Evaluation of Anatomic Changes in Young Children with Natural Rotavirus Infection: Is Intussusception Biologically Plausible?

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**Background.** Several studies have shown an association between vaccination with the rotavirus vaccine and the development of intussusception. We evaluated the plausibility of a causal association between natural rotavirus infection and intussusception.

**Methods.** We performed ultrasound measurements, in infants with confirmed rotavirus infection and in healthy control subjects, of the ileum wall thickness and mesenteric lymph nodes, at enrollment and 1 month later.

**Results.** Thirteen pairs of patients with rotavirus infection and control subjects were enrolled. The mean distal ileum wall thickness at the first examination was 3.0 mm in patients with rotavirus infection and 2.0 mm in control subjects \(P = .037\). The maximum lymph node size in patients with rotavirus infection was 11.6 mm at the first examination and 7.4 mm at the second examination \(P = .017\). Nodal aggregates and free fluid were also observed more commonly among patients with rotavirus infection (54\% vs. 9\%; \(P = .033\) for both).

**Conclusion.** Rotavirus infection was associated with increased distal ileum wall thickness and lymphadenopathy during the illness period. These changes suggest a plausible mechanism by which rotavirus infection could cause intussusception.

Rotavirus is a common cause of gastrointestinal illness in young children. Peak illness rates occur in children aged 4–23 months. By age 5 years, nearly all children test seropositive for rotavirus, which indicates previous infection with this ubiquitous virus. Annually, rotavirus infections cause 800,000 deaths worldwide and cost the US economy an estimated $250 million in hospitalization costs [1]. At least 6 different rotavirus vaccine strains that use rhesus, bovine, recombinant human and rhesus, and human infectious strains have been developed. One strain, rhesus-human reassortant tetravalent vaccine (RRV-TV; Wyeth-Ayerst), was withdrawn from the market after a reported increase in the incidence of intussusception in the vaccine recipients [2]. Recent commentaries have discussed the hypothesis that RRV-TV is associated with intussusception during the immediate postimmunization period but protects against intussusception that occurs after subsequent infection with wild-type rotavirus [3, 4]. This hypothesis gains credibility if biological plausibility can be established whereby natural rotavirus infections could lead to intussusception.

Intussusception is the most common cause of intestinal obstruction in young children [5]. Although it is believed to be associated with hypertrophy of Peyer patches and mesenteric lymphadenopathy, intussusception in this age group is idiopathic in the vast majority of cases. The results of virus studies have indicated that ~50\% of children with intussusception shed virus in stool, with adenovirus, enterovirus, and cytomegalovirus being the most commonly implicated viruses [6–11]. Before the experience with RRV-TV vaccine, natural rotavirus infection had not been shown to be definitively associated with intussusception. However, few researchers had attempted to identify rotavirus using rotavirus-antigen detection or virus-isolation procedures. Of note, a comprehensive review of previously published data by Rennels et al. [6] in 1998 concluded...
that the 3 small case series that reported the identification of rotavirus infection in children with intussusception constituted insufficient evidence to conclude that the infection caused intussusception. These case series lacked control subjects and predated the development of rotavirus-antigen detection procedures and culture methods.

The purpose of our pilot study was to explore possible mechanisms by which natural rotavirus infection might cause intussusception by using ultrasound to define bowel-wall thickening and mesenteric lymphadenopathy in rotavirus-infected patients and in healthy age- and sex-matched control subjects. Ultrasound offers an ideal method to evaluate alterations in the bowel and mesentery that might be induced by a variety of disease processes. It is also highly sensitive in detecting intussusception [5] and has been used to measure bowel-wall thickness in a variety of disorders, including inflammatory bowel disease, cystic fibrosis, and Yersinia and Salmonella infection [12, 13].

SUBJECTS AND METHODS

Study design and population. This was a prospective matched-cohort study that enrolled children aged 0–13 months. Children with symptomatic rotavirus infection were identified in a variety of clinic, emergency department, and inpatient care settings. Asymptomatic, healthy control children were identified contemporaneously at 3 general pediatric clinics in the area during well-child visits. They were matched, by age and sex, to existing patients with rotavirus infection and had no gastrointestinal symptoms at the time of enrollment. Children considered for either group who had a preexisting medical condition affecting the bowel—such as cystic fibrosis, inflammatory bowel disease, or prior gastrointestinal surgical procedures—were ineligible for the study. Informed consent was obtained from the parents or guardians of all subjects and control subjects. The study was approved by Vanderbilt University’s institutional review board.

