Epidemiology and clinical characteristics of human coronaviruses OC43, 229E, NL63, and HKU1: a study of hospitalized children with acute respiratory tract infection in Guangzhou, China

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Abstract

Human coronaviruses (HCoV) OC43, 229E, NL63, and HKU1 are common respiratory viruses which cause various respiratory diseases, including pneumonia. There is a paucity of evidence on the epidemiology and clinical manifestations of these four HCoV strains worldwide. We collected 11,399 throat swabs from hospitalized children with acute respiratory tract infection from July 2009 to June 2016 in Guangzhou, China. These were tested for four strains of HCoV infection using real-time polymerase chain reaction (PCR). HCoV-positive patients were then tested for 11 other respiratory pathogens. 4.3% (489/11399) of patients were positive for HCoV, of which 3.0% were positive for OC43 (346/11399), 0.6% for 229E (65/11399), 0.5% for NL63 (60/11399), and 0.3% for HKU1 (38/11399). Patients aged 7–12 months had the highest prevalence of HCoV and OC43 when compared with other age groups (p < 0.001). The peak seasons of infection varied depending on the HCoV strain. Patients infected with a single strain of HCoV infection were less likely to present fever (≥38 °C) (p = 0.014) and more likely to present pulmonary rales (p = 0.043) than those co-infected with more than one HCoV strain or other respiratory pathogens. There were also significant differences in the prevalence of certain symptoms, including coughing (p = 0.032), pneumonia (p = 0.026), and abnormal pulmonary rales (p = 0.002) according to the strain of HCoV detected. This retrospective study of the prevalence of four HCoV strains and clinical signs among a large population of pediatric patients in a subtropical region of China provides further insight into the epidemiology and clinical features of HCoV.

Introduction

Respiratory viral infections in humans, which can vary from common colds to severe respiratory disease, represent a significant global health burden and a pressing public health challenge in developing countries and among socioeconomically disadvantaged children in particular. Human coronaviruses (HCoV) OC43, 229E, NL63, and HKU1 are associated with a range of upper respiratory tract infections (URTI) and, occasionally, lower respiratory tract infections (LRTI), including pneumonia and bronchiolitis [1–4], particularly in children [5]. Although HCoV is widespread globally [6–8], the frequency of detection of its four major strains varies significantly both by geography and over time [9–13]. Despite these features of its epidemiology, few long-term studies of the prevalence of HCoV strains and their clinical manifestations have been undertaken [14–16]. This paucity of evidence has led to an incomplete characterization of the epidemiology and clinical presentation of HCoV across different contexts.

To expand the existing evidence base and provide new insights into the epidemiology and clinical manifestations of HCoV in a subtropical region, we performed a 7-year study of four HCoV strains among hospitalized pediatric patients with acute respiratory tract infection (ARTI) in Guangzhou, China.
Materials and methods

Sample collection

Throat swabs were collected from pediatric patients (≤ 14 years old) hospitalized with ARTI at The First Affiliated Hospital of Guangzhou Medical University and Sun Yat-Sen Memorial Hospital from July 2009 to June 2016. Both hospitals, each with nearly 2000 beds, were located in urban areas in Guangzhou, the capital city of a province with a humid subtropical climate. A case of HCoV was defined when a patient presented at least two of the following symptoms: cough, pharyngeal discomfort, rhinobyon, rhinorrhea, sneeze, dyspnea, or diagnosed with pneumonia by chest radiography during the previous week. The respiratory samples were refrigerated at 2–8 °C in viral transport medium, transported on ice to the State Key Laboratory of Respiratory Diseases, and analyzed immediately or stored at −80 °C before analysis.

Cases were retrospectively categorized into three groups according to their clinical symptoms: URTI, influenza-like symptoms, and LRTI. Patients presenting with cough, expectoration, rhinorrhea, sneeze, pharyngeal discomfort, or trachyphonia were classified as having URTI. Patients with fever (≥ 38 °C), chills, dizziness, headache, myalgia, or debilitation were classified as having influenza-like symptoms. Patients with bronchopneumonia, pneumonia, asthma, shortness of breath, chest tightness, chest pain, or abnormal pulmonary rales were classified as having LRTI. Bronchopneumonia and pneumonia were diagnosed with chest radiography. Other clinical symptoms were identified by common medical examinations and clinical descriptions.

