Incidence of Enteric Adenoviruses among Children in Thailand and
the Significance of These Viruses in Gastroenteritis

JOHN E. HERRMANN, NEIL R. BLACKLOW, DOROTHY M. PERRON-HENRY, EMILY CLEMENTS,
DAVID N. TAYLOR, and PETER ECHEVERRIA

Division of Infectious Diseases, University of Massachusetts Medical School, Worcester, Massachusetts 01655, and
Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Received 5 April 1988/Accepted 31 May 1988

In countries with temperate climates, enteric adenoviruses have been shown to be a substantial cause of
diarrheal gastroenteritis. To determine the incidence of adenovirus infection in a tropical climate, stools were
collected from children under age 7 during a 1-year period at an outpatient clinic in Bangkok, Thailand. Stools
from 1,114 children with gastroenteritis and from 947 children without gastroenteritis were tested. Each stool
was tested for adenovirus group antigen and for specific enteric adenovirus types (Ad40 and Ad41) by
monoclonal antibody enzyme immunoassays. We found that 4.4% (49 of 1,114) of children with gastroenteritis
and 1.8% (17 of 947) of children without gastroenteritis were positive for adenovirus group antigen. In tests for
specific enteric adenovirus types, 2.0% (22 of 1,114) of the tests were positive in children with gastroenteritis,
and 0.6% (6 of 947) were positive in children without gastroenteritis. There was a significant correlation ($P < 0.02$)
of gastroenteritis with nonenteric adenovirus types (27 of 1,114) as well as with specific enteric adenovirus
types ($P < 0.01$). By comparison, 19.7% of children with gastroenteritis and 0.7% of those without gastroenteritis
were positive for rotavirus infection. In the adenovirus-infected children with gastroenteritis, there were coinfections with rotavirus only in those with nonenteric adenovirus infection (7 of 27 children).
There were no significant differences in the association of bacterial or parasitic infections with either enteric or
nonenteric adenovirus infections in either group of children studied. These data demonstrate that Ad40 and
Ad41 are causes of gastroenteritis in this population, but among the spectrum of diarrheal etiologies, they may
be proportionately less important than they are in countries with temperate climates.

It is now well established that two distinct serotypes of adenovirus, types 40 and 41, are the major types involved in
the etiology of adenoviral gastroenteritis. These enteric adenoviruses have been frequently identified in the stools of
infants and young children with diarrhea in countries which have temperate climates (1, 2, 7, 8, 16, 17), and among
viruses studied, they may be second in importance only to rotaviruses (16). The percentage of cases of gastroenteritis
due to enteric adenoviruses has been variable in these countries, with an approximate range of 6 to 8% (13). Studies
concerning the incidence of enteric adenovirus have utilized either type-specific identification techniques or, more
frequently, presumptive methods. In the latter methods, electron microscopy (EM) is usually used in tandem with growth
characteristics of enteric adenoviruses in cell cultures. The most comprehensive study which utilized the type-specific
identification methods was done by Uhnoo et al. (17) in Uppsala, Sweden, who reported a 7.9% (33 of 416) incidence of
Ad40 or Ad41 in samples collected during a 1-year period. In studies using presumptive methods, Cevenini et al. (5)
found an infection rate of 8.3% (325 of 3,932) for enteric adenovirus during a 5-year period in Bologna, Italy, and
Leite et al. (12) reported a 1.9% (14 of 746) rate in a 2-year study done in Rio de Janeiro, Brazil. A study in Osaka,
Japan, which used only EM, found that 6% (23 of 395) of diarrheic samples contained adenovirus particles (14). EM
detects both enteric and nonenteric adenovirus, but in stools from symptomatic cases, it is likely that 60 to 80% of the
adenovirus detected by EM are enteric types (2, 16). Thus, use of EM alone can give a general indication of enteric
adenovirus incidence. Extensive studies by Brandt et al. (1)

* Corresponding author.

using EM alone have shown that over an 8-year period, the
detection of adenovirus particles in stools was quite variable
from year to year, ranging from 1.4 to 10.7% of the samples
examined.

Both types of enteric adenovirus have been isolated from
cases of gastroenteritis in countries with tropical climates,
but no epidemiological studies on their incidence have been
reported. In this study, we have examined 1,114 stools
collected from children with gastroenteritis and 947 stools
from children without gastroenteritis who were seen in an
outpatient clinic in Bangkok, Thailand, for the presence of
enteric and nonenteric adenoviruses. The stools were also
examined for the presence of rotavirus to determine the
relative frequency of each of the viruses as etiologic agents
of gastroenteritis in this population.

