

Characterization of rotavirus strains detected among children and adults with acute gastroenteritis in Gizan, Saudi Arabia

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ABSTRACT

الخلفية العلمية: تعتبر فيروسات الروتا من المسببات الأساسية للالتهابات المعوية في المملكة، واستشرافا لدخول التطعيمات المضادة للفيروس في البلاد تم عمل دراسة قصيرة لبحث سلالات الفيروس على حالات من الأطفال والبالغين المصابين بالتهابات معوية لعدد من المراجعين لمستشفيات المنطقة.

الطريقة: تم إجراء دراسة مقطعية في خمسة مستشفيات بمنطقة جازان، جمعت خلالها عينات براز من مرضى مصابين بإسهال حاد خلال الفترة من نوفمبر ٢٠٠٤ إلى مارس ٢٠٠٥. تم الكشف على فيروس الروتا باستخدام طريقة المقايضة الإنزيمية المناعية (الإليزا) كما تم تعريف سلالات الفيروس باستخدام طريقة سلسلة تفاعلات إنزيم البلمرة (بي سي آر) وطريقة الرحلان الكهربائي (الإلكتروفوريسز).

النتائج: أظهرت الدراسة وجود الفيروس في 54 حالة من أصل 454 عينة جمعت بنسبة إصابة بلغت 12% في حالات تتراوح أعمارهم بين 15 يوم إلى 20 سنة وسجلت أعلى نسبة إصابة بين الفئة العمرية (59-48 شهر). بشكل عام تم تحديد نوعية 93% من العينات مع وجود النوع (ج 1 ب 8) في 89% من الحالات كما عزل النوع (ج 9 ب 8) والنوع (ج 2 ب 4) في عينة لكل منهما.

خاتمة: هذه الدراسة هي الأولى في المملكة التي حددت النوع البائي للفيروس وعزل فيها لأول مرة النوع ج 9. أظهرت الدراسة أن التوزيع السنوي الغير طبيعي لحالات الإصابة بفيروسات الروتا يستدعي دراسات مستقبلية بشكل أكبر توقعاً لدخول التطعيم.

Objectives: To assess the circulating rotavirus strains among hospitalized children and adults in Gizan City.

Methods: This cross-sectional study was based in 5 hospitals in the Gizan area. Stool samples were collected between November 2004 and March 2005, from sequential patients with acute, dehydrating diarrhea. Rotavirus antigen was detected in stool by enzyme-linked immunosorbent assay. The diversity of rotavirus strains was investigated using electropherotyping and reverse

transcription-polymerase chain reaction amplification of the VP7 and VP4 genes (G and P genotyping).

Results: Rotavirus was detected in 54 of 454 (12%) subjects. The ages of those infected with rotavirus ranged from 15 days to 20 years, with a median age of 36 months. The highest rotavirus detection rate (24%) occurred in children aged 48-59 months. Overall, 50 (93%) of strains could be assigned both a G- and P-type; G1P[8] was the most frequently detected strain type (n=48, 89%) with one rotavirus each of G2P[4] and G9P[8].

Conclusion. Rotavirus strains circulating in Gizan would be well covered by current rotavirus vaccines. Rotavirus serotype G9 has been detected in Saudi Arabia for the first time. Continued surveillance of rotavirus strains is required.

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Rotavirus is recognized as a leading cause of severe gastroenteritis resulting in hospital admission and death in infants and young children worldwide.¹ Globally, an estimated 527,000 deaths each year are attributed to rotavirus infection among children under 5 years of age, and these deaths mainly occur in the developing countries.² Two rotavirus vaccines, namely

Rotarix, a human monovalent rotavirus vaccine (GSK Biologicals, Rixensart, Belgium) and RotaTeq, a human-bovine multivalent rotavirus vaccine (Merck Research Laboratories, Philadelphia, Pennsylvania, USA) have proven highly effective in preventing severe rotavirus gastroenteritis, and are now entering childhood immunization programmes in the Americas and in Europe.³⁻⁵ Rotaviruses are triple-layered icosahedral particles and their genomes consist of 11 segments of double-stranded RNA. Based on epitopes on the inner capsid, rotaviruses are divided into 7 groups (A-G) but most human infections belong to group A. Rotaviruses are further classified according to the genetic and antigenic diversity of the 2 outer capsid proteins, VP4 and VP7. To date, 15 G serotypes and 26 P types have been defined.^{6,7} Initially, these were defined as serotypes by panels of hyperimmune sera and monoclonal antibodies, but more recently genotyping has been possible using the reverse transcription - polymerase chain reaction (RT-PCR).^{8,9} The G-serotype and G-genotype designations coincide, but those of P-types do not: the P-genotype is therefore indicated in a square bracket. As the segregation of VP7 genes and VP4 genes occurs independently, many G and P combinations are theoretically possible. Globally, however, there are 5 common rotavirus strains: these comprise serotypes G1, G3, G4, and G9 with P[8] VP4 specificity, and G2P[4] strains.^{10,11} Rotavirus infection is a major cause of childhood morbidity in Saudi Arabia.¹² However, only 4 studies in Saudi have undertaken G-typing¹³⁻¹⁶ and none has undertaken P-typing. It is considered important to describe the prevalent rotavirus strain types in a country before and after the introduction of rotavirus vaccines. In order to further investigate the diversity of circulating rotavirus strains in Saudi Arabia, we therefore conducted a 5-month study of rotavirus gastroenteritis in Gizan City.

