The potential rise in the incidence of Rotavirus G3[P8] in Kuwait

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Introduction

Human rotavirus infection is associated with severe diarrhea in children, and accounts for 39% of hospitalizations. Children below the age of two years are most commonly affected. In temperate climates, the peak prevalence of the disease generally occurs during winter [1]. The RotaTeq vaccine was recently included in the Kuwait national immunization program, in a three-dose schedule at 2, 4 and 6 months of age. The last molecular epidemiological study in Kuwait on rotavirus infection dated back to 2005-2006 showing the predominance of rotavirus G1P[8] [2]. This led us to investigate a potential change in the prevalence of rotavirus genotypes circulating in Kuwait, and to compare the VP4/VP7 subgenomic lineages of circulating rotavirus strains to those of Rotarix and RotaTeq vaccines.

Methodology

The study population consisted of 101 infants and children under 5 years of age with severe acute gastroenteritis. The study period was one year, between January and December 2016. Total RNA was extracted from 200 µl of 10% stool suspension using an automated MagNa Pure LC 2.0 system. Two reverse transcription-polymerase chain reactions (RT-PCR) were carried out, one to amplify a 905 bp of VP7-coding gene (G-typing) using 9con1 and 9con2 primers [3], and one to amplify a 876 bp of VP4-coding gene (P-typing) using con2 and con3 primers [4]. The nucleotide sequences of both DNA strands were then determined by performing direct double-strand DNA cycle sequencing using the ABI PRISM® BigDye® Terminator Cycle Sequencing v3.1 Kit on an ABI 3500 Genetic Analyzer. Phylogenetic trees were constructed using neighbor-joining method, with evolutionary distances computed using the Maximum Composite Likelihood method.

Results

Rotavirus dsRNA was detected in fecal samples from 25 (24.7%) children between February and May, with peak in March (Figure 1). All rotavirus cases were admitted to pediatric casualty wards with a diagnosis of severe diarrhea. The median age was 1 year (range: 1 to 36 months; IQR: 7 to 18 months). Most cases (n = 19, 76%) were children under 2 years of age. Sixty percent of rotavirus cases were males, but there was no significant difference in the distribution of rotavirus positivity by gender (p-value = 0.185).

Figure 1

Good quality of nucleotide sequence information was obtained for 19 out of 25 rotavirus cases. According to the VP4 and VP7 phylogenetic analyses (Figures 2 and 3), the most prevalent rotavirus type was G3P[8] (n=9, 47%), followed by G1P[8] (n=5, 26%), G9P[8] (n=2, 10.5%), G4P[8] (n=2, 10.5%), and G9P[4] (n=1, 5%). Some strains formed clusters with strong bootstrap support (>90%). However, no identical rotavirus VP7 or VP4 sequences could be identified. The nucleotide sequence identities in the amplified VP7 region between different strains were 91-98% for G1 type, 89-99% for G3 type, 95% for G4 type, and 92% for G9 type, whereas the nucleotide sequence identities between different strains of P[8] type in the amplified VP4 region were between 85 and 99%. There was no significant difference in the distribution of rotavirus G types between males and females (p-value = 0.69). In addition, the median age of the children did not vary among different rotavirus G types (p-value = 0.99).

Figure 2

None of the VP7 and VP4 nucleotide sequences of rotavirus strains were identical to those in Rotarix and RotaTeq vaccines. All VP7 nucleotide sequences of rotavirus G1 type clustered in lineage 1 compared to lineage 2 for Rotarix VP7, and lineage 3 for RotaTeq VP7, with nucleotide sequence identities to Rotarix and RotaTeq VP7 ranging from 91.1 to 95.8%, and 88.3 to 91.3%, respectively (Figure 2). VP7 nucleotide sequences of rotavirus G3 type clustered in the same lineage 2 of RotaTeq VP7, with nucleotide sequence identities to RotaTeq VP7 ranging from 88.5 to 93.4%. VP7 nucleotide sequences of rotavirus G4 type clustered in the same lineage 1 of RotaTeq VP7, with nucleotide sequence identities to RotaTeq VP7 ranging from 92.7 to 95.4%. VP7 nucleotide sequences of rotavirus G9 strains clustered in lineage 3. All VP4 nucleotide sequences of rotavirus P[8] type but one clustered in lineage 3 compared to lineage 1 for Rotarix VP4, and lineage 2 for RotaTeq VP4, with nucleotide sequence identities to Rotarix and RotaTeq VP4 ranging from 89.6 to 91.9%, and 89.9 to 94.9%, respectively (Figure 3). The VP4 nucleotide sequence of the strain 6076 clustered in P[4] lineage 3, and showed 83.5% nucleotide sequence similarity to Rotarix VP4 (P[8]), and 82.6% to RotaTeq VP4 (P[8]).

Figure 3

Conclusion

The present study provides evidence of the rise in the incidence of rotavirus G3P[8] in Kuwait, and highlights the circulation of genotypes and genetic lineages that are not included in the Rotarix or RotaTeq vaccine.

References