	*	*	*	*	*	N	R	*	*	D	*	*	*	*	*
SG1[P8] (n=9)	Unvaccinated	*	S	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
SG1[P4] (n=3)	Vaccinated (two dose)	*	G	*	S	*	N	*	D	*	*	*	*	*	S	*	T	N	N	E	*	S	D	*	*	*	D	*
SG1[P4] (n=2)	Vaccinated (one dose)	*	G	*	S	*	N	*	D	*	*	*	*	*	S	*	T	N	N	E	*	S	D	*	*	*	D	*
SG1[P4] (n=1)	Unvaccinated	*	S	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	S	*	*	*	*	*	*	*	*	*

Rotarix (RVA) vaccine

Different from both Rotarix

Figure 3: (A) Deduced amino acid sequences of approximately 110 residues were obtained from RV strains circulating in Qatar (2015-2019) and compared with Rotarix® vaccine strain (green). Identical amino acids with Rotarix® strain in each isolate are identified by dots and the mutated residues are denoted in red color (B) Deduced amino acid sequences of approximately 102 residues were obtained from RV strains circulating in Qatar (2015-2019) and compared with Rotarix® vaccine strain (green). Identical amino acids with Rotarix® strain in each isolate are identified by dots and the mutated residues are denoted in red color

Protein structure analysis of VP7 modeled with Rotarix® strain



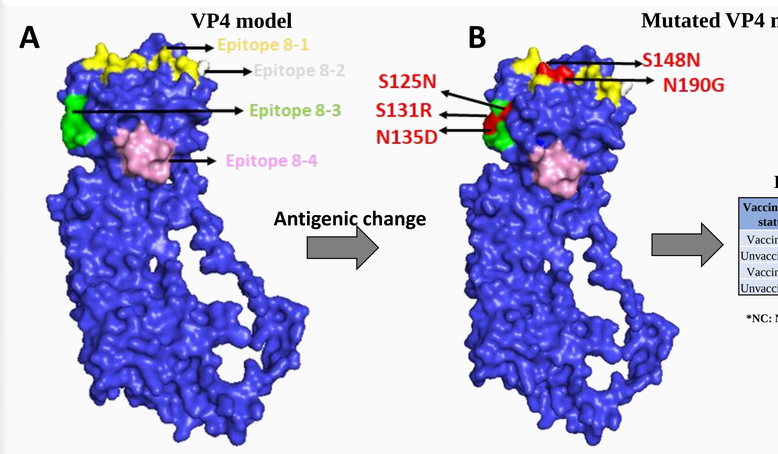
Percentage of mutations in the antigenic epitopes

Vaccination status	Genotype	Rotarix vaccine (G1P[8])	Mutations		
			N94S	S123N	M217T
Vaccinated	G1P[8]	Rotarix-A41CB052A	80%	80%	80%
Unvaccinated	G1P[8]	Rotarix-A41CB052A	NC*	NC	NC
Vaccinated	G1P[4]	Rotarix-A41CB052A	50%	50%	50%
Unvaccinated	G1P[4]	Rotarix-A41CB052A	NC	NC	NC

*NC: No change

Figure 4: (A)) Protein structure analysis and modeling of VP7 from Qatar RV G1P[8] strains. The model was built using the UCSF Chimera-Molecular Modeling System v1.11. (B) Residues that differ between the VP7 protein from Qatar RV strains and the Rotarix® vaccine strain are indicated in red color. (C) The table denotes percentage of mutations in the antigenic epitopes between vaccinated and unvaccinated G1 circulating strains with the Rotarix® strain

Protein structure analysis of VP4 modeled with Rotarix® strain



Percentage of mutations in the antigenic epitopes

Vaccination status	Genotype	Rotarix vaccine (G1P[8])	Mutations				
			S148N	N190G	S125N	S131R	N135D
Vaccinated	G1P[8]	Rotarix-A41CB052A	80%	75%	80%	80%	75%
Unvaccinated	G1P[8]	Rotarix-A41CB052A	NC	NC	NC	NC	NC
Vaccinated	G1P[4]	Rotarix-A41CB052A	25%	25%	25%	35%	80%
Unvaccinated	G1P[4]	Rotarix-A41CB052A	NC	NC	NC	NC	NC

*NC: No change

Figure 5: (A)) Protein structure analysis and modeling of VP4 from Qatar RV G1P[8] strains. The model was built using the UCSF Chimera-Molecular Modeling System v1.11. (B) Residues that differ between the VP4 protein from Qatar RV strains and the Rotarix® vaccine strain are indicated in red color. (C) The table denotes percentage of mutations in the antigenic epitopes between vaccinated and unvaccinated P[8] and P[4] circulating strains with the Rotarix® strain

CONCLUSION

- RV infections occurred throughout the year, with a noticeable increase in summer (42.8 %) and a drop in winter (20.1%).
- The dominant RV strains during the study period were G3P[8] (30.8%), G2P[8] (12.3%), G4P[8] (11.7%) and G1P[8] (10.4%).
- Among the RV-positive cases, 135 (59.3%) had been vaccinated using either of the RV vaccines available.
- The percentage reduction of disease in a vaccinated group of pediatrics compared to an unvaccinated group of pediatrics was 25%. Of these, 108 (78.2%) experienced diarrhea for less than three days, and only eight (6.7%) had diarrhea for more than five days
- Eighty percent (n=8) of the G1 genotype specimens harbored three amino acid substitutions (N94S, S123N, and M217T) in 7-1a and 7-2b antigenic sites in comparison to the Rotarix® vaccine
- The P[8] and P[4] strains with G1 counterpart showed the highest degree of variation (S148N, N190G, S125N, S131R, N135D) in their VP4 antigenic epitopes when compared with the P[8] component of the Rotarix® vaccine.

ACKNOWLEDGMENT

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