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# BMJ Open

## Epidemiology and clinical features of human metapneumovirus in children hospitalized with acute respiratory tract infection from 2013 to 2016: a cross-sectional study



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Complete List of Authors:	Zhang, Ling; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Liu, Donglan; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Chen, Dehui; Department of Pediatrics, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Tan, Weiping ; Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University Qiu, Shuyan; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Xu, Duo; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Li, Xiao; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Liu, Tiantian; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Liu, Wenkuan; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Zhou, Rong; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University
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15 Ling Zhang<sup>1†</sup>, Donglan Liu<sup>1†</sup>, Dehui Chen<sup>2</sup>, Weiping Tan<sup>3</sup>, Shuyan Qiu<sup>1</sup>, Duo Xu<sup>1</sup>, Xiao  
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17 Li<sup>1</sup>, Tiantian Liu<sup>1</sup>, Wenkuan Liu<sup>1\*</sup>, Rong Zhou<sup>1\*</sup>  
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22 <sup>1</sup>State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of  
23  
24 Guangzhou Medical University, Guangzhou Medical University, Guangzhou,  
25  
26 People's Republic of China.  
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29  
30 <sup>2</sup>Department of Pediatrics, The First Affiliated Hospital of Guangzhou Medical  
31  
32 University, Guangzhou Medical University, Guangzhou, People's Republic of China.  
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35 <sup>3</sup>Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, People's  
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37 Republic of China.  
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42 \*Correspondence to Professor Rong Zhou; [zhourong@gird.cn](mailto:zhourong@gird.cn) and Wenkuan Liu;  
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44 [ahlwk2000-2004@163.com](mailto:ahlwk2000-2004@163.com);  
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47 <sup>†</sup>Ling Zhang, Donglan Liu contributed equally to this work.  
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19 **Abstract**

20 **Objectives:** Human metapneumovirus (HMPV) is one of the most important  
21 respiratory viral pathogens among infants and children worldwide. To analyze  
22 epidemiologic and clinical characteristics of HMPV, patients hospitalized with acute  
23 respiratory illness (ARI) were studied in Guangzhou, southern China.

24 **Study design:** A cross-sectional study.

25 **Setting:** Two hospitals in Guangzhou.

26 **Participants and methods:** Throat swabs (n=5133) were collected and tested from  
27 pediatric patients ( $\leq 14$  years) hospitalized with ARI over a three-year period, and 101  
28 of 103 (98.1) HMPV-positive patients' clinical presentations were recorded for  
29 further analysis.

30 **Results:** Of the 5,133 patients, 103 (2.0%) were positive for HMPV. More frequency  
31 of HMPV infection occurred in patients  $\leq 5$  year-old (2.2%, 98/4399) than in patients  $> 5$   
32 year-old (0.7%, 5/734) ( $p=0.004$ ), and mostly distributed in children with the age  
33 groups of 3–6 months (2.4%, 10/423),  $> 6$ –12 months (2.2%, 30/1342),  $> 1$ –2 years  
34 (2.8%, 21/752) and  $> 2$ –5 years (2.3%, 32/1403) ( $p=0.865$ ). Two seasonal HMPV  
35 frequency peaks were found every year, and mainly occurred in spring and early  
36 summer. Nineteen of 103 (18.4%) HMPV-positive patients had co-infection with other  
37 pathogens, and most common co-pathogen was respiratory syncytial virus (36.8%,  
38 7/19). HMPV infection led to a wide spectrum of symptoms, and the main  
39 symptoms included cough (100.0%, 101/101), abnormal pulmonary breath sound  
40 (91.1%, 92/101), fever (88.1%, 89/101), expectoration (77.2%, 78/101), bronchopn-

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4 41 eumonia (55.4%, 56/101), coryza (50.5%, 51/101) and wheezing (46.5%, 47/101).

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6 42 More fever ( $\geq 38^{\circ}\text{C}$ ) cases (71.6%, 76/83) were found among mono-infected patients

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9 43 than co-infected patients (72.2%, 13/18) ( $p=0.037$ ). While diarrhea was found more

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11 44 frequently in HMPV co-infected patients (22.2%, 4/18) than mono-infected patients

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13 45 (3.6%, 3/83) ( $p=0.018$ ).

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16 46 **Conclusions:** This study provides valuable insight into HMPV epidemiology and

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18 47 clinical characteristics, which may be helpful in the control and prevention of infectious

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21 48 diseases.

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26 50 **Strengths and limitations of this study**

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28 51 1 .The study investigate the epidemiological and clinical features of human

29  
30 52 metapneumovirus (HMPV) infection in pediatric patients hospitalized with acute

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32 53 respiratory illness, which may aid in diagnosis, control and prevention of infectious

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34 54 diseases.

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36 55 2. The study of relatively large sample size is helpful to the accurate study of HMPV

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38 56 characteristics.

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40 57 3. The study had some limitations: the characteristics of HMPV-negative patients were

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42 58 not further analyzed, and bacterial pathogens were not detected in HMPV-infection

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44 59 patients. These defects may affect our comprehensive understanding of HMPV

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46 60 infection in epidemiological and clinical features.

## 61 Introduction

62 Human metapneumovirus (HMPV) is a nonsegmented, negative-sense  
63 single-stranded RNA virus, which belongs to the *Paramyxoviridae* family,  
64 *Pneumovirinae* subfamily, and *Metapneumovirus* genus.<sup>1</sup> HMPV was first discovered  
65 in 2001 in the Netherlands and was isolated from a pediatric patient with acute  
66 respiratory illness (ARI).<sup>2</sup> Since then, HMPV has been associated with acute  
67 respiratory disease in individuals of all ages worldwide, but it is more common in  
68 children, elderly, and immunocompromised adults.<sup>3-5</sup> Young children aged  $\leq 5$  years  
69 seem to be particularly susceptible to HMPV infection. In previous studies,<sup>6-12</sup> which  
70 mostly detected in developed countries, approximately 2.5–11.3% of respiratory  
71 samples were positive for HMPV in children  $\leq 5$  years and 90% of individuals have  
72 positive serology for HMPV by 5 years of age.<sup>13</sup>

73 HMPV causes a variety of clinical symptoms ranging from a mild upper  
74 respiratory tract infection (URTI) to life-threatening lower respiratory tract infection  
75 (LRTI).<sup>3 14-16</sup> And until now, there is no effective vaccine and drug treatment for HMPV.  
76 Thus, more studies are needed all over the world, especially in developing countries,  
77 where only a limited number of reports about HMPV.

78 In this study, we investigated the epidemiological and clinical features of HMPV  
79 infection in pediatric patients ( $\leq 14$  years) hospitalized with ARI in Guangzhou,  
80 southern China, between July 2013 and June 2016 using real-time PCR. The findings  
81 of this study will be helpful in understanding the distribution of HMPV in subtropical  
82 region and providing the data for developing effective control and prevention

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4 83 strategies. Our results also provide valuable insight into the clinical features of HMPV,  
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6 84 which will be helpful in early clinical diagnosis.  
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## 85 **Methods**

### 86 **Study design and respiratory sample collection**

87 This was a cross-sectional study at The First Affiliated Hospital of Guangzhou  
88 Medical University and Sun Yat-Sen Memorial Hospital in Guangzhou. Samples in this  
89 study were taken as part of standard care. Throat swab samples (n=5133) from  
90 pediatric patients ( $\leq 14$  years) hospitalized with ARI (presenting with at least two of the  
91 following symptoms: cough, pharyngeal discomfort, nasal obstruction, coryza, sneeze,  
92 dyspnoea or diagnosed with pneumonia by chest radiography during the previous  
93 week) were collected at two hospitals between July 2013 and June 2016. The  
94 samples were collected with established clinical protocols.<sup>17</sup> The samples were  
95 refrigerated at 2–8°C in viral transport medium, transported on ice to the State Key  
96 Laboratory of Respiratory Diseases, and analyzed immediately or stored at -80°C  
97 before analysis as previously described.<sup>18</sup>

98 The patients' clinical symptoms were extracted from their medical records  
99 using designed presentation cards and were retrospectively categorized into the  
100 following four groups: URTI, LRTI, systemic influenza-like symptoms, and  
101 gastrointestinal illness. Patients with nasal obstruction, coryza, sneezing, coughing,  
102 pharyngeal discomfort, or hoarseness were categorized as having URTI. Patients with  
103 pneumonia, bronchiolitis, increased lung markings, dyspnea, or an abnormal  
104 pulmonary breath sound were categorized as having LRTI. Patients with high fever  
105 ( $\geq 38^\circ\text{C}$ ), chills, dizziness, headache, myalgia, or debility were categorized as having  
106 systemic influenza-like symptoms. Patients with vomiting, poor appetite, or diarrhea

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4 107 were categorized as having gastrointestinal illness. Some patients were assigned to  
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6 108 several clinical presentation groups. Patients with incomplete clinical data were  
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9 109 excluded from this analysis. Increased lung markings, bronchopneumonia,  
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11 110 pneumonia, and bronchiolitis were diagnosed by chest radiography. Abnormal  
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13 111 pulmonary breath sounds included phlegmatic rales, wheezy rales, bubbling rales,  
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15 112 and moist rales. Other clinical symptoms were identified by a general medical  
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17 113 examination and clinical descriptions as previously reported.<sup>19</sup>  
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#### 115 **Real-time RT-PCR for HMPV detection**

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26 116 RNA were extracted from the respiratory samples with the QIAamp Viral RNA  
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28 117 Mini Kit (Qiagen, Shanghai, China), respectively, according to the manufacturer's  
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30 118 protocols. HMPV was determined by using TaqMan Real-time PCR assays, as  
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32 119 previously reported,<sup>18</sup> using kits from Guangzhou HuYanSuo Medical Technology Co.,  
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34 120 Ltd according to the manufacturer's protocols.  
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#### 122 **Detection of common respiratory pathogens in HMPV-positive patients**

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43 123 HMPV-positive samples were simultaneously tested by using TaqMan Real-time  
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45 124 PCR assays for the following 17 respiratory pathogens : respiratory syncytial virus  
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47 125 (RSV), parainfluenza virus types 1–4 (PIV1–4), influenza A and B viruses (InfA, InfB),  
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49 126 adenovirus (ADV), enterovirus (EV), human coronaviruses (HCoV-229E, HCoV-OC43,  
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51 127 HCoV-NL63, HCoV-HKU1), human rhinovirus (HRV), human bocavirus (HBoV),  
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55 128 *Mycoplasma pneumoniae* (MP), and *Chlamydomphila pneumoniae* (CP). The testing  
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4 129 procedure was conducted using kits from Guangzhou HuYanSuo Medical Technology  
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6 130 Co., Ltd as previously described.<sup>18</sup>  
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11 132 **Statistical analysis**

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13 133 All statistical analyses were performed with SPSS statistical software (version  
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16 134 19.0; SPSS Inc., Chicago, IL, USA). To compare categorical data, the  $\chi^2$  test and  
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19 135 Fisher's exact test were used as appropriate. All tests were two-tailed, and  $p < 0.05$   
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21 136 was considered statistically significant.  
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## 137 **Results**

### 138 **Detection of HMPV from patients with ARI**

139 In total, 5133 pediatric patients ( $\leq 14$  years) hospitalized with ARI between July  
140 2013 and June 2016 were enrolled in this study. The male-to-female ratio was 1.75  
141 (3269:1864) and the median age was 2.76 years (interquartile range, 0.75–4.00  
142 years). Of the 5133 patients, 103 (2.0%) were positive for HMPV. The male-to-female  
143 ratio was 1.86:1 (67:36) in HMPV-positive patients and 1.75:1 (3202:1828) in  
144 HMPV-negative patients ( $p=0.771$ ). The median age of HMPV-positive patients was  
145 1.95 years (interquartile range, 0.83–3.00 years).

### 147 **Co-infection with common respiratory pathogens**

148 In this study, we tested HMPV-positive patients for 17 other common  
149 respiratory pathogens. Of the 103 HMPV-positive patients, 84 (81.6%) patients had  
150 HMPV mono-infection and 19 (18.4%) patients had HMPV co-infection with  $\geq 1$  other  
151 pathogen. The male-to-female ratio was 2:1 (56:28) in HMPV mono-infected patients  
152 and 1.38:1 (11:8) in HMPV co-infected patients ( $p=0.469$ ). Nine of these 17 pathogens  
153 (52.9%) were detected, and the most frequent co-pathogens were RSV (36.8%, 7/19),  
154 HCoV-OC43 (15.8%, 3/19), MP (15.8%, 3/19), and HBoV (15.8%, 3/19) (**Table 1**).

### 156 **Age distributions of HMPV-positive patients**

157 Overall, a significant difference were found between the distribution of  
158 HMPV-positive patients aged  $>5$ –14 years (0.7%, 5/734) and those aged  $\leq 5$  years

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4 159 (2.2%, 98/4399) ( $p=0.004$ ), for detail, the patients were divided into six age groups:  
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6 160 0–3 months, >3–6 months, >6–12 months, >1–2 years, >2–5 years, and >5–14 years.  
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9 161 The distribution of HMPV-infected patients in each group was significantly different  
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11 162 ( $p=0.03$ ) (**Figure 1**). HMPV infection most frequently occurred in children aged >1–2  
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14 163 years (2.8%, 21/752) (**Figure 1**). However, there were no significant difference in  
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16 164 frequency of HMPV infection among patients aged >1–2 years, >3–6 months (2.4%,  
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18 165 10/423), >6–12 months (2.2%, 30/1342), and >2–5 years (2.3%, 32/1403) ( $p=0.865$ ).  
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### 22 23 24 167 **Seasonal distribution of HMPV infection**

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26 168 In general, the frequency of HMPV infections peaked twice every year (**Figure**  
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28 169 **2**). Large peaks in the HMPV detection rate occurred in March 2014 (8%, 16/200),  
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31 170 May 2015 (7.6%, 8/105), and February 2016 (8.7%, 9/103). Small peaks in the HMPV  
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34 171 detection rates occurred in November 2014 (1.7%, 2/121), September 2015 (2.8%,  
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36 172 2/72), and May 2016 (1.5%, 2/135) (**Figure 2**).  
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### 40 41 174 **Clinical presentations of HMPV-positive patients**

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44 175 We analyzed the clinical presentations of 101 of 103 (98.1%) HMPV-positive  
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46 176 patients. Patients with incomplete clinical data were excluded from this analysis ( $n=2$ ).  
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49 177 The main symptoms of HMPV infection included cough (100.0%, 101/101), abnormal  
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51 178 pulmonary breath sound (91.1%, 92/101), fever (88.1%, 89/101), expectoration  
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54 179 (77.2%, 78/101), bronchopneumonia (55.4%, 56/101), coryza (50.5%, 51/101) and  
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56 180 wheezing (46.5%, 47/101) (**Table 2**). We also compared the clinical features of  
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4 181 patients with HMPV mono-infection and co-infections, and significant differences were  
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6 182 found in patients with fever ( $\geq 38^{\circ}\text{C}$ ) ( $p=0.037$ ) and diarrhea ( $p=0.018$ ), which  
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9 183 observed in 91.6% (76/83), 3.6% (3/83) patients with single infection and 72.2%  
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11 184 (13/18), 22.2% (4/18) patients with co-infection, respectively (**Table 2**).

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4 185 **Discussion**

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6 186 Viruses are the most frequent cause of respiratory infection.<sup>20</sup> HMPV has been  
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9 187 recognized as an important respiratory pathogen causing ARI since its discovery in  
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11 188 2001. It has been reported that HMPV was responsible for approximately 5–10% of  
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14 189 hospitalizations of children suffering from ARI,<sup>14</sup> which has led to considerable clinical  
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16 190 and economic burden worldwide. HMPV detection rates vary according to geographic  
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19 191 location, and the incidence of HMPV may show seasonal or annual patterns in the  
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21 192 same area. In previous studies,<sup>21-30</sup> the HMPV detection rate was approximately  
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24 193 1–17% of ARI. In this 3-year study, 103 of 5133 (2.0%) patients were positive for  
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26 194 HMPV, which is similar to previous reports of HMPV in patients with ARI in southern  
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29 195 China<sup>31</sup> and Japan.<sup>32</sup>

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31 196 HMPV is commonly found in the pediatric population, with children under 5  
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34 197 year-old most susceptible to and children under 2 year-old at the greatest risk for  
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36 198 serious HMPV infections.<sup>15</sup> In our study, more frequency of HMPV infection occurred  
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39 199 in patients  $\leq 5$  year-old (2.2%, 98/4399) than in patients  $> 5$  year-old (0.7%, 5/734)  
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41 200 ( $p=0.004$ ), which is consistent with most previous studies.<sup>3 30 33 34</sup> In detail, HMPV  
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44 201 infection were found high frequency in children with the age groups of 3–6  
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46 202 months,  $> 6$ –12 months,  $> 1$ –2 years and  $> 2$ –5 years ( $p=0.865$ ) (**Figure 1**). Therefore,  
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49 203 children of this age need more attention to prevent HMPV infection. Moreover, our  
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51 204 findings suggested a male predominance, there was no significant difference in the  
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54 205 HMPV positivity rate between males and females ( $p=0.771$ ), which is consistent with  
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56 206 previous studies.<sup>35 36</sup>

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4 207 HMPV has a typical seasonal distribution. The activity of HMPV is largely  
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6 208 affected by different climate factors, which are locally present. The activity of HMPV in  
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9 209 temperate climates peaks at the end of winter or in early spring.<sup>37 38</sup> In this study,  
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11 210 seasonal peaks of HMPV infection were detected in February 2016, March 2014, and  
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13 211 May 2015 (**Figure 2**). This finding indicates that in Guangzhou, HMPV circulates  
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15 212 primarily during the spring and early summer, as previously reported in other  
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18 213 subtropical areas.<sup>22 39</sup> Furthermore, we also found small peaks in November 2014,  
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20 214 September 2015, and May 2016 (**Figure 2**). Generally, the seasonal distribution  
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22 215 pattern of HMPV overlaps that of RSV in this area.<sup>19</sup> These data might be useful for  
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24 216 control and prevention strategies of HMPV.

