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Detection of human coronaviruses in children with acute gastroenteritis

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A B S T R A C T
Background: Human coronaviruses (HCoVs) are known respiratory pathogens. Moreover, coronavirus-
like particles have been seen by electron microscope in stools, and SARS-HCoV has been isolated from
intestinal tissue and detected in stool samples.
Objectives: To find out if HCoVs can be found in stools of children with acute gastroenteritis and to assess
the significance of HCoVs in the etiology of acute gastroenteritis in children.
Study design: 878 stool specimens from children with acute gastroenteritis and 112 from control children
were tested by RT-PCR to detect HCoV groups 1B, 2A and SARS. HCoVs were typed by sequencing all PCR
positive samples.
Results: Twenty-two (2.5%) of the 878 stool specimens of children with acute gastroenteritis were positive
for HCoVs. The following HCoV types were detected: OC43 (10 cases, 45.5%), HKU1 (6 cases, 27.3%), 229E
(2 cases, 9.1%) and NL63 (4 cases, 18.2%). In 4 of the cases a HCoV was the only detected virus; in the
remaining cases rotavirus or norovirus was found in the same sample. In control groups there were two
HCoV positive samples of 112 tested.
Conclusions: This study shows that all known non-SARS HCoVs can be found in stools of children with
acute gastroenteritis. On the basis of this study, the significance of coronaviruses as gastrointestinal
pathogens in children appears minor, since most of the coronavirus findings were co-infections with
known gastroenteritis viruses.

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1. Background

The first human coronaviruses (HCoVs) 229E and OC43 were identified in the 1960s.1–4 These viruses are common causes of
upper respiratory tract infections5,6 but also have association with lower respiratory tract disease especially in patients with under-
lying disease.7–9 Coronavirus-like particles have also been seen by electron microscope (EM) in stool samples of both diarrheic
and healthy patients evoking discussion about human enteric coronaviruses.10 The clinical significance of these findings has
been unclear and there are findings showing that such putative enteric coronaviruses are antigenically unrelated to OC43 and 229E
viruses.11

In 2003 interest towards coronaviruses increased when a new coronavirus was found to be a causative agent of SARS (severe
acute respiratory syndrome).12–14 SARS-HCoV caused a serious lower respiratory tract infection with high mortality.15–17 The main
symptoms were fever, chills, myalgia, cough and headache, but also diarrhea was common and in one study registered in 38.4%
of patients. In the same study SARS-HCoV was also isolated from intestinal tissue and viral RNA was found in 16% of stool samples,
which was comparable to the detection rate in nasopharyngeal aspirates.18 In another cohort of patients diarrhea was seen in 73% of
patients and viral RNA was detected in 97% of stool specimens. In this cohort it was suggested that the outbreak was caused by faulty
sewage system whereby the transmission might been fecal–oral rather than respiratory.18 No association between the presence of
diarrhea and mortality in SARS cases has been observed, however.20

A fourth human coronavirus, HCoV-NL63, was identified in 2004 in the Netherlands. It was isolated from a 7-month-old child suf-
f ering from bronchiolitis and conjunctivitis, and was categorized to be a new group 1 coronavirus.21 Soon after, another group in
the Netherlands independently detected the same virus in an 8-month-old child with pneumonia.22 Since the discovery, NL63 has been
detected in patients with respiratory tract infection in several countries around the world, including Canada, Australia, Korea and France.23–26 NL63 has been associated with severe lower respiratory tract infection and croup.24,27 The seroprevalence in
6–12-month-old children for NL63 is 28.6–40.0%.28

Less than a year later a second new coronavirus, HCoV-HKU1, was discovered in Hong Kong. This group 2 coronavirus was
detected in a 71-year-old man with pneumonia.29


2. Objectives

Considering the EM findings, presence of gastrointestinal symptoms in SARS, and the findings of HKU1 in stools, we wanted to find out if non-SARS human coronaviruses could be detected in stool samples of children with acute gastroenteritis using RT-PCR assay in order to assess the potential significance of coronaviruses in the etiology of acute gastroenteritis in children.

3. Study design

The clinical material for the study was collected in a prospective study of acute gastroenteritis in children Tampere and Kuopio University Hospital during a 2-year period 2006–2008 (Räsänen et al., unpublished). Healthy children (N = 36), children with indeterminate fever and vomiting (N = 43), and children with respiratory tract infection (N = 33) were used as control groups. Also group A rotavirus, calicivirus (including norovirus genogroups I and II, and sapovirus), aichiavirus and human bocavirus were studied from the same material. Adenovirus was not tested systematically but one of 101 nucleotide. The produced cDNA was stored at −70 °C until used.

Reverse transcription was done using random primers as previously described by Pang et al. (2005) except that reaction contained 1 First Strand Buffer (Invitrogen, USA) and the final concentration of dNTPs (Promega, USA) was 375 nM per each nucleotide. The produced cDNA was stored at −20 °C.

