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Characterisation of a G9P[8] rotavirus strain identified during a gastroenteritis outbreak in Alice Springs, Australia post Rotarix™ vaccine introduction

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ABSTRACT

A large rotavirus gastroenteritis outbreak occurred in the Alice Springs region of the Northern Territory, Australia from the 12th of March until the 11th of July 2007. The outbreak occurred five months after the introduction of the Rotarix™ vaccine. Electropherotype and sequence analysis demonstrated that a single G9P[8] strain was responsible for the outbreak and that the strain remained highly conserved during the outbreak period. The outbreak strain contained amino acid changes in regions of the VP7 and NSP4 genes, with known biological function, when compared to previously characterised G9P[8] strains from Australia and other international locations. The recent vaccine introduction was unlikely to have influenced genotype selection in this setting. Importantly, Rotarix™ vaccine was highly effective against the G9P[8] outbreak strain.

1. Introduction

Rotavirus, first identified in 1973 by Bishop et al. in Melbourne Australia, is recognised as the principle aetiological agent of acute gastroenteritis in young children worldwide [1,2]. A considerable burden of disease can be attributed to rotavirus in both developing and developed nations. Rotavirus causes 114 million episodes of diarrhoea annually worldwide, resulting in 24 million clinic visits and 2.4 million hospitalisations in children under five years of age [2]. The mortality rates associated with rotavirus disease are unevenly distributed; of the estimated 527,000 annual rotavirus deaths, the overwhelming majority occur in developing nations in Asia and Sub-Saharan Africa [3].

Rotavirus belongs to the *Reoviridae* virus family and has an 11 segment double-stranded RNA (dsRNA) genome that encodes six structural viral proteins (VP1–4, VP6, VP7) and six non-structural proteins (NSP1–6). The RNA genome is encased in three concentric layers of protein consisting of a core, inner and outer capsid [4]. Rotavirus can be classified into seven groups (Group A–G) based on the genetic characteristics and antigenicity of the inner capsid protein VP6. Group A rotaviruses are the most common cause of symptomatic disease in humans. The two outer capsid proteins VP7 and VP4 elicit type-specific and cross-reactive neutralising antibody responses, and are used to classify Group A rotavirus strains into G (glycoprotein, VP7) and P (protease sensitive, VP4) genotypes, respectively [4,5].

Of the 24 G genotypes and 33 P genotypes described to date, 12 G and 15 P genotypes are known to infect humans [6,7]. Genotype G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] strains cause over 90% of rotavirus disease worldwide. In North America, Europe and Australia they represent over 90% of characterised isolates, but in South America and Africa they represent 83% and 55% of isolates respectively [8]. Genotype G9 strains were initially identified in the USA, and Japan in the 1983–1984 [9,10]. Genotype G9 strains re-emerged in early to mid 1990s and the global prevalence has increased, such that G9 in combination with P[8], P[4] and P[6] have been detected in over 55 countries in Europe, Asia, Africa, South and North America and represent the dominant genotype in some regions during the past decade [5,8].

The development and implementation of efficacious vaccination programs against rotavirus are a global priority. Two live-oral vaccines are currently available on the global market; Rotarix™ and RotaTeq™, and are licensed in over 100 and 85 countries worldwide respectively. They are included in the routine vaccination programs of many countries including the USA, Brazil, Panama, Venezuela, Belgium and Australia [11].

Rotarix™ is a live-attenuated monovalent vaccine, possessing a genotype G1P[8] strain, while RotaTeq™ is a live-attenuated pentavalent vaccine that contains five genetically distinct human-bovine reassortant virus strains [12,13]. Each reassortant strain

Abbreviations: PAGE, Polyacrylamide Gel Electrophoresis; RT–PCR, reverse transcription/polymerase chain reaction.

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contains a human gene encoding one of the outer capsid proteins within a bovine WC3 strain backbone (G6P[5]). Four of the reassortant strains have a VP7 gene encoding G1, G2, G3 or G4 and one reassortant strain carries the VP4 gene encoding P[8] [13].

Rotarix™ and RotaTeq™ became commercially available in Australia in early 2006 and were introduced into the National Immunisation Program in July 2007, with the exception of the Northern Territory which introduced routine vaccination in October 2006 due to a high rotavirus disease burden [14]. Each state and territory independently evaluated which vaccine to implement. Victoria, Queensland, Western Australia and South Australia currently use RotaTeq™, New South Wales, the Northern Territory, Tasmania and the Australian Capital Territory use Rotarix™ [15]. Prior to vaccine introduction in Australia, 115,000 GP consults, 22,000 emergency department presentations and 10,000 hospitalisations in children under five years of age could be attributed to rotavirus infection annually [16].

In this study we report the characterisation and molecular analysis of a G9P[8] strain responsible for a large outbreak of rotavirus gastroenteritis in the Northern Territory of Australia in 2007, five months after the commencement of the Rotarix™ vaccination program.

