Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Short communication

Human coronaviruses are uncommon in patients with gastrointestinal illness

Frank Espera, Zhen Ou, Yung T. Huang

Department of Pediatrics, University Hospitals Case Medical Center, Cleveland, OH 44106, United States
Case Western Reserve University, 11100 Euclid Avenue, Cleveland, OH 44106, United States
Department of Pathology, University Hospitals Case Medical Center, Cleveland, OH 44106, United States

Abstract

Background: Coronaviruses infect numerous animal species causing a variety of illnesses including respiratory, neurologic and enteric disease. Human coronaviruses (HCoV) are mainly associated with respiratory tract disease but have been implicated in enteric disease.

Objectives: To investigate the frequency of coronaviruses in stool samples from children and adults with gastrointestinal illness by RT-PCR.

Study design: Clinical samples submitted for infectious diarrhea testing were collected from December 2007 through March 2008. RNA extraction and RT-PCR was performed for stools negative for Clostridium difficile using primer sets against HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1. Clinical data from samples positive for coronaviruses were reviewed and recorded.

Results: Samples from 479 patients were collected including 151 pediatric (≤18 years), and 328 adults (>18 years). Of these samples, 4 patients (1.3%, 2 adult; 2 pediatric) screened positive for the presence of a coronavirus. All detected coronaviruses were identified as HCoV-HKU1. No stools screened positive for either HCoV-229E, HCoV-NL63 or HCoV-OC43. All HCoV-HKU1 positive samples occurred between mid-January to mid-February. Clinical manifestations from HCoV-HKU1 positive patients included diarrhea, emesis and respiratory complaints. Three (75%) patients were admitted to the hospital with a median length of stay of 6 days.

Conclusions: Coronaviruses as a group are not commonly identified in stool samples of patients presenting with gastrointestinal illness. HCoV-HKU1 can be identified in stool samples from children and adults with gastrointestinal disease, with most individuals having respiratory findings as well. No stool samples screened positive for HCoV-NL63, HCoV-229E, or HCoV-OC43.

1. Background

Gastroenteritis is a significant cause of morbidity and mortality worldwide in both children and adults. Viruses recognized as important enteric pathogens include rotavirus, noroviruses, astroviruses, sapoviruses, and enteric adenovirus. Other viruses implicated in human gastroenteritis include coronaviruses, toroviruses, human bocaviruses, picornoviruses, pestivirus, and breda virus. However, the role of these viruses in gastrointestinal illness remains unclear. Even with sensitive molecular diagnostic techniques, a substantial percentage of gastrointestinal illness has no identifiable etiology. This suggests the presence of unrecognized pathogens.

Coronaviruses are enveloped, plus-sense RNA viruses recognized as a cause of respiratory disease since the 1970s. Coronavirus infections occur throughout the year, often with a wintertime predominance in temperate climates. Human coronaviruses (HCoV) can be divided into 2 serogroups with HCoV-229E and HCoV-NL63 falling into serogroup 1 and HCoV-OC43 and HCoV-HKU1 residing in serogroup 2. Severe acute respiratory syndrome associated coronavirus (SARS-CoV) has tentatively been regarded as a member of serogroup 2.

It has been hypothesized that human coronaviruses may play a role in enteric disease. Coronaviruses are associated with diarrheal disease in many animal species and early studies implicated coronaviruses with human gastrointestinal illnesses. Rouset et al. found coronavirus-like particles by electron microscopy in stool of children with diarrhea and infants with necrotizing enterocolitis. Resta et al. demonstrated that a higher proportion of children with gastroenteritis had an antibody response to coronaviruses. During the SARS-CoV 2002–2003 outbreak, enteric involvement was reported in 38–70% of patients and was detected frequently in stool samples col-

© 2010 Elsevier B.V. All rights reserved.
lected from infected individuals. Studies of newly recognized coronaviruses report affected patients have evidence of gastrointestinal involvement. Recently, Vabret et al identified HCoV-HKU1 from stools in pediatric patients whose respiratory samples screened positive for HCoV-HKU1. However, large studies investigating coronaviruses in patients with gastrointestinal illness are lacking.

2. Objectives

We screened stool samples from children and adults with gastrointestinal illness for evidence of human coronaviruses: HCoV-NL63, HCoV-HKU1, HCoV229E, and HCoV-OC43.