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28 217 HMPV co-infection with other respiratory pathogens has been reported in  
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30 218 many studies, including RSV,<sup>40</sup> InfA,<sup>41</sup> InfB,<sup>42</sup> PIV,<sup>43</sup> ADV,<sup>43 44</sup> HBoV,<sup>45</sup> HCoV,<sup>43 46</sup> RV,<sup>43</sup>  
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32 219 EV,<sup>43</sup> MP,<sup>41</sup> and CP.<sup>41</sup> In this study, most HMPV-positive patients had mono-infection  
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34 220 (81.6%, 84/103), and there was no significant difference in the HMPV positivity rate  
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36 221 according to sex between HMPV mono- and co-infected patients ( $p=0.469$ ). The  
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38 222 co-infection rate was 18.4% (19/103), and the most frequently detected co-pathogen  
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40 223 was RSV (36.8%, 7/19) (**Table 1**), which might be due to the overlapping seasonal  
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42 224 distribution of HMPV and RSV.<sup>47</sup> Co-infection with HCoV-OC43, MP, HBoV, ADV, RV,  
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44 225 InfA, PIV2, and PIV3 occurred at rates >5%, suggesting a broad range of  
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46 226 co-pathogens in HMPV infection (**Table 1**).

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49 227 HMPV most commonly causes URTI and LRTI in young children. The clinical  
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51 228 manifestations of HMPV infection are similar to those of RSV infection, especially in  
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4 229 young children,<sup>48-50</sup> and HMPV has been reported that the most frequent diagnoses of  
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6 230 children hospitalized with HMPV infection were pneumonitis and bronchiolitis.<sup>3 51 52</sup> In  
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9 231 some studies, bronchiolitis and recurrent wheezing/pneumonia were the main clinical  
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11 232 diagnosis,<sup>7 22 53 54</sup> while in other studies, bronchopneumonia, bronchiolitis, and  
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14 233 bronchial asthma exacerbation were the main clinical diagnosis.<sup>34</sup> In this study, we  
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16 234 analyzed the clinical presentations of 101 HMPV-positive patients (**Table 2**), and  
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19 235 55.4%, 7.9%, and 10.9% of patients were diagnosed with bronchopneumonia,  
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21 236 bronchiolitis, and pneumonia by chest radiography, respectively. Of all clinical  
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24 237 presentations, cough (100.0%), abnormal pulmonary breath sound (91.1%), fever  
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26 238 (88.1%), expectoration (77.2%), bronchopneumonia (55.4%), coryza (50.5%), and  
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29 239 wheezing (46.5%) occurred frequently in HMPV-positive patients (Table 2), similar to  
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31 240 those reported previously.<sup>16 29 52 55</sup>

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34 241 When we compared the clinical features between HMPV mono- and  
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36 242 co-infected patients, more fever cases ( $p=0.037$ ) were found among mono-infected  
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39 243 patients than co-infected patients, suggesting that fever was the key clinical  
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41 244 presentation caused by HMPV infection (**Table 2**). Diarrhea was found more  
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44 245 frequently in HMPV co-infected patients than mono-infected patients ( $p=0.018$ ),  
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46 246 suggested that diarrhea is not the main manifestation of HMPV infection, but may be  
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49 247 caused by other pathogens. No significant difference was found in the other clinical  
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51 248 presentations between HMPV mono- and co-infected patients, similar to the results of  
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53  
54 249 a previous study.<sup>51</sup> In this study, most frequent co-pathogen in HMPV-positive patients  
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56 250 were RSV (36.8%) (**Table 1**). Whether HMPV/RSV co-infection is more severe than  
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4 251 the respective mono-infections remains unclear. A previous study showed that  
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6 252 children with HMPV/RSV co-infection were more likely to develop pneumonia,  
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9 253 however, disease severity was not increased.<sup>53</sup> Conversely, Semple et al.<sup>56</sup> reported  
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11 254 that HMPV/RSV co-infection can cause more severe bronchiolitis in patients. In our  
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14 255 study, no significant difference in the pneumonia rate was observed between HMPV  
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16 256 mono- and co-infected patients. It has been confirmed that a history of prematurity,  
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19 257 particular age groups, and the presence of chronic diseases increases the risk of  
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21 258 severe LRTI among HMPV- and RSV-infected children.<sup>51</sup> The clinical manifestations  
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24 259 of HMPV infection are complex and diverse; the data in this work might be helpful in  
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26 260 the diagnosis of HMPV. In this study, the characteristics of HMPV-negative patients  
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29 261 were not further analyzed, and bacterial pathogens were not detected in  
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31 262 HMPV-infection patients. These defects may affect our comprehensive understanding  
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34 263 of HMPV infection in epidemiological and clinical features.  
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4 264 **Conclusions**  
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6 265 In this study, we analyzed the epidemiology and clinical manifestations of  
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9 266 HMPV infection in hospitalized children under 14 years old over a three-year period in  
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11 267 Guangzhou, China. Our results provide valuable insight into the epidemiology and  
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13 268 clinical features of HMPV infection in subtropical region, which may be helpful in  
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16 269 diagnosis, prevention of HMPV infection.  
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16 275 **Contributors**  
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20 277 performed pathogen testing. DHC and WPT collected clinical data. All authors  
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22 278 participated in the data analysis. LZ, DLL, RZ and WKL drafted the manuscript. All  
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24 279 authors read and approved the final version of this manuscript.  
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48 288 **Competing interests**  
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50 289 None declared.  
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56 291 **Ethics approval**  
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292 The study was approved by The First Affiliated Hospital of Guangzhou Medical  
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294 legal guardians.

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296 **Data sharing statement**

297 No additional data are available.

For peer review only

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452 **Table 1. Distribution of co-pathogens in 19 HMPV-positive patients**

Co-pathogens <sup>a</sup>	Patient No. (%)
RSV/HMPV	7(36.8)
HCoV-OC43/ HMPV	3(15.8)
MP/ HMPV	3(15.8)
HBoV/ HMPV	3(15.8)
ADV/ HMPV	2(10.5)
HRV/ HMPV	2(10.5)
InfA/ HMPV	2(10.5)
PIV2/ HMPV	1(5.3)
PIV3/ HMPV	1(5.3)

453 <sup>a</sup>HMPV-positive patients were tested for 17 common respiratory pathogens.

454 InfB, PIV1, PIV4, EV, HCoV-HKU1, HCoV-229E, HCoV-NL63, CP were not detected.

455 Percentages sum to >100% because some patients were co-infected with more than  
 456 two viruses.

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Table 2. Clinical presentations in HMPV-positive patients

Characteristics	Total HMPV infection (n=101)	Patients with single HMPV and co-pathogens		p value <sup>b</sup>
		Single HMPV (n = 83)	Co-pathogens (n= 18)	
<b>URTI</b>				
Nasal obstruction	40(39.6)	34(41.0)	6(33.3)	0.605
Coryza	51(50.5)	44(53.0)	7(38.9)	0.309
Sneeze	1(1.0)	1(1.2)	0(0)	— <sup>c</sup>
Cough	101(100)	83(100)	18(100)	— <sup>d</sup>
Expectoration	78(77.2)	66(79.5)	12(66.7)	0.351
Pharyngeal discomfort	2(2.0)	2(2.4)	0(0)	— <sup>c</sup>
Hoarseness	1(1.0)	1(1.2)	0(0)	— <sup>c</sup>
<b>LRTI</b>				
Abnormal pulmonary breath sound <sup>a</sup>	92(91.1)	75(90.4)	17(94.4)	0.582
Increasing lung markings	6(6.0)	4(4.8)	2(11.1)	0.29
Bronchiolitis	8(7.9)	7(8.4)	1(5.6)	0.682
Pneumonia	11(10.9)	10(12.1)	1(5.6)	0.684
bronchopneumonia	56(55.4)	46(55.4)	10(55.6)	0.992
wheezing	47(46.5)	40(48.2)	7(38.9)	0.473
anhelation	24(23.8)	21(25.3)	3(16.7)	0.551
<b>Systemic influenza-like symptoms</b>				
Fever (≥38°C)	89(88.1)	76(91.6)	13(72.2)	<b>0.037</b>
Chill	7(6.9)	6(7.2)	1(5.6)	0.8
Debilitation	11(10.9)	11(13.3)	0(0)	— <sup>c</sup>
<b>Gastrointestinal illness</b>				
Vomiting	21(20.8)	16(19.3)	5(27.8)	0.522
Poor appetite	19(18.8)	17(20.5)	2(11.1)	0.513
Diarrhea	7(7.0)	3(3.6)	4(22.2)	<b>0.018</b>

458 Data are presented as No. (%) of each group.

459 Percentages sum to >100% because some patients had >1 clinical presentations.

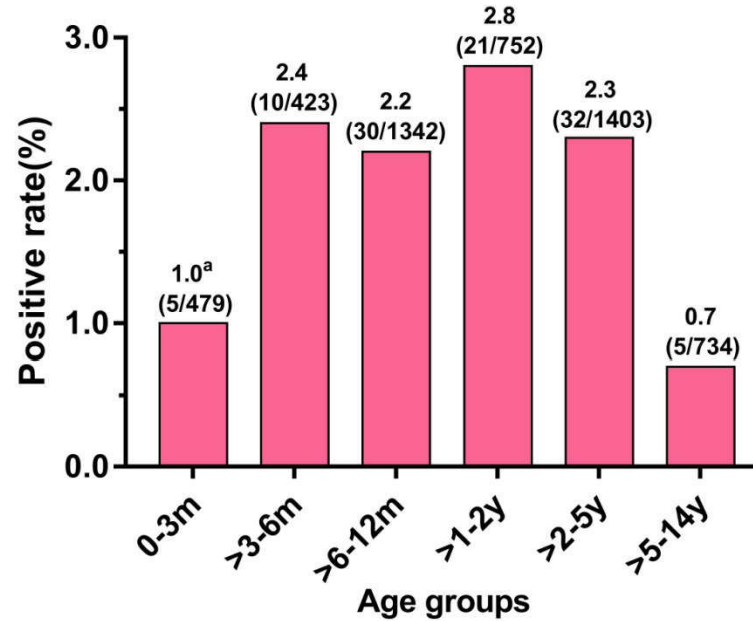
460 <sup>a</sup>Including phlegmatic rales, wheezing rales, bubbling rales, and moist rales.

461 <sup>b</sup>Two-tailed  $\chi^2$  test comparing the distribution of each illness or diagnosis between  
 462 HMPV mono- and co-infected patients.

463 <sup>c</sup>Not tested because the number of positive samples obtained was small.

464 <sup>d</sup>Not tested because the rate of positive samples obtained was 100%.

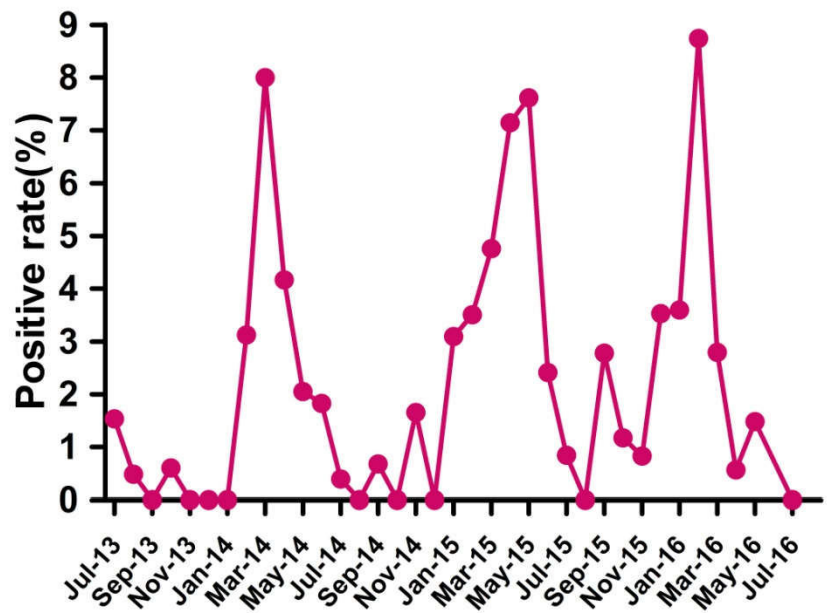
## Figure 1



**Figure 1** Age distributions of patients with HMPV.

<sup>a</sup>Data were presented as positive rate(positive number/total number) in each group; m: month(s); y: year(s).

Figure 2



**Figure 2** Seasonal distribution of HMPV infection in pediatric patients hospitalized with acute respiratory infection from July 2013 to June 2016 in Guangzhou.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4-5
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8
		(b) Describe any methods used to examine subgroups and interactions	8
		(c) Explain how missing data were addressed	7
		(d) If applicable, describe analytical methods taking account of sampling strategy	8
		(e) Describe any sensitivity analyses	
<b>Results</b>			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9-11
		(b) Indicate number of participants with missing data for each variable of interest	9
Outcome data	15*	Report numbers of outcome events or summary measures	9-11
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-11
		(b) Report category boundaries when continuous variables were categorized	9-10
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	12-15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-15
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).

# BMJ Open

## Epidemiology and clinical features of human metapneumovirus in children hospitalized with acute respiratory illness from 2013 to 2016: a cross-sectional study in Guangzhou, southern China

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Complete List of Authors:	Zhang, Ling; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Liu, Donglan; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Chen, Dehui; Department of Pediatrics, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Tan, Weiping ; Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University Qiu, Shuyan; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Xu, Duo; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Li, Xiao; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Liu, Tiantian; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Liu, Wenkuan; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Zhou, Rong; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University
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Secondary Subject Heading:	Epidemiology, Paediatrics, Infectious diseases
Keywords:	Human metapneumovirus, Epidemiology < TROPICAL MEDICINE, Clinical feature, Acute respiratory illness



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8 **3 study in Guangzhou, southern China**  
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15 Ling Zhang<sup>1†</sup>, Donglan Liu<sup>1†</sup>, Dehui Chen<sup>2</sup>, Weiping Tan<sup>3</sup>, Shuyan Qiu<sup>1</sup>, Duo Xu<sup>1</sup>, Xiao  
16 Li<sup>1</sup>, Tiantian Liu<sup>1</sup>, Wenkuan Liu<sup>1\*</sup>, Rong Zhou<sup>1\*</sup>  
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22 <sup>1</sup>State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of  
23 Guangzhou Medical University, Guangzhou Medical University, Guangzhou,  
24 People's Republic of China.  
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29 <sup>2</sup>Department of Pediatrics, The First Affiliated Hospital of Guangzhou Medical  
30 University, Guangzhou Medical University, Guangzhou, People's Republic of China.  
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34 <sup>3</sup>Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, People's  
35 Republic of China.  
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41 \*Correspondence to Wenkuan Liu; [ahlwk2000-2004@163.com](mailto:ahlwk2000-2004@163.com) and Rong Zhou;  
42 [zhourong@gird.cn](mailto:zhourong@gird.cn)  
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46 <sup>†</sup>Ling Zhang, Donglan Liu contributed equally to this work.  
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19 **Abstract**

20 **Objectives:** Human metapneumovirus (HMPV) is one of the most important  
21 respiratory viral pathogens among infants and children worldwide. Our study was  
22 designed to analyze epidemiological and clinical characteristics of HMPV present in  
23 patients hospitalized with acute respiratory illness (ARI) in Guangzhou, southern  
24 China.

25 **Study design:** A cross-sectional study.

26 **Setting:** Two tertiary hospitals in Guangzhou.

27 **Participants and methods:** Throat swabs were collected and tested from pediatric  
28 patients ( $\leq 14$  years,  $n=5133$ ) hospitalized with ARI over a three-year period, and 101  
29 of 103 (98.1%) HMPV-positive patients' clinical presentations were recorded for  
30 further analysis.

