Original Article

Rotavirus-Associated Acute Gastroenteritis Hospitalizations among Japanese Children Aged <5 Years: Active Rotavirus Surveillance in Mie Prefecture, Japan

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SUMMARY: Two effective vaccines for rotavirus infection will be available near future in Japan and data on the burden of rotavirus disease and the circulating rotavirus strains are urgently needed. To obtain these data, we set up active rotavirus hospitalization surveillance in three cities, Tsu, Matsusaka, and Ise in Mie Prefecture, Japan. From November 1, 2007 through October 31, 2009, we enrolled children <5 years of age who were hospitalized with a diagnosis of acute gastroenteritis (AGE) and collected information on age, sex, month of admission, city of residence, and symptoms at the time of hospitalization. Stool samples were also obtained for rotavirus testing and genotype investigation. Rotavirus infection accounted for approximately 40% to 50% of hospitalized AGE cases in each city, and approximately 63% of those hospitalized were 2 years of age or younger. Matsusaka had the highest incidence rate at 4.7 rotavirus hospitalizations per 1,000 children <5 years of age (95% confidence interval [CI]: 3.6–5.9), followed by Tsu City (4.4 per 1,000; 95% CI: 3.6–5.3), and Ise City (2.8 per 1,000; 95% CI: 2.0–4.0). The most dominant rotavirus genotype was G3P[8], which accounted for 73.1% of cases. Our findings confirm the substantial health burden of rotavirus AGE hospitalization among Japanese children <5 years of age.

INTRODUCTION

Acute gastroenteritis (AGE) is among the leading causes of childhood morbidity and mortality worldwide, accounting for an estimated 1.3 million deaths in children <5 years of age (1). Rotavirus is the leading cause of severe childhood AGE worldwide, and it is estimated to account for more than half a million deaths each year (2-4). Although deaths due to rotavirus infection are uncommon in industrialized countries, rotavirus infection remains an important cause of morbidity in young children in developing countries (5,6).

Since 2006, two safe and effective rotavirus vaccines, Rotarix® (GlaxoSmithKline Biologicals, Rixensart, Belgium) and RotaTeq® (Merck and Company, Whitehouse Station, N.J., USA), have been licensed in >100 countries worldwide (7,8). Both vaccines have undergone clinical testing in Japan. Rotarix® was approved for licensure in July 2010 (9), and licensure approval for RotaTeq® was filed in 2010 (10). Since we are on the verge of rotavirus vaccine introduction in Japan, data on the burden of rotavirus disease and circulating rotavirus strains are urgently needed to help inform policy decisions on the use of rotavirus vaccines and to help evaluate the impact of vaccine on rotavirus disease burden after their introduction.

Previously, our group studied the disease burden of rotavirus infection in two sentinel hospitals in central Japan from 2003 to 2007 (11). This retrospective evaluation relied on the results of rotavirus testing as part of routine clinical care, and our findings may have been biased since rotavirus testing was performed at the discretion of the physician, and the factors that prompted testing could not be fully ascertained. In addition, because fecal specimens were not available, we were unable to characterize rotavirus strains in positive specimens.

Since November 2007, we conducted active surveillance in the same area to gain further understanding of rotavirus infection in Japan and to overcome some of the limitations of our previous work. This paper describes the findings from our active surveillance of rotavirus AGE hospitalizations in central Japan, including data on circulating rotavirus strains.
MATERIALS AND METHODS

Study hospitals: We conducted active surveillance of AGE hospitalizations at five hospitals in four cities in Mie Prefecture, Japan: Mie National Hospital and Mie Chuo Medical Center in Tsu City, Mie Chuo Medical Center in Matusaka City, Yamada Red Cross Hospital in Ise City, and Suzuka Chuo Hospital in Suzuka City. We chose these hospitals because patients with severe diarrhea requiring hospitalization in these cities are exclusively admitted to these hospitals, thereby allowing us to calculate the rates of rotavirus AGE hospitalizations for these populations. At Suzuka Chuo Hospital, which also admits patients from other cities, we included only patients who resided in the cities of Tsu, Matusaka, and Ise. Institutional review board approval was obtained from every hospital.

Patient data: From November 1, 2007 through October 31, 2009, we enrolled children <5 years of age who were hospitalized with a diagnosis of AGE, which was defined as three or more watery stools or at least one episode of vomiting during the 24 h prior to hospital admission. For all AGE patients, we obtained information on age, sex, month of admission, city of residence, and symptoms at the time of hospitalization.