Data collection. At enrollment, each subject’s parent or guardian was interviewed, to obtain demographic data, the extent and duration of gastrointestinal symptoms (if the children had rotavirus infection), and general contact information. All subjects also submitted a stool sample at enrollment to be tested for the presence of rotavirus antigen and other intestinal viruses. Rotavirus status was verified in all study enrollees using stool samples tested with either Rotaclone or Immuno-Rotastat cards (Meridian Bioscience), both at enrollment and at the follow-up visit 1 month later. The enrollment and follow-up stool samples were also tested for the presence of anti–rotavirus IgA and were cultured for common gastrointestinal viruses (adenovirus, enterovirus, and rotavirus). Patient follow-up ended at the time of the second ultrasound examination, 1 month after enrollment.

All subjects underwent ultrasound examination performed by a single board- and Certificate of Added Qualification–certified pediatric radiologist blinded to the rotavirus infection status of the patients. Ultrasound examinations were performed at enrollment and 1 month later at the follow-up visit. The examination was done using ATL 5000 equipment (Bothell) or Sequoia (Acuson), with a linear transducer operating at a variable frequency of 7.5–12 MHz. The examinations were performed with graded compression, beginning at the inferior surface of the liver and identifying the ascending portion of the colon and the small-bowel loops in the right abdomen and right lower quadrant. The terminal ileum (TI) was considered to have been identified only if it was observed inserting into the cecum via the ileocecal valve (figure 1). A distended diameter was defined as the noncompressed outer-wall-to-outert-wall diameter of a loop of bowel (figure 2). Wall thickness was defined as one-half of the outer-wall-to-outer-wall diameter of a completely compressed loop (figure 3). Nodes (figure 4) and nodal aggregates (figure 5) were identified and measured at the longest axis (figure 4).

Because intussusception may begin in the distal ileum rather than in the ileocecal valve area, other loops of bowel in the distal right lower quadrant, in addition to the TI, were selected and measured in the same manner. These were designated as “distal ileum” loops; both the noncompressed diameter and compressed wall thickness were measured in these areas and recorded. Mesenteric lymph nodes, singly and in aggregate, were measured when they were present. The presence of free intraperitoneal fluid was also recorded.

The primary study end points were the measurement of the diameter and wall thickness of the TI. The secondary study

![Figure 1. Right lower quadrant compression image outlining the terminal ileum at the ileocecal valve of an 11-month-old patient with rotavirus infection. The distance between the calipers indicates the outer-wall-to-outter-wall bowel diameter of the terminal ileum (1.19 cm). Single wall thickness, 6 mm. Arrows outline the cecum clasping the terminal ileum at the ileocecal valve.](https://academic.oup.com/jid/article-abstract/189/8/1382/819483)
end points were the diameter and wall thickness of small-bowel loops, lymph node size, and the presence and extent of aggregate lymph nodes. Group means were compared using the Mann-Whitney U test, and comparisons between first and second ultrasound results were done using the Wilcoxon signed rank test for each group. Group comparisons included the results of (1) the first examination of a patient with rotavirus infection versus that of a control subject, (2) the second examination of a patient with rotavirus infection versus that of a control subject, (3) the first versus the second examination of a patient with rotavirus infection, and (4) the first versus the second examination of a control subject. Categorical variables were compared using the $\chi^2$ or Fisher’s exact test, as appropriate. Missing data were excluded on an analysis-by-analysis basis. Data were analyzed using SPSS software.

RESULTS

Study population. We identified 14 children with rotavirus infection and enrolled age- and sex-matched healthy control subjects for 13 of them. A total of 13 pairs of subjects had both first and second ultrasound examinations, which formed the basis of the comparative analyses.