31 **Results:** Of the 5133 patients, 103 (2.0%) were positive for HMPV. HMPV mostly  
32 distributed in children with the age groups of  $>3-6$  months (2.4%, 10/423),  $>6-12$   
33 months (2.2%, 30/1342),  $>1-2$  years (2.8%, 21/752) and  $>2-5$  years (2.3%, 32/1403)  
34 ( $p=0.865$ ). Two seasonal HMPV frequency peaks were found every year, and mainly  
35 occurred in spring and early summer. Nineteen of 103 (18.4%) HMPV-positive  
36 patients were co-detected with other pathogens, and the most common co-detected  
37 pathogen was respiratory syncytial virus (36.8%, 7/19). HMPV-positive patients  
38 presented a wide spectrum of symptoms, including cough (100.0%, 101/101),  
39 abnormal pulmonary breath sound (91.1%, 92/101), fever (88.1%, 89/101),  
40 expectoration (77.2%, 78/101), coryza (50.5%, 51/101) and wheezing (46.5%,

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4 41 47/101). The major diagnosis of HMPV-positive patients was bronchopneumonia  
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6 42 (55.4%, 56/101). Fever ( $\geq 38^{\circ}\text{C}$ ) (91.6%, 76/83) was detected more often in single  
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8 43 HMPV-positive patients than in co-pathogens-positive patients (72.2%, 13/18)  
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11 44 ( $p=0.037$ ), whereas diarrhea was presented more common in co-pathogens-positive  
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13 45 patients (22.2%, 4/18) than single HMPV-positive patients (3.6%, 3/83) ( $p=0.018$ ).  
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16 **Conclusions:** HMPV is a common respiratory pathogen in children with ARI in  
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18 47 Guangzhou, particularly in patients from 3 months to 5 years old. HMPV epidemic has  
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21 48 a seasonal variation. Bronchopneumonia is the major diagnosis in HMPV-positive  
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23 49 patients.  
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#### 26 27 28 51 **Strengths and limitations of this study**

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30 ■ This is the study that lasted three years consecutively and had a large sample  
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32 size (more than five thousands). Patients were enrolled from two large  
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34 municipal tertiary hospitals (hospital beds > 1000). In addition, patients  
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36 54 enrolled in the study had a large age spanning from infancy to juveniles. Thus,  
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38 55 our results possess representative characteristics of HMPV presence in children  
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40 56 with ARI in this area.  
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47 ■ The study had some limitations: the characteristics of HMPV-negative patients  
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49 were not further analyzed, and bacterial pathogens were not detected in  
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51 HMPV-positive patients. Lack of these data may affect our comprehensively  
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53 61 understanding the clinical characteristics caused by this pathogen.  
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## 65 Introduction

66 Human metapneumovirus (HMPV) is a nonsegmented, negative-sense  
67 single-stranded RNA virus, which belongs to the *Paramyxoviridae* family,  
68 *Pneumovirinae* subfamily, and *Metapneumovirus* genus.<sup>1</sup> HMPV was first discovered  
69 in 2001 in the Netherlands, and isolated from a pediatric patient with acute respiratory  
70 illness (ARI).<sup>2</sup> Since then, HMPV has been found in associated with acute respiratory  
71 disease in individuals of all ages worldwide. Children, elderly, and  
72 immunocompromised adults are more easily to contract the virus.<sup>3-5</sup> Children younger  
73 than 5 years of age seem to be particularly susceptible to HMPV. Previous studies,<sup>6-12</sup>  
74 which mainly conducting in developed countries, have revealed that approximately  
75 2.5–11.3% of respiratory samples were positive for HMPV in children  $\leq 5$  years, and  
76 90% of individuals have positive serology for HMPV by 5 years of age.<sup>13</sup>

77 HMPV causes a variety of clinical symptoms ranging from a mild upper  
78 respiratory tract infection (URTI) to life-threatening lower respiratory tract infection  
79 (LRTI).<sup>3 14-16</sup> However, there is so far no effective vaccine and medication either for  
80 prevention or treatment available for HMPV infection. Thus, it is imperative to conduct  
81 more studies, especially in developing and undeveloped countries, to understand this  
82 pathogen in different areas and populations.

83 In this study, we consecutively investigated, from July 2013 to June 2016, the  
84 epidemiological and clinical features of HMPV present in pediatric patients ( $\leq 14$  years)

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4 85 hospitalized with ARI in Guangzhou, southern China, using real-time PCR. The  
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6 86 findings of this study will be helpful in understanding the distribution of HMPV in  
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8 87 subtropical region. Our results also provide valuable insight into the clinical features of  
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11 88 HMPV, which will be helpful in early clinical diagnosis.  
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## 89 **Methods**

### 90 **Study design and respiratory sample collection**

91 This was a cross-sectional study at two tertiary hospitals, the First Affiliated  
92 Hospital of Guangzhou Medical University and Sun Yat-Sen Memorial Hospital in  
93 Guangzhou. Samples in this study were taken as part of standard care. Throat swab  
94 samples (n=5133) from pediatric patients ( $\leq 14$  years) hospitalized with ARI were  
95 collected at two hospitals between July 2013 and June 2016. ARI was defined as an  
96 illness that presented with at least two of the following symptoms: cough, pharyngeal  
97 discomfort, nasal obstruction, coryza, sneeze, dyspnoea, or diagnosed with  
98 pneumonia by chest radiography during the previous week. Chest radiography was  
99 performed based on the clinical situation of the patients. The samples were collected  
100 according to established clinical protocols.<sup>17</sup> The samples were refrigerated at 2–8°C  
101 in viral transport medium, transported on ice to the State Key Laboratory of  
102 Respiratory Diseases, and analyzed immediately or stored at -80°C before analysis  
103 as previously described.<sup>18</sup>

104 The patients' clinical presentations or diagnoses were extracted from their  
105 medical records using designed presentation cards and were retrospectively  
106 categorized into the following four groups: URTI, LRTI, systemic influenza-like  
107 symptoms, and gastrointestinal illness. Patients with nasal obstruction, coryza,  
108 sneezing, coughing, expectoration, pharyngeal discomfort, or hoarseness were  
109 categorized as having URTI. Patients with bronchiolitis, pneumonia,  
110 bronchopneumonia, increased lung markings, dyspnea, or an abnormal pulmonary

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4 111 breath sound were categorized as having LRTI. Patients with high fever ( $\geq 38^{\circ}\text{C}$ ),  
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6 112 chills, dizziness,, headache, myalgia, or debilitation were categorized as having  
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8 113 systemic influenza-like symptoms. Patients with vomiting, poor appetite, or diarrhea  
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10 114 were categorized as having gastrointestinal illness. Some patients were assigned to  
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12 115 several clinical presentation groups. Patients with incomplete clinical data were  
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14 116 excluded from this analysis. Increased lung markings, bronchopneumonia,  
15  
16 117 pneumonia, and bronchiolitis were diagnosed by chest radiography. Abnormal  
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18 118 pulmonary breath sounds included phlegmatic rales, wheezy rales, bubbling rales,  
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20 119 and moist rales. Other clinical symptoms were identified by a general medical  
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22 120 examination and clinical descriptions as previously reported.<sup>19</sup>  
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#### 30 122 **Real-time RT-PCR for HMPV detection**

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33 123 RNA were extracted from the throat swab samples with the QIAamp Viral RNA  
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35 124 Mini Kit (Qiagen, Shanghai, China), according to the manufacturer's protocols. HMPV  
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37 125 was determined by using TaqMan Real-time PCR assays, as previously reported,<sup>18</sup>  
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39 126 using kits from Guangzhou HuYanSuo Medical Technology Co., Ltd according to the  
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41 127 manufacturer's protocols. In brief, 50  $\mu\text{l}$  RNA were extracted from 200  $\mu\text{l}$  sample, and  
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43 128 real-time PCR was conducted using 25  $\mu\text{l}$  reaction mix containing M-MLV, Taq  
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45 129 polymerase and 5  $\mu\text{l}$  extracted RNA. Cycling conditions included an initial reverse  
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47 130 transcription at  $55^{\circ}\text{C}$  for 10 min incubation at  $94^{\circ}\text{C}$  for 2 min, followed by 40 cycles of  
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49 131  $94^{\circ}\text{C}$  for 10 sec and  $55^{\circ}\text{C}$  for 35 sec (ABI-7500 real-time PCR instrument, Life  
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53 132 Technologies, Singapore).  
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6 134 **Detection of common respiratory pathogens in HMPV-positive patients**

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8 135 HMPV-positive samples were simultaneously tested by using TaqMan Real-time  
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10 136 PCR assays for the following 17 respiratory pathogens : respiratory syncytial virus  
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12 137 (RSV), parainfluenza virus types 1–4 (PIV1–4), influenza A and B viruses (InfA, InfB),  
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14 138 adenovirus (ADV), enterovirus (EV), human coronaviruses (HCoV-229E, HCoV-OC43,  
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16 139 HCoV-NL63, HCoV-HKU1), human rhinovirus (HRV), human bocavirus (HBoV),  
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18 139 *Mycoplasma pneumoniae* (MP), and *Chlamydomphila pneumoniae* (CP). The testing  
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20 140 procedure was conducted using kits from Guangzhou HuYanSuo Medical Technology  
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22 141 Co., Ltd as previously described.<sup>18</sup>

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30 144 **Statistical analysis**

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32 145 All statistical analyses were performed with SPSS statistical software (version  
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34 146 19.0; SPSS Inc., Chicago, IL, USA). To compare categorical data, the  $\chi^2$  test and  
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36 147 Fisher's exact test were used as appropriate. All tests were two-tailed, and  $p < 0.05$   
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38 148 was considered statistically significant.

## 149 **Results**

### 150 **Detection of HMPV from patients with ARI**

151 In total, 5133 pediatric patients aging from infants to 14 years old, hospitalized  
152 with ARI between July 2013 and June 2016 were enrolled in this study. The  
153 male-to-female ratio was 1.75 (3269:1864) and the median age in months was 33  
154 months (interquartile range, 9–48 months). Of the 5133 patients, 103 (2.0%) were  
155 positive for HMPV. The male-to-female ratio was 1.86:1 (67:36) in HMPV-positive  
156 patients and 1.75:1 (3202:1828) in HMPV-negative patients ( $p=0.771$ ). The median  
157 age in months of HMPV-positive patients was 23.5 months (interquartile range, 10–36  
158 months).

### 160 **Co-detection with common respiratory pathogens in HMPV-positive patients**

161 In this study, we also tested HMPV-positive samples for other 17 common  
162 respiratory pathogens. Of the 103 HMPV-positive patients, 84 (81.6%) patients had  
163 single HMPV detection and 19 (18.4%) patients had co-detected other respiratory  
164 pathogens. The male-to-female ratio was 2:1 (56:28) in single HMPV-positive patients  
165 and 1.38:1 (11:8) in co-pathogen-positive patients ( $p=0.469$ ). Nine out of 17  
166 respiratory pathogens (52.9%) were detected, and the most common co-detection  
167 pathogens were RSV (36.8%, 7/19), HCoV-OC43 (15.8%, 3/19), MP (15.8%, 3/19),  
168 and HBoV (15.8%, 3/19) (**Table 1**).

### 170 **Age distributions of HMPV-positive patients**

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4 171 Overall, a significant difference of HMPV detection rate was found between  
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6 172 patients >5–14 years (0.7%, 5/734) and those ≤5 years (2.2%, 98/4399) ( $p=0.004$ ).  
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8 173 For detail, the patients were divided into six age groups: 0–3 months, >3–6  
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10 174 months, >6–12 months, >1–2 years, >2–5 years, and >5–14 years. The distribution of  
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12 175 HMPV-positive rates among these age groups was significantly different ( $p=0.03$ )  
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14 176 (**Figure 1**). HMPV was most frequently detected in children >1–2 years (2.8%,  
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16 177 21/752). However, there was no significant difference in the frequency of HMPV  
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18 178 detection in the following groups: 2.4% in >3–6 months (10/423), 2.2% in >6–12  
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20 179 months (30/1342), 2.8% in >1–2 years (21/752), and 2.3% in >2–5 years (32/1403)  
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22 180 ( $p=0.865$ ) (**Figure 1**). Thus, as a whole, HMPV prevalence obviously had a differential  
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24 181 age distribution in ARI pediatric patients, predominant in patients from 3 months to 5  
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26 182 years old (2.4%, 93/3920)..  
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### 35 184 **Seasonal distribution of HMPV**

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37 185 Over the 3-year study period, the prevalence of HMPV peaked twice every  
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39 186 year (**Figure 2**). Large peaks occurred in March 2014 (8%, 16/200), May 2015 (7.6%,  
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41 187 8/105), and February 2016 (8.7%, 9/103). Small peaks occurred in November 2014  
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43 188 (1.7%, 2/121), September 2015 (2.8%, 2/72), and May 2016 (1.5%, 2/135) (**Figure 2**).  
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### 50 190 **Clinical presentations of HMPV-positive patients**

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52 191 We analyzed the clinical presentations of 101 of 103 (98.1%) HMPV-positive  
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54 192 patients, the other two patients were excluded from this analysis due to incomplete  
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4 193 clinical data. The main symptoms of HMPV-positive patients included cough (100.0%,  
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6 101/101), abnormal pulmonary breath sound (91.1%, 92/101), fever (88.1%, 89/101),  
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8 195 expectoration (77.2%, 78/101), coryza (50.5%, 51/101) and wheezing (46.5%,  
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11 196 47/101), the main diagnosis by chest radiography was bronchopneumonia (55.4%,  
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13 197 56/101), followed by pneumonia (10.9%, 11/101) and bronchiolitis (7.9%, 8/101).  
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16 198 (**Table 2**). We also compared the clinical features of single HMPV-positive patients  
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18 199 with those of the co-pathogens-positive patients. Fever and diarrhea were two  
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21 200 symptoms that showed statistically different between these two groups; fever ( $\geq 38^{\circ}\text{C}$ )  
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23 201 was seen more common in most of the single HMPV-positive cases (91.6%, 76/83) ,  
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26 202 whereas only in 72.2% (13/18) of the multi-pathogen-positive patients ( $p=0.037$ ).  
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28 203 Conversely, Diarrhea appeared more often in multi-pathogen-positive patients (22.2%,  
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31 204 4/18) than in single HMPV-positive patients (3.6%, 3/83) ( $p=0.018$ ) (**Table 2**).

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205 **Discussion**

206 Viruses are the most frequent cause of respiratory infection.<sup>20</sup> HMPV has been  
207 recognized as an important respiratory pathogen causing ARI since its discovery in  
208 2001. It has been reported that HMPV was responsible for approximately 5–10% of  
209 hospitalizations of children suffering from ARI,<sup>14</sup> creating considerable clinical and  
210 economic burden worldwide. HMPV detection rates vary according to geographic  
211 location, and the incidence of HMPV may show seasonal or annual patterns in the  
212 same area. In the previous studies,<sup>21-30</sup> the HMPV detection rate was approximately  
213 1–17% of ARI cases. In this 3-year study, 103 of 5133 (2.0%) patients were positive  
214 for HMPV, which is similar to previous reports of HMPV in patients with ARI in  
215 southern China<sup>31</sup> and Japan.<sup>32</sup>

216 HMPV is commonly found in the pediatric population; Children younger than 5  
217 years of age are most susceptible to HMPV infection, and those younger than 2 years  
218 of age at the greatest risk of developing serious conditions.<sup>15</sup> In our study, HMPV  
219 positive cases appeared more frequently in patients ≤5 year-old (2.2%, 98/4399) than  
220 in patients >5 year-old (0.7%, 5/734) ( $p=0.004$ ), which is consistent with many  
221 previous studies.<sup>3 30 33 34</sup> In detail, HMPV positive rate was high in children of 3 months  
222 to 5 years (2.4%, 93/3920) (**Figure 1**). This may be explained by that the immunity  
223 against HMPV passed down from mother gradually subsided as time goes on;  
224 moreover, children were more contact with outside world as they grew older.  
225 Therefore, children of this age need more attention to prevent HMPV infection.  
226 Moreover, our findings suggested no gender predominance, there was no significant

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4 227 difference in the HMPV positive rate between males and females ( $p=0.771$ ), which is  
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6 228 consistent with previous studies.<sup>35 36</sup>  
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9 229 HMPV has a typical seasonal distribution. The activity of HMPV is largely  
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11 230 affected by different climate factors locally. The activity of HMPV in temperate  
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13 231 climates peaks at the end of winter or in early spring.<sup>37 38</sup> In this study, seasonal peaks  
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15 232 of HMPV were detected in February 2016, March 2014, and May 2015 (**Figure 2**).  
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17 233 This finding indicates that HMPV circulates primarily during the spring and early  
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19 234 summer in Guangzhou, as the previous reports in other subtropical areas.<sup>22 39</sup>  
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21 235 Furthermore, we also found small peaks in November 2014, September 2015, and  
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23 236 May 2016 (**Figure 2**), the reason for this is unknown. It should be pointed out that the  
24  
25 237 seasonal distribution pattern of HMPV overlaps with that of RSV in this area.<sup>19</sup>  
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27 238 Knowing the epidemical characteristics of HMPV could be helpful for public health  
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29 239 authority and medical community to lay down strategies for better controlling HMPV  
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31 240 infection.  
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38 241 HMPV co-infection with other respiratory pathogens has been reported in  
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40 242 many studies, including RSV,<sup>40</sup> InfA,<sup>41</sup> InfB,<sup>42</sup> PIV,<sup>43</sup> ADV,<sup>43 44</sup> HBoV,<sup>45</sup> HCoV,<sup>43 46</sup>  
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42 243 HRV,<sup>43</sup> EV,<sup>43</sup> MP,<sup>41</sup> and CP.<sup>41</sup> In this study, most HMPV-positive patients had single  
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44 244 HMPV-detection (81.6%, 84/103), and there was no significant difference in the  
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46 245 HMPV positivity rate according to sex between single HMPV-positive and  
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48 246 co-pathogens-positive patients ( $p=0.469$ ). The co-detection rate was 18.4% (19/103),  
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50 247 and RSV (36.8%, 7/19) was the most frequently co-detected pathogen (**Table 1**),  
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52 248 which might be due to overlapping of seasonal distribution of these two pathogens.<sup>47</sup>  
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4 249 Co-detection rates of HCoV-OC43, MP, HBoV, ADV, HRV, InfA, PIV2, and PIV3 were  
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6 250 over 5%, suggesting a broad range of respiratory pathogens could co-exist with  
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8 251 HMPV in ARI pediatric patients (**Table 1**).