Laboratory data: Stool samples were obtained for rotavirus testing. For those children from whom we were unable to collect stool samples at the time of hospitalization, we attempted to collect stool samples during hospitalization. For those children from whom we were unable to collect any stool samples, we collected only personal data. At the two hospitals in Tsu City and at Matusaka Chuo General Hospital, stool samples were tested using a commercially available enzyme immunoassay (Rapid-testa; Daiichi Pharmaceutical Co. [now Sekisui Medical Co., Tokyo, Japan]); the sensitivity and specificity of this immunoassay are approximately 92% and 100%, respectively, when compared with electron microscopy (12). At Yamada Red Cross Hospital, samples were tested with a commercial latex agglutination test (Rotaclone Adeno Dry; Daiichi Pharmaceutical Co. [now Sekisui Medical Co.]); the sensitivity and specificity of this test are approximately 94% and 99%, respectively, when compared with electron microscopy (data from package insert). During the first year of surveillance, we performed rotavirus testing on all stool samples using a microplate Enzyme-Immunoassay (Rapid-testa; Daiichi Pharmaceutical Co. [now Sekisui Medical Co.], Tokyo, Japan); the sensitivity and specificity of this immunoassay are approximately 92% and 100%, respectively, when compared with electron microscopy (12).

For G typing, the full-length VP7 gene was amplified using a pair of primers, 5'-GGCTTTAAAAAGAGAAGTTTCG-3' (T31) and 5'-GGTCACATGCATAATTCATGAG-3' (T32), corresponding to the common ends of the VP7 gene, respectively. In the second PCR amplification, the T32 primer was used along with G1, G2, G3, G4, G8, and G9 genotype-specific primers in order to identify G types. For P typing, a pair of primers, 5'-TTGCTTCGGTTTATGAGACAGAC-3' and 5'-CAAATTCATCCACAGCGAC-3', corresponding to the common ends of the VP8 gene, including nucleotides 11–32 and 1,072–1,094, respectively, was used for the first amplification; a mixture of primers specific to each of the variable regions: P[8], P[4], P[6], and P[9], along with a primer corresponding to nucleotides 11–32 were employed for the second amplification. PCR products were electrophoresed in 1% agarose gels, and stained with ethidium bromide.
**RESULTS**

**Demographic data:** The distributions of all AGE cases, rotavirus-positive AGE cases, and %rotavirus AGE cases from each of the three cities are summarized according to age, sex, and gender in Table 1. Rotavirus infection accounted for approximately 40% to 50% of hospitalized AGE cases in each city. In Tsu City, 229 children <5 years old were hospitalized with AGE during the study period. Among these children, 111 (48.5%) tested positive for rotavirus. In Matsusaka City, 148 children were hospitalized with AGE, and 70 (47.3%) tested positive for rotavirus. In Ise City, 84 children were hospitalized with AGE, and 33 (39.2%) tested positive for rotavirus. The cases by city did not differ significantly in the distribution of sex; however, most of them were hospitalized in the first half of the year.

**Age distribution of rotavirus AGE hospitalizations:**
The cumulative age distribution of children <5 years of age hospitalized for rotavirus AGE in Tsu City, Matsusaka City, and Ise City is shown in Fig. 1. A similar pattern was observed in each of the three cities. Of all rotavirus AGE hospitalizations in this study, only 4.2% (9/214) occurred in children <6 months of age. Approximately 24.8% (53/214) of the hospitalized children were <1 year of age, and 63.5% (136/214) were <2 years of age.

**Seasonality of rotavirus AGE hospitalizations:** The seasonal trends in rotavirus-positive AGE hospitalization in the three cities are shown in Fig. 2. The peak months for rotavirus hospitalization were February through May, and were similar among the three cities. Rotavirus hospitalizations returned to baseline from July to November. In Tsu City, the number of cases during the months with peak rotavirus activity decreased from 27 cases in 2007/2008 to 14 in 2008/2009, while in Matsusaka City and Ise City, the number of cases increased from 12 to 19 and 6 to 8, respectively.