3. Study design

3.1. Sample collection

From December 1, 2007 to March 31, 2008, stool samples were collected from the core laboratory at University Hospitals—Case Medical Center of Cleveland. Samples were submitted to the core laboratory at the discretion of the primary medical teams. Submitted samples originated from the emergency department, inpatient wards, intensive care units, and hospital-affiliated primary care outpatient clinics. We obtained all clinical specimens from children and adults that screened negative for Clostridium difficile A and B toxin by enzyme immunoassay (Meridian Bioscience, Cincinnati, OH). Each month, a minimum of 100 stool specimens were randomly selected for coronavirus screening. Samples were reviewed to ensure an adequate sampling of pediatric patients. Other than age no selection criteria was used.

3.2. RNA extraction and reverse transcriptase/polymerase chain reaction (RT-PCR)

Nucleic acid from each stool specimen was extracted with the MagMAX™ Total Nucleic Acid Isolation Kit (Applied Biosystems, Foster City, CA) according to the manufacturer’s protocol. Random hexamer primers (Invitrogen Carlsbad, CA), were used to create a cDNA library for each specimen using M-MLV RT (Invitrogen, Carlsbad, CA) according to the manufacturer’s specification. Primers used to screen stool specimens originate from published reports. Amplification protocol for all reactions were as follows: 95 °C for 3 min; followed by 40 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 30 s; and completed with a final extension cycle of 72 °C for 10 min. Coronavirus positive isolates were confirmed by sequence analysis and screened for the presence of common gastrointestinal viruses including adenovirus, rotavirus, noroviruses, and human bocaviruses by RT-PCR.

3.3. Clinical data

Medical records of all coronavirus positive patients were reviewed. Demographic data, history of illness, results of clinical examination, and laboratory studies were recorded on a standard collection form.

4. Results

Between December 1, 2007 through March 31, 2008 479 stool samples were selected for coronavirus screening. Of these, 328 (68%) were obtained from adult patients (age ≥ 18 years) and 151 (32%) from pediatric patients (age < 18 years). Four (0.8%) samples screened positive for the presence of a coronavirus including 2 (0.6%) adult patients and 2 (1.3%) pediatric patients (Table 1). All four coronavirus isolates were identified as HCoV-HKU1. No samples screened positive for either HCoV-229E, HCoV-OC43, or HCoV-NL63.

All HCoV-HKU1 samples were detected between January 15, 2007 and February 17, 2008. Three (75%) of which occurred in January representing 2.5% of all samples screened. No samples screened positive for coronaviruses during the months of December or March. All HCoV-HKU1 positive stools screened negative for the presence of adenovirus, rotavirus, noroviruses, and human bocaviruses by RT-PCR.

The most common gastrointestinal symptoms reported include emesis (75%) and diarrhea (75%) (Table 1). In addition, many patients (75%) had associated respiratory findings. Three patients (75%) were admitted to the hospital whereas one was seen and discharged from the emergency department. Median length of hospitalization was 6 days. Encounter diagnoses include gastroenteritis (50%), pneumonia (25%), and fussiness (25%). Three patients, including both adults, had underlying illnesses including diabetes, COPD, and congenital heart disease. No coronavirus positive patients had underlying gastrointestinal comorbidities.

5. Discussion

Coronaviruses are common human pathogens affecting children and adults worldwide with most individuals becoming infected in the first few years of life. In patients with respiratory disease, coronaviruses have been identified in up to 13% of respiratory samples. Nearly 25% of patients with HCoV-NL63 and close to 50% of patients with HCoV-HKU1 having associated gastrointestinal findings. Our knowledge of animal coronaviruses and SARS-CoV demonstrate these viruses may transit and thrive within the gastrointestinal system.

This is the first study targeting known human coronaviruses from a large number of patients with gastrointestinal illness. We demonstrate the identification of human coronavirus HKU1 RNA and note the absence of other recognized coronavirus pathogens. The lack of HCoV-229E, HCoV-NL63 and HCoV-OC43 in this study is surprising but is not conclusive of their absence. Because of year

### Table 1: Clinical characteristics of patients with coronavirus positive stool samples.

| Patient | Gender | Hospitalized | Duration of hospitalization (days) | GI symptoms | Respiratory symptoms | Underlying conditions |
|---------|--------|--------------|-----------------------------------|-------------|----------------------|----------------------|
| 1       | F      | Y            | 5                                 | Nausea, emesis, abdominal pain | Dyspnea, wheezing | Diabetes, COPD |
| 2       | F      | Y            | 7                                 | Diarrhea    | Dyspnea, rhonchorous breath sounds, hypoxia | Diabetes |
| 3       | M      | N            | –                                 | Fever, nausea, emesis, diarrhea | None | None |
| 4       | M      | Y            | 22                                | Fever, emesis | Rhinorhea | Congenital heart disease |