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10 252 HMPV most commonly causes URTI and LRTI in young children. The clinical  
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13 253 manifestations of HMPV-positive patients are similar to those of RSV-positive patients,  
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15 254 especially in young children,<sup>48-50</sup> and HMPV has been reported that the most frequent  
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18 255 diagnoses of children hospitalized with HMPV infection were pneumonitis and  
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20 256 bronchiolitis.<sup>3 51 52</sup> In some studies, bronchiolitis and recurrent wheezing/pneumonia  
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23 257 were the main clinical diagnosis,<sup>7 22 53 54</sup> while in other studies, bronchopneumonia,  
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25 258 bronchiolitis, and bronchial asthma exacerbation were the main clinical diagnosis.<sup>34</sup>  
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28 259 Our study showed that 55.4% (56/101), 7.9% (8/101), and 10.9% (11/101) of  
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30 260 HMPV-positive patients were diagnosed with bronchopneumonia, bronchiolitis, and  
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33 261 pneumonia by chest radiography, respectively. Of all clinical presentations, cough  
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35 262 (100.0%, 101/101), abnormal pulmonary breath sound (91.1%, 92/101), fever (88.1%,  
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38 263 89/101), expectoration (77.2%, 78/101), coryza (50.5%, 51/101), and wheezing  
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40 264 (46.5%, 47/101) occurred frequently in HMPV-positive patients (**Table 2**), similar to  
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42 265 those reported previously.<sup>16 29 52 55</sup>

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45 266 Most of the HMPV-positive patients had a fever (88.1%, 89/101); it appeared in  
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47 267 the single HMPV-positive patients more frequently than in the co-pathogens-positive  
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49 268 patients ( $p=0.037$ ) (**Table 2**). Although diarrhea was not a major symptom in  
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52 269 HMPV-positive patients (7.0%, 7/101), but it had a higher incidence rate in  
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55 270 co-pathogen-positive patients than in single HMPV-positive patients ( $p=0.018$ ),  
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4 271 suggesting that diarrhea could be caused by other pathogens. No significant  
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6 272 difference was found in the other clinical presentations between single HMPV-positive  
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8 273 and co-pathogens-positive patients, similar to the results of a previous study.<sup>51</sup>  
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11 274 In this study, RSV (36.8%, 7/19) was the most frequently co-detected pathogen  
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13 275 in HMPV-positive patients (**Table 1**). Whether HMPV/RSV co-infection is more severe  
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15 276 than the respective mono-infections remains unclear. A previous study showed that  
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17 277 children with HMPV/RSV co-infection were more likely to develop pneumonia;  
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19 278 however, disease severity was not increased.<sup>53</sup> Conversely, Semple et al.<sup>56</sup> reported  
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21 279 that HMPV/RSV co-infection can cause more severe bronchiolitis in patients. In our  
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23 280 study, no significant difference in the diagnosis of pneumonia was observed between  
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25 281 single HMPV-positive and co-pathogens-positive patients. It has been confirmed that  
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27 282 a history of prematurity, particular age groups, and the presence of chronic diseases  
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29 283 increases the risk of severe LRTI among HMPV- and RSV-infected children.<sup>51</sup> Our  
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31 284 study also suggested that the clinical manifestations of HMPV infection are complex  
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33 285 and diverse; the data might be helpful in the diagnosis of HMPV.  
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40 286 The study had some limitations. Firstly, collection of symptoms and physical  
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42 287 findings in infants and young children often requires the experience of medical staff,  
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44 288 patient's cooperation, and the knowledge of patient's guardian; but it is still possible to  
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46 289 have incomplete or even false description of patients' manifestations. Secondly,  
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48 290 because our study mainly focused on HMPV, other common respiratory pathogens  
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50 291 including bacterial pathogens were not tested in all samples, and accordingly, our  
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52 292 study could not give a fully picture of respiratory pathogen infection in hospitalized  
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3 293 pediatric patients with ARI. Thirdly, the characteristics of HMPV-negative patients  
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6 294 were not further analyzed because our study only focused on the epidemical and  
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9 295 clinical features of HMPV positive cases. That might affect our understanding of the  
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11 296 clinical feature of HMPV-positive patients. Despite these shortcomings, our results  
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13 297 provide valuable insight into the epidemiological and clinical characteristics of HMPV  
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16 298 present in patients hospitalized with acute respiratory illness (ARI) in a subtropical  
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18 299 region, Guangzhou, southern China.  
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300 **Conclusions**

301 HMPV is a common respiratory pathogen in children with ARI in Guangzhou ,  
302 China, particularly in children from 3 month to 5 years old. HMPV epidemic has a  
303 seasonal variation. The clinical characteristics of HMPV infection has its own pattern  
304 in children aged from infants to juveniles. Bronchopneumonia is the major clinical  
305 diagnosis. In the future, our data should be taken into account when local public  
306 health authority and medical community try to manage HMPV infection in children.

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16 312 **Contributors**  
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18 313 RZ, WKL, LZ and DLL designed the study. LZ, DLL, WKL, SYQ, DX, XL and LTT  
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20 314 performed pathogen testing. DHC and WPT collected clinical data. All authors  
21  
22 315 participated in the data analysis. LZ, DLL, RZ and WKL drafted the manuscript. All  
23  
24 316 authors read and approved the final version of this manuscript.  
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47 325 **Competing interests**  
48

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54 328 **Ethics approval**  
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4 329 The study was approved by The First Affiliated Hospital of Guangzhou Medical  
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6 330 University Ethics Committee. Informed written consent was obtained from parents or  
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8 331 legal guardians.  
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13 333 **Data sharing statement**  
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16 334 No additional data are available.  
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4 492 **Figure legends**

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8 494 **Figure 1** Age distributions of patients with HMPV.

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10 495 <sup>a</sup>Date were presented as HMPV positive rate (number of HMPV-positive  
11 patients/number of patients in each age group); m: month(s); y: year(s).  
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18 498 **Figure 2** Seasonal distribution of HMPV infection in pediatric patients hospitalized  
19 with acute respiratory infection from July 2013 to June 2016 in Guangzhou.  
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**Table 1. Distribution of co-pathogens in 19 HMPV-positive patients**

Co-pathogens <sup>a</sup>	Patient No.
RSV/HMPV	4
HCoV-OC43/HMPV	3
ADV/HMPV	2
HRV/HMPV	2
MP/HMPV	1
PIV2/HMPV	1
PIV3/HMPV	1
RSV/MP/HMPV	1
RSV/InfA/HMPV	1
RSV/HBoV/HMPV	1
MP/HBoV/HMPV	1
InfA/HBoV/HMPV	1

501 <sup>a</sup>HMPV-positive patients were tested for 17 common respiratory pathogens.

502 InfB, PIV1, PIV4, EV, HCoV-HKU1, HCoV-229E, HCoV-NL63, CP were not detected.

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**Table 2. Clinical presentations in HMPV-positive patients**

Diagnosis/Symptom	Total HMPV (n=101)	Patients with single HMPV and co-pathogens		
		Single HMPV (n = 83)	Co-pathogens (n= 18)	p value <sup>b</sup>
<b>URTI</b>				
Nasal obstruction	40(39.6)	34(41.0)	6(33.3)	0.605
Coryza	51(50.5)	44(53.0)	7(38.9)	0.309
Sneeze	1(1.0)	1(1.2)	0(0)	— <sup>c</sup>
Cough	101(100)	83(100)	18(100)	— <sup>d</sup>
Expectoration	78(77.2)	66(79.5)	12(66.7)	0.351
Pharyngeal discomfort	2(2.0)	2(2.4)	0(0)	— <sup>c</sup>
Hoarseness	1(1.0)	1(1.2)	0(0)	— <sup>c</sup>
<b>LRTI</b>				
Abnormal pulmonary breath sound <sup>a</sup>	92(91.1)	75(90.4)	17(94.4)	0.582
Increasing lung markings	6(6.0)	4(4.8)	2(11.1)	0.29
wheezing	47(46.5)	40(48.2)	7(38.9)	0.473
anhelation	24(23.8)	21(25.3)	3(16.7)	0.551
Bronchiolitis	8(7.9)	7(8.4)	1(5.6)	0.682
Pneumonia	11(10.9)	10(12.1)	1(5.6)	0.684
bronchopneumonia	56(55.4)	46(55.4)	10(55.6)	0.992
<b>Systemic influenza-like symptoms</b>				
Fever (≥38°C)	89(88.1)	76(91.6)	13(72.2)	<b>0.037</b>
Chill	7(6.9)	6(7.2)	1(5.6)	0.8
Debilitation	11(10.9)	11(13.3)	0(0)	— <sup>c</sup>
<b>Gastrointestinal illness</b>				
Vomiting	21(20.8)	16(19.3)	5(27.8)	0.522
Poor appetite	19(18.8)	17(20.5)	2(11.1)	0.513
Diarrhea	7(7.0)	3(3.6)	4(22.2)	<b>0.018</b>

506 Data are presented as No. (%) of each group.

507 Percentages sum to >100% because some patients had >1 clinical presentations.

508 <sup>a</sup>Including phlegmatic rales, wheezing rales, bubbling rales, and moist rales.

509 <sup>b</sup>Two-tailed  $\chi^2$  test comparing the distribution of each illness or diagnosis between  
510 HMPV mono- and co-infected patients.

511 <sup>c</sup>Not tested because the number of positive samples obtained was small.

512 <sup>d</sup>Not tested because the rate of positive samples obtained was 100%.

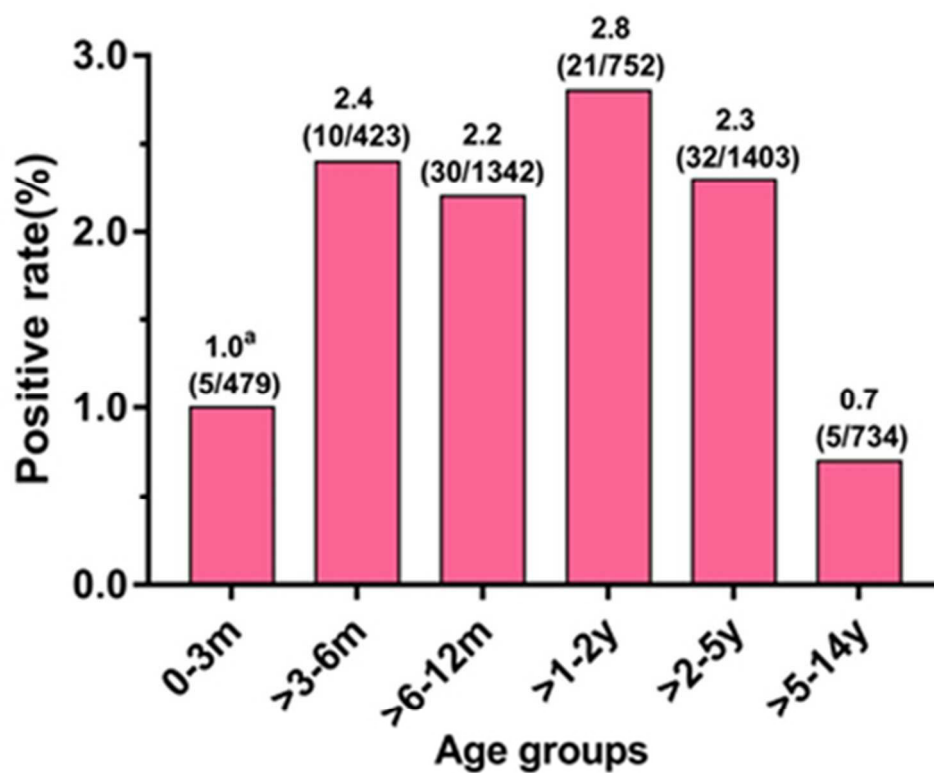


Figure 1 Age distributions of patients with HMPV.

<sup>a</sup>Data were presented as HMPV positive rate (number of HMPV-positive patients/number of patients in each age group); m: month(s); y: year(s).

42x35mm (300 x 300 DPI)

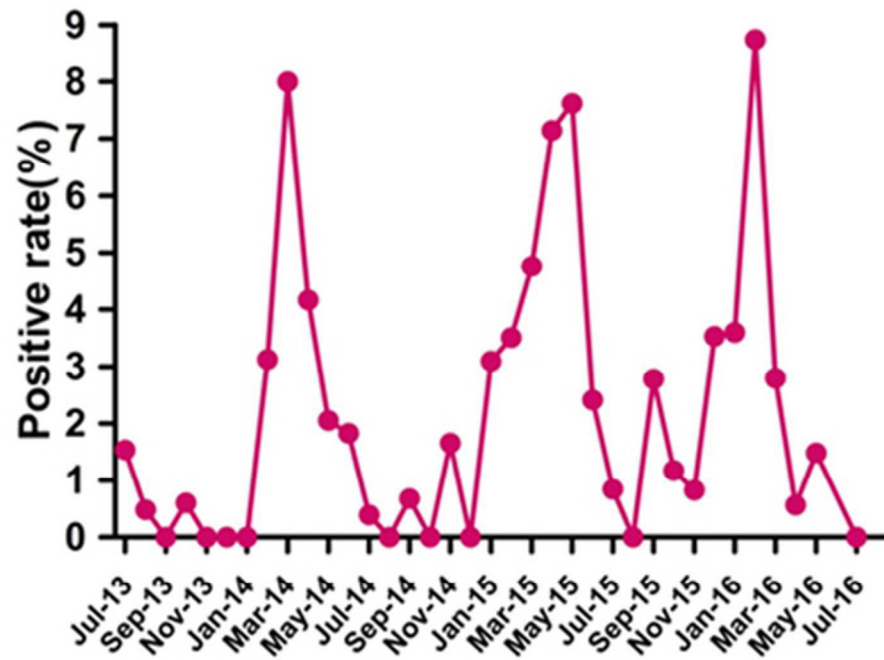


Figure 2 Seasonal distribution of HMPV infection in pediatric patients hospitalized with acute respiratory infection from July 2013 to June 2016 in Guangzhou.

39x31mm (300 x 300 DPI)

**STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies***

Section/Topic	Item #	Recommendation	Reported on page #
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4-5
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8
		(b) Describe any methods used to examine subgroups and interactions	8
		(c) Explain how missing data were addressed	7
		(d) If applicable, describe analytical methods taking account of sampling strategy	8
		(e) Describe any sensitivity analyses	
<b>Results</b>			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9-11
		(b) Indicate number of participants with missing data for each variable of interest	9
Outcome data	15*	Report numbers of outcome events or summary measures	9-11
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-11
		(b) Report category boundaries when continuous variables were categorized	9-10
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	12-15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15-16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-16
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).

# BMJ Open

## Epidemiology and clinical features of human metapneumovirus in hospitalized pediatric patients with acute respiratory illness: a cross-sectional study in southern China, from 2013 to 2016

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Complete List of Authors:	Zhang, Ling; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Liu, Donglan; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Chen, Dehui; Department of Pediatrics, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Tan, Weiping ; Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University Qiu, Shuyan; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Xu, Duo; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Li, Xiao; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Liu, Tiantian; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Liu, Wenkuan; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University Zhou, Rong; State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University
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Keywords:	Human metapneumovirus, Epidemiology < TROPICAL MEDICINE, Clinical feature, Acute respiratory illness



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4 1 **Epidemiology and clinical features of human metapneumovirus in hospitalized**  
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6 2 **pediatric patients with acute respiratory illness: a cross-sectional study in**  
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8 3 **southern China, from 2013 to 2016**  
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14 5 Ling Zhang<sup>1†</sup>, Donglan Liu<sup>1†</sup>, Dehui Chen<sup>2</sup>, Weiping Tan<sup>3</sup>, Shuyan Qiu<sup>1</sup>, Duo Xu<sup>1</sup>, Xiao  
15  
16 6 Li<sup>1</sup>, Tiantian Liu<sup>1</sup>, Wenkuan Liu<sup>1\*</sup>, Rong Zhou<sup>1\*</sup>  
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20  
21 8 <sup>1</sup>State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of  
22  
23 9 Guangzhou Medical University, Guangzhou Medical University, Guangzhou,  
24  
25 10 People's Republic of China.  
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27

28  
29 11 <sup>2</sup>Department of Pediatrics, The First Affiliated Hospital of Guangzhou Medical  
30  
31 12 University, Guangzhou Medical University, Guangzhou, People's Republic of China.  
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34 13 <sup>3</sup>Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, People's  
35  
36 14 Republic of China.  
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41 16 \*Correspondence to: Wenkuan Liu; [ahlwk2000-2004@163.com](mailto:ahlwk2000-2004@163.com) and Rong Zhou;  
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43 17 [zhourong@gird.cn](mailto:zhourong@gird.cn)  
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46 18 <sup>†</sup>Ling Zhang and Donglan Liu contributed equally to this work.  
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19 **Abstract**

20 **Objectives:** Human metapneumovirus (HMPV) is one of the most important  
21 respiratory viral pathogens affecting infants and children worldwide. Our study was  
22 describes the epidemiological and clinical characteristics of HMPV present in patients  
23 hospitalized with acute respiratory illness (ARI) in Guangzhou, southern China.

