Epidemiology of Rotavirus Gastroenteritis among Children <5 Years of Age in Morocco during 1 Year of Sentinel Hospital Surveillance, June 2006–May 2007

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Background. In anticipation of vaccine introduction, we assessed the epidemiology, burden, and genotype of infecting strains of rotavirus disease among Moroccan children hospitalized for acute gastroenteritis.

Methods. From June 2006 through May 2007, 345 children <5 years of age who had acute gastroenteritis and were admitted to 4 sentinel hospitals in different regions of Morocco were enrolled in this surveillance study, and stool specimens were tested for the presence of rotavirus with use of enzyme immunoassay. RNA from positive samples was genotyped by reverse-transcriptase polymerase chain reaction.

Results. Overall, 314 children had complete data available, and among these, 138 (44%) tested positive for rotavirus. Rotavirus infection was most common among children <24 months of age (95% of all hospitalizations for rotavirus infection). Rotavirus infection was detected year-round at all 4 sites but was most prevalent from September through January. Genotype analysis demonstrated that 30.6% of samples were G1[P8], 26% were G9[P8], 7.5% were G2[P6], 3.7% were G1[P6], and 0.7% were G2[P8]. Nucleotide sequencing analysis of G- or P-untypeable strains showed that 4.5% were G9[P8], 2.2% were G1[8], 2.2% were G2[P6], and 1.5% were G2[P4]. A high frequency of mixed infection (21%) was found, of which G1G2[P8] accounted for the majority (16.4%).

Conclusions. Rotavirus was responsible for 44% of all hospitalizations for diarrhea among young children at these 4 separate sites in Morocco. These data will help inform a decision on the introduction of rotavirus vaccine in Morocco. Continued and extended surveillance in Morocco will be important to monitor changes in the epidemiology of rotavirus disease and the impact of vaccination after introduction.

Diarrheal disease remains one of the most important global public health problems and is a leading cause of morbidity and mortality among children <5 years of age in developing countries. Annually, diarrheal disease accounts for up to 2.5 million deaths among children <5 years of age, representing 21% of all deaths in this age group [1–3].

Severe diarrhea due to rotavirus causes an estimated 527,000 deaths among children <5 years of age [4]. In Africa alone, it has been estimated that 150,000–200,000 children <5 years of age annually die of rotavirus infection [5]. New, safe, and efficacious rotavirus vaccines have been developed and introduced in several countries around the world and, when launched in Africa, will likely greatly reduce diarrhea-associated morbidity and mortality.

The population of Morocco is ~30 million, and children <5 years of age represent ~10% of the population.
According to the last national health survey, performed in 1998, diarrheal diseases represented the second leading cause of death among children <5 years of age, accounting for 6000 deaths or 33% of all-cause mortality in this age group during 1997 [6].

Very few data on illness caused by rotavirus in Morocco have been published, and these studies were conducted predominantly in only a single large hospital in Casablanca >20 years ago [7]. The genotypic characteristics of circulating isolates of rotavirus in Morocco have not yet been identified.

The objectives of this study were to describe for the first time the epidemiological profile of rotavirus gastroenteritis at 4 surveillance sites and to characterize the circulating rotavirus strains during 1 year of sentinel hospital surveillance for rotavirus diarrhea. Information on rotavirus disease burden and strain diversity is important to make decisions on the introduction of rotavirus vaccines in the national immunization program and will constitute the baseline data necessary for measuring the impact of rotavirus vaccine after its introduction.

**MATERIALS AND METHODS**

*Study sites and population.* This national surveillance program was established as part of the regional rotavirus gastroenteritis surveillance network of the eastern Mediterranean region of the World Health Organization (WHO). Sentinel hospital surveillance was established at 4 hospitals located in 4 geographically different regions of Morocco with support from the regional WHO. Two of the sites were coastal, including University Children’s Hospital in Rabat (western region) and Mohamed V Hospital in Tanger (northern region), and 2 sites were inland, including Al Farabi Hospital in Oujda (eastern region) and Prefectoral Hospital in Benimellal (central region) (Figure 1). The study population was children <5 years of age who were hospitalized for acute gastroenteritis at the selected sentinel hospitals.