**AGE admissions and rotavirus testing:** In Tsu City, rotavirus testing was not conducted in 43 (18.7%) of the children hospitalized with AGE because stool samples were not collected. Similarly, testing was not conducted for 22 (14.9%) and 8 (9.5%) children hospitalized with AGE in Matsusaka City and Ise City, respectively. Therefore, to obtain a more precise number of cases with rotavirus AGE, we applied age-specific and admission month-specific proportions of rotavirus-positive tests to the corresponding number of untested children hospitalized in each age group and each month. Using this method, we estimated 10 additional cases of rotavirus AGE in Tsu, 11 additional cases in Matsusaka, and 3 additional cases in Ise.

**Incidence and risk of rotavirus AGE hospitalization:**
Based exclusively on the number of laboratory-confirmed rotavirus cases identified through active surveillance in the three cities, Matsusaka City had the highest incidence rate at 4.7 rotavirus hospitalizations per 1,000 children <5 years of age (95% CI: 3.6–5.7), followed by Tsu City with 4.4 per 1,000 (95% CI: 3.6–5.3), and Ise had 3.1 per 1,000 (95% CI: 2.1–4.3) (Table 2). When the incidence rates for rotavirus AGE were adjusted accounting for the estimated number of rotavirus-positive cases among untested children, Matsusaka had 81 cases, with an incidence rate of 4.8 per 1,000 (95% CI: 4.3–6.7), Tsu had 121 cases, with an incidence rate of 4.8 per 1,000 (95% CI: 4.0–5.7), and Ise had 36 cases, with an incidence rate of 3.1 per 1,000 (95% CI: 2.1–4.3). This means that by 5 years of age, 1 in 37 children born in Matsusaka, 1 in 40 children born in Tsu, and 1 in 61 children born in Ise will be hospitalized with rotavirus AGE.

Extrapolation of the mean incidence of rotavirus hospitalization in the three cities to the national popula-

<table>
<thead>
<tr>
<th>Admission month</th>
<th>Tsu</th>
<th>Matsusaka</th>
<th>Ise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan.–Mar.</td>
<td>93</td>
<td>65</td>
<td>70</td>
</tr>
<tr>
<td>Apr.–Jun.</td>
<td>59</td>
<td>45</td>
<td>76</td>
</tr>
<tr>
<td>Jul.–Aug.</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oct.–Dec.</td>
<td>70</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>Tsu</th>
<th>Matsusaka</th>
<th>Ise</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5</td>
<td>6</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>6–11</td>
<td>51</td>
<td>28</td>
<td>55</td>
</tr>
<tr>
<td>12–23</td>
<td>87</td>
<td>40</td>
<td>46</td>
</tr>
<tr>
<td>24–35</td>
<td>51</td>
<td>29</td>
<td>57</td>
</tr>
<tr>
<td>36–59</td>
<td>34</td>
<td>11</td>
<td>32</td>
</tr>
<tr>
<td>Total</td>
<td>229</td>
<td>111</td>
<td>48</td>
</tr>
</tbody>
</table>

**Table 1. Characteristics (age, sex, admission month) of children hospitalized for acute gastroenteritis by city (Tsu, Matsusaka, and Ise) and its distribution of percentage rotavirus positive**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Tsu</th>
<th>Matsusaka</th>
<th>Ise</th>
</tr>
</thead>
<tbody>
<tr>
<td>male</td>
<td>118</td>
<td>63</td>
<td>53</td>
</tr>
<tr>
<td>female</td>
<td>111</td>
<td>48</td>
<td>43</td>
</tr>
</tbody>
</table>

*P = 0.13*  
*P = 0.13*  
*P = 0.91*

*Mantel-Hansel chi square test.  
AGE, acute gastroenteritis; RV, rotavirus.
distribution data for children <5 years of age (5,578,087) yielded an annual estimate of approximately 30,000 hospitalizations for rotavirus AGE among Japanese children <5 years of age.

Distribution of genotypes: Of the 214 rotavirus-positive cases, 160 (74.4%) stool samples were randomly selected for G and P typing by nested PCR (Table 3). The most dominant genotype was G3P[8], which accounted for 73.1% of specimens. The second highest was G1P[8] (14.4%), followed by G9P[8] (10.0%). This trend was consistent among the three cities. The distribution of G2P[4] strains was very low.