4. Laboratory methods

Before RNA extraction the stool samples were diluted in phosphate-buffered saline (PBS) creating 10% stool suspension. Viral RNA was extracted using QIAamp Viral RNA Mini Kit (QIAGEN, Germany) according to the manufacturer’s protocol. Extracted RNA was stored at −70 °C until used.

Forward primer primer pair (fwd-GWTTGGAYTATCNCNNARTCTGTA and rev-YRTCTACATCAATCTCATAGM) for 35 cycles of amplification (30 s at 94 °C, 30 s at 54 °C, 1 min at 72 °C) and final extension at 72 °C for 5 min.

PCR-products were separated and recognized by gel electrophoresis, and all positive PCR products were confirmed to be coronaviruses and the specific type defined by sequencing. ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems, USA) was used in sequencing. Sequences were aligned and confirmed by using Sequencher™ 4.8 program (Gene Codes Corporation, USA) and confirmed sequences were compared to reference strains by NCBI Blast® program.

5. Results

Twenty-two (2.5%) of the 878 stool specimens of children with acute gastroenteritis were positive for HCoVs. A HCoV as a single pathogen was detected in only four of the samples (18.2% of the positive samples). In the remaining cases either norovirus or rotavirus was detected in the same sample (Table 1). In eleven (50%) of the 22 coronavirus positive cases there were symptoms of respiratory tract infection at the same time with gastroenteritis, or respiratory symptoms had been present before symptoms of gastroenteritis.

Three of the 4 patients with coronavirus as a single pathogen in stool sample had respiratory tract related symptoms. Patient 1 (Table 1) had cough and rhinitis, and had just recovered from otitis media. Patient 7 (Table 1) did not have any respiratory tract symp-
toms. Patient 11 (Table 1) had tonsillitis and patient 19 (Table 1) had headache and dizziness in addition to symptoms of respiratory tract infection.

All non-SARS human coronavirus types were found, members of group 2A; OC43 (10 of the cases, 45.5%) and HKU1 (6 of the cases, 27.3%) were most common, whereas group 1B viruses 229E and NL63 were found only in 2 and 4 cases, respectively. No SARS or SARS-like viruses were found. Still there might be unknown coronaviruses that our PCR method did not detect in spite of the universal primers in the 1st PCR. Most HCoV positive cases were found from January to April (Fig. 1).

The age distribution of the coronavirus infected children was 9–75 months (median, 19.5 months), whereas in the total material the youngest child was 14 days and the oldest 14 years and 4 months (median, 17 months). Of the coronavirus positive children 59% were males.

Within the control groups two (1.8%) of the 112 stool samples were positive for HCoV. One of the cases was a 3-year-old female with pneumonia. OC43 was detected as the only pathogen in her stool samples on February 2007. Two days after sample collection she also developed symptoms of gastroenteritis. The second patient was a healthy female aged 2 years and 11 months tested on July 2007. OC43 was again detected in a stool sample as a single pathogen.

6. Discussion

Our study shows that human coronaviruses OC43, HKU1, 229E and NL63 can be found in stool samples of children with acute gastroenteritis. The significance of coronaviruses as gastrointestinal pathogens seems at most marginal, even though it is also possible that our PCR method did not detect all existing coronaviruses and there still might be unknown coronaviruses related to diarrheal disease. Most of the coronavirus findings were co-infections with well known enteric pathogens, norovirus and rotavirus. Furthermore, half of the patients with coronavirus in stools had symptoms related to respiratory tract infection and, therefore, HCoV found in the stools could have originated from respiratory tract. Unfortunately, no specimens from respiratory tract were collected to confirm the presence of coronaviruses in the respiratory tract in these patients, and further studies are needed to evaluate simultaneous presence of HCoV in stools and respiratory tract. Even if coronaviruses were found in respiratory tract in cases of acute gastroenteritis it would be difficult to determine whether they were primarily causing the respiratory or gastrointestinal symptom.

Studies with SARS have shown that RNA of SARS coronavirus can be detected in stool samples for more than 10 weeks after symptom onset.18 This elicits the question whether also non-SARS coronaviruses might be detected after a prolonged time from the original infection. Previous studies with EM showed that coronavirus-like particles can be seen in stools of both diarrheic and healthy patients.10 In our study one of the 36 healthy control patients had coronavirus detected in stool specimen and thus, there was no difference in the HCoV detection rate between the cases of acute gastroenteritis and control children. This study was hospital based and did not include mild cases of gastroenteritis treated at home or healthcare centers. Future studies should investigate such mild cases for HCoVs.

In conclusion, non-SARS human coronaviruses can be found in stool samples of children with acute gastroenteritis. However, such findings are rare and occur usually with other well established gastroenteritis viruses. HCoVs may also be found in occasional stool samples of children without gastroenteritis. Taken together, it appears that known HCoVs may at most have a minor etiologic role in the acute gastroenteritis of children.

Ethical approval

The study protocol and consent forms had been approved by the Ethics Committee of the Pirkanmaa Hospital District in 2006.

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Conflict of interest

None declared.

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