Serotyping of Group A Rotaviruses in Egyptian Neonates and Infants Less than 1 Year Old with Acute Diarrhea

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Received 21 January 1997/Returned for modification 8 April 1997/Accepted 19 August 1997

Group A human rotavirus G serotypes were detected in stool specimens from neonates and infants with and without acute diarrhea in Cairo by using monoclonal antibodies in an enzyme-linked immunosorbent assay. Serotypes G1 and G4 predominated in all age groups. Mixed (G1 plus G4) and nontypeable specimens represented 16.1 and 38.7% of the total number serotyped, respectively.

Of 1.3 billion cases of acute diarrhea in children under the age of 5 years (14), more than 140 million cases were due to rotavirus, and 1 million children died from the effects of rotavirus infection in the Third World in 1986 (4, 11).

In a hospital-based study in Cairo (17), rotavirus was detected in 33% of infants with acute, complicated (associated with severe dehydration, active bleeding, acute renal failure, pneumonia, or seizures) diarrhea. In rural Egypt (16, 21), 44% of children experienced at least one bout of rotavirus diarrhea by the age of 3 years.

Group A human rotavirus (HRV) is the most common cause of acute gastroenteritis in infants worldwide (9). To date, seven G serotypes of group A HRV have been identified by neutralization tests; four serotypes (G1 through G4) have a global distribution.

In this study, enzyme immunoassay (EIA) has been used to detect, for the first time in Egypt, the relative frequency and temporal distribution of HRV G serotypes 1 to 4 among the community of neonates and infants with and without acute diarrhea who attended Cairo University Children’s Hospital between August 1992 and October 1993.

Patient selection and rotavirus screening. Fecal samples were collected from 20 neonates and 109 infants less than 1 year old with acute diarrhea and from 20 neonates and 30 infants without acute diarrhea, all of whom attended Cairo University Children’s Hospital between August 1992 and October 1993.

Serotyping EIA for HRV in stools. The EIA used in the present study is a modification of one that was described previously (7). Brieﬂy, ﬂat-bottomed polystyrene 96-well microtiter plates (F-8 Immunomodules; Nunc, Roskilde, Denmark) were coated with polyclonal sheep immunoglobulin G against a mixture of rotaviruses representing HRV G serotypes 1 to 4 and were incubated overnight at 4°C. A 10% (wt/vol) fecal suspension in phosphate-buffered saline (PBS) was clarified by centrifugation at 2,000×g for 10 min. Then the supernatant or control virus (Wa, DS-1, P, or VA-70 for serotype G1, G2, G3, or G4, respectively) was added to wells containing PBS-Tween 20–Ca with 2.5% (wt/vol) skim milk powder. After incubation at 37°C for 3 h, murine monoclonal antibodies (RV 4:2 for G1, RV 5:3 for G2, RV 3:1 for G3, and ST 3:1 for G4) diluted 1:100 in PBS–Tween 20–1% bovine serum albumin (BSA) were added to wells, followed by incubation at 37°C for 2 h. Horseradish peroxidase-conjugated sheep anti-mouse immunoglobulin G diluted 1:1,000 in PBS–Tween 20–1% BSA was then added, followed by incubation for 1 1/2 h. Finally, 2,2’-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) substrate solution was added. Plates were kept at room temperature for 20 to 30 min in the dark and were then read on a spectrophotometer (Titertek, Multiscan MCC 340; Flow Laboratories, Inc., McLean, Va.) by using a 417-nm-pore-size ﬁlter.

Reactions were considered positive if the optical density (OD) was at least double the OD of the negative control and was ≥0.15. For each control virus, the OD had to be 3 times background with its speciﬁc serotyping monoclonal antibody and ≤1.5 times background with a heterologous serotyping monoclonal antibody.

Cross-reactivity. If the OD of a positive reaction to a heterologous serotyping monoclonal antibody was >50% of the value for the monoclonal antibody giving the highest reaction, stool samples were further diluted to 7.5% (wt/vol) in PBS instead of 10% and wells were incubated with PBS–Tween 20–1% BSA for 1/2 h prior to addition of detector antibody (20). In this way, cross-reactivity was resolved for some specimens.

Rotavirus was identiﬁed in 64 (35.6%) of the 180 neonates and infants less than 1 year old with and without acute diarrhea who were examined by EIA for the presence of rotavirus antigen in their stools over a 15-month period from August 1992 to October 1993. Of these, 21 were neonates (15 with and 6 without diarrhea) and 43 were in the 1- to 12-month age group.

<table>
<thead>
<tr>
<th>Serotype*</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>11 (17.7)</td>
</tr>
<tr>
<td>G2</td>
<td>2 (3.2)</td>
</tr>
<tr>
<td>G3</td>
<td>4 (6.5)</td>
</tr>
<tr>
<td>G4</td>
<td>11 (17.7)</td>
</tr>
<tr>
<td>M</td>
<td>10 (16.1)</td>
</tr>
<tr>
<td>NT</td>
<td>24 (38.7)</td>
</tr>
</tbody>
</table>

* M, mixed; NT, nontypeable.
Rotavirus infection peaked from August to December. Serotype analysis. Of the 62 rotavirus-positive specimens serotyped with an EIA using monoclonal antibodies to VP7 of HRV in the present study, 11 each (17.7%) were determined to belong to serotypes G1 and G4, 4 (6.5%) belonged to serotype G3, 2 (3.2%) belonged to serotype G2, and 10 (16.1%) were mixed (recognized by both serotype G1- and serotype G4-specific monoclonal antibodies). The remaining 24 specimens (38.7%) were nontypeable with the monoclonal antibodies used in the present study. These findings are shown in Table 1.