Methods. This study was based in 5 hospitals in Gizan City and surrounding areas. The city is one of the largest cities in Saudi Arabia and is situated in the southern part of the kingdom. The climate is hot and humid for most of the year extending from April to October, followed by a milder season between December and February. Stool samples were collected from sequential patients with acute, dehydrating diarrhea who were either admitted to hospital, or who were given oral rehydration therapy as outpatients, from November 2004 to March 2005, thus covering both climatic seasons. All cases with acute watery diarrhea of less than 2 weeks were included. Information regarding age and gender of the patient, date of sample collection and whether the patient was treated as an in-patient or an out-patient were obtained. Any case without complete data was excluded. All stool

samples were stored at -20°C until transported frozen to the Department of Medical Microbiology, University of Liverpool and stored at -80°C until further analysis. Ethical approval was given by the Research Ethics Committee, General Directory of Health Affairs in Gizan, Ministry of Health, Kingdom of Saudi Arabia. Verbal consent was obtained from the subjects.

Rotavirus antigen was detected in faeces by using the Rotaclone EIA kit (Meridian Diagnostics, Cincinnati, OH). The EIA positive faecal samples were suspended in phosphate buffered saline at a concentration of 10%. Suspensions were clarified by centrifugation and rotavirus double-stranded RNA (dsRNA) was extracted by using a guanidine isothiocyanate/silica method.¹⁷ Rotavirus electropherotypes were determined by polyacrylamide gel electrophoresis (PAGE) of rotavirus dsRNA followed by silver staining as previously described.¹⁸ Rotavirus genome profiles that could not be clearly categorized as short or long electropherotypes were labelled positive. Rotavirus G and P typing was determined using a hemi-nested, multiplex RT-PCR and used consensus and type-specific primers described previously for G-serotypes G1-5, G8, G9, G12 and P-types P[4], P[6], P[8], P[9] and P[10].^{8,9,19} The RT-PCR products were resolved by electrophoresis on a 2% agarose gel, stained with ethidium bromide and visualized by UV trans-illumination. Samples co-migrating with reference strains of known genotype were assigned to that genotype. Samples failing to G or P type were re-tested with additional G1 and P[8] typing primers NAC 9 and NAC 10.²⁰

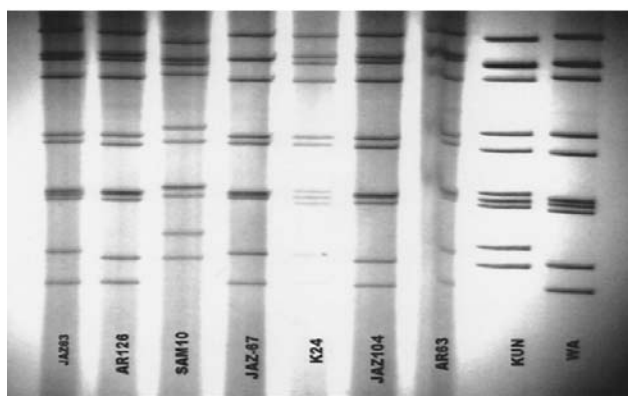
Statistical tests were performed by EPIInfo version 6.0. The difference in prevalence between 2 groups was calculated by the Chi square test. A *p* value of <0.05 was considered significant.

Table 1 - Rotavirus detection rate by age.

Age (months)	No. of samples of gastroenteritis cases (%)	No. of rotavirus positive of age group (%)	Relative (%) distribution of rotavirus cases among those <5 years old (n=39)
0-5	25 (6)	2 (4)	5
6-11	27 (6)	4 (7)	10
12-23	73 (16)	9 (12)	23
24-35	60 (13)	9 (15)	23
36-47	64 (14)	8 (13)	21
48-59	29 (6)	7 (24)	18
≥60	176 (39)	15 (9)	
Total	454 (100)	54 (11.9)	

Table 2 - Electropherotypes and G and P types of rotaviruses from Gizan.