*Case definition.* A case of acute gastroenteritis was defined as acute occurrence of at least 3 looser than normal or watery stools in a 24-h period or ≥2 episodes of vomiting unexplained by other reasons. Acute occurrence was defined as symptom(s) onset ≤7 days before the medical consultation.

*Surveillance methodology.* Patients were enrolled from June 2006 through May 2007. For each patient with diarrhea, health care workers collected demographic, clinical, and laboratory information on a case report form and obtained a stool specimen for rotavirus laboratory diagnosis. This form was sent by each hospital to the provincial epidemiological surveillance department, where data were entered using EpiInfo software (version 6.0). Data were sent monthly by each sentinel site to the Epidemiology and Diseases Control Department in Rabat (Central Epidemiological Surveillance Department). The Vesikari scale [8] was used to categorize the severity of episodes of diarrheal disease.

*Laboratory diagnosis of rotavirus infection.* At 3 sentinel hospitals (Oujda, Tanger, and Benimellal), stool samples were routinely sent to the local hospital laboratory for rotavirus de-
tection by use of a commercial enzyme immunoassay (EIA; IDEIA). All positive samples and 20% of the negative samples were sent quarterly to the National Institute of Hygiene, Central Virology Laboratory in Rabat, for quality control. Stool samples obtained from children at the University Children’s Hospital, Rabat, were sent to the National Institute of Hygiene for rotavirus testing by EIA (IDEIA). Rotavirus-positive samples collected from the 4 sentinel hospitals were stored at −70°C at the National Institute of Hygiene until molecular characterization.

At the US Centers for Disease Control and Prevention, all specimens positive for rotavirus by enzyme-linked immunosorbent assay were subjected to a second enzyme-linked immunosorbent assay (Rotacpclone kit; Meridian Diagnostics). Concordant samples (>97% of the samples) that were positive by both assays were subjected to G and P genotyping using previously described techniques for reverse-transcription polymerase chain reaction (RT-PCR) [9, 10].

The genotypes detected by RT-PCR were G1–G4, G9, P[4], P[6], P[8], P[9], and P[10]. Samples in which the P or G type could not be determined by RT-PCR were subjected to nucleotide sequence analysis. Fragments of the VP7 (904 base pairs) or VP4 genes (876 base pairs) were amplified using 9 con1-L and VP7-R primers [11, 12] and con2 and con3 primers [9], respectively. The nucleotide sequence of each PCR product was determined using the Big-Dye terminator cycle sequencing kit and an automated DNA sequencer (ABI 3130XL; Perkin Elmer-Applied Biosystem). The sequences were compared with rotavirus sequences in the GenBank database with use of the Blast program, and genotypes were assigned by relatedness to reference VP7 or VP4 genes.

Data analysis. Proportions were compared using the χ² test for unequal odds, and median values were compared using the Wilcoxon rank sum test. The level of statistical significance was set at 95%.

RESULTS

Hospital surveillance. From June 2006 through May 2007, 345 children <5 years of age who had acute gastroenteritis were enrolled at the 4 hospitals and provided stool samples for rotavirus screening. Of these, 314 children (91%) had complete epidemiological, clinical, and laboratory data available. Overall, 138 (44%) of the 314 children had samples positive for rotavirus by EIA. Rates of rotavirus detection at the sentinel hospitals ranged from 38% in Rabat to 48% in Oujda (Figure 1).

Clinical and epidemiological analysis. Rotavirus was detected in samples from children in all age groups tested but predominantly in samples from children <12 months of age, who accounted for 76% of all cases of rotavirus infection. The prevalence of rotavirus gastroenteritis was low during the first 3 months of age, peaked at 6–11 months of age, and decreased after 12 months of age (Figure 2).

Boys predominated among enrolled patients, compared with girls (63% vs 37% of hospital admissions; P < .05). However, the percentage of patients testing positive for rotavirus was similar (91 [46%] of 199 vs 47 [41%] of 115; P = .47)

During this period of surveillance, rotavirus infection was detected year-round at all sites, but it was particularly frequent from September through January (Figure 3). Geographically, we observed no major differences in seasonal trends among the 4 sentinel hospitals or between coastal and desert climates, nor did we observe any differences in the percentage of rotavirus positivity between children classified as living in rural districts and children living in urban districts (data not shown).

