Electrophoretic typing of rotavirus at a
Tertiary Care Centre, Saudi Arabia

Javed Akhter, Bsc(Hons), FIMLS,
S. M. Hussain Qadri, Ph D, FRCPath, Diplomat ABMM, FAAM,

Abstract Objective: To determine the epidemiologic features of rotavirus serotypes in Saudi Arabia.

Design: RNA genome analysis was performed on 200 rotavirus positive patients by polyacrylamide gel electrophoresis (PAGE).

Setting: King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia.

Subjects: 200 patients with rotavirus diarrhea.

Results: Seven patterns were found of which 5 were long and 2 were short. The distribution pattern showed that one of the long electrophorotypes dominated in 159 patients. Most patients (153) were under 5 years of age; of the 200 patients there were 111 males and 89 females. Of these 118 were outpatients and 88 inpatients with approximately 75% of admission being referrals from other parts of the Kingdom.

Conclusions: The greatest variations in mobility were found in segments 2, 3, 6, 8 and 9. No correlation was found with age or sex of patients. Modifications in running current and gel thickness were found to improve separation of bands.

Keywords: Electrophorotyping, rotavirus, Saudi Arabia

Human rotavirus is a major aetiological agent of infantile gastroenteritis. Group A rotavirus is known to be the most common cause of severe diarrhea among children in North America and Europe. A new group of rotavirus (group B) has been found to cause large outbreaks of severe diarrhea in China but has not been found frequently elsewhere.1 Rotavirus infects virtually every child in the U.S.A. by the age of 4 years and causes potentially lethal dehydration in 0.75% of children less than 2 years of age.2 The problem of acute gastroenteritis in developing countries is of much greater magnitude where approximately 5 million deaths occur annually in Africa, Asia and Latin America alone, rotavirus being responsible for one fifth of them.3,4

Rotaviruses are difficult to cultivate and characterize. This has hampered understanding of the epidemiological features of individual serotypes prevailing worldwide. This is particularly true of developing countries where efforts are being made to develop an effective vaccine. Group A rotaviruses have three major antigens, the subgroup antigen, the VP7 serotype antigen, and the VP4 serotype antigen. The rotavirus genome consists of 11 double-stranded RNA segments and at least two distinct subgroups I and II. Seven different VP7 serotype antigens have been distinguished so far.5

Molecular techniques such as the analysis of the eletrophoretic mobility of the 11 double-stranded ribonucleic acid (RNA) segments of rotavirus by polyacrylamide gel electrophoresis (PAGE) are now being used for epidemiological studies.6 This is useful in determining the different electrophorotypes circulating in different geographic areas. They can be classified as L(long) or S(short), depending on the migration of segments 10 and 11. Rotavirus strains with short eletrophorotypes have been found to be associated with subgroup I and serotype 2 and 8, whereas strains with long electrophorotypes are associated with subgroup II and serotypes.1,3,6

Comparison of migration patterns have been used as epidemiological markers to study prevalence of different rotavirus strains. It is also a useful technique for detecting and differentiating

From the King Faisal Specialist Hospital and Research Centre, Riyadh (AKHTER, QADRI)

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Address correspondence and reprint request to: Dr. S.M. Hussain Qadri, Head of Microbiology Laboratory, King Faisal Specialist Hospital & Research Centre, MBC 10, PO Box 3354, Riyadh 11211, Saudi Arabia
Rotaviruses in outbreaks, mixed infections and atypical strains. Hence, this study was carried out to determine the epidemiological features of rotavirus serotypes at a tertiary referral center in Riyadh.

**Materials and methods** Diarrheal specimens from 200 patients were collected between 1993-1994 at King Fahad Specialist Hospital and Research Centre, a 550-bed referral center in Riyadh. Approximately seventy-five percent of admissions were from patients outside Riyadh.

Ribonucleic acid (RNA) genome analysis was performed on 200 rotavirus positive specimens by enzyme-immunoassay (EIA) and/or electron microscopy (EM). The EIA detected only group A rotavirus and EM detected both group A and non-group A rotaviruses.

