

ORIGINAL ARTICLES

The importance of gastrointestinal presentation for understanding respiratory virus infection in patients with acute respiratory illness: A cross-sectional study in Guangzhou

Wen-Kuan Liu¹, Qian Liu², De-Hui Chen¹, Wei-Ping Tan³, Shu-Yan Qiu¹, Duo Xu¹, Chi Li¹, Shu-Jun Gu¹, Rong Zhou*¹

¹State Key Laboratory of Respiratory Diseases, National Clinical Research Center for Respiratory Disease, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University, Guangzhou, China

²Central Laboratory, The First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou, China

³Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, China

Received: January 29, 2018

Accepted: May 16, 2018

Online Published: May 23, 2018

DOI: 10.5430/jer.v4n2p19

URL: <https://doi.org/10.5430/jer.v4n2p19>

ABSTRACT

Background: Respiratory virus infections often cause a wide spectrum of symptoms including gastrointestinal presentations (GP). The epidemiology of respiratory viruses in patients with GP needs to be better described.

Methods: Throat swabs were collected and tested for 15 respiratory viruses from pediatric patients (≤ 14 years old) with acute respiratory illness in Guangzhou over a 3-year period. The features of respiratory virus infections were analyzed among those with GP.

Results: Of 4,242 patients enrolled, 1,223 (28.8%) had GP. Among those, 647 (52.9%) were positive with one or more of the 15 tested respiratory viruses. The most frequently detected viruses were respiratory syncytial virus (RSV) (21.1%, 258), enterovirus (EV) (10.1%, 124), influenza A virus (infA) (7.8%, 95), adenovirus (ADV) (5%, 61), human metapneumovirus (HMPV) (4.1%, 50), and human bocavirus (HBoV) (3.5%, 43). More RSV ($p = .001$) and EV ($p < .001$) infections were found in patients with GP than in patients without GP. 734 (60.0%) patients with GP presented with "Poor appetite", 480 (39.2%) with "Vomiting", 301 (24.6%) with "Diarrhea" and 73 (6.0%) with "Stomachache". Significant differences in the virus positivity rate were found for RSV ($p < .001$), EV ($p = .002$) and PIV3 ($p = .037$). 90.6% (1,108/1,223) of patients with GP were under 5 years old. Among different age groups, significant differences in the virus positivity rate were found for infA ($p = .005$), influenza B virus (infB) ($p = .006$), RSV ($p < .001$), parainfluenza virus type 3 (PIV3) ($p = .019$), ADV ($p < .001$), and HBoV ($p = .009$). RSV was mostly detected in patients under 2 years old (90.3%, 233/258) with frequency declining with age, while frequency of infA and infB increased with age. ADV, HBoV, and PIV3 reached their highest peaks in the age groups of 6-10 years old (11%), 7-12 months (8%) and 4-6 months (5.8%), respectively. In general, sample positivity rates in patients with GP increased when seasons changed. RSV, EV, infA, ADV, HMPV, and HBoV formed the bulk of the positive samples.

Conclusion: In this study, the epidemiology of respiratory virus infections in patients with GP was analyzed. This information increases our understanding of respiratory virus infections and may help in clinical diagnosis of these viruses.

Key Words: Respiratory virus, Acute respiratory illness, Gastrointestinal presentation, Epidemiology

*Correspondence: Rong Zhou; Email: zhou3218@yahoo.com; Address: State Key Laboratory of Respiratory Diseases, National Clinical Research Center for Respiratory Disease, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University, Guangzhou, China.

1. INTRODUCTION

Respiratory viruses are important pathogens of acute respiratory illness (ARI) which lead to 4 to 5 million deaths each year in low-income countries,^[1-4] and mostly occurred in children.^[1,5] The studies, which conducted in developed countries using viral culture diagnosis, have estimated that infants and preschoolers experience 6-10 viral infections annually and school-age children and adolescents experience 3-5 illnesses annually.^[6,7]

Viral ARI is very complex in etiology and diverse in clinical presentations, and has a close relationship with gastrointestinal illness. Respiratory virus infection usually leads to gastrointestinal presentation (GP) as well as ARI. However, limited data have been obtained about the relationship between respiratory virus infection and GP.^[8-13] To answer the question of whether GP is important for understanding respiratory virus infection, the overall epidemic characteristics of respiratory virus needed to be evaluated. In this work, which took place in Guangzhou over a 3-year period, we studied the characteristics of 15 respiratory virus infections and GP in children with ARI. Our results have provided a more complete understanding of the symptoms of respiratory virus infection.

2. METHODS

2.1 Ethics statement

The study was approved by The First Affiliated Hospital of Guangzhou Medical University Ethics Committee for research on human beings, and the next of kin, caretakers, or guardians gave signed informed consent on behalf of the minors/children for participation in the study.

2.2 Sample collection

The study was conducted in three tertiary hospitals between July 2009 and June 2012 in Guangzhou, southern China. The throat swab samples were collected from pediatric patients (≤ 14 years old) who first presented with ARI. ARI criteria were defined as presenting with at least two of the following symptoms: cough, pharyngeal discomfort, nasal congestion, rhinorrhea, sneeze, or dyspnea during the previous week. Patients, who were diagnosed with pneumonia by chest radiography during the previous week, were also