**DISCUSSION**

In the three cities in central Japan included in our study, active surveillance showed that rotavirus accounted for approximately 40%–50% of hospitalized AGE cases among children <5 years of age, with the peak of rotavirus AGE hospitalization occurring between February and May. Based on the incidence rates of rotavirus AGE hospitalization ranging from 3.3–5.8 per 1,000 children in Tsu City, Matsusaka City, and Ise City, we estimated that 1 in 40 children born in Tsu, 1 in 37 children born in Matsusaka, and 1 in 61 children...
vaccination.

demonstrated the potential health benefits of rotavirus AGE among Japanese children and confirmed a substantial health burden of hospitalization in a different region of Japan (15). Therefore, our study is the same as those reported by Ito et al. in a study conducted following routine childhood vaccination in socioeconomic settings similar to Japan and from observational studies conducted following routine childhood vaccination in the United States have demonstrated sustained protection against most cases rotavirus AGE hospitalization. Second, approximately 40% of all rotavirus AGE hospitalizations in children under the age of 5 occurred in the second year of life, while another 37% occurred in children between 2 and 5 years of age, thereby demonstrating the need for sustained protection from vaccination in the first few years of life. In this regard, it is reassuring that data from long-term follow-up of children in the pivotal clinical trials of rotavirus vaccines conducted in socioeconomic settings similar to Japan and from observational studies conducted following routine childhood vaccination in the United States have demonstrated sustained protection by vaccination until 2–3 years of age (20).

We found that G3P[8] was the most dominant genotype (73.1%) in this region during the study period. According to the Infectious Agents Surveillance Report, in Japan, G3 was the dominant strain during the 2008/2009 season, while G9 was dominant during the 2007/2008 season (21). According to reports from local laboratories in other parts of Japan, the dominant strain during the same seasons was different. For instance, the prefectural laboratory in Aichi, which joins Mie, reported that G9 was dominant in 2007 and 2008 and G1 was dominant in 2009. This indicates that there may be both regional and temporal variations in rotavirus strain predominance, which will be important to consider when assessing the impact of vaccination on rotavirus strains. Furthermore, continued rotavirus strain surveillance after vaccine introduction will allow for epidemiologic assessment of vaccine effectiveness against the range of currently circulating strains and other potential strains that may be identified in the future.

Some limitations should be considered when interpreting our results. First, it is possible that some of the children living in the three cities sought care for AGE at other hospitals in adjacent cities. If so, this would have led us to underestimate the incidence of rotavirus AGE hospitalization. Second, due to substantial variation in recording practices at the surveillance hospitals, we were unable to determine the age and sex of patients or the specific details of their hospitalization. This could potentially result in misclassification of cases and affect the accuracy of our estimates. Therefore, it is important to consider these limitations when interpreting our findings and further research is needed to address these issues.

### Table 2. Population-based figures for hospitalization for rotavirus-positive acute gastroenteritis: Tsu, Matsusaka, and Ise cities in Mie Prefecture, Japan, November 2007–October 2009

<table>
<thead>
<tr>
<th>City</th>
<th>Population&lt;5</th>
<th>Crude no. of hospitalization by rotavirus AGE (for 2 years)</th>
<th>Crude incidence OR (95% CI) per 1,000 person-years</th>
<th>Adjusted no. of hospitalization by rotavirus AGE (for 2 years)</th>
<th>Adjusted incidence OR (95% CI) per 1,000 person-years</th>
<th>Risk of hospitalization per child by age 5&lt;sup&gt;7b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tsu</td>
<td>12,549</td>
<td>111</td>
<td>4.4 (95% CI: 3.6–5.3)</td>
<td>121</td>
<td>4.8 (95% CI: 4.0–5.7)</td>
<td>1 in 40</td>
</tr>
<tr>
<td>Matsusaka</td>
<td>7,487</td>
<td>70</td>
<td>4.7 (95% CI: 3.6–5.9)</td>
<td>81</td>
<td>5.4 (95% CI: 4.3–6.7)</td>
<td>1 in 37</td>
</tr>
<tr>
<td>Ise</td>
<td>5,775</td>
<td>33</td>
<td>2.8 (95% CI: 2.0–4.0)</td>
<td>36</td>
<td>3.1 (95% CI: 2.1–4.3)</td>
<td>1 in 61</td>
</tr>
</tbody>
</table>

<sup>7b</sup>: Numbers are from 2005 Japanese census data.

<sup>7c</sup>: By Fisher’s exact test.

<sup>7d</sup>: Birth cohort in 2007 for Tsu (2,434), Matsusaka (1,517), and Ise (1,097) are used.