Clinical presentation. The average age at enrollment was 7.2 months for patients with rotavirus infection and 7.3 months for control subjects, with an average time of 60 h from initial enrollment and collection of stool sample to the completion of the first ultrasound examination. A total of 57% of patients with rotavirus infection were male, and 54% of control subjects were male. None of the control subjects attended day care, in contrast to 36% of patients with rotavirus infection. Of the patients with rotavirus infection, 36% were evaluated in an emergency room setting and received intravenous (iv) rehydration therapy, 43% were admitted as an inpatient to the hospital for iv rehydration therapy, and 21% were seen in an outpatient clinic and did not receive iv rehydration therapy. Symptoms in rotavirus-infected children were abdominal pain (71%); loose stools (100%; mean, 17 stools/24 h); vomiting (86%; mean, 5.2 times/24 h); signs of dehydration, such as sunken fontanel, reduced tear production, and dry mucous membranes (57%); and prodrome of respiratory symptoms, including cough and/or coryza (64%). None of these parameters was found to correlate statistically with the degree of bowel-wall thickening, bowel distension, or lymphadenopathy.

One of our study patients, who was hospitalized for severe rotavirus infection, had a transient ileocolic intussusception that was imaged during the real-time ultrasound test (figure 6). This intussusception was initially 3 cm in length, reduced to 1.4 cm, and then resolved without intervention. Clinically, the patient did not develop further symptoms.

TI end points. In the first ultrasound examination, the TI was visualized in 46% of patients with rotavirus infection and in 62% of control subjects. At the second examination, 1 month later, the TI was visualized in 64% of patients with rotavirus infection and in 46% of control subjects. The mean TI diameter (table 1) was 6.6 mm in patients with rotavirus infection and 5.9 mm in control subjects ($P = .755$). The TI wall thickness was 3.4 mm in patients with rotavirus infection and 2.8 mm in control subjects ($P = .470$). The mean TI diameter and wall thickness did not change significantly between the first and second examination in either patients with rotavirus infection or control subjects.

Small-bowel end points. Distal ileum loops were visualized in all study subjects. There were no significant differences in

Figure 2. Right lower quadrant noncompression image outlining fluid-filled, distended loops of the bowel in a 7-month-old patient with rotavirus infection. The distance between the calipers indicates the outer-wall-to-outer-wall bowel diameter of the terminal ileum (1.35 cm). Note that there is similar distension of adjoining loops (arrows) that contain more echogenic fluid.

Figure 3. Right lower quadrant compression image outlining normal loops of bowel in a 12-month-old control subject. The distance between the calipers indicates the outer-wall-to-outter-wall bowel diameter (5.5 mm). Single wall thickness, 2.75 mm; P, transverse axis of the right psoas muscle.
distal ileum distention between patients with rotavirus infection and control subjects at either examination, and no significant change was seen in this measurement for patients with rotavirus infection between the first and second examinations (table 1). The mean small-bowel loop wall thickness was 3.0 mm in patients with rotavirus infection at the time of the first ultrasound, versus 2.0 mm in control subjects ($P = .037$). Wall thickness declined to 2.0 mm by the second examination in the patients with rotavirus infection ($P = .123$ vs. the first examination).

**Adenopathy.** Nodes were visualized in 92% of patients with rotavirus infection at the first examination and in 82% at the second, whereas nodes were visualized in 62% of healthy control subjects at the first examination and in 69% at the second. Nodes were measured at the longest diameter (see table 1). Patients with rotavirus infection had a larger maximum lymph node size at the first examination than did control subjects—at the first examination, the maximum lymph node size in patients with rotavirus infection was 11.6 mm, compared with 8.0 mm in control subjects ($P = .057$); this value decreased to 7.4 mm at the second examination ($P = .017$).

Patients with rotavirus infection more commonly had nodal aggregates, compared with control subjects; at the first examination, aggregates were seen in 54% of patients with rotavirus infection, compared with 15% of control subjects at the first examination ($P = .097$). Nodal aggregates were also visualized more frequently at the first examination than at the second, with aggregates seen in only 9% of patients with rotavirus infection at the second examination ($P = .033$). The mean maximum diameter of lymph node aggregates at the first examination was 27.7 mm in patients with rotavirus infection, compared with 22.0 mm in control subjects ($P = .889$). At the second examination, the size of lymph node aggregates had decreased to a maximum of 15.0 mm in patients with rotavirus infection, compared with 21.0 mm in control subjects ($P = .667$). $P$ values for intragroup comparisons were incalculable, because no patient had aggregates at both examinations.

**Free fluid in the abdomen.** Free fluid in the abdomen was detected at the first ultrasound examination in 54% of patients with rotavirus infection, compared with 23% of control subjects ($P = .107$). At the second examination, free fluid was detected in 9% of patients with rotavirus infection ($P = .033$ vs. the first examination).