Compared with rotavirus-negative patients (median Vesikari score, 13), patients with illness due to rotavirus infection (median Vesikari score, 14) experienced slightly but significantly more severe disease (P = .03) (Table 1). No difference in severity was found among patients infected with different P or G types (data not shown). Two enrolled children died during the period of surveillance, but neither was rotavirus positive.

Rotavirus strain distribution. A total of 134 rotavirus iso-
lates were characterized for G and P genotypes by RT-PCR, and isolates untypeable for G or P were characterized by sequencing analysis (Table 2). Three G genotypes were found during this study: G1, G2, and G9. Genotype G1 (36.5%) was the most prevalent, followed by G9 (30.5%) and G2 (11.6%). Mixed infection with 2 G genotypes was detected at a high frequency (20.9%).

Two P genotypes, P[8] and P[6], were detected using RT-PCR, and P[4] genotype was detected by sequencing analysis of P-untypeable strains. P[8] (81.4%) was the most prevalent, followed by P[6] (16.4%) and P[4] (1.5%). Mixed P types were detected in only 1 positive specimen (0.7%).

The most common genotype combination of rotavirus strains was G1[P8] (33%), followed by G9[P8] (30.5%), G2[P6] (9%), G1[P6] (3.7%), and G2[P8] (0.7%). We found a high prevalence of mixed strains (21% of the total) and a high frequency of G1G2P[8] mixed infections (16.4% of the positive samples).

Statistically significant differences were found in the geographical distribution of genotypes among the 4 sentinel sites. G9 was most prevalent in Benimellal (47.8% of all isolates) and Oujda (42%), and G1 was the most common strain detected in Tanger (46%) and Rabat (33%). Mixed G1/G2 infections were detected with a high frequency in Rabat (44.4%) and

### Table 1. Characteristics of Acute Gastroenteritis (AGE)–Associated Hospitalizations of Children <5 Years of Age at 4 Sentinel Hospitals in Morocco, June 2006–May 2007

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Children with rotavirus AGE (n = 138)</th>
<th>Children with nonrotavirus AGE (n = 176)</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of episodes of vomiting per day</td>
<td></td>
<td></td>
<td>.03</td>
</tr>
<tr>
<td>0</td>
<td>6 (4)</td>
<td>24 (14)</td>
<td></td>
</tr>
<tr>
<td>1–3</td>
<td>44 (32)</td>
<td>60 (34)</td>
<td></td>
</tr>
<tr>
<td>4–10</td>
<td>87 (63)</td>
<td>90 (51)</td>
<td></td>
</tr>
<tr>
<td>&gt;10</td>
<td>1 (&lt;1)</td>
<td>2 (1)</td>
<td></td>
</tr>
<tr>
<td>No. of episodes of diarrhea per day</td>
<td></td>
<td></td>
<td>.19</td>
</tr>
<tr>
<td>&lt;3</td>
<td>25 (18)</td>
<td>39 (22)</td>
<td></td>
</tr>
<tr>
<td>4–10</td>
<td>109 (79)</td>
<td>136 (77)</td>
<td></td>
</tr>
<tr>
<td>&gt;10</td>
<td>4 (3)</td>
<td>1 (&lt;1)</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td></td>
<td></td>
<td>.88</td>
</tr>
<tr>
<td>Yes</td>
<td>38 (28)</td>
<td>46 (26)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>100 (72)</td>
<td>130 (74)</td>
<td></td>
</tr>
<tr>
<td>Duration of illness, days</td>
<td></td>
<td></td>
<td>&lt;.001</td>
</tr>
<tr>
<td>&lt;3</td>
<td>79 (57)</td>
<td>111 (63)</td>
<td></td>
</tr>
<tr>
<td>4–6</td>
<td>57 (41)</td>
<td>47 (27)</td>
<td></td>
</tr>
<tr>
<td>&gt;6</td>
<td>2 (2)</td>
<td>18 (10)</td>
<td></td>
</tr>
<tr>
<td>Dehydration</td>
<td></td>
<td></td>
<td>.24</td>
</tr>
<tr>
<td>None</td>
<td>16 (12)</td>
<td>31 (18)</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>100 (72)</td>
<td>113 (64)</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>22 (16)</td>
<td>32 (18)</td>
<td></td>
</tr>
<tr>
<td>Rehydration</td>
<td></td>
<td></td>
<td>.64</td>
</tr>
<tr>
<td>Oral</td>
<td>14 (10)</td>
<td>22 (13)</td>
<td></td>
</tr>
<tr>
<td>Intravenous</td>
<td>124 (90)</td>
<td>154 (87)</td>
<td></td>
</tr>
<tr>
<td>Median Vesikari score</td>
<td>14</td>
<td>13</td>
<td>.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> By χ<sup>2</sup> test for unequal odds, unless otherwise noted.
<sup>b</sup> By Wilcoxon rank sum test for median values.