MATERIALS AND METHODS

Stool specimens. Stools were obtained from children under
age 7 during a 1-year period at an outpatient clinic in
Bangkok, Thailand. Of 2,061 stool samples collected, 1,114
were obtained from children with gastroenteritis, and 947
were obtained from children of similar ages who visited the
clinic for reasons other than gastroenteritis. Stools from
patients with gastroenteritis were tested for parasites and for
bacterial pathogens, including Salmonella spp., Shigella
spp., enterotoxigenic Escherichia coli, Campylobacter jejuni,
Plesiomonas shigelloides, and Vibrio spp., by methods
previously described (15). Stools were frozen and stored at
–70°C for later viral testing.

Detection of adenoviruses and rotavirus. Stool samples
were tested as 10% (wt/vol) suspensions in phosphate-
buffered saline (PBS), pH 7.0, for adenovirus group antigen,
Ad40, Ad41, and group A rotavirus by monoclonal antibody-
TABLE 1. Incidence of enteric adenoviruses, nonenteric adenoviruses, and group A rotavirus in stools from children with and without gastroenteritis

<table>
<thead>
<tr>
<th>Condition of children</th>
<th>No. of children tested</th>
<th>No. (%) of children virus positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastroenteritis</td>
<td>1,114</td>
<td>22 (2.0)</td>
</tr>
<tr>
<td>No gastroenteritis</td>
<td>947</td>
<td>6 (0.61)</td>
</tr>
</tbody>
</table>

- *P < 0.01.
- *P < 0.02.

ENZYMELINKED IMMUNOSORBENT ASSAYS (ELISAS). The ELISAs for the enteric adenovirus types were performed with a mixture of monoclonal antibodies to each type (11) in a format previously described and validated by us (10). In brief, wells of microdilution plates were coated either with guinea pig anti-adenovirus type 7, which contained antibody to adenovirus group antigen, or with rabbit anti-rotavirus SA11 for the rotavirus assay (9). Antibodies were diluted in 0.01 M PBS, pH 7.0, to contain approximately 10 μg/ml. Stool suspensions (10% [wt/vol] in PBS) were added (0.05 ml) and incubated 1 h at 37°C for the rotavirus assay or overnight at 20 to 22°C for the adenovirus tests. The plates were washed five times with PBS here and after subsequent steps. A mixture of monoclonal antibodies to Ad40 and Ad41 or to rotavirus VP6 antigen (6) was added to the wells and incubated 1 h at 37°C. Peroxidase-labeled goat antimouse immunoglobulin G, heavy and light chains (Kirkegaard and Perry Laboratories, Gaithersburg, Md.), at 1 μg/ml was added and incubated 1 h at 37°C. The diluent used for antibody and conjugate was 0.1 M Tris hydrochloride buffer, pH 7.2, with 50% fetal calf serum and 0.15% Tween 20 added. The plates were washed, soaked for 30 s with PBS containing 0.05% Tween 20, and washed again. All plate washing was done with an automatic plate washer (Skatron, Lier, Norway). Substrate for peroxidase was added (0.1 ml of o-phenylenediamine–H₂O₂; Abbott Laboratories, North Chicago, Ill.) for 10 min, and the reaction was stopped with 0.2 ml of 1 N H₂SO₄. The A₄₉₀ of the solution was measured in a plate reader spectrophotometer. Samples were considered positive for virus antigen if the absorbance value was >0.1 and more than three times that of the negative control (negative stool in PBS).

For detection of adenovirus group antigen, a monoclonal antibody to adenovirus hexon group antigen (4), obtained from the American Type Culture Collection, Rockville, Md., was substituted for the Ad40 and Ad41 mixture of monoclonal antibodies in the ELISA.

RESULTS

The detection rates for each of the virus groups assayed in children with and without gastroenteritis are given in Table 1. Although rotavirus was clearly the major viral pathogen detected, there was a significantly higher (P < 0.01) detection rate of enteric adenovirus in those children with diarrhea than in those without diarrhea. There was also a significant (P < 0.02) association of nonenteric adenovirus with gastroenteritis. The Fisher exact test was used for these and subsequent determinations of significance.

The age distribution of children from each group infected with either enteric or nonenteric adenovirus is shown in Fig. 1. In the gastroenteritis group, 86% (19 of 22) of children with enteric adenovirus and 70% (19 of 27) of those with nonenteric adenovirus were 18 months of age or younger. Of children without gastroenteritis, 67% (4 of 6) of those with enteric adenovirus and 73% (8 of 11) of those with nonenteric adenovirus were 18 months of age or younger. There were no apparent seasonal trends with either enteric or nonenteric adenovirus, nor were there significant differences in respiratory symptoms in those with either enteric or nonenteric adenovirus.