**Extraction** Extraction was carried out by the method of Herring et al. Briefly, double stranded genome of rotavirus present in stool specimens was extracted as follows. A heavy suspension of faecal material was suspended on 0.5 ml of sodium acetate buffer (pH 5.0) containing 1% sodium dodecyl sulphate. An equal volume of phenol: chloroform:isomyl alcohol (25:24:1) was added to the suspension. The mixture was shaken vigorously in a 1.5 ml Eppendorf tube for 1 minute on a Vortex mixer. This was followed by centrifugation at 6,000 rpm for 5 min. This was followed by configuration at 6,000 rpm for 5 min. The clear upper layer containing double stranded RNA was removed and a 40 μl aliquot was mixed with 15 μl of loading buffer containing 0.5 M Tris (pH 6.8), 25% glycerol, and 0.25% bromophenol blue.

**Page** Electrophoresis was carried out in a slab polyacrylamide gel with SDS omitted from all buffers. An 8% polyacrylamide gel was used and 50 μl of each RNA preparation was carefully loaded into each well. Electrophoresis was carried out at room temperature at a constant voltage of 70 V. All solutions were made from sterile distilled water and degassed for 15 minutes before use.

**Silver staining** The gels were washed with 10% ethanol-0.5% acetic acid for 30 mins. and then soaked in 0.011 M silver nitrate for 2 hours. The gel was washed in distilled water and the reduction step performed with a solution of 0.75 M sodium hydroxide containing 0.1 M formaldehyde and 0.0023 M sodium borohydride. Once the bands were visible the gels were placed in a solution of 5% acetic acid for 30 mins. followed by 0.007 M sodium carbonate.

**Classification** To facilitate systematic comparison of the viral RNA patterns encountered, the method of Lourenco et al. was used in which 11 RNA segments after resolution were divided into 4 groups and eletrophoretotypes identified as combinations of variations within each group.

**Results** Electrophoretotypes of 200 isolates collected at King Faisal Specialist Hospital and Research Centre revealed 7 patterns of which 5 were long and 2 short (Fig. 1). One of the long electrophoretotypes (A) dominated for the whole investigation period. No specific eletrophoretotype correlated with patient age. Mixed infection was also identified (Fig. 2). The greatest variations in morbidity were found in segments 2, 3, 6, 8 and 9; segment 1, 4 and 10 showed most unchangeable mobility. The distribution of electrophoretotypes showed there were 159 with pattern A, 21 with B, 3 with C, 6 with D, 2 with E, 2 with F and 3 with pattern G.

Analysis of patients showed that 77% of infections were in children 5 years or less. There were also a large number of infections in children under 1 year old (35%) and this may be due to a greater number of immunocompromised patients at this hospital (Table 1). There was also a seasonal variation with more infections in the cooler months.

This simple method was found to separate rotaviral RNA bands. However, it was found that by increasing the current to 200 mA separation of bands could be improved (Fig. 3). Using this, modification gels could be run for 5 hours and stained the same day. Any increase of current above this caused overheating and buckling of the gels. Also, by using a 1 mm thick gel instead of a 1.5 mm thick gel also improved the separation of the bands slightly. Initially gels were produced with a 3% stacking gel, however, this did not offer any advantages and so a continuous 8% gel was used subsequently, which also improved the rapidity of the results.

**Discussion** Rotavirus has a global distribution and affects mainly infants aged 6-24 months, with a peak incidence at 9-12 months. The prevalence has been determined mainly from hospital based
studies which have shown rotavirus in up to 50% of patients with acute enteritis. Rotavirus is most prevalent during the winter months in temperate regions but seasonal variation is less evident in tropical regions.

Gastroenteritis among children in the Middle East is common. Dutta et al. reported that in children under 5 years in Bahrain, rotavirus was the most common enteropathogen, representing 69% of the total positives. It was detected most frequently in the age group 6-11 months. Prevalence rates of 10 to 46% have been found in Saudi Arabia. In this study the prevalence rate was 12% and 77% of rotavirus infections were in children 5 years or less. There was an indication of seasonal variation with more infections in the cooler months. The fact that approximately 75% of admissions are from patients outside Riyadh indicates that these electropherotypes are found throughout the Kingdom.