24 **Study design:** A cross-sectional study.

25 **Setting:** Two tertiary hospitals in Guangzhou.

26 **Participants and methods:** Throat swabs were collected over a 3-year period from  
27 5133 pediatric patients ( $\leq 14$  years) hospitalized with ARI. HMPV-positive patients'  
28 clinical presentations (101/103) were recorded for further analysis.

29 **Results:** Of the 5133 patients included in the study, 103 (2.0%) were positive for  
30 HMPV. HMPV was more prevalent in children  $\leq 5$  years (2.2%, 98/4399) compared  
31 with older children ( $>5$ –14 years) (0.7%, 5/734) ( $p=0.004$ ). Two seasonal HMPV  
32 peaks were observed each year and mainly occurred in spring and early summer.  
33 Overall, 18.4% (19/103) of HMPV-positive patients were co-detected with other  
34 pathogens, most frequently respiratory syncytial virus (36.8%, 7/19). HMPV-positive  
35 patients presented with a wide spectrum of clinical features, including cough (100.0%,  
36 101/101), abnormal pulmonary breath sound (91.1%, 92/101), fever (88.1%, 89/101),  
37 expectoration (77.2%, 78/101), coryza (50.5%, 51/101) and wheezing (46.5%,  
38 47/101). The main diagnosis of HMPV-positive patients was bronchopneumonia  
39 (55.4%, 56/101). Fever ( $\geq 38^{\circ}\text{C}$ ) (91.6%, 76/83) was detected more often in patients  
40 with only HMPV detected than in patients with HMPV plus other pathogen(s) detected

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4 41 (72.2%, 13/18) ( $p=0.037$ ), whereas diarrhea was more common in patients with  
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6 42 HMPV plus other pathogen(s) detected (22.2%, 4/18), compared with patients with  
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8 43 HMPV only (3.6%, 3/83) ( $p=0.018$ ).

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11 44 **Conclusions:** HMPV is an important respiratory pathogen in children with ARI in  
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13 45 Guangzhou, particularly in children  $\leq 5$  years old. HMPV has a seasonal variation.  
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16 46 Bronchopneumonia is a major diagnosis in HMPV-positive patients.  
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#### 18 19 20 21 48 **Strengths and limitations of this study**

- 22  
23 49 ● 5133 patients hospitalized with acute respiratory illness at two large municipal  
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25 50 tertiary hospitals (hospital beds > 1000) were enrolled on the study over 3 years.
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28 51 ● Patients aged from 1 day to 14 years old were tested for HMPV using Taqman  
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30 52 real-time PCR.
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33 53 ● Clinical characteristics of patients with HMPV-positive were recorded.
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35 54 ● There was incomplete HMPV co-pathogen detection, because bacterial  
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37 55 pathogens were not detected for HMPV-positive patients.
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40 56 ● The clinical characteristics of HMPV-negative patients and outcome data  
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42 57 (discharge/death) for the HMPV-positive patients were not collected for further  
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44 58 analysis, limiting our understanding of this pathogen.

#### 45 46 47 48 49 50 60 **Introduction**

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52 61 Human metapneumovirus (HMPV) is a nonsegmented, negative-sense  
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54 62 single-stranded RNA virus, which belongs to the *Paramyxoviridae* family.<sup>1</sup> HMPV was

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4 63 first discovered in 2001 in the Netherlands, after being isolated from a pediatric patient  
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6 64 with acute respiratory illness (ARI).<sup>2</sup> Since then, HMPV has been associated with  
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8 65 acute respiratory disease in individuals of all ages worldwide. Children, elderly and  
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10 66 immunocompromised adults are most at risk of contracting the virus.<sup>3-5</sup> Children  
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12 67 younger than 5 years of age seem to be particularly susceptible to HMPV. Previous  
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14 68 studies,<sup>6-12</sup> which were mainly conducted in developed countries, have revealed that  
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16 69 approximately 2.5%–11.3% of respiratory samples were positive for HMPV in children  
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18 70 ≤5 years, and 90% of individuals were seropositive for HMPV by 5 years of age.<sup>13</sup>  
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23 HMPV causes a variety of clinical symptoms ranging from a mild upper  
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25 72 respiratory tract infection (URTI) to life-threatening lower respiratory tract infection  
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28 73 (LRTI).<sup>3 14-16</sup> However, to date, there is no effective vaccine or specific medication  
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30 74 either for prevention or treatment of HMPV infection. Consequently, it is imperative to  
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33 75 conduct more studies, especially in low and middle-income countries, to understand  
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35 76 this pathogen in different areas and populations.  
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38 In this study, we investigated the epidemiological and clinical features of HMPV  
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40 78 in pediatric patients, from July 2013 to June 2016. The findings of this study will help  
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43 79 understand the distribution of HMPV in a subtropical region. Our results also provide a  
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45 80 valuable insight into the clinical features of HMPV, which will improve early clinical  
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48 81 diagnosis.  
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## 82 **Methods**

### 83 **Study design and respiratory sample collection**

84 We conducted a cross-sectional study at two tertiary hospitals in Guangzhou,  
85 southern China. Throat swab samples (n=5133) from pediatric patients ( $\leq 14$  years)  
86 hospitalized with ARI, were collected at two hospitals between July 2013 and June  
87 2016. ARI was defined as an illness that presented with at least two of the following  
88 clinical presentations: cough, nasal obstruction, coryza, sneeze, dyspnoea during the  
89 previous week. Patients, who were diagnosed with pneumonia by chest radiography  
90 during the previous week, were also included in the study, even if they did not show  
91 the clinical features described above. Some patients, who had been cured and  
92 discharged some time ago and readmitted because of new episodes of ARI, if met the  
93 recruitment criteria, were included in the study as new cases, otherwise excluded.  
94 Chest radiography was performed based on the clinical situation of the patients. The  
95 samples were collected according to established clinical protocols.<sup>17</sup> The samples  
96 were refrigerated at 2–8°C in viral transport medium, transported on ice to the State  
97 Key Laboratory of Respiratory Diseases, and analyzed immediately or stored at -80°C  
98 before analysis as previously described.<sup>18</sup>

99 The patients' clinical presentations or diagnoses were recorded from patients'  
100 medical records by attending physicians, using designed presentation cards and were  
101 categorized retrospectively into the following four groups: URTI, LRTI, systemic  
102 influenza-like symptoms, and gastrointestinal illness. Patients with nasal obstruction,  
103 coryza, sneezing, coughing, expectoration, or hoarseness were categorized as

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3 104 having URTI. Patients with bronchiolitis, pneumonia, bronchopneumonia, increased  
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6 105 lung markings, dyspnea, or an abnormal pulmonary breath sound were categorized  
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9 106 as having LRTI. Patients with a high fever ( $\geq 38^{\circ}\text{C}$ ), chills, or debilitation were  
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11 107 categorized as having systemic influenza-like symptoms. Patients with vomiting, poor  
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13 108 appetite, or diarrhea were categorized as having gastrointestinal illness. Some  
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16 109 patients were assigned to several clinical presentation groups. Patients with  
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18 110 incomplete clinical data were excluded from this analysis. Increased lung markings,  
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21 111 bronchopneumonia, pneumonia, and bronchiolitis were diagnosed by chest  
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23 112 radiography. Abnormal pulmonary breath sounds included phlegmatic, wheezy,  
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26 113 bubbling, and moist rales. Other clinical symptoms were identified by a general  
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28 114 medical examination and clinical descriptions, as previously reported.<sup>19</sup>

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### 32 33 116 **Real-time PCR for HMPV detection**

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35 117 RNA was extracted from the throat swab samples with the QIAamp Viral RNA  
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38 118 Mini Kit (Qiagen, Shanghai, China), according to the manufacturer's protocols. HMPV  
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40 119 was identified by using TaqMan Real-time PCR assays, as previously reported,<sup>18</sup>  
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43 120 using kits from Guangzhou HuYanSuo Medical Technology Co., Ltd according to the  
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46 121 manufacturer's protocols. In brief, 50  $\mu\text{l}$  RNA were extracted from a 200  $\mu\text{l}$  sample,  
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48 122 and real-time PCR was conducted using 25  $\mu\text{l}$  reaction mix, containing M-MLV, Taq  
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51 123 polymerase and 5  $\mu\text{l}$  extracted RNA. Cycling conditions included an initial reverse  
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53 124 transcription at  $55^{\circ}\text{C}$  for 10 min, incubation at  $94^{\circ}\text{C}$  for 2 min, followed by 40 cycles of  
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55 125  $94^{\circ}\text{C}$  for 10 sec and  $55^{\circ}\text{C}$  for 35 sec (ABI-7500 real-time PCR instrument, Life

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### 11 128 **Detection of common respiratory pathogens in HMPV-positive patients**

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13 129 HMPV-positive samples were tested simultaneously using TaqMan Real-time  
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15 130 PCR assays for the following 17 respiratory pathogens: respiratory syncytial virus  
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17 131 (RSV); parainfluenza virus types 1–4 (PIV1–4); influenza A and B viruses (InfA, InfB);  
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19 132 adenovirus (ADV); enterovirus (EV); human coronaviruses (HCoV-229E, HCoV-OC43,  
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21 133 HCoV-NL63, HCoV-HKU1); human rhinovirus (HRV); human bocavirus (HBoV);  
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23 134 *Mycoplasma pneumoniae* (MP); and *Chlamydomphila pneumoniae* (CP); The testing  
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25 135 procedure was conducted using kits from Guangzhou HuYanSuo Medical Technology  
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28 136 Co., Ltd as previously described.<sup>18</sup>  
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### 33 138 **Statistical analysis**

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35 139 All statistical analyses were performed with SPSS statistical software (version  
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37 140 19.0; SPSS Inc., Chicago, IL, USA). To compare categorical data,  $\chi^2$  and Fisher's  
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39 141 exact tests were used, as appropriate. All tests were two-tailed, and  $p < 0.05$  was  
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42 142 considered statistically significant.  
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## 143 **Results**

### 144 **Detection of HMPV from patients with ARI**

145 In total, 5133 pediatric patients, ranging from 1 day to 14 years old were  
146 enrolled in this study. The male-to-female ratio was 1.75 (3269:1864) and the median  
147 age was 33 months (interquartile range, 9–48 months). Of the 5133 patients, 103  
148 (2.0%) were positive for HMPV. The male-to-female ratio was 1.86:1 (67:36) in  
149 HMPV-positive patients and 1.75:1 (3202:1828) in HMPV-negative patients ( $p=0.771$ ).  
150 The median age in months of HMPV-positive patients was 23.5 months (interquartile  
151 range, 10–36 months).

### 153 **Co-detection with common respiratory pathogens in HMPV-positive patients**

154 We also tested HMPV-positive samples for 17 common respiratory pathogens.  
155 Of the 103 HMPV-positive patients, 84 (81.6%) patients had only HMPV detection,  
156 and 19 (18.4%) patients had HMPV plus other pathogen(s) detected. Nine out of 17  
157 respiratory pathogens (52.9%) were detected and the most common co-detection  
158 pathogens were RSV (36.8%, 7/19), HCoV-OC43 (15.8%, 3/19), MP (15.8%, 3/19),  
159 and HBoV (15.8%, 3/19) (**Table 1**). The male-to-female ratio was 2:1 (56:28) in  
160 patients with only HMPV detected and 1.38:1 (11:8) in patients with HMPV plus other  
161 pathogen(s) detected ( $p=0.469$ ).

### 163 **Age distribution of HMPV-positive patients**

164 Overall, there was a significant difference in HMPV prevalence between

165 patients >5–14 years (0.7%, 5/734) and those ≤5 years (2.2%, 98/4399) ( $p=0.004$ ).

166 The patients were divided into six age groups: 0–3 months, >3–6 months, >6–12  
167 months, >1–2 years, >2–5 years, and >5–14 years. The distribution of HMPV  
168 prevalence between these age groups was significantly different ( $p=0.03$ ), and  
169 children >1–2 years had the highest prevalence (2.8%, 21/752) (**Figure 1**).

### 171 **Seasonal distribution of HMPV**

172 Over the 3-year study period, the prevalence of HMPV peaked twice every  
173 year (**Figure 2**). Large peaks occurred in March 2014 (8%, 16/200), May 2015 (7.6%,  
174 8/105) and February 2016 (8.7%, 9/103). Small peaks occurred in November 2014  
175 (1.7%, 2/121), September 2015 (2.8%, 2/72) and May 2016 (1.5%, 2/135) (**Figure 2**).

### 177 **Clinical presentation of HMPV-positive patients**

178 We analyzed the clinical presentation of 101 of the 103 (98.1%) HMPV-positive  
179 patients, the other two patients were excluded from this analysis because of  
180 incomplete clinical data. The main clinical features of HMPV-positive patients included  
181 cough (100.0%, 101/101), abnormal pulmonary breath sound (91.1%, 92/101), fever  
182 (88.1%, 89/101), expectoration (77.2%, 78/101), coryza (50.5%, 51/101) and  
183 wheezing (46.5%, 47/101). The main diagnosis by chest radiography was  
184 bronchopneumonia (55.4%, 56/101), followed by pneumonia (10.9%, 11/101) and  
185 bronchiolitis (7.9%, 8/101) (**Table 2**). We also compared the clinical features of  
186 patients with only HMPV detected and those of the patients with HMPV plus other

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4 187 pathogen(s) detected. Fever and diarrhea were the two symptoms that were identified  
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6 188 as statistically different between these two groups; A fever ( $\geq 38^{\circ}\text{C}$ ) was seen more  
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8 189 common in patients with only HMPV detected (91.6%, 76/83) , compared with 72.2%  
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10 190 (13/18) in patients with HMPV plus other pathogen(s) detected ( $p=0.037$ ). Conversely,  
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13 191 diarrhea appeared more often in patients with HMPV plus other pathogen(s) detected  
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15 192 (22.2%, 4/18) than in the patients with only HMPV detected (3.6%, 3/83) ( $p=0.018$ )  
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18 193 (**Table 2**).

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4 194 **Discussion**

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6 195 Viruses are the most frequent cause of respiratory infections.<sup>20</sup> HMPV has  
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8 196 been recognized as an important cause of ARI since its discovery in 2001. A previous  
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10 197 study reported HMPV was responsible for approximately 5–10% of hospitalizations in  
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12 198 children suffering from ARI,<sup>14</sup> creating a considerable clinical and economic burden  
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14 199 worldwide. HMPV detection rates vary according to geographic location, and the  
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16 200 incidence of HMPV may show seasonal or annual patterns in the same area. In  
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18 201 previous studies,<sup>21-30</sup> HMPV was detected in approximately 1%–17% of ARI cases. In  
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20 202 this 3 year study, 103 of 5133 (2.0%) patients were positive for HMPV, which is similar  
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22 203 to previous reports of HMPV in patients with ARI in southern China<sup>31</sup> and Japan.<sup>32</sup>

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25 204 Children under 5 years of age are most susceptible to HMPV infection, and  
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27 205 those younger than 2 years of age are at the greatest risk of developing serious  
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29 206 conditions.<sup>15</sup> In our study, HMPV-positive cases appeared more frequently in patients  
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31 207 ≤5 year-old (2.2%, 98/4399) than in patients >5 year-old (0.7%, 5/734) ( $p=0.004$ ),  
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33 208 which is consistent with many previous studies.<sup>3 30 33 34</sup> Specifically, HMPV prevalence  
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35 209 was highest in children >1–2 years (2.8%, 21/752). This may be explained by the  
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37 210 immunity against HMPV, which is passed down from mother-to-child, gradually  
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39 211 subsiding over time. Moreover, children were more contact with outside world as they  
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41 212 grew older. Therefore, children of this age need more attention to prevent HMPV  
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43 213 infection. Moreover, our findings suggested no difference in risk between sex, there  
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45 214 was no significant difference in the HMPV prevalence between male and female  
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47 215 patients ( $p=0.771$ ), which is consistent with previous studies.<sup>35 36</sup>

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4 216 HMPV has a typical seasonal distribution. HMPV activity is largely affected by  
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6 217 different local climate factors. The prevalence of HMPV in temperate climates peaks  
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8 218 at the end of winter or in early spring.<sup>37 38</sup> In our study, seasonal peaks of HMPV were  
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10 219 detected in February 2016, March 2014 and May 2015. This finding indicates that  
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12 220 HMPV circulates primarily during the spring and early summer in Guangzhou, this  
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14 221 pattern is similar to previous reports from other subtropical areas.<sup>22 39</sup> Furthermore, we  
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16 222 also found small peaks in November 2014, September 2015, and May 2016, the  
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18 223 reason for this is unknown. It should be noted that the seasonal distribution of HMPV  
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20 224 overlaps with RSV in this geographical area.<sup>19</sup> Knowing the epidemiological  
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22 225 characteristics of HMPV will help public health authorities and clinicians to improve  
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24 226 strategies for controlling HMPV infection.