Assays for bacterial and parasitic pathogens were performed on samples from the groups with and without gastroenteritis. In the group with gastroenteritis, children with viral infections frequently had coinfections with other pathogens as well (Table 2). The coinfecting agents most frequently involved with rotavirus and all adenovirus types were Salmonella spp. (5 to 14%), enteropathogenic E. coli (7 to 10%), and C. jejuni (8 to 10%). Giardia lamblia was recovered from 8% of the samples with the enteric adenovirus types but from less than 1% of those with nonenteric adenovirus or rotavirus. P. shigelloides was isolated from 5% of children with nonenteric adenovirus. There were fewer children without gastroenteritis who had rotavirus or adenovirus infections, but of those infected, Salmonella spp., enteropathogenic E. coli, and C. jejuni were the major agents isolated. There were no significant differences in the number of coinfections of enteric or nonenteric adenovirus with bacteria or parasites in children with or without gastroenteritis (P = 0.32 and 0.35 for enteric and nonenteric adenovirus, respectively).

Most of the major pathogens in children without a detectable virus infection were the same as those in each group of infected children, with Salmonella spp. occurring in 10 to 13% and C. jejuni occurring in 9 to 11%. However, Shigella spp. were isolated from 14% of the children with gastroen-
Enteric adenovirus the infections children in with virus compared with the in (P with the analyzed, but missed by types culture. Also, culture of cell-cultivated particles more, it positive samples that test children with The = different infection adenovirus with a and Ad4l and Ad4l that been infection, gastroenteritis, > 40.7% in stools those positive samples and cell determined nonenteric patients other infections, and 3% in children with rotavirus infection. Rotavirus coinfections occurred in 7 of 27 children in the diarrheic, nonenteric adenovirus group compared with 0 of 22 in the enteric adenovirus group (P = 0.02).

**DISCUSSION**

The results of the investigation of a large number of stool samples demonstrate that enteric adenovirus types are significantly associated (P < 0.01) with pediatric gastroenteritis in the Thai population studied. There was also a significant association (P < 0.02) of nonenteric adenovirus infection with gastroenteritis (27 patients). Of these 27, 7 were coinfected with rotavirus, which may have been a factor in their illness. The specific adenovirus serotypes involved were not analyzed, but we assume that they do not represent enteric types missed by the ELISA used for their detection. We have shown previously that our ELISA for enteric adenovirus detected 57 of 59 samples positive by EM and cell culture. Also, the ELISA values for the group antigen test were generally the same as or lower than those obtained for samples positive by the enteric type-specific ELISA, and the ELISA group antigen gave lower endpoints by serial dilution of cell-cultivated Ad40 and Ad41 (data not shown). Furthermore, it has been demonstrated that the quantity of enteric adenovirus particles in stools as determined by EM is usually far higher than that of the nonenteric adenovirus types, a finding which can be used for presumptive diagnosis of enteric adenovirus types by EM (3). Thus, it is unlikely that samples were missed by the specific enteric adenovirus test and detected by the group antigen test.

Infection with nonviral pathogens was common among children with gastroenteritis, but excluding the rates for *Shigella* spp. infection, infection rates were not significantly different (P > 0.05) from those among children without gastroenteritis. The incidence of coinfection was no higher (P = 0.25) among those with gastroenteritis and enteric adenovirus infection than among those with other adenoviruses (Table 2). Thus, the gastroenteritis seen in children with adenovirus infection can be considered, in most cases, a result of viral infection alone.

The pattern of infection with enteric and nonenteric adenoviruses, association of these viruses with gastroenteritis, and coinfection of patients with other pathogens is similar to the pattern found in a study of 416 ill children in a temperate climate in Uppsala, Sweden (17). The percentage of total adenovirus specimens identified as enteric was somewhat higher in that study (59% [33 of 56] compared with the 45% [22 of 49] that we found in Thailand), and the overall incidence of enteric adenovirus as well as the total adenovirus was higher in that population than in the one we studied (7.9 and 13.5% versus 2.0 and 4.4%, respectively).

We conclude that the enteric adenovirus types are a cause of gastroenteritis in the Thai children studied. However, as a proportion of total diarrheal diseases, the incidence of enteric adenovirus in this group was less than that found in most reports to date from areas with temperate climates.

**ACKNOWLEDGMENT**

This work was supported by contract DAMD 17-86-C6196 from the U.S. Army Medical Research and Development Command.

**LITERATURE CITED**


**TABLE 2. Coinfection of children with viral and bacterial or parasitic pathogens**

<table>
<thead>
<tr>
<th>Virus detecteda</th>
<th>% of children (no. positive/total no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With diarrhea</td>
</tr>
<tr>
<td>Enteric adenovirus</td>
<td>54.6 (12/22)</td>
</tr>
<tr>
<td>Nonenteric adenovirus</td>
<td>40.7 (13/27)</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>35.5 (7/22)</td>
</tr>
<tr>
<td>None</td>
<td>48.3 (41/851)</td>
</tr>
</tbody>
</table>

a The total number of diarrheic children tested was 1,114. However, because there were six coinfections of rotavirus and nonenteric adenovirus, the sum of the denominators for children tested in this group is 1,120.

b With bacteria or parasites.

c P = 0.25.
d P = 0.32.
e P = 0.35.

Vol. 26, 1988

**ENTERIC ADENOVIRUSES IN GASTROENTERITIS**

1785


