Detection of Viruses in the Stools of Thai Children with Gastroenteritis by Electron Microscopy

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OBJECTIVES:

1. To evaluate the frequency with which viruses can be visualized in stools of children with gastroenteritis in Bangkok.

2. To obtain serum and stool specimens from children with gastroenteritis to be used in the development and evaluation of assays for the diagnosis of viral gastroenteritis.

BACKGROUND: With the past 5 years a variety of viruses (rotaviruses, parvo-like viruses, non-cultivatable adenoviruses) have been implicated as pathogens in acute non-bacterial gastroenteritis of humans (1). Evidence for infection with rotaviruses has been reported, in several large series, in over 40% to almost 90% of children with gastroenteritis (2–4). Although the overall role of viruses in gastroenteritis in adults is less well defined, both rotaviruses and parvo-like viruses appear to be well established as causes of acute outbreaks (5–8).

In 1976, workers at the IVR in Bangkok in collaboration with investigators from Ramathibodi Hospital detected rotaviruses in 39 of 100 stool specimens from small children by electron-microscopy (9). The results of this study were not published and no attempt was made to isolate and characterize the observed particles.

On the basis of preliminary cross-neutralizing antibody tests of rotavirus strains grown in LLC-Mk2 tissue cultures and detected by the fluorescent-focus method, Flewett et al (10) reported at least 2 and probably four serotypes of rotaviruses. Given the existence of multiple serotypes and the observation that

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pre-existing group-specific serum antibody is not protective, the level of
immunity to rotaviruses in a community must be verified by neutralization of
the locally prevalent serotypes. To do this, local strains of rotavirus must
first be identified and collected.

METHODS:

Patients: Children less than 2 years old presenting to the Pramongkut-
klao Hospital or Children's Hospital clinics between 1 December 1978 and 1
April 1979 were candidates for study if they had gastroenteritis (nausea,
vomiting, diarrhea) as judged by the hospital physician. No other restric-
tions were placed on entry into the study. Specifically, severity of disease
was not a criterion.

Clinical specimens collected: The following were obtained from each
patient: an acute blood specimen, an acute stool or diaper scraping, a 10-14
day convalescent blood, and a simple questionnaire.

Laboratory studies (Acute stool): 1. Routine culture for easily identified
bacterial enteric pathogens (Salmonella and Shigella). 2. Electron microscopy:
The method of Zissis et al. (11) was followed. Briefly, stool specimens were
suspended about 30% (v/v) in PBS and centrifuged at 10,000 rev/minute for 30
minutes at 4°C in conical centrifuge tubes. The supernatants were again
centrifuged at 10,000 rev/min for 30 minutes at 4°C. Five ml of the clarified
supernatant were centrifuged at 250,000 g for one hour in a Beckman centrifuge
(SW 50/rotor), and the pellet resuspended in 5 drops of distilled water.
Electron-microscope grids, covered by a formular membrane, were placed on a
drop of the suspension for 15 minutes, and the virus allowed to absorb to the
membrane. After the membranes were dried, they were rinsed four times in a drop
of saline, blotted dry after each dip, then negatively stained with 2% (v/v)
uranyl acetate or sodium phosphotungstate. After drying, the grids were
examined with the Hitachi (H-11C) electron-microscope at the IVR.

RESULTS: Virus particles were detected in 13 of the 44 specimens examined by
electron-microscopy. Table 1 summarizes the number of different morphologic
types seen.

Of twelve specimens collected at the RTAH, none were positive for rota-
viruses, one was positive for adenovirus. Of the 32 specimens collected at
Children's Hospital, 9 (28%) were positive for rotaviruses, one for adenovirus,
and one for mixed adenovirus and parvovirus-like particles. The number of rota-
virus particles visualized varied from <1 to more than 100 per grid square.

REFERENCES


Table 1. Virus-like Particles Visualized in Stool Specimens from Bangkok Children with Gastroenteritis.

<table>
<thead>
<tr>
<th></th>
<th>Age (months)</th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
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<tr>
<td></td>
<td>0-5</td>
<td>6-11</td>
<td>12-17</td>
<td>18-24+</td>
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<tr>
<td>Rotavirus</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>9</td>
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<tr>
<td>Adenovirus</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>? Enterovirus</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Adenovirus + ? Parvovirus</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>No particles seen</td>
<td>14</td>
<td>9</td>
<td>3</td>
<td>5</td>
<td>31</td>
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<tr>
<td></td>
<td><strong>20</strong></td>
<td><strong>13</strong></td>
<td><strong>4</strong></td>
<td><strong>7</strong></td>
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