**DISCUSSION**

We undertook this pilot study to define more clearly the effect of natural rotavirus infection on bowel anatomy, with the purpose of exploring whether natural rotavirus infection–induced changes might predispose patients toward intussusception. Our results establish that rotavirus infection in young children affects the bowel wall, most notably by increasing wall thickness in the distal ileum and by enlarging mesenteric lymph node size. The effect of rotavirus on the bowel wall is a possible mechanism for virally induced intussusception, as has been postulated in studies of adenovirus and of intussusception [14–20]. These prior studies documented the presence of adenovirus in the mesenteric lymph nodes of patients with intussusception, and one documented the presence of adenovirus in the appendiceal tissue within the bowel wall [24]. Our present findings show that rotavirus similarly affects the bowel wall, but its role in inducing intussusception remains unclear.

Much of the published data that have examined the role of rotavirus in triggering intussusception predate current rotavirus detection and culturing methods. Three case series that analyzed the role of rotavirus in triggering intussusception were undertaken in the late 1970s and early 1980s and used electron microscopy (EM), ELISA, or immunofluorescence to identify rotavirus in stool samples from young children. The first study, conducted by Konno et al. [8] in Japan, identified rotavirus in...
Figure 6. Right abdominal compression image illustrating incidental ileocecal intussusception occurring coincidentally at the time of imaging in a 4-month-old patient with rotavirus infection. Transverse (left) and longitudinal (right) images at the level of the hepatic flexure outline the diagnostic bowel-within-bowel appearance. Arrowheads, intussusceptum; arrows, intussuscipiens; P, long axis of the psoas muscle.

11 of 30 patients (using EM) who presented with intussusception over an unstated time frame. Nine of the patients who tested positive for rotavirus presented with intussusception during the winter months, the seasonal peak for rotavirus infections in Japan [8]. In the same study, adenovirus was isolated in 9 of 30 patients, with no dual infections reported [8]. The second study, which was conducted by Nicolas et al. [7] in France, used ELISA to identify increases in rotavirus antibody titers in 6 (9%) of 64 patients with intussusception; 4 of these patients also demonstrated serologic increases in hemagglutination inhibition titers to adenovirus, which suggested the possibility of dual infection and resulted in 2 (3%) of 64 having rotavirus infection alone. The third study, conducted by Mulcahy et al. [9] in Australia, used EM, ELISA, and immunofluorescence of fecal extracts to identify rotavirus infection. Two (2%) of 120 children with intussusception showed evidence of current or recent rotavirus infection [9].

Are there varying degrees of intussusception, some of which “stick” and require clinical or surgical management and others that transiently resolve and do not require clinical attention? By chance, we observed by ultrasound, during the present study, a transient intussusception in a child with rotavirus infection

Table 1. Characteristics of patients with rotavirus infection vs. control subjects, during 2 examinations.