### Table 2. Distribution of Rotavirus G and P Genotypes among Children <5 Years of Age in Morocco, June 2006–May 2007

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>44 (33)</td>
<td>5 (3.7)</td>
<td>…</td>
<td>…</td>
<td>49 (36.6)</td>
</tr>
<tr>
<td>G2</td>
<td>1 (0.7)</td>
<td>12 (9)</td>
<td>2 (1.5)</td>
<td>1 (0.7)</td>
<td>16 (11.9)</td>
</tr>
<tr>
<td>G9</td>
<td>41 (30.5)</td>
<td>…</td>
<td>…</td>
<td>41 (30.6)</td>
<td></td>
</tr>
<tr>
<td>Mixed&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23 (17.2)</td>
<td>5 (3.7)</td>
<td>…</td>
<td>…</td>
<td>28 (20.9)</td>
</tr>
<tr>
<td>Total</td>
<td>109 (81.4)</td>
<td>22 (16.4)</td>
<td>2 (1.5)</td>
<td>1 (0.7)</td>
<td>134 (100)</td>
</tr>
</tbody>
</table>

<sup>a</sup> The mixed P type found in this study was P6P8 (0.7%).
<sup>b</sup> The mixed G types found in this study were G1G2 (22%), G9G2 (2.2%), and G9G1 (1.5%).
Tanger (20.8%). Mixed G infections in Oujda included G9G1 (6.4%) and G9G2 (6.4%). The proportion of mixed G infections in Benimellal was low, accounting for only 4.8% of samples. G2 strains were found at the 4 hospital sites in different age groups. No difference in severity was found among these strains.

**Sequence analysis of P- and G-untypeable strains.** Nine P (6.7%) and 6 G (4.5%) strains that could not initially be genotyped using RT-PCR were assigned on the basis of nucleotide sequence analysis. Their VP4 and VP7 nucleotide sequences were compared with those of reference strains available in GenBank by using Blast. Thus, after sequence analysis, 4 P-untypeable strains were P[8] associated with either G9 or G1, 3 strains were P[6] associated with G2, and 2 strains were P[4] combined with G2. Sequence analysis of G-untypeable strains revealed that 4 were G9 and 2 were G1; both were associated with P[8].

**DISCUSSION**

This article presents the results of the first national hospital-based sentinel surveillance program in Morocco and documents the burden of rotavirus diarrhea and the distribution of circulating genotypes. We found that rotavirus may be responsible for almost one-half (44%) of all hospitalizations for diarrhea in children <5 years of age in Morocco, is common in both coastal and inland areas, and is particularly a burden among young children. More than 75% of episodes of rotavirus diarrhea occurred among children during their first year of life. Our estimated prevalence of rotavirus infection is higher than that in a study in Casablanca, which found that 20% of hospitalizations for diarrhea were attributable to rotavirus among children <5 years of age [7]. This is consistent with the concept that, with sanitation improvements, morbidity associated with diarrhea caused by bacteria has decreased more quickly than has morbidity associated with diarrhea caused by rotavirus over time, leading to an increase in the prevalence of rotavirus infection. During the year of sentinel hospital surveillance, rotavirus infection occurred throughout the year with a seasonal pattern typical of that seen in other temperate climates; activity peaked in autumn and winter, including a defined peak in October. This finding is similar to those of a long-term study in Casablanca in 1988 [7] and to seasonal trends seen in other North African countries, such as Tunisia [13] and Egypt [14]. During this surveillance study, male children predominated among enrolled patients, compared with female children; we have no clear explanation why more male children than female children were enrolled in our surveillance, although we speculate that families may be more likely to take male children than female children to the hospital.