Electropherotype	P[8]		P[4]	P[NT]		Total (%)
	G1	G9	G2	G1	GNT	
Long	35	1				36 (67)
Short						
Positive	6		1			7 (13)
Negative	6			3	1	10 (19)
Mixed	1					1 (2)
Total	48	1	1	3	1	54

**Figure 1** - Polyacrylamide gel electrophoresis and silver staining of rotavirus dsRNA genome. WA and KUN - Standard strains showing long electropherotype profile (strain WA), and short electropherotype profile (strain KUN). AR63 - Saudi field strain showing positive electropherotypes profiles G1P[8]. JAZ 104 and AR126 - Saudi field strains showing long electropherotypes profiles (L1) (G1P[8]). K24 and JAZ 63 - Saudi field strains showing long electropherotypes profiles (L2) (G1P[8]). JAZ 67 - Saudi field strain showing long electropherotypes profiles (L3) (G9P[8]). SAM10 - Saudi field strain showing short electropherotypes profiles (G2P[4]).

Results. A total of 54/454 (12%) specimens were positive for group A rotavirus, which was detected throughout the 5 months study period. The ages of subjects with rotavirus infection ranged from 15 days to 20 years (median age 36 months). The majority of rotavirus infections (39/54 [72%]) were identified among children under 5 years of age and nearly half (24/54 [44%]) were among children under age 3 years (Table 1). The highest rotavirus detection rate (7/29 [24%]) was observed in subjects aged 48-59 months, followed by children aged 60-72 months (11/53 [21%]), although differences in rotavirus detection rates did not reach statistical significance between age groups. Four (7%) of the 54 rotavirus infections occurred in subjects

aged 9, 16, 19, and 20 years. Among the 54 rotaviruses identified, G- and P- types were determined for (53/54 [98%]) and (50/54 [93%]) rotavirus strains; 50 (93%) of rotaviruses could be assigned both a G- and a P-type. Of these, G1P[8] was the most common strain identified (48/54 [89%]), with one G2P[4] and one G9P[8] strain each detected. Fifteen of the G1 strains, and 14 of P[8] strains, were typeable only with the G1 primer NAC9 and P[8] primer NAC10, respectively. Ribonucleic acid bands could not be visualized for any of the 4 non-typeable samples using polyacrylamide gel electrophoresis. Overall, 36 (67%) of the 54 rotaviruses could be assigned an electropherotype (long); 7 were not interpretable (positive), one showed a mixed pattern and 10 were PAGE negative (Table 2). Among the long electropherotype strains 3 different patterns were identified (L1, L2, L3). The G1 strains produced patterns L1 and L2 and the G9 pattern L3 (Figure 1).

Discussion. The monthly prevalence of rotavirus gastroenteritis did not alter over the study period, which spanned the only seasonal changes in the region. Nevertheless, to properly assess seasonality at least 12 months surveillance would be needed. As expected, most cases of rotavirus gastroenteritis were in children under age 5 years. The slightly higher rate of rotavirus detection reported in the current study among children age >48 months is of interest, since the highest detection rates are normally seen in children <2 years of age.¹ Larger studies are required to determine whether these age-specific trends are real, or have occurred by chance. Fifteen (28%) of rotavirus infections were detected in subjects aged 5 years and older with the eldest being 9, 16, 19 and 20 years. These 4 infections were each with G1P[8] strains, which suggests that either immunity to this strain has waned over time, or perhaps that such strains were not circulating in Gizan whilst the subjects were infants. A similar situation has previously been described for serotype G1, G4, and G9 rotaviruses causing gastroenteritis in adults.²¹⁻²³ Detected strains of rotavirus were remarkably uniform, 89% of strains belonged to serotype G1P[8]. We identified only a single G2P[4] strain and one G9P[8] strain. However, 15 of the G1 strains were typeable only by using additional G1 primer NAC9.²⁰ In addition, a small number of strains could be neither G- nor P- typed (1 and 4 respectively). This could be explained by the presence of insufficient amounts of RNA in the non-typeable samples (these strains had negative PAGE profiles). A total of 22 studies of rotavirus gastroenteritis have been published from Saudi Arabia¹² with rotavirus prevalences ranging from 10-46%, with a median rotavirus detection rate of 30%. The only other study to be undertaken in Gizan

was from October 1983 to February 1984 which found a rotavirus prevalence of 16%.²⁴ Only 4 previous studies have provided data on G-serotypes in Saudi Arabia.¹³⁻¹⁶ In each study, G1 was the predominant serotype with prevalences ranging from 39.6-53% and followed in decreasing order by G4, G3, and G2. This is the first report of G9P[8] rotavirus from Saudi Arabia and adds another country to the widening geographical distribution of this strain.²⁵

Although this study is limited by a small sample size and short duration, it was described for the first time both P- and G- genotypes, and the occurrence of G9 rotavirus in Saudi Arabia. While available rotavirus vaccines would be expected to provide good coverage among currently circulating strains, continued rotavirus surveillance in the country is necessary to detect any changes in strain types pre- and post- rotavirus vaccine introduction.

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