<table>
<thead>
<tr>
<th>Characteristic visualized</th>
<th>Patients with rotavirus, examination</th>
<th>Control subjects, examination</th>
<th>Between-group P for examination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>Within-group P</td>
</tr>
<tr>
<td>TI, %</td>
<td>46</td>
<td>64</td>
<td>.444</td>
</tr>
<tr>
<td>Distended, mm</td>
<td>6.6 (2.7)</td>
<td>4.0 (2.4)</td>
<td>.109</td>
</tr>
<tr>
<td>Wall thickness, mm</td>
<td>3.4 (1.5)</td>
<td>2.6 (1.4)</td>
<td>.109</td>
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<tr>
<td>Distal ileum, %</td>
<td>100</td>
<td>100</td>
<td>1.000</td>
</tr>
<tr>
<td>Distended, mm</td>
<td>10.4 (4.5)</td>
<td>4.6 (3.3)</td>
<td>.138</td>
</tr>
<tr>
<td>Wall thickness, mm</td>
<td>3.0 (1.4)</td>
<td>2.1 (0.8)</td>
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<tr>
<td>Nodes, %</td>
<td>92</td>
<td>82</td>
<td>.576</td>
</tr>
<tr>
<td>Maximum size, mm</td>
<td>11.6 (4.2)</td>
<td>7.4 (2.1)</td>
<td>.017</td>
</tr>
<tr>
<td>Nodal aggregates, %</td>
<td>54</td>
<td>9</td>
<td>.033</td>
</tr>
<tr>
<td>Maximum size, mm</td>
<td>27.7 (12.3)</td>
<td>15 (%*b)</td>
<td>...b</td>
</tr>
<tr>
<td>Free fluid, %</td>
<td>54</td>
<td>9</td>
<td>.033</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean millimeters (SD), unless otherwise noted. The first examination was done at enrollment, and the second was done at the follow-up visit 1 month later. All measurements were made using ATL 5000 or Acuson Sequoia ultrasound by a board- or Certificate of Added Qualification–certified pediatric radiologist blinded to group assignment. Distal ileum measurements were taken from a representative loop of bowel in the right lower quadrant. Wall thickness was defined as one-half of the outer-wall-to-outer-wall diameter of a completely compressed loop. The distended diameter was defined as the noncompressed outer-wall-to-outer-wall diameter of a loop of bowel. Lymph nodes and lymph node aggregates were measured at their longest diameter. The Mann-Whitney U test was used to make intergroup comparisons, and the Wilcoxon signed rank test was used to make intragroup comparisons. Categorical variables were compared using the χ² or Fisher’s exact test, as appropriate. Missing data were excluded on a case-by-case basis. Values given in bold are statistically significant. TI, terminal ilium.

* No SD is given for this measure because only 1 value is represented.

b No P value is given because no patient had nodal aggregates at both the first and second examinations.
(figure 6). Published data [14, 21] and anecdotal reports from pediatric surgeons support the notion that the bowel tissue in young children transiently telescopes without leading to clinical intussusception. Our results suggest that this may occur in rotavirus infection. Enlarged mesenteric nodes and nodal aggregates are often observed during the surgical correction of intussusception [14, 17, 19]. Konno et al. [22], using autopsy material, reported that hyperplasia of the intestinal lymphoid tissue occurs in acute gastroenteritis caused by rotavirus infection. Pathological data on resected bowel from intussusceptions associated with the now-withdrawn rotavirus vaccine identified lymphoid hyperplasia as possible lead points [23]. Furthermore, Berrebi et al. [24] reported the isolation of adenovirus in resected appendiceal tissue from children with acute intussusception. Thus, these findings support a role for viral infections, specifically natural rotavirus infection, in the creation of potential lead points for intussusception, as was observed in our study.

Although this was only a pilot study to evaluate the relationship between rotavirus and intussusception, our findings are novel and suggest that this association needs to be investigated further. Ecological studies conducted in the United States have not suggested a link between rotavirus and intussusception [6]. However, ecological approaches are not sensitive to strong associations that are often observed during the surgical correction of intussusception [14, 17, 19]. Konno et al. [22], using autopsy material, reported that hyperplasia of the intestinal lymphoid tissue occurs in acute gastroenteritis caused by rotavirus infection. Pathological data on resected bowel from intussusceptions associated with the now-withdrawn rotavirus vaccine identified lymphoid hyperplasia as possible lead points [23]. Furthermore, Berrebi et al. [24] reported the isolation of adenovirus in resected appendiceal tissue from children with acute intussusception. Thus, these findings support a role for viral infections, specifically natural rotavirus infection, in the creation of potential lead points for intussusception, as was observed in our study.

Our study aimed to answer the question of whether there is any relationship between rotavirus infection and bowel changes. However, our study was not designed to answer the greater question of the relationship between rotavirus infection and intussusception. Our pilot study was limited by its small size, the difficulty in positively identifying the TI in all patients studied, and the wide variance in bowel measurements among patients with rotavirus infection. This variance could have resulted from differences in the clinical severity of rotavirus infection or the time of presentation for clinical care.

An inherent difficulty in designing the study was ascertaining what the "normal" bowel wall thickness and diameter are in young children. Further complicating the design was the dearth of prior scientific literature regarding viral causes of intussusception. To our knowledge, questions such as to what extent the TI would need to thicken or distend to provoke an intussusception that requires clinical attention have not been addressed. Our findings point to a normal value of ~2 mm for wall thickness in the distal ileum and an uncomprssed loop diameter of 3.5–4 mm in the distal ileum. Further study is needed to better define these "normal" values and to understand the role of rotavirus infection in intussusception.

References