Epidemiology of eletrophoretic patterns of segmented viral RNA by polyacrylamide gel electrophoresis is another useful tool in the epidemiology of rotavirus infections. The pattern
and range of eletrophoretotypes have differed greatly throughout the world. Studies in Bangladesh have shown 11 migration patterns\textsuperscript{13} whereas 108 eletrophoretotypes have been observed in England.\textsuperscript{14} Of the rotavirus positive samples 5 had long eletrophoretic pattern characteristics of human rotavirus subgroup 2 and only two short patterns were observed. These results are similar to a study in Tbilisi\textsuperscript{15} where 7 eletrophoretotypes were also noted over an 18 month period.

Mohammed et al\textsuperscript{16} using enzyme-immunoassay and monoclonal antibodies have carried out subgrouping and serotyping of rotaviruses in Saudi Arabia. They found that subgroup II was predominant and represented 61.3\% of cases subgrouped. Subgroup I comprised 26.3\% of cases. All subgroup I were of serotype 2 and all subgroup II belonged to serotypes 1 (54.7\%) and 4 (9.4\%). The remainder were untypeable. Serotyping of 355 rotaviruses revealed a distribution profile of serotype 1, 53.5\%; serotype 2, 6.8\%; serotype 3, 5.9\%; and serotype 4, 22.8\%. The eletrophoretotypes in this study were also mostly indicative of subgroup II and serotypes 1, 3 and 4.

The high prevalence of rotavirus shows the need to combat the spread of this virus through vehicles such as water, hands, fomites and food, and especially the nosocomial transmission of the infection. Such measures will greatly help to contain this relatively resistant and ubiquitous virus. Further epidemiological studies nationwide will also be useful for future vaccine development.

Table 1 - Characteristics of 200 diarrheal patients with rotavirus infection at KFSH & RC

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Number</th>
</tr>
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<tbody>
<tr>
<td>less than 1</td>
<td>70</td>
</tr>
<tr>
<td>1-5</td>
<td>85</td>
</tr>
<tr>
<td>6-19</td>
<td>23</td>
</tr>
<tr>
<td>20-55</td>
<td>17</td>
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<tr>
<td>Over 55</td>
<td>5</td>
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<tr>
<th>Male:Female</th>
<th>111 : 89</th>
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</table>

| Inpatient:Outpatient | 88 : 112 |

References
الغرض من الدراسة:

تحديد الخصائص الوبائية للأنيات المصلية للفيروسات الأمريكية باللبنان العربي بألمانيا السعودية.

التصميم:

أجريت دراسة تحليلية لمراجعة الفيروسات المشتركة في 200 مريض حاملين للفيروس الأمريكي باستخدام الرحلات الكهربائية للأكريلاميد الهلامي المتعدد (PAGE).

الموقع:

مستشفى الملك فصل التخصصي ومركز الأبحاث، الرياض، المملكة العربية السعودية.

الموضوع:

توليد امرأة مريضة مصابة بإسهال ناجم عن الفيروس الأمريكي.

النتائج:

وجدت سبعة أنواع خمسة منها طويلة وأثنان قصيرة. أظهر نموذج التوزيع أن أحد الأشعة الطويلة كان سائدا في 159 مريضا. كانت غالبية أعمار المرضى بالعمر 6 (159 مريضا) دون تسع سنوات. بلغ عدد المرضى البالغ عددهم 111 مريضا، و98.2% من المرضى الذين تجاوزوا 88 مريضا والداخليين 88 مريضا، مع نسبة 20% من المرضى الذين لم يكونوا من مناطق مختلفة من المملكة.

الخلاصة:

ظهرت الدراسات الكبرى في المحافظة على متوسط 6، 7، 8، 9، 10، و11. لم تجد علاقة بين أعمار وأجناس المرضى. ينبغي إجراء تعديلات في الوسائط الحالية وتكافه الهلام لتحسين عزل الشراط.