This study is also the first to document the circulating genotypes in Morocco and extends the data for rotavirus strain diversity in Africa, especially in North Africa, where only data from Libya and Tunisia have been published [13, 15]. G1 rotavirus strains were the most common in this study, which reflects the global predominance of these strains [16]. Genotype G9, which is a globally emergent strain, was reported in this study as the second most common genotype identified. G9 rotavirus has been reported from various studies in Africa [15–20] and around the world [16, 21]. Genotype P[8] was, by far, the most predominant; genotype [P4], which was detected by nucleotide sequencing of untypeable strains accounted for 1.5%; and genotype P[6] had a prevalence of >14%, significantly higher than its estimated global prevalence of 4%–5% [16, 22]. This finding is consistent with the significantly higher detection rate of P[6] in many regions in Africa [20]. The degree of vaccine-generated cross-protection between different G and P types is not known, but a high prevalence of P[6] antigen, which is not included in either of the commercially available vaccines, highlights the need for strain surveillance before and after vaccine introduction. We found an unusually high proportion (16.4%) of G1G2P[8] mixed infections and a single G2P[8] reassortant strain. Such mixed infections could predispose to reassortment of viruses and may pose challenges for the effectiveness of rotavirus vaccines. High proportions of mixed infection have been documented in many developing countries [23–27]. Of interest, mixed infections overall are less common in populations in developed countries [28, 29], although with some exceptions. Studies conducted in Denmark in 2002 [30] and Ireland in 2001–2004 [31] showed high proportions of mixed infections (20% and 28.2%, respectively). In the current study, we found notable variations in the proportions of mixed infections among the 4 sentinel hospitals. For example, G mixed infection was detected with a higher frequency at 2 hospitals (Rabat and Tanger), but we have no clear explanation as to why this occurred. We detected only a single G2[P8] strain. The remainder were mixed infections (G1G2P[8]) that may have been primarily mixed infections between G2[P6] (or G2[P4]) with G1P[8] strains.

Although no children died in our study, hospitalization data can be used as a proxy to estimate rotavirus-associated mortality nationally and globally [2] by applying the percentage of hospitalizations for diarrhea due to rotavirus among all deaths due to diarrhea. The WHO rotavirus-associated mortality estimate for Morocco is based on a regional estimate of 30% of hospitalizations for diarrhea due to rotavirus; therefore, the same proportion of deaths due to diarrhea is likely to be attributable to the virus, for an estimated total of 1400 deaths due to rotavirus infection in Morocco in 2004 [4]. However, if the proportion of hospitalizations for diarrhea due to rotavirus among children is similar to that of death due to diarrhea, our data on hospitalizations suggest that 40%–50% of deaths due to diarrhea among children <5 years of age in Morocco may be attributable to rotavirus. Unfortunately, we do not have recent routinely collected data on the frequency or etiology of deaths
due to all-cause diarrhea among children in Morocco. A national study in 1998 indicated that 6000 children <5 years of age died of diarrhea during 1997 [6]; however, because the number of deaths due to diarrhea and the proportion of these deaths that are attributable to rotavirus have likely changed since 1997, we cannot reliably apply the percentage of rotavirus infection from this study to this estimate of mortality associated with all-cause diarrhea from >10 years earlier to estimate the number of deaths due to rotavirus infection in Morocco.

The findings of this study highlight the need for continuing and extending surveillance at other sites in Morocco to monitor changes in the epidemiology of rotavirus disease and to identify dynamic shifts in circulating rotavirus strains. These data will be critical for making an informed decision about the introduction of rotavirus vaccine in Morocco and will provide a baseline against which the impact of vaccine introduction can be measured in the future.

Acknowledgments

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References