### Table 3. Genotype distribution of rotavirus acute gastroenteritis hospitalization cases in Mie Prefecture (Tsu, Matsusaka, and Ise cities), Japan, 2007–2009

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tsu</th>
<th>Matsusaka</th>
<th>Ise</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1P[8]</td>
<td>16</td>
<td>3</td>
<td>4</td>
<td>23 (14.4)</td>
</tr>
<tr>
<td>G2P[4]</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>4 (2.5)</td>
</tr>
<tr>
<td>G3P[8]</td>
<td>56</td>
<td>35</td>
<td>26</td>
<td>117 (73.1)</td>
</tr>
<tr>
<td>G9P[8]</td>
<td>7</td>
<td>0</td>
<td>9</td>
<td>16 (10.0)</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>40</td>
<td>39</td>
<td>160 (100)</td>
</tr>
</tbody>
</table>

born in Ise will be hospitalized with rotavirus AGE by the age of 5 years. By extrapolating our calculated incidence to the Japanese population, we estimate that 30,000 Japanese children <5 years of age are hospitalized annually for rotavirus AGE. Applying the newly reported average direct medical costs for a single rotavirus AGE hospitalization in Japan ($221,000 or US$1,888) (15), the direct costs of 30,000 rotavirus hospitalizations would be approximately ¥6.6 billion or US$57 million. The estimated number of hospitalizations and the estimated direct costs in our study are the same as those reported by Ito et al. in a study conducted in a different region of Japan (15). Therefore, our study confirmed a substantial health burden of hospitalization for rotavirus AGE among Japanese children and demonstrated the potential health benefits of rotavirus vaccination.

The 40%–50% rotavirus detection rate observed among children hospitalized with AGE is similar to figures reported in other studies from high-income countries (6,16). The incidence rates of rotavirus AGE hospitalization in the three study cities are also comparable with rates reported from other industrialized countries such as Sweden, Denmark, and the United States at 3.7, 2.4, and 2.7 hospitalizations per 1,000 person years, respectively (17–19). The rates of rotavirus hospitalization of children in Tsu and Ise from the present active surveillance were also quite similar to those estimated in our previous retrospective study (4.9 [95% CI: 3.4, 7.0] and 3.8 [95% CI: 2.8, 5.1], respectively). This indicates that in a setting of limited resources, conducting similar retrospective studies in other areas may provide comparable estimates of the true disease burden of rotavirus AGE hospitalizations.
unable to collect detailed data on the clinical features and severity of AGE cases. Third, clinical protocols for the admission of patients with AGE may have differed between hospitals, and possibly lead to underestimates of disease. For example, in some hospitals, patients who might otherwise have been admitted for treatment at a different hospital were treated with intravenous fluids in the emergency room and/or outpatient clinic due to staffing issues. Finally, data from these three cities may not be representative of all of Japan, which could limit the generalizability of our results to the national population. However, the consistency of retrospective and prospective study in same area demonstrate the substantial burden of rotavirus among children hospitalized with AGE in Japan and the potential value of rotavirus vaccination.

In conclusion, our findings confirm the substantial health burden of rotavirus AGE hospitalization among Japanese children <5 years of age. These data should help pediatricians and policy makers assess the potential benefits of introducing a rotavirus vaccine in Japan. The fact that the majority of rotavirus disease occurred in children >6 months of age, which is after the completion of routine rotavirus vaccination, indicates that most rotavirus AGE hospitalizations should be preventable by the standard rotavirus vaccination schedule. Implementing the new rotavirus vaccines, which have a demonstrated efficacy of 80%-98% against severe rotavirus disease in high-income settings (8,22,23) and have already shown tremendous public health impact in many countries that have implemented routine childhood vaccination, could prove to be a potentially useful strategy to improve and protect the health of children in Japan.

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Conflict of interest None to declare.

Appendix Space limitations preclude the inclusion as authors of the following members of the Rotavirus Epidemiology Study Group: Drs Toshiaki Ihara, Takao Fujisawa, Shigeru Suga, Ryouji Ichimi, Kazutoyo Asada, Takaaki Tanaka (Matsusaka Chuo General Hospital), Masaru Ido, Shigeki Tanaka (Mie Chuo Medical Center), Kazutoyo Asada, Takaaki Tanaka (Matsusaka Chuo General Hospital), Masakazu Inoue, Masamune Higashikawa, Takashi Fujwara (Yamada Red Cross Hospital), and Ms. Manami Negoro and Ms. Maiko Kinoshita (Mie National Hospital).

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