Real-time PCR for HCoV detection

RNA was extracted from throat swab samples using the QIAamp Viral RNA Mini Kit (Qiagen, Shanghai, China), according to the manufacturer’s protocols. Samples were tested for four HCoV strains (HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1) using the TaqMan real-time PCR testing kit (Guangzhou HuYanSuo Medical Technology Co., Ltd.), as previously reported [17].

Detection of common respiratory pathogens in HCoV-positive patients

HCoV-positive samples were simultaneously tested using TaqMan real-time PCR assays (Guangzhou HuYanSuo Medical Technology Co., Ltd.) for the following 11 respiratory pathogens: influenza A virus (Flu A), influenza B virus (Flu B), respiratory syncytial virus (RSV), human bocavirus (HBoV), adenovirus (ADV), human rhinovirus (HRV), human metapneumovirus (HMPV), enterovirus (EV), Mycoplasma pneumoniae (MP), Chlamydia pneumoniae (CP), and four types of human parainfluenza virus (HPIV).

Statistical analysis

All data were analyzed with SPSS statistical software (version 19.0; SPSS Inc., Chicago, IL), as described previously [18]. The χ² test and Fisher’s exact test were used for comparisons of data. All tests were two tailed and p < 0.05 was considered statistically significant.

Results

Detection of HCoV among patients with ARTI

A total of 11,399 hospitalized pediatric patients (≤ 14 years old) with ARTI were enrolled in this study between July 2009 and June 2016. The median age of the patients was 1.75 years (interquartile range, 0.75–3.83) and the male to female ratio was 1.82:1 (7361:4038). We found that 489 out of the 11,399 patients (4.3%) tested positive for HCoV. Of these, 346 (3%) were positive for HCoV-OC43, 65 (0.6%) for HCoV-229E, 60 (0.5%) for HCoV-NL63, and 38 (0.3%) for HCoV-HKU1. The median age of HCoV-positive patients was 1.25 years (interquartile range, 0.75–3) and the male to female ratio was 1.67:1 (306:183).

Co-infection in HCoV-positive patients

Samples from HCoV-positive patients were also tested for 11 other common respiratory pathogens. Of the 489 HCoV-positive patients, 258 (52.8%) were infected with only one HCoV strain, while 231 (47.2%) were found to be co-infected with one or more additional strains of HCoV or another respiratory pathogen (Table 1). Of these, the most frequently identified pathogens were Flu A (21.6%, 50/231) and RSV (21.6%, 50/231).

Age distribution of HCoV-positive patients

In this study, patients were divided into seven age groups: 0–3 months, 4–6 months, 7–12 months, 1–2 years, 3–5 years, 6–10 years, and 11–14 years. There were statistically significant differences in the prevalence of overall HCoV and of HCoV-OC43 by age group (p < 0.001). Patients aged 7–12 months had the highest prevalence of both overall HCoV (5.9%, 71/1203) and HCoV-OC43 (4.1%, 49/1203) compared with the other age groups (Fig. 1). There were no significant differences in the prevalence of HCoV-229E (p = 0.429) or HCoV-NL63 (p = 0.437). Too few cases of HCoV-HKU1 were identified to assess the age distribution for this strain.
There was a clear seasonal pattern in the presentation of HCoV cases over the 7-year period (Fig. 2). The overall prevalence of HCoV among attending patients tended to be highest in the spring and autumn. During the study period, the months with the highest recorded prevalences were February 2011 (11.7%, 9/77), April 2011 (13.2%, 14/106), April 2012 (15.3%, 25/163), August 2012 (13.4%, 19/142), July 2013 (11.7%, 23/196), and January 2014 (11.0%, 17/154). These seasonal trends were primarily driven by cases of HCoV-OC43. The other strains had different seasonal patterns, with smaller, more sporadic outbreaks.

Clinical presentations of HCoV-positive patients

Table 2 shows the prevalence of clinical symptoms among HCoV-positive patients (n = 489) according to whether they had a single HCoV infection (n = 258) or were co-infected (n = 231), and according to the strain of HCoV detected.

There were statistically significant differences in the prevalence of pulmonary rales and fever according to whether a patient was co-infected. While the prevalence of pulmonary rales was higher among patients with a single HCoV infection (63.6%, 164/258) than among co-infected patients (54.5%, 126/231) (p = 0.043), fever was more prevalent among co-infected patients (66.2%, 153/231) than those with only one HCoV strain (55.4%, 143/258) (p = 0.014).