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30 227 HMPV co-infection with other respiratory pathogens has been reported in  
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32 228 many studies, including RSV,<sup>40</sup> InfA,<sup>41</sup> InfB,<sup>42</sup> PIV,<sup>43</sup> ADV,<sup>43 44</sup> HBoV,<sup>45</sup> HCoV,<sup>43 46</sup>  
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34 229 HRV,<sup>43</sup> EV,<sup>43</sup> MP,<sup>41</sup> and CP.<sup>41</sup> In our study, most HMPV-positive patients only had  
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36 230 HMPV (81.6%, 84/103), and there was no significant difference in the HMPV  
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38 231 prevalence according to sex between the patients with only HMPV detected and the  
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40 232 patients with HMPV plus other pathogen(s) detected ( $p=0.469$ ). The co-detection rate  
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42 233 was 18.4% (19/103), and RSV (36.8%, 7/19) was the most frequently co-detected  
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44 234 pathogen, which might be because of the overlapping seasonal distribution of these  
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46 235 two pathogens.<sup>47</sup> Co-detection rates of HCoV-OC43, MP, HBoV, ADV, HRV, InfA, PIV2,  
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48 236 and PIV3 were over 5%, suggesting a broad range of respiratory pathogens could  
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50 237 co-exist with HMPV in ARI pediatric patients.

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4 238 HMPV most commonly causes URTI and LRTI in young children. The clinical  
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6 239 manifestations of HMPV-positive patients are similar to those of RSV-positive patients,  
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8 240 especially in young children.<sup>48-50</sup> In previous studies, the most frequent diagnoses of  
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10 241 children hospitalized with HMPV infection were pneumonitis and bronchiolitis.<sup>3 51 52</sup> In  
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12 242 some studies, bronchiolitis and recurrent wheezing/pneumonia were the main clinical  
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14 243 diagnoses,<sup>7 22 53 54</sup> while in other studies, bronchopneumonia, bronchiolitis, and  
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16 244 bronchial asthma exacerbation were the main clinical diagnoses.<sup>34</sup> Our study showed  
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18 245 that 55.4% (56/101), 7.9% (8/101) and 10.9% (11/101) of HMPV-positive patients  
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20 246 were diagnosed with bronchopneumonia, bronchiolitis, and pneumonia by chest  
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22 247 radiography, respectively. Of all the clinical features recorded, cough (100.0%,  
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24 248 101/101), abnormal pulmonary breath sound (91.1%, 92/101), fever (88.1%, 89/101),  
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26 249 expectoration (77.2%, 78/101), coryza (50.5%, 51/101), and wheezing (46.5%,  
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28 250 47/101) occurred frequently in HMPV-positive patients, similar to those reported  
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30 251 previously.<sup>16 29 52 55</sup>

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37 252 Most of the HMPV-positive patients had a fever (88.1%, 89/101); it appeared  
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39 253 more frequently in patients with only HMPV detected than in the patients with HMPV  
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41 254 plus other pathogen(s) detected ( $p=0.037$ ). Diarrhea was not a major symptom in  
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43 255 HMPV-positive patients (7.0%, 7/101), but it had a higher prevalence in patients with  
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45 256 HMPV plus other pathogen(s) detected than in the patients with only HMPV detected  
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47 257 ( $p=0.018$ ), suggesting that diarrhea is probably caused by other pathogens. No  
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49 258 significant difference was found in the other clinical features between the patients with  
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51 259 only HMPV detected and the patients with HMPV plus other pathogen(s) detected,  
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3 260 similar to the results of a previous study.<sup>51</sup>  
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6 261 In our study, RSV (36.8%, 7/19) was the most frequently co-detected pathogen  
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8 262 in HMPV-positive patients. Whether HMPV/RSV co-infection is more severe than the  
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10 263 respective mono-infections remains unclear. A previous study showed that children  
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12 264 with HMPV/RSV co-infection were more likely to develop pneumonia; however,  
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14 265 disease severity was not increased.<sup>53</sup> Conversely, Semple et al.<sup>56</sup> reported that  
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16 266 HMPV/RSV co-infection can cause more severe bronchiolitis in patients. In our study,  
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18 267 no significant difference in the diagnosis of pneumonia was observed between the  
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20 268 patients with only HMPV detected and the patients with HMPV plus other pathogen(s)  
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22 269 detected. It has been confirmed that a history of prematurity, particular age groups,  
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24 270 and the presence of chronic diseases increases the risk of severe LRTI among  
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26 271 HMPV- and RSV-infected children.<sup>51</sup> Our study suggests that the clinical  
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28 272 manifestations of HMPV infection are complex and diverse; our data will be helpful in  
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30 273 the diagnosis of HMPV.  
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38 274 The study had some limitations. First, collection of data on symptoms and  
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40 275 physical findings in infants and young children requires experienced medical staff,  
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42 276 patient's cooperation, and consent from the patient's guardian. Consequently, it is  
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44 277 possible we will have incomplete or even an inaccurate description of patients'  
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46 278 manifestations. Second, because our study mainly focused on HMPV, other common  
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48 279 respiratory pathogens, including bacterial pathogens, were not tested and  
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50 280 subsequently our study does not give a fully account of respiratory pathogen infection  
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52 281 in hospitalized pediatric patients with ARI. Third, the characteristics of HMPV-negative  
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3 282 patients and outcome data (discharge/death) were not analyzed further, because our  
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6 283 study only focused on the epidemiological and clinical features of HMPV positive  
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8 284 cases. This might affect our understanding of the clinical features of HMPV-positive  
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11 285 patients. Despite these shortcomings, our results provide a valuable insight into the  
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13 286 epidemiological and clinical characteristics of HMPV present in patients hospitalized  
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16 287 with ARI in Guangzhou, southern China.  
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4 288 **Conclusions**

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6 289 HMPV is an important respiratory pathogen in children with ARI in Guangzhou ,  
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8 290 China, particularly in children under 5 years old. In future, our data can be used by  
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10 291 public health authorities and clinicians to improve the management of HMPV infection  
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13 292 in children.  
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297

298 **Contributors**

299 RZ, WKL, LZ and DLL designed the study. LZ, DLL, WKL, SYQ, DX, XL and LTT  
300 performed pathogen testing. DHC and WPT collected the clinical data. All authors  
301 participated in the data analysis. LZ, DLL, RZ and WKL drafted the manuscript. All  
302 authors read and approved the final version of this manuscript.

303

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311 **Competing interests**

312 None declared.

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314 **Ethics approval**

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3 315 The study was approved by The First Affiliated Hospital of Guangzhou Medical  
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6 316 University Ethics Committee. Informed written consent was obtained from parents or  
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8 317 legal guardians.  
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13 319 **Data sharing statement**  
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16 320 No additional data are available.  
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4 510 **Figure legends**

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8 512 **Figure 1** Age distributions of patients with HMPV.

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11 513 <sup>a</sup>Date were presented as HMPV positive rate (number of HMPV-positive  
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13 514 patients/number of patients in each age group); m: month(s); y: year(s).

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18 516 **Figure 2** Seasonal distribution of HMPV infection in pediatric patients hospitalized with  
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20 517 acute respiratory infection from July 2013 to June 2016 in Guangzhou.

518 **Table 1. Distribution of co-pathogens in 19 HMPV-positive patients**

Co-pathogens <sup>a</sup>	Patient No.
RSV/HMPV	4
HCoV-OC43/HMPV	3
ADV/HMPV	2
HRV/HMPV	2
MP/HMPV	1
PIV2/HMPV	1
PIV3/HMPV	1
RSV/MP/HMPV	1
RSV/InfA/HMPV	1
RSV/HBoV/HMPV	1
MP/HBoV/HMPV	1
InfA/HBoV/HMPV	1

519 <sup>a</sup>HMPV-positive patients were tested for 17 common respiratory pathogens.

520 RSV: respiratory syncytial virus; PIV: parainfluenza virus; InfA: influenza A virus; ADV:

521 adenovirus; HCoV: human coronaviruses; HRV: human rhinovirus; HBoV: human

522 bocavirus; MP: *Mycoplasma pneumoniae*.

523 Influenza B viruses, parainfluenza virus types 1 and 4, enterovirus, human

524 coronaviruses-HKU1, human coronaviruses-229E, human coronaviruses-NL63, and

525 *Chlamydomphila pneumoniae* were not detected.

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527

**Table 2. Clinical presentations of HMPV-positive patients**

Diagnosis/Symptom	Total HMPV (n=101)	Patients with only HMPV and HMPV plus other pathogen(s) detected.		
		Single HMPV (n = 83)	Co-pathogens (n= 18)	p value <sup>b</sup>
<b>Upper respiratory tract infection</b>				
Nasal obstruction	40(39.6)	34(41.0)	6(33.3)	0.605
Coryza	51(50.5)	44(53.0)	7(38.9)	0.309
Sneeze	1(1.0)	1(1.2)	0(0)	— <sup>c</sup>
Cough	101(100)	83(100)	18(100)	— <sup>d</sup>
Expectoration	78(77.2)	66(79.5)	12(66.7)	0.351
Hoarseness	1(1.0)	1(1.2)	0(0)	— <sup>c</sup>
<b>Lower respiratory tract infection</b>				
Abnormal pulmonary breath sound <sup>a</sup>	92(91.1)	75(90.4)	17(94.4)	0.582
Increasing lung markings	6(6.0)	4(4.8)	2(11.1)	0.29
wheezing	47(46.5)	40(48.2)	7(38.9)	0.473
anhelation	24(23.8)	21(25.3)	3(16.7)	0.551
Bronchiolitis	8(7.9)	7(8.4)	1(5.6)	0.682
Pneumonia	11(10.9)	10(12.1)	1(5.6)	0.684
bronchopneumonia	56(55.4)	46(55.4)	10(55.6)	0.992
<b>Systemic influenza-like symptoms</b>				
Fever (≥38°C)	89(88.1)	76(91.6)	13(72.2)	<b>0.037</b>
Chill	7(6.9)	6(7.2)	1(5.6)	0.8
Debilitation	11(10.9)	11(13.3)	0(0)	— <sup>c</sup>
<b>Gastrointestinal illness</b>				
Vomiting	21(20.8)	16(19.3)	5(27.8)	0.522
Poor appetite	19(18.8)	17(20.5)	2(11.1)	0.513
Diarrhea	7(7.0)	3(3.6)	4(22.2)	<b>0.018</b>

528 Data are presented as No. (%) of each group.

529 Percentages sum to >100% because some patients had >1 clinical presentations.

530 <sup>a</sup>Including phlegmatic, wheezing, bubbling, and moist rales.

531 <sup>b</sup>Two-tailed  $\chi^2$  test comparing the distribution of each illness or diagnosis between  
532 patients with only HMPV and HMPV plus other pathogen(s) detected.

533 <sup>c</sup>Not tested because the number of positive samples obtained was too small.

534 <sup>d</sup>Not tested because the prevalence was 100%.

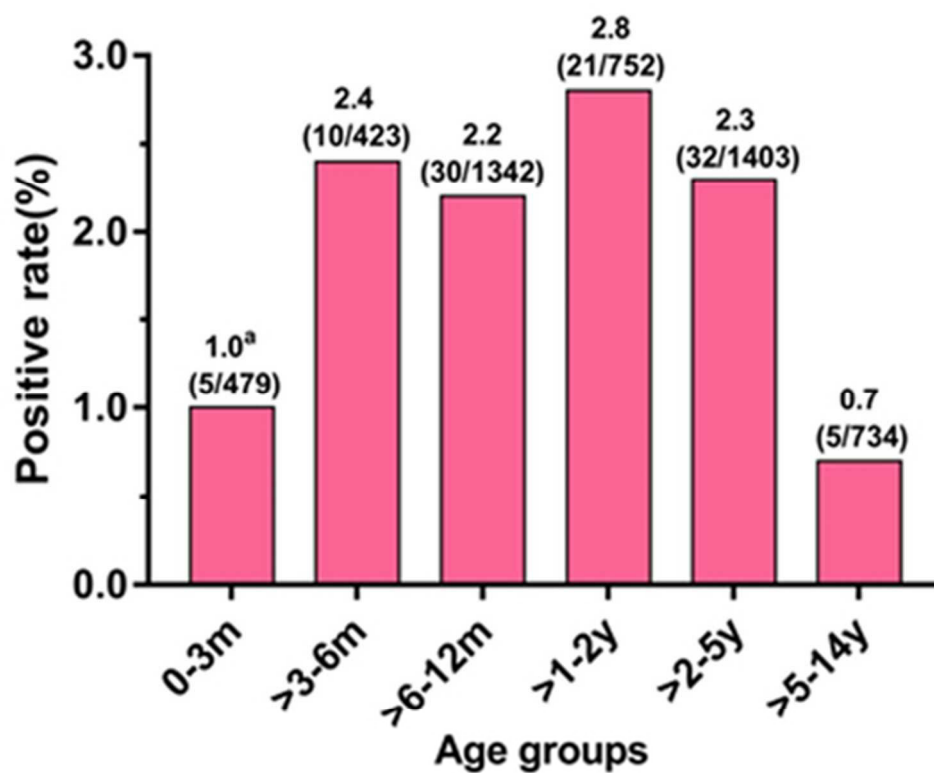


Figure 1 Age distributions of patients with HMPV.

<sup>a</sup>Data were presented as HMPV positive rate (number of HMPV-positive patients/number of patients in each age group); m: month(s); y: year(s).

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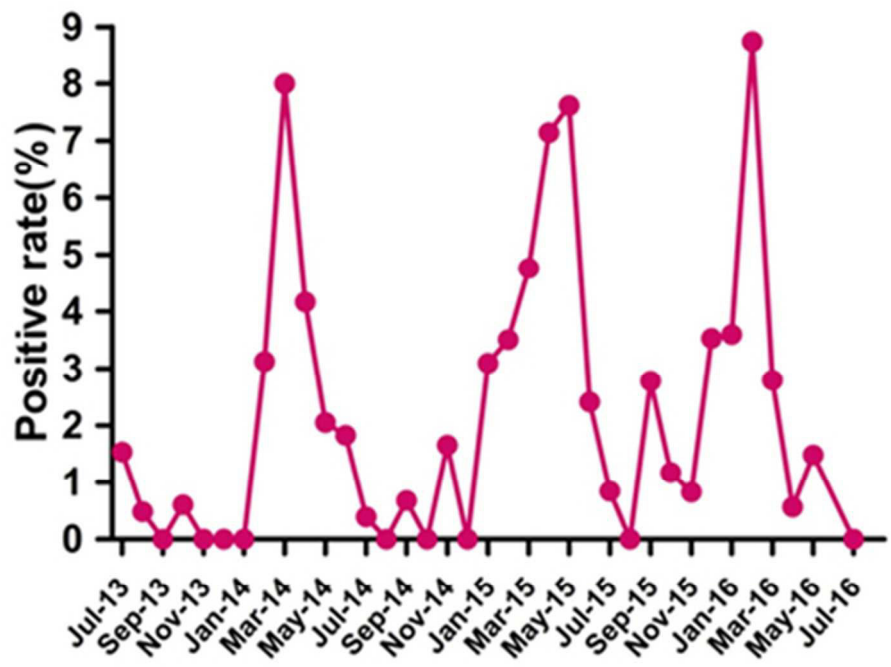


Figure 2 Seasonal distribution of HMPV infection in pediatric patients hospitalized with acute respiratory infection from July 2013 to June 2016 in Guangzhou.

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**STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies***

Section/Topic	Item #	Recommendation	Reported on page #
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4-5
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8
		(b) Describe any methods used to examine subgroups and interactions	8
		(c) Explain how missing data were addressed	7
		(d) If applicable, describe analytical methods taking account of sampling strategy	8
		(e) Describe any sensitivity analyses	
<b>Results</b>			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9-11
		(b) Indicate number of participants with missing data for each variable of interest	9
Outcome data	15*	Report numbers of outcome events or summary measures	9-11
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-11
		(b) Report category boundaries when continuous variables were categorized	9-10
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	12-15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15-16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-16
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).

# BMJ Open

## Epidemiology and clinical features of human metapneumovirus in hospitalized pediatric patients with acute respiratory illness: a cross-sectional study in southern China, from 2013 to 2016

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Keywords:	Respiratory infections < THORACIC MEDICINE, Epidemiology < INFECTIOUS DISEASES, VIROLOGY, Diagnostic microbiology < INFECTIOUS DISEASES

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4 1 **Epidemiology and clinical features of human metapneumovirus in hospitalized**  
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6 2 **pediatric patients with acute respiratory illness: a cross-sectional study in**  
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8 3 **southern China, from 2013 to 2016**  
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14 5 Ling Zhang<sup>1†</sup>, Wenkuan Liu<sup>1†</sup>, Donglan Liu<sup>1†</sup>, Dehui Chen<sup>2</sup>, Weiping Tan<sup>3</sup>, Shuyan  
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16 6 Qiu<sup>1</sup>, Duo Xu<sup>1</sup>, Xiao Li<sup>1</sup>, Tiantian Liu<sup>1</sup>, Rong Zhou<sup>1\*</sup>  
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20  
21 8 <sup>1</sup>State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of  
22  
23 9 Guangzhou Medical University, Guangzhou Medical University, Guangzhou,  
24  
25 10 People's Republic of China.  
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29 11 <sup>2</sup>Department of Pediatrics, The First Affiliated Hospital of Guangzhou Medical  
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31 12 University, Guangzhou Medical University, Guangzhou, People's Republic of China.  
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34 13 <sup>3</sup>Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, People's  
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41 16 <sup>†</sup>Ling Zhang, Wenkuan Liu and Donglan Liu contributed equally to this work.  
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46 18 \*Correspondence to Rong Zhou, e-mail: [zhourong@gird.cn](mailto:zhourong@gird.cn)  
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4 19 **Abstract**

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6 20 **Objectives:** Human metapneumovirus (HMPV) is one of the most important  
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8 21 respiratory viral pathogens affecting infants and children worldwide. Our study  
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10 22 describes the epidemiological and clinical characteristics of HMPV present in patients  
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12 23 hospitalized with acute respiratory illness (ARI) in Guangzhou, southern China.