2. Materials and methods

2.1. Stool specimens

A total of 107 stool samples were collected from paediatric patients hospitalised with severe gastroenteritis during a rotavirus outbreak in the Alice Springs region of the Northern Territory between the 12th of March and the 11th of July 2007. Patient information including date of birth, date of sample collection, sex and rotavirus immunisation status was obtained. Samples were stored frozen and forwarded to the Australian Rotavirus Reference Centre (ARRC) in Melbourne. Ninety-nine samples had adequate sample for analysis and were characterised using a combination of serotyping EIA and hemi-nested multiplex RT-PCR. Seventy-eight samples were found to be rotavirus positive and typed as G9P[8] and were analysed further in this study [25].

2.2. Nucleic acid extraction

Rotavirus dsRNA was extracted from clarified 20% faecal suspensions using a RNA extraction Kit (QIAamp® Viral RNA mini Kit (spin protocol), Qiagen, Inc., Hilden, Germany) in accordance with the manufacturer's instructions for use in RT-PCR. Rotavirus dsRNA was extracted from 20% faecal suspensions using phenol-chloroform extraction and purified using hydroxyapatite as previously described for use in Polyacrylamide Gel Electrophoresis (PAGE) [17].

2.3. PAGE

The dsRNA genome segments were separated on 10% (w/v) polyacrylamide gel and the genome migration patterns (electropherotypes) were visualised by silver staining according to the established protocol [18,19].

2.4. Amplification of VP4, VP7 and NSP4 genes of G9 strains

Of the 78 rotavirus positive samples collected during the outbreak, 14 were selected for further analysis including five from vaccinated patients. Samples were evenly selected during the outbreak period. Portions of gene segment 4 (VP4), 9 (VP7) and 10 (NSP4) were reverse transcribed and amplified by PCR using the Superscript III One Step RT-PCR with Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA). RNA was denatured and reverse transcribed at 45 °C for 30 min followed by PCR activation at 95 °C for 15 min. Forty cycles of amplification of 95 °C for 45 s, 45° for 45 s and 70 °C for 3 min was followed by a final extension at 70 °C for 7 min. The standard primer sets VP7F/R and Beg9/End9 were used to amplify VP7, VP4F/R and Con2/Con3 to amplify the VP8* subunit of the VP4 gene and 10.1/10.2 to amplify NSP4 [20–22].

2.5. Sequence analysis

PCR amplicons were purified using the QIAquick Gel extraction Kit (Qiagen, Inc., Hilden, Germany) according to the manufacturer's protocol. Purified cDNA was sequenced using BigDye Sequencing Kit version 3.1 (Applied Biosystems; Foster City, CA, USA) in both directions using the oligonucleotide primer sets used in the gene amplification PCR protocol. The thermal cycling reaction consisted of 30 cycles of 96 °C for 15 s, 50 °C for 10 s and 60 °C for 4 min and the products purified by ethanol precipitation. The nucleotide sequence was determined by Applied Genetic Diagnostics (University of Melbourne, Victoria, Australia), using an ABI 3130xl Genetic Analyser (Applied Biosystems, Foster City, CA, USA).

Sequence data were analysed utilising the Sequencher® Software program version 4.1 (Gene Codes Corp Inc., Ann Arbor, MI, USA). Sequence identity was determined using the BLAST (Basic Local Alignment Search Tool) server on the GenBank database at the National Centre for Biotechnology Information, USA (www.ncbi.nlm.nih.gov). Sequences from this study and those obtained from the GenBank database were aligned using ClustalW [23]. Genetic distances were calculated using the Kimura-2 correction parameters at the nucleotide level and phylogenetic trees were constructed using the Neighbor-joining method with 500 bootstrap replicates utilising the program MEGA version 4 [24].

The nucleic acid sequences for genes described in this study have been deposited in GenBank (Accession numbers JN377704-JN377721). For simplicity, samples will be referred to by abbreviate common names, AS07-obV2, AS07-obV12, AS07-obV18, AS07-obV37, AS07-obV42, AS07-obV50, AS07-obV57, AS07-obV75, AS07-obV93, and AS07-obV121.

3. Results

3.1. Strain characterisation

A total of 107 stool samples were collected during a large gastroenteritis outbreak in Alice Springs (Northern Territory) were sent to the Australian Rotavirus Reference Centre for genotype analysis. Seventy-eight samples were found to be rotavirus positive.

Sixty-five samples were analysed by PAGE and silver stained to allow the visualisation and comparison of the electrophoretic pattern. An RNA electropherotype was visible in 57 samples; all samples displayed an identical long electropherotype (data not shown).

3.2. Sequence analysis of VP7 gene of G9P[8] strains

Fourteen G9P[8] samples from the outbreak were selected for sequence analysis, including two samples from vaccinated infants. The coding region of the VP7 gene was determined for each of the 14 samples, and revealed a highly conserved gene, which displayed 99.9–100% nucleotide homology and 99.6–100% amino acid identity to each other. No conserved amino acid changes were observed between samples obtained from vaccinated and non-vaccinated patients.

The nucleotide sequence of the VP7 coding region of the G9P[8] 2007 outbreak strains were compared phylogenetically to previously published human strains with a G9 specificity. As depicted