Temporal distribution. Serotypes G1 and G4 were detected in specimens from all age groups with nearly equal frequency; in infants 6 to 12 months old, serotype G3 was the third most common type. Of the six HRV-positive specimens from asymptomatic neonates, one belonged to serotype G1, two belonged to serotype G4, and three were nontypeable. The age group from birth to 12 months was selected because in Egypt, infants in this group showed the highest incidence of rotavirus infection (16). Serotypes G1 and G4 predominated (17.7% each) in Egyptian neonates and infants less than 1 year old with and without acute diarrhea, followed by serotypes G3 (6.5%) and G2 (3.2%). Interestingly, serotypes G1 and G4 were the predominant serotypes in other countries situated between transverse lines 20 and 40°N, such as Saudi Arabia (13), Israel (8), Mexico (15), and India (5). Serotype G1 appears to be the most prevalent serotype worldwide (3).

The seasonal peak of rotavirus infection in Egypt tends to shift over consecutive years. In the present random study, this peak occurred from August to December. Rotavirus infection peaked from September to April in a previous hospital-based study (10) and from November to April in a community-based study (21).

In the present study, 45.2% of the rotavirus-positive stool specimens from neonates and infants could be serotyped. The success rate of serotyping of HRV in stools with monoclonal antibodies in an EIA system has been extremely variable, ranging from 100% in Sweden (3) to only 29% in Bangladesh (1). Likewise, 75 to 90% of rotavirus-positive specimens from inpatients at Royal Children’s Hospital in Victoria, Australia, are typeable, compared to only 40 to 50% of specimens from overseas sources (mainly Indonesia) (6). Inability to type 38.7% of the rotavirus-positive specimens in the present study may be due to the absence of epitopes recognizable by the particular monoclonal antibodies used due to strain variations or to the presence of inhibitory factors in stools (19). On the other hand, coinfection with two serotypes may explain mixed infections.

### Table 1: Age distribution of rotavirus serotypes in Egyptian neonates and infants

<table>
<thead>
<tr>
<th>Serotype</th>
<th>0-1</th>
<th>1-6</th>
<th>6-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>3</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>G2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>G3</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>G4</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>M</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>NT</td>
<td>9</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>15</td>
<td>26</td>
</tr>
</tbody>
</table>

* M, mixed; NT, nontypeable.

Of positive specimens from asymptomatic neonates, one belonged to serotype G1, two belonged to serotype G4, and three were nontypeable.

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**Development of vaccines against rotavirus infection is considered a high priority in developing countries.** Prior to the present report, the HRV serotypes circulating in the community of Egyptian neonates and infants were unknown. In this study, group A HRV G serotypes 1 to 4 were detected in stool specimens by using serotype-specific neutralizing monoclonal antibodies to VP7 in a double-sandwich EIA. The age group from birth to 12 months was selected because in Egypt, infants in this group showed the highest incidence of rotavirus infection (16). Serotypes G1 and G4 predominated (17.7% each) in Egyptian neonates and infants less than 1 year old with and without acute diarrhea, followed by serotypes G3 (6.5%) and G2 (3.2%). Interestingly, serotypes G1 and G4 were the predominant serotypes in other countries situated between transverse lines 20 and 40°N, such as Saudi Arabia (13), Israel (8), Mexico (15), and India (5). Serotype G1 appears to be the most prevalent serotype worldwide (3).

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In contrast with another study (18), only 28.5% of neonates in the present study were asymptomatic; however, most (84%) asymptomatic infections occurred in neonates. The same two serotypes (G1 and G4) predominated in typeable neonatal infections as in infections of older infants in the present study, in contrast to a single serotype, as reported previously (2).

In conclusion, serotypes G1 and G4 of HRV must be included in any candidate vaccine to be tried against rotavirus diarrhea in Egyptian neonates and infants in order for the vaccine to be successful. Such a vaccine would be cost-effective, especially because rotavirus infection persists among infants in developing countries (12). Further studies are needed to determine if specimens not typed in the present study belong to a serotype other than G1 through G4.

Special thanks are due to Barbara S. Coulson of the Gastroenterology Department, Royal Children’s Hospital, Victoria, Australia, who donated the ascitic fluids containing the monoclonal antibodies against HRV serotypes G1 to G4 that were used in our research. We also thank Richard L. Ward of the James N. Gamble Institute of Medical Research, Cincinnati, Ohio, for providing the positive-control viruses Wa, DS-1, P, and VA-70. The staff of the Central Research Laboratory at Namru-3 in Cairo, headed by M. K. Kamal, provided valuable technical guidance for the performance of the serotyping procedure.

REFERENCES