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16 24 **Study design:** A cross-sectional study.

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18 25 **Setting:** Two tertiary hospitals in Guangzhou.

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20 26 **Participants and methods:** Throat swabs were collected over a 3-year period from  
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22 27 5133 pediatric patients ( $\leq 14$  years) hospitalized with ARI. HMPV-positive patients'  
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24 28 clinical presentations (101/103) were recorded for further analysis.

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28 29 **Results:** Of the 5133 patients included in the study, 103 (2.0%) were positive for  
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30 30 HMPV. HMPV was more prevalent in children  $\leq 5$  years (2.2%, 98/4399) compared  
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32 31 with older children ( $>5$ –14 years) (0.7%, 5/734) ( $p=0.004$ ). Two seasonal HMPV  
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34 32 peaks were observed each year and mainly occurred in spring and early summer.  
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36 33 Overall, 18.4% (19/103) of HMPV-positive patients were co-detected with other  
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38 34 pathogens, most frequently respiratory syncytial virus (36.8%, 7/19). HMPV-positive  
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40 35 patients presented with a wide spectrum of clinical features, including cough (100.0%,  
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42 36 101/101), abnormal pulmonary breath sound (91.1%, 92/101), fever (88.1%, 89/101),  
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44 37 expectoration (77.2%, 78/101), coryza (50.5%, 51/101) and wheezing (46.5%,  
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46 38 47/101). The main diagnosis of HMPV-positive patients was bronchopneumonia  
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48 39 (66.7%, 56/84). Fever ( $\geq 38^{\circ}\text{C}$ ) (91.6%, 76/83) was detected more often in patients  
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50 40 with only HMPV detected than in patients with HMPV plus other pathogen(s) detected  
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4 41 (72.2%, 13/18) ( $p=0.037$ ), whereas diarrhea was more common in patients with  
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6 42 HMPV plus other pathogen(s) detected (22.2%, 4/18), compared with patients with  
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8 43 HMPV only (3.6%, 3/83) ( $p=0.018$ ).

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11 44 **Conclusions:** HMPV is an important respiratory pathogen in children with ARI in  
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13 45 Guangzhou, particularly in children  $\leq 5$  years old. HMPV has a seasonal variation.  
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16 46 Bronchopneumonia is a major diagnosis in HMPV-positive patients.

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21 48 **Strengths and limitations of this study**

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23 49 ● 5133 patients hospitalized with acute respiratory illness at two large municipal  
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25 50 tertiary hospitals (hospital beds  $> 1000$ ) were enrolled on the study over 3 years.  
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28 51 ● Patients aged from 1 day to 14 years old were tested for HMPV using Taqman  
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30 52 real-time PCR.  
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33 53 ● Clinical characteristics of patients with HMPV-positive were recorded.  
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35 54 ● There was incomplete HMPV co-pathogen detection, because bacterial  
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37 55 pathogens were not detected for HMPV-positive patients.  
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40 56 ● The clinical characteristics of HMPV-negative patients and outcome data  
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42 57 (discharge/death) for the HMPV-positive patients were not collected for further  
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44 58 analysis, limiting our understanding of this pathogen.

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50 60 **Introduction**

51  
52 61 Human metapneumovirus (HMPV) is a nonsegmented, negative-sense  
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54 62 single-stranded RNA virus, which belongs to the *Paramyxoviridae* family.<sup>1</sup> HMPV was



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4 63 first discovered in 2001 in the Netherlands, after being isolated from a pediatric patient  
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6 64 with acute respiratory illness (ARI).<sup>2</sup> Since then, HMPV has been associated with  
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8 65 acute respiratory disease in individuals of all ages worldwide. Children, elderly and  
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10 66 immunocompromised adults are most at risk of contracting the virus.<sup>3-5</sup> Children  
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12 67 younger than 5 years of age seem to be particularly susceptible to HMPV. Previous  
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14 68 studies,<sup>6-12</sup> which were mainly conducted in developed countries, have revealed that  
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16 69 approximately 2.5%–11.3% of respiratory samples were positive for HMPV in children  
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18 70 ≤5 years, and 90% of individuals were seropositive for HMPV by 5 years of age.<sup>13</sup>  
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23 71 HMPV causes a variety of clinical symptoms ranging from a mild upper  
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25 72 respiratory tract infection (URTI) to life-threatening lower respiratory tract infection  
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27 73 (LRTI).<sup>3 14-16</sup> However, to date, there is no effective vaccine or specific medication  
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29 74 either for prevention or treatment of HMPV infection. Consequently, it is imperative to  
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31 75 conduct more studies, especially in low and middle-income countries, to understand  
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33 76 this pathogen in different areas and populations.  
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38 77 In this study, we investigated the epidemiological and clinical features of HMPV  
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40 78 in pediatric patients, from July 2013 to June 2016. The findings of this study will help  
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42 79 understand the distribution of HMPV in a subtropical region. Our results also provide a  
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44 80 valuable insight into the clinical features of HMPV, which will improve early clinical  
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46 81 diagnosis.  
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## 82 **Methods**

### 83 **Study design and respiratory sample collection**

84 We conducted a cross-sectional study at two tertiary hospitals in Guangzhou,  
85 southern China. Throat swab samples (n=5133) from pediatric patients ( $\leq 14$  years)  
86 hospitalized with ARI, were collected at two hospitals between July 2013 and June  
87 2016. ARI was defined as an illness that presented with at least two of the following  
88 clinical presentations: cough, nasal obstruction, coryza, sneeze, dyspnoea during the  
89 previous week. Patients, who were diagnosed with pneumonia by chest radiography  
90 during the previous week, were also included in the study, even if they did not show  
91 the clinical features described above. Some patients, who had been cured and  
92 discharged some time ago and readmitted because of new episodes of ARI, if met the  
93 recruitment criteria, were included in the study as new cases, otherwise excluded.  
94 Chest radiography was performed based on the clinical situation of the patients. The  
95 samples were collected according to established clinical protocols.<sup>17</sup> The samples  
96 were refrigerated at 2–8°C in viral transport medium, transported on ice to the State  
97 Key Laboratory of Respiratory Diseases, and analyzed immediately or stored at -80°C  
98 before analysis as previously described.<sup>18</sup>

99 The patients' clinical presentations or diagnoses were recorded from patients'  
100 medical records by attending physicians, using designed presentation cards and were  
101 categorized retrospectively into the following four groups: URTI, LRTI, systemic  
102 influenza-like symptoms, and gastrointestinal illness. Patients with nasal obstruction,  
103 coryza, sneezing, coughing, expectoration, or hoarseness were categorized as

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3 104 having URTI. Patients with bronchiolitis, pneumonia, bronchopneumonia, increased  
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6 105 lung markings, dyspnea, or an abnormal pulmonary breath sound were categorized  
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9 106 as having LRTI. Patients with a high fever ( $\geq 38^{\circ}\text{C}$ ), chills, or debilitation were  
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11 107 categorized as having systemic influenza-like symptoms. Patients with vomiting, poor  
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13 108 appetite, or diarrhea were categorized as having gastrointestinal illness. Some  
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16 109 patients were assigned to several clinical presentation groups. Patients with  
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18 110 incomplete clinical data were excluded from this analysis. Increased lung markings,  
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21 111 bronchopneumonia, pneumonia, and bronchiolitis were diagnosed by chest  
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23 112 radiography. Abnormal pulmonary breath sounds included phlegmatic, wheezy,  
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26 113 bubbling, and moist rales. Other clinical symptoms were identified by a general  
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28 114 medical examination and clinical descriptions, as previously reported.<sup>19</sup>

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### 32 116 **Real-time PCR for HMPV detection**

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35 117 RNA was extracted from the throat swab samples with the QIAamp Viral RNA  
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38 118 Mini Kit (Qiagen, Shanghai, China), according to the manufacturer's protocols. HMPV  
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40 119 was identified by using TaqMan Real-time PCR assays, as previously reported,<sup>18</sup>  
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43 120 using kits from Guangzhou HuYanSuo Medical Technology Co., Ltd according to the  
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46 121 manufacturer's protocols. In brief, 50  $\mu\text{l}$  RNA were extracted from a 200  $\mu\text{l}$  sample,  
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48 122 and real-time PCR was conducted using 25  $\mu\text{l}$  reaction mix, containing M-MLV, Taq  
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51 123 polymerase and 5  $\mu\text{l}$  extracted RNA. Cycling conditions included an initial reverse  
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53 124 transcription at  $55^{\circ}\text{C}$  for 10 min, incubation at  $94^{\circ}\text{C}$  for 2 min, followed by 40 cycles of  
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55 125  $94^{\circ}\text{C}$  for 10 sec and  $55^{\circ}\text{C}$  for 35 sec (ABI-7500 real-time PCR instrument, Life

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4 126 Technologies, Singapore).  
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8 128 **Detection of common respiratory pathogens in HMPV-positive patients**  
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10 129 HMPV-positive samples were tested simultaneously using TaqMan Real-time  
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12 130 PCR assays for the following 17 respiratory pathogens: respiratory syncytial virus  
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14 131 (RSV); parainfluenza virus types 1–4 (PIV1–4); influenza A and B viruses (InfA, InfB);  
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16 132 adenovirus (ADV); enterovirus (EV); human coronaviruses (HCoV-229E, HCoV-OC43,  
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18 133 HCoV-NL63, HCoV-HKU1); human rhinovirus (HRV); human bocavirus (HBoV);  
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20 134 *Mycoplasma pneumoniae* (MP); and *Chlamydomphila pneumoniae* (CP); The testing  
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22 135 procedure was conducted using kits from Guangzhou HuYanSuo Medical Technology  
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24 136 Co., Ltd as previously described.<sup>18</sup>  
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32 138 **Statistical analysis**  
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35 139 All statistical analyses were performed with SPSS statistical software (version  
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37 140 19.0; SPSS Inc., Chicago, IL, USA). To compare categorical data,  $\chi^2$  and Fisher's  
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39 141 exact tests were used, as appropriate. All tests were two-tailed, and  $p < 0.05$  was  
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41 142 considered statistically significant.  
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## 143 **Results**

### 144 **Detection of HMPV from patients with ARI**

145 In total, 5133 pediatric patients, ranging from 1 day to 14 years old were  
146 enrolled in this study. The male-to-female ratio was 1.75 (3269:1864) and the median  
147 age was 33 months (interquartile range, 9–48 months). Of the 5133 patients, 103  
148 (2.0%) were positive for HMPV. The male-to-female ratio was 1.86:1 (67:36) in  
149 HMPV-positive patients and 1.75:1 (3202:1828) in HMPV-negative patients ( $p=0.771$ ).  
150 The median age in months of HMPV-positive patients was 23.5 months (interquartile  
151 range, 10–36 months).

### 153 **Co-detection with common respiratory pathogens in HMPV-positive patients**

154 We also tested HMPV-positive samples for 17 common respiratory pathogens.  
155 Of the 103 HMPV-positive patients, 84 (81.6%) patients had only HMPV detection,  
156 and 19 (18.4%) patients had HMPV plus other pathogen(s) detected. Nine out of 17  
157 respiratory pathogens (52.9%) were detected and the most common co-detection  
158 pathogens were RSV (36.8%, 7/19), HCoV-OC43 (15.8%, 3/19), MP (15.8%, 3/19),  
159 and HBoV (15.8%, 3/19) (**Table 1**). The male-to-female ratio was 2:1 (56:28) in  
160 patients with only HMPV detected and 1.38:1 (11:8) in patients with HMPV plus other  
161 pathogen(s) detected ( $p=0.469$ ).

### 163 **Age distribution of HMPV-positive patients**

164 Overall, there was a significant difference in HMPV prevalence between

165 patients >5–14 years (0.7%, 5/734) and those ≤5 years (2.2%, 98/4399) ( $p=0.004$ ).

166 The patients were divided into six age groups: 0–3 months, >3–6 months, >6–12

167 months, >1–2 years, >2–5 years, and >5–14 years. The distribution of HMPV

168 prevalence between these age groups was significantly different ( $p=0.03$ ), and

169 children >1–2 years had the highest prevalence (2.8%, 21/752) (**Figure 1**).

170

### 171 **Seasonal distribution of HMPV**

172 Over the 3-year study period, the prevalence of HMPV peaked twice every

173 year (**Figure 2**). Large peaks occurred in March 2014 (8%, 16/200), May 2015 (7.6%,

174 8/105) and February 2016 (8.7%, 9/103). Small peaks occurred in November 2014

175 (1.7%, 2/121), September 2015 (2.8%, 2/72) and May 2016 (1.5%, 2/135) (**Figure 2**).

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### 177 **Clinical presentation of HMPV-positive patients**

178 We analyzed the clinical presentation of 101 of the 103 (98.1%) HMPV-positive

179 patients, the other two patients were excluded from this analysis because of

180 incomplete clinical data. The main clinical features of HMPV-positive patients included

181 cough (100.0%, 101/101), abnormal pulmonary breath sound (91.1%, 92/101), fever

182 (88.1%, 89/101), expectoration (77.2%, 78/101), coryza (50.5%, 51/101) and

183 wheezing (46.5%, 47/101). Of 84 with a chest radiography, 56 (66.7%) were

184 diagnosed with bronchopneumonia, 11 (13.1%) were pneumonia and 8 (9.5%) were

185 bronchiolitis (**Table 2**). We also compared the clinical features of patients with only

186 HMPV detected and those of the patients with HMPV plus other pathogen(s) detected.

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4 187 Fever and diarrhea were the two symptoms that were identified as statistically  
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6 188 different between these two groups; A fever ( $\geq 38^{\circ}\text{C}$ ) was seen more common in  
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8 189 patients with only HMPV detected (91.6%, 76/83) , compared with 72.2% (13/18) in  
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10 190 patients with HMPV plus other pathogen(s) detected ( $p=0.037$ ). Conversely, diarrhea  
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12 191 appeared more often in patients with HMPV plus other pathogen(s) detected (22.2%,  
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14 192 4/18) than in the patients with only HMPV detected (3.6%, 3/83) (**Table 2**).

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193 **Discussion**

194 Viruses are the most frequent cause of respiratory infections.<sup>20</sup> HMPV has  
195 been recognized as an important cause of ARI since its discovery in 2001. A previous  
196 study reported HMPV was responsible for approximately 5–10% of hospitalizations in  
197 children suffering from ARI,<sup>14</sup> creating a considerable clinical and economic burden  
198 worldwide. HMPV detection rates vary according to geographic location, and the  
199 incidence of HMPV may show seasonal or annual patterns in the same area. In  
200 previous studies,<sup>21–30</sup> HMPV was detected in approximately 1%–17% of ARI cases. In  
201 this 3 year study, 103 of 5133 (2.0%) patients were positive for HMPV, which is similar  
202 to previous reports of HMPV in patients with ARI in southern China<sup>31</sup> and Japan.<sup>32</sup>

203 Children under 5 years of age are most susceptible to HMPV infection, and  
204 those younger than 2 years of age are at the greatest risk of developing serious  
205 conditions.<sup>15</sup> In our study, HMPV-positive cases appeared more frequently in patients  
206 ≤5 year-old (2.2%, 98/4399) than in patients >5 year-old (0.7%, 5/734) ( $p=0.004$ ),  
207 which is consistent with many previous studies.<sup>3 30 33 34</sup> Specifically, HMPV prevalence  
208 was highest in children >1–2 years (2.8%, 21/752). This may be explained by the  
209 immunity against HMPV, which is passed down from mother-to-child, gradually  
210 subsiding over time. Moreover, children were more contact with outside world as they  
211 grew older. Therefore, children of this age need more attention to prevent HMPV  
212 infection. Moreover, our findings suggested no difference in risk between sex, there  
213 was no significant difference in the HMPV prevalence between male and female  
214 patients ( $p=0.771$ ), which is consistent with previous studies.<sup>35 36</sup>



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4 215 HMPV has a typical seasonal distribution. HMPV activity is largely affected by  
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6 216 different local climate factors. The prevalence of HMPV in temperate climates peaks  
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8 217 at the end of winter or in early spring.<sup>37 38</sup> In our study, seasonal peaks of HMPV were  
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10 218 detected in February 2016, March 2014 and May 2015. This finding indicates that  
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12 219 HMPV circulates primarily during the spring and early summer in Guangzhou, this  
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14 220 pattern is similar to previous reports from other subtropical areas.<sup>22 39</sup> Furthermore, we  
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16 221 also found small peaks in November 2014, September 2015, and May 2016, the  
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18 222 reason for this is unknown. It should be noted that the seasonal distribution of HMPV  
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20 223 overlaps with RSV in this geographical area.<sup>19</sup> Knowing the epidemiological  
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22 224 characteristics of HMPV will help public health authorities and clinicians to improve  
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24 225 strategies for controlling HMPV infection.