There were also significant differences in the prevalence of cough (p = 0.032), pneumonia (p = 0.026), and abnormal pulmonary rales (p = 0.002) according to the strain of HCoV detected.

Data are presented as no. (%) of each group. Percentages sum to over 100% because some patients had more than one diagnosis:

- Fluv A, influenza A virus; Fluv B, influenza B virus; ADV, adenovirus; HRV, human rhinovirus; HMPV, human metapneumovirus; EV, enterovirus; MP, Mycoplasma pneumoniae; RSV, respiratory syncytial virus; HBoV, human bocavirus; HPIV, human parainfluenza virus; CP, Chlamydia pneumoniae; HCoV, human coronavirus

b Detection of more than one strain of HCoV

c Not detected
Discussion

This retrospective study analyzed data from 11,399 hospitalized children (≤ 14 years old) presenting with ARTI in two large municipal hospitals over a 7-year period in Guangzhou, China. Given the present study’s duration and large sample size, our results represent an important addition to the evidence base on the epidemiology and clinical manifestations of HCoV. Of the 11,399 patients tested, we found that 489 (4.3%) were HCoV-positive and that the most prevalent strain of HCoV was OC43 (3.0%), followed by 229E (0.6%), NL63 (0.5%), and HKU1 (0.3%). These findings are consistent with
the results of other studies around the world [11, 13, 19]. The most common co-infecting pathogens among HCoV-positive patients were Flu A and RSV (Table 1). Other recent studies have also reported that RSV, Flu A, and rhinoviruses are the most common pathogens that co-occur with HCoV, and that co-infection may influence the clinical presentation of HCoV-positive patients [4, 5, 19–21].

Consistent with studies conducted in other contexts, including America and Slovenia [16, 20], our results showed that the prevalence of HCoV was highest among patients aged 7–12 months (Fig. 1). This increased vulnerability to respiratory pathogens may be attributable to increased contact with pathogens as infants begin to explore their environment or the waning of maternal antibody levels in infants while the immune system remains underdeveloped [22–24].

HCoV is widespread globally and patterns of outbreaks vary according to locations and seasonal factors [4]. Our study found that HCoV prevalence among patients presenting with ARTI in Guangzhou over a 7-year period was highest in the spring and autumn (Fig. 2). This stands in contrast to other studies which find higher prevalence of HCoV infection in winter and spring [16, 20]. We also found different seasonal prevalence patterns for each of the four HCoV strains, with peak frequencies of 229E, NL63, and OC43 occurring mostly in the spring and autumn in Guangzhou, although OC43 had lower peaks appearing in July 2012 and 2013, however (Fig.