* M, Male; F, Female; COPD, chronic obstructive pulmonary disease.
to year variation, circulation during the study period may have been minimal. Surveillance encompassing all seasons over several years would aid in this regard. The presence of HCoV-HKU1 genetic sequences in stool samples from patients with gastrointestinal illness suggests this coronavirus may play a role in enteric disease. However, causality cannot be addressed through our study’s design. Prospective, population-based studies are required.

This study contains several shortcomings. The most notable is the lack of a control group without gastrointestinal disease. Further studies including an asymptomatic control group and paired respiratory sample analysis is warranted. In addition, by selecting specimens acquired through the core laboratories of the regional referral hospital, a bias towards finding individuals with more severe disease may occur. Investigations focusing on mild gastroenteritis outside the hospital should be undertaken. Despite these shortcomings, the paucity of individuals who screened positive for coronavirus suggests that these viruses likely play a minor role in human gastroenteritis requiring medical evaluation.

In conclusion, we identified the human coronavirus HKU1 in stool samples from patients with gastrointestinal symptoms. Shedding of HCoV-HKU1 in stool may play a role in this virus’s transmission. Other common human coronaviruses including HCoV-NL63, HCoV-OC43 and HCoV-229E were notably absent suggesting that these coronaviruses may play a lesser role in severe gastrointestinal disease. Further investigation into the role of coronaviruses in human disease outside the respiratory tract will lead to better understanding of these viral pathogens.

Conflicts of interest statement

None of the authors report conflicts of interest.

Acknowledgments

Support: This study is supported by the National Institute of Allergy And Infectious Diseases K23 AI065829-01. Internal Review Board: Collection of specimens and clinical data were approved by the University Hospitals Human Investigation Committee.