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30 226 HMPV co-infection with other respiratory pathogens has been reported in  
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32 227 many studies, including RSV,<sup>40</sup> InfA,<sup>41</sup> InfB,<sup>42</sup> PIV,<sup>43</sup> ADV,<sup>43 44</sup> HBoV,<sup>45</sup> HCoV,<sup>43 46</sup>  
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34 228 HRV,<sup>43</sup> EV,<sup>43</sup> MP,<sup>41</sup> and CP.<sup>41</sup> In our study, most HMPV-positive patients only had  
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36 229 HMPV (81.6%, 84/103), and there was no significant difference in the HMPV  
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38 230 prevalence according to sex between the patients with only HMPV detected and the  
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40 231 patients with HMPV plus other pathogen(s) detected ( $p=0.469$ ). The co-detection rate  
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42 232 was 18.4% (19/103), and RSV (36.8%, 7/19) was the most frequently co-detected  
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44 233 pathogen, which might be because of the overlapping seasonal distribution of these  
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46 234 two pathogens.<sup>47</sup> Co-detection rates of HCoV-OC43, MP, HBoV, ADV, HRV, InfA, PIV2,  
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48 235 and PIV3 were over 5%, suggesting a broad range of respiratory pathogens could  
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50 236 co-exist with HMPV in ARI pediatric patients.

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4 237 HMPV most commonly causes URTI and LRTI in young children. The clinical  
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6 238 manifestations of HMPV-positive patients are similar to those of RSV-positive patients,  
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8 239 especially in young children.<sup>48-50</sup> In previous studies, the most frequent diagnoses of  
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10 240 children hospitalized with HMPV infection were pneumonitis and bronchiolitis.<sup>3 51 52</sup> In  
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12 241 some studies, bronchiolitis and recurrent wheezing/pneumonia were the main clinical  
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14 242 diagnoses,<sup>7 22 53 54</sup> while in other studies, bronchopneumonia, bronchiolitis, and  
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16 243 bronchial asthma exacerbation were the main clinical diagnoses.<sup>34</sup> Our study showed  
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18 244 that 66.7% (56/84), 9.5% (8/84) and 13.1% (11/84) of HMPV-positive patients were  
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20 245 diagnosed with bronchopneumonia, bronchiolitis, and pneumonia by chest  
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22 246 radiography, respectively. Of all the clinical features recorded, cough (100.0%,  
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24 247 101/101), abnormal pulmonary breath sound (91.1%, 92/101), fever (88.1%, 89/101),  
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26 248 expectoration (77.2%, 78/101), coryza (50.5%, 51/101), and wheezing (46.5%,  
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28 249 47/101) occurred frequently in HMPV-positive patients, similar to those reported  
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30 250 previously.<sup>16 29 52 55</sup>

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37 251 Most of the HMPV-positive patients had a fever (88.1%, 89/101); it appeared  
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39 252 more frequently in patients with only HMPV detected than in the patients with HMPV  
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41 253 plus other pathogen(s) detected ( $p=0.037$ ). Diarrhea was not a major symptom in  
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43 254 HMPV-positive patients (7.0%, 7/101), but it had a higher prevalence in patients with  
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45 255 HMPV plus other pathogen(s) detected than in the patients with only HMPV detected  
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47 256 ( $p=0.018$ ), suggesting that diarrhea is probably caused by other pathogens. No  
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49 257 significant difference was found in the other clinical features between the patients with  
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51 258 only HMPV detected and the patients with HMPV plus other pathogen(s) detected,  
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3 259 similar to the results of a previous study.<sup>51</sup>  
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6 260 In our study, RSV (36.8%, 7/19) was the most frequently co-detected pathogen  
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8 261 in HMPV-positive patients. Whether HMPV/RSV co-infection is more severe than the  
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10 262 respective mono-infections remains unclear. A previous study showed that children  
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12 263 with HMPV/RSV co-infection were more likely to develop pneumonia; however,  
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14 264 disease severity was not increased.<sup>53</sup> Conversely, Semple et al.<sup>56</sup> reported that  
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16 265 HMPV/RSV co-infection can cause more severe bronchiolitis in patients. In our study,  
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18 266 no significant difference in the diagnosis of pneumonia was observed between the  
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20 267 patients with only HMPV detected and the patients with HMPV plus other pathogen(s)  
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22 268 detected. It has been confirmed that a history of prematurity, particular age groups,  
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24 269 and the presence of chronic diseases increases the risk of severe LRTI among  
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26 270 HMPV- and RSV-infected children.<sup>51</sup> Our study suggests that the clinical  
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28 271 manifestations of HMPV infection are complex and diverse; our data will be helpful in  
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30 272 the diagnosis of HMPV.  
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38 273 In this study, we analyzed the epidemiological characteristics and clinical  
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40 274 characteristics of HMPV in more than 5000 children with ARI in two tertiary hospitals  
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42 275 in Guangzhou, which is an international metropolis and the most important political,  
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44 276 economic and cultural center in southern China. Therefore, the results are not limited  
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46 277 to the two hospital cases, but also represent and reflect HMPV infection in children  
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48 278 with ARI in south China and play a positive role in the prevention and diagnosis of  
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50 279 HMPV infection in the area.  
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54 280 The study had some limitations. First, collection of data on symptoms and  
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4 281 physical findings in infants and young children requires experienced medical staff,  
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6 282 patient's cooperation, and consent from the patient's guardian. Consequently, it is  
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8 283 possible we will have incomplete or even an inaccurate description of patients'  
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10 284 manifestations. Second, because our study mainly focused on HMPV, other common  
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12 285 respiratory pathogens, including bacterial pathogens, were not tested and  
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14 286 subsequently our study does not give a fully account of respiratory pathogen infection  
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16 287 in hospitalized pediatric patients with ARI. Third, the characteristics of HMPV-negative  
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18 288 patients and outcome data (discharge/death) were not analyzed further, because our  
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20 289 study only focused on the epidemiological and clinical features of HMPV positive  
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22 290 cases. This might affect our understanding of the clinical features of HMPV-positive  
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24 291 patients. Despite these shortcomings, our results provide a valuable insight into the  
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26 292 epidemiological and clinical characteristics of HMPV present in patients hospitalized  
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28 293 with ARI in Guangzhou, southern China.  
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4 294 **Conclusions**

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6 295 HMPV is an important respiratory pathogen in children with ARI in Guangzhou ,  
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8 296 China, particularly in children under 5 years old. In future, our data can be used by  
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10 297 public health authorities and clinicians to improve the management of HMPV infection  
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13 298 in children.  
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16 304 **Contributors**  
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18 305 RZ, WKL, LZ and DLL designed the study. LZ, DLL, WKL, SYQ, DX, XL and LTT  
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20 306 performed pathogen testing. DHC and WPT collected the clinical data. All authors  
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22 307 participated in the data analysis. LZ, DLL, RZ and WKL drafted the manuscript. All  
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24 308 authors read and approved the final version of this manuscript.  
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41 315 analysis, decision to publish, or preparation of the manuscript.  
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47 317 **Competing interests**  
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49 318 None declared.  
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55 320 **Ethics approval**  
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3 321 The study was approved by The First Affiliated Hospital of Guangzhou Medical  
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6 322 University Ethics Committee. Informed written consent was obtained from parents or  
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8 323 legal guardians.  
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13 325 **Data sharing statement**  
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16 326 No additional data are available.  
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4 516 **Figure legends**

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8 518 **Figure 1** Age distributions of patients with HMPV.

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11 519 <sup>a</sup>Date were presented as HMPV positive rate (number of HMPV-positive  
12 patients/number of patients in each age group); m: month(s); y: year(s).

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18 522 **Figure 2** Seasonal distribution of HMPV infection in pediatric patients hospitalized with  
19 acute respiratory infection from July 2013 to June 2016 in Guangzhou.  
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**Table 1. Distribution of co-pathogens in 19 HMPV-positive patients**

Co-pathogens <sup>a</sup>	Patient No.
RSV/HMPV	4
HCoV-OC43/HMPV	3
ADV/HMPV	2
HRV/HMPV	2
MP/HMPV	1
PIV2/HMPV	1
PIV3/HMPV	1
RSV/MP/HMPV	1
RSV/InfA/HMPV	1
RSV/HBoV/HMPV	1
MP/HBoV/HMPV	1
InfA/HBoV/HMPV	1

525 <sup>a</sup>HMPV-positive patients were tested for 17 common respiratory pathogens.

526 RSV: respiratory syncytial virus; PIV: parainfluenza virus; InfA: influenza A virus; ADV:

527 adenovirus; HCoV: human coronaviruses; HRV: human rhinovirus; HBoV: human

528 bocavirus; MP: *Mycoplasma pneumoniae*.

529 Influenza B viruses, parainfluenza virus types 1 and 4, enterovirus, human

530 coronaviruses-HKU1, human coronaviruses-229E, human coronaviruses-NL63, and

531 *Chlamydomphila pneumoniae* were not detected.

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**Table 2. Clinical presentations of HMPV-positive patients**

Diagnosis/Symptom	Total HMPV (n=101)	Patients with only HMPV and HMPV plus other pathogen(s) detected.		
		Single HMPV (n = 83)	Co-pathogens (n= 18)	p value <sup>b</sup>
<b>Upper respiratory tract infection</b>				
Nasal obstruction	40(39.6)	34(41.0)	6(33.3)	0.605
Coryza	51(50.5)	44(53.0)	7(38.9)	0.309
Sneeze	1(1.0)	1(1.2)	0(0)	— <sup>c</sup>
Cough	101(100)	83(100)	18(100)	— <sup>d</sup>
Expectoration	78(77.2)	66(79.5)	12(66.7)	0.351
Hoarseness	1(1.0)	1(1.2)	0(0)	— <sup>c</sup>
<b>Lower respiratory tract infection</b>				
Abnormal pulmonary breath sound <sup>a</sup>	92(91.1)	75(90.4)	17(94.4)	0.582
Wheezing	47(46.5)	40(48.2)	7(38.9)	0.473
Anhelation	24(23.8)	21(25.3)	3(16.7)	0.551
Increasing lung markings <sup>e</sup>	6(7.1)	4(5.8)	2(13.3)	0.304
Bronchiolitis <sup>e</sup>	8(9.5)	7(10.1)	1(6.7)	0.677
Pneumonia <sup>e</sup>	11(13.1)	10(14.5)	1(6.7)	0.415
Bronchopneumonia <sup>e</sup>	56(66.7)	46(66.7)	10(66.7)	0.626
<b>Systemic influenza-like symptoms</b>				
Fever (≥38°C)	89(88.1)	76(91.6)	13(72.2)	<b>0.037</b>
Chill	7(6.9)	6(7.2)	1(5.6)	0.8
Debilitation	11(10.9)	11(13.3)	0(0)	— <sup>c</sup>
<b>Gastrointestinal illness</b>				
Vomiting	21(20.8)	16(19.3)	5(27.8)	0.522
Poor appetite	19(18.8)	17(20.5)	2(11.1)	0.513
Diarrhea	7(7.0)	3(3.6)	4(22.2)	<b>0.018</b>

534 Data are presented as No. (%) of each group.

535 Percentages sum to >100% because some patients had >1 clinical presentations.

536 <sup>a</sup>Including phlegmatic, wheezing, bubbling, and moist rales.

537 <sup>b</sup>Two-tailed  $\chi^2$  test comparing the distribution of each illness or diagnosis between  
538 patients with only HMPV and HMPV plus other pathogen(s) detected.

539 <sup>c</sup>Not tested because the number of positive samples obtained was too small.

540 <sup>d</sup>Not tested because the prevalence was 100%.

541 <sup>e</sup>Diagnosed by chest radiography, and a total of 84 HMPV-positive patients (69

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3 542 patients with only HMPV detected and 15 patients with HMPV plus other pathogen(s)  
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5 543 detected) were examined.  
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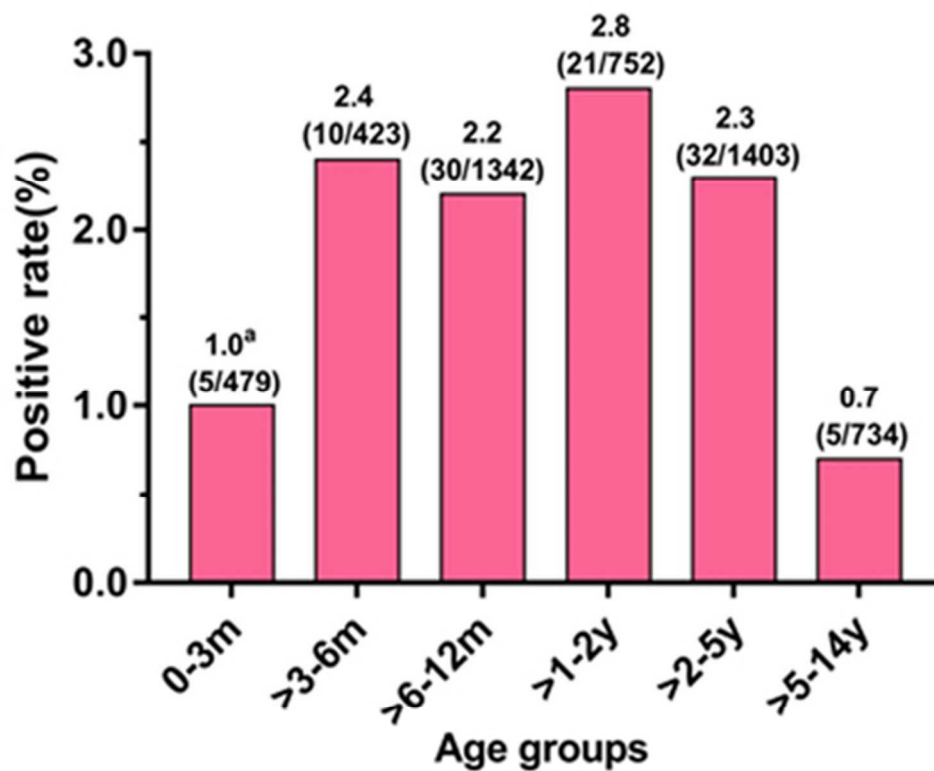


Figure 1 Age distributions of patients with HMPV.

<sup>a</sup>Data were presented as HMPV positive rate (number of HMPV-positive patients/number of patients in each age group); m: month(s); y: year(s).

42x35mm (300 x 300 DPI)

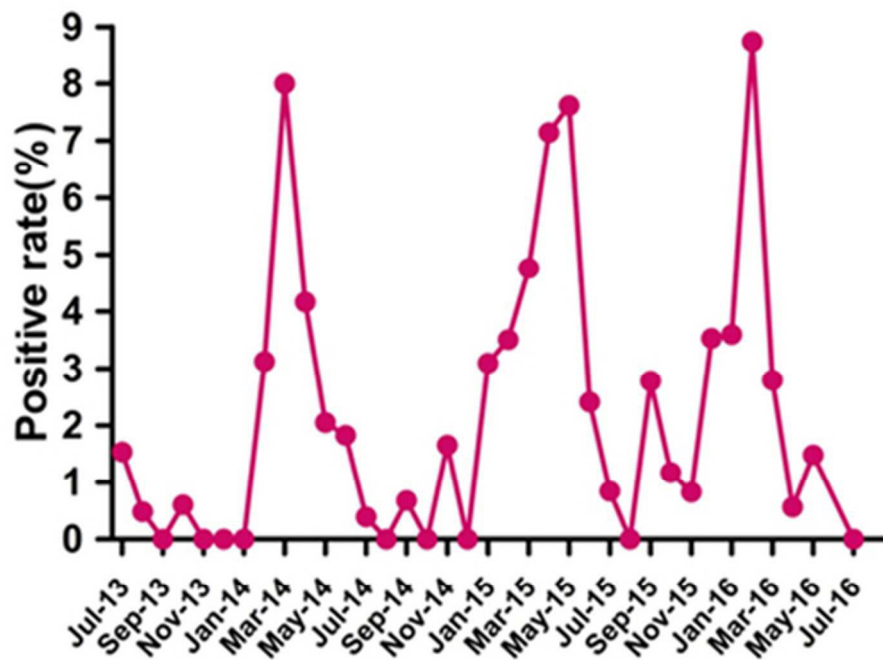


Figure 2 Seasonal distribution of HMPV infection in pediatric patients hospitalized with acute respiratory infection from July 2013 to June 2016 in Guangzhou.

39x31mm (300 x 300 DPI)

**STROBE 2007 (v4)Statement—Checklist of items that should be included in reports of *cross-sectional studies***

Section/Topic	Item #	Recommendation	Reported on page #
<b>Title and abstract</b>	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4-5
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8
		(b) Describe any methods used to examine subgroups and interactions	8
		(c) Explain how missing data were addressed	7
		(d) If applicable, describe analytical methods taking account of sampling strategy	8
		(e) Describe any sensitivity analyses	
<b>Results</b>			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9-11
		(b) Indicate number of participants with missing data for each variable of interest	9
Outcome data	15*	Report numbers of outcome events or summary measures	9-11
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-11
		(b) Report category boundaries when continuous variables were categorized	9-10
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	12-15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15-16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-16
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).