### Table 2: Clinical characteristics of human coronavirus-positive patients

| Clinical presentation | Infection with HCoV | Distributions of HCoV strain |
|-----------------------|---------------------|------------------------------|
|                       | Single HCoV (n = 258) | Co-pathogen (n = 231) | p-Value<sup>a</sup> | 229E (n = 65) | OC43 (n = 346) | NL63 (n = 60) | HKU1 (n = 38) | p-Value<sup>b</sup> |
| Upper respiratory tract infection | | | | | | | | |
| Cough                 | 214 (82.9)          | 198 (85.7)          | 0.401             | 52 (80.0)       | 298 (86.1)       | 43 (71.7)       | 33 (86.9)       | 0.032                  |
| Expectoration         | 87 (33.7)           | 81 (35.0)           | 0.755             | 20 (30.8)       | 119 (34.4)       | 15 (25.0)       | 18 (47.4)       | 0.137                  |
| Rhinorrhea            | 87 (33.7)           | 87 (37.7)           | 0.363             | 24 (36.9)       | 123 (35.6)       | 21 (35.0)       | 13 (34.2)       | 0.993                  |
| Rhinobyon             | 79 (30.6)           | 69 (29.9)           | 0.857             | 22 (33.9)       | 106 (30.6)       | 15 (25.0)       | 12 (31.6)       | 0.747                  |
| Sneeze                | 10 (3.9)            | 10 (4.3)            | 0.801             | 4 (6.2)         | 16 (4.6)         | 2 (3.4)         | 0 (0)           | –                      |
| Pharyngeal discomfort<sup>c</sup> | 17 (6.6)           | 15 (6.5)           | 0.966             | 8 (12.3)        | 22 (6.4)         | 4 (6.8)         | 3 (7.9)         | 0.381                  |
| Trachyphonia          | 9 (3.5)             | 0 (0)               | –<sup>e</sup>     | 1 (1.5)         | 3 (0.9)         | 5 (8.3)         | 0 (0)           | –                      |
| Influenza-like symptoms | | | | | | | | |
| Fever (≥ 38 °C)       | 143 (55.4)          | 153 (66.2)          | 0.014             | 38 (58.5)       | 213 (61.6)       | 32 (53.3)       | 23 (60.5)       | 0.662                  |
| Chills                | 8 (3.1)             | 11 (4.8)            | 0.343             | 3 (4.6)         | 16 (4.6)         | 1 (1.7)         | 1 (2.6)         | 0.859                  |
| Dizziness             | 0 (0)               | 1 (0.5)             | –                  | 0 (0)           | 1 (0.3)         | 0 (0)           | 0 (0)           | –                      |
| Headache              | 2 (0.8)             | 1 (0.5)             | 0.923             | 0 (0)           | 3 (0.9)         | 0 (0)           | 0 (0)           | –                      |
| Myalgia               | 0 (0)               | 0 (0)               | –                  | 0 (0)           | 0 (0)           | 0 (0)           | 0 (0)           | –                      |
| Debilitation          | 1 (0.4)             | 2 (0.9)             | 0.923             | 0 (0)           | 3 (0.9)         | 0 (0)           | 0 (0)           | –                      |
| Lower respiratory tract infection | | | | | | | | |
| Bronchopneumonia      | 47 (18.2)           | 52 (22.5)           | 0.238             | 12 (18.5)       | 79 (22.8)       | 8 (13.3)       | 6 (15.8)       | 0.295                  |
| Pneumonia             | 26 (10.1)           | 32 (13.9)           | 0.197             | 13 (20.0)       | 34 (9.8)       | 12 (20.0)       | 6 (15.8)       | 0.026                  |
| Asthma                | 70 (27.1)           | 64 (27.7)           | 0.887             | 16 (24.6)       | 99 (28.6)       | 13 (21.7)       | 9 (23.7)       | 0.632                  |
| Shortness of breath   | 35 (13.6)           | 28 (12.1)           | 0.634             | 7 (10.8)       | 42 (12.1)       | 9 (15.0)       | 6 (15.8)       | 0.776                  |
| Chest tightness       | 1 (0.4)             | 0 (0)               | –                  | 0 (0)           | 1 (0.3)         | 0 (0)           | 0 (0)           | –                      |
| Chest pain            | 1 (0.4)             | 1 (0.4)             | 0.926             | 0 (0)           | 2 (0.5)         | 0 (0)           | 0 (0)           | –                      |
| Abnormal pulmonary rales<sup>d</sup> | 164 (63.6)       | 126 (54.5)          | 0.043             | 30 (46.2)       | 193 (55.8)       | 18 (30.0)       | 19 (50.0)       | 0.002                  |

Data are presented as no. (%) of each group. Percentages sum to over 100% because some patients had more than one diagnosis. Significant differences in bold.

<sup>a</sup> Two-tailed χ² test, testing the distribution of each illness or diagnosis between patients infected with a single HCoV type and those co-infected with other type of HCoV or other respiratory pathogen.

<sup>b</sup> Two-tailed χ² test, testing the distribution of each illness or diagnosis between the four HCoV types.

<sup>c</sup> Including pharyngeal dryness and pharyngalgia.

<sup>d</sup> Including phlegm rale, wheeze rale, bubbling rale, moist rale, and laryngeal stridor.

<sup>e</sup> Not performed due to small sample size.
HCoV-NL63 and HCoV-OC43 are associated with LRTI in consist with the findings of Lee and Storch [13] that fever, cough, sore throat, and headache [1, 19], and that LRTI [26]. Recent studies have also shown that the most RSV may be more strongly implicated in cases of severe some symptoms related to LRTI, other pathogens such as though HCoV-OC43 may be associated with asthma and even pneumonia. The present study underscores the importance of HCoV infection in the etiology of pediatric ARTI, its relevance in clinical practice, and the pressing need to improve surveillance and detection in developing country contexts.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of The First Affiliated Hospital of Guangzhou Medical University Ethics Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent This study was a retrospective study, so formal consent was not required.

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