References

1. Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis 2003;9(May (5)):565–72.
2. Jin Y, Cheng WX, Yang XM, et al. Viral agents associated with acute gastroenteritis in children hospitalized with diarrhea in Lanzhou, China. J Clin Virol 2009;44(March (3)):238–41.
3. Jamieson FB, Wang EE, Bain C, Good J, Duckmann I, Petric M. Human torovirus: a new nosocomial gastrointestinal pathogen. J Infect Dis 1998;178(November (5)):1263–9.
4. Yamashita T, Sakae K, Tsuzuki H, et al. Complete nucleotide sequence and genetic organization of Aichi virus, a distinct member of the Picornaviridae associated with acute gastroenteritis in humans. J Virol 1998;72(October (10)):8408–12.
5. Cheng WX, Jin Y, Duan ZJ, et al. Human bocavirus in children hospitalized for acute gastroenteritis: a case–control study. Clin Infect Dis 2008;47(July (2)):161–7.
6. Yu JM, Li DD, Xu ZQ, et al. Human bocavirus infection in children hospitalized with acute gastroenteritis in China. J Clin Virol 2008;42(July (3)):280–5.
7. Chow BD, Ou Z, Esper FP. Newly recognized bocaviruses (HBoV, HBoV2) in children and adults with gastrointestinal illness in the United States. J Clin Virol 2010;47(February (2)):143–7.
8. Harvala H, Simmonds P. Human parechoviruses: biology, epidemiology and clinical significance. J Clin Virol 2009;45(May (1)):1–9.
9. Bradburne AF, Bynoe ML, Tyrrell DA. Effects of a “new” human respiratory virus in volunteers. Br Med J 1967;3(September (5568)):767–9.
10. Michael Lai SF, Anderson L. Coronavirus. 5th ed. Philadelphia: Lippincott-Raven; 2007.
11. Vabret A, Mouthon F, Mourou T, Gouari S, Petitjean J, Fremuth F. Direct diagnosis of human respiratory coronaviruses 229E and OC43 by the polymerase chain reaction. J Virol Methods 2001;97(September (1–2)):50–66.
12. Vabret A, Dina J, Gouari S, et al. Human (non-severe acute respiratory syndrome) coronavirus infections in hospitalised children in France. J Paediatr Child Health 2008;44(April (4)):176–81.
13. Dare RK, Fry AM, Chittagangpatt M, Sawanpanyalert P, Olsen SJ, Erdman DD. Human coronavirus infections in rural Thailand: a comprehensive study using real-time reverse-transcription polymerase chain reaction assays. J Infect Dis 2007;196(November (5)):1321–8.
14. Esper F, Weibel C, Ferguson D, Landry ML, Kahn JS. Evidence of a novel human coronavirus that is associated with respiratory tract disease in infants and young children. J Infect Dis 2005;191(February (4)):492–8.
15. Pedersen NC, Allen CE, Lyons LA. Pathogenesis of feline enteric coronavirus infection. J Feline Med Surg 2008;(June).
16. Tsunemitsu H, Yonenich H, Hiraiz T, et al. Isolation of bovine coronavirus from feces and nasal swabs of calves with diarrhea. J Vet Med Sci 1991;53(September (3)):433–7.
17. Resta S, Luby JP, Rosenfeld CR, Siegel JD. Isolation and propagation of a human enteric coronavirus. Science 1985;229(September (4717)):978–81.
18. Roussot S, Moscovic I, Lebon P, et al. Intestinal lesions containing coronavirus-like particles in neonatal necrotizing enterocolitis: an ultrastructural analysis. Pediatrics 1984;73(February (2)):218–24.
19. Gerna G, Passarini N, Battaglia M, Revello MG, Torre D, Cereda PM. Coronaviruses and gastroenteritis: evidence of antigenic relatedness between human enteric coronavirus strains and human coronavirus OC43. Microbiology 1984;70(October (4)):315–22.
20. Leung WK, To KF, Chan PK, et al. Enteric involvement of severe acute respiratory syndrome–associated coronavirus infection. Gastroenterology 2003;125(October (4)):1011–7.
21. Cheng PK, Wong DA, Tong KL, et al. Viral shedding patterns of coronavirus in patients with probable severe acute respiratory syndrome. Lancet 2004;363(May (9422)):1699–700.
22. Esper F, Weibel C, Ferguson D, Landry ML, Kahn JS. Coronavirus HKU1 infection in the United States. Emerg Infect Dis 2006;12(May (5)):775–9.
23. Vabret A, Dina J, Gouari S, Petitjean J, Corbet S, Fremuth F. Detection of the new human coronavirus HKU1: a report of 6 cases. Clin Infect Dis 2006;42(March (5)):634–9.
24. Nokso-Koivisto J, Pitkaranta A, Blomqvist S, Kilpi T, Hovi T. Coronavirus infections in children younger than two years of age. Pediatr Infect Dis J 2000;19(February (2)):164–6.
25. Osowy C. Direct detection of respiratory syncytial virus, parainfluenza virus, and adenovirus in clinical respiratory specimens by a multiplex reverse transcription–PCR assay. J Clin Microbiol 1998;36(November (11)):3409–54.
26. Park MS, Lee R, Bournemouth N, Leblanc B, Preiksaitis JK, Yu IP CC. Increased detection of rotavirus using a real time reverse transcription–polymerase chain reaction (RT–PCR) assay in stool specimens from children with diarrhea. J Med Virol 2004;72(March (3)):496–501.
27. Attaran RL, Estes MK. Diagnosis of noncultivable gastrointestinal viruses, the human calciviruses. Clin Microbiol Rev 2001;14(January (1)):15–37.
28. Dijkman R, Jehmfink MF, El Idriessi NB, et al. Human coronavirus NL63 and 229E serocconversion in children. J Clin Microbiol 2008;46(February (7)):2368–73.
29. Shao X, Guo X, Esper F, Weibel C, Kahn JS. Seroepidemiology of group I human coronaviruses in children. J Clin Virol 2007;40(November (3)):207–13.
30. van Elden LJ, van Loon AM, van Alphen F, et al. Frequent detection of human coronavirus in clinical specimens from patients with respiratory tract infection by use of a novel real-time reverse-transcriptase polymerase chain reaction. J Infect Dis 2004;189(February (4)):652–7.
31. van der Hoek L. Human coronaviruses: what do they cause? Antivir Ther 2007;12(4 Pt B):651–8.
32. Lau SK, Woo PC, Yip CC, et al. Coronavirus HKU1 and other coronavirus infections in Hong Kong. J Clin Microbiol 2006;44(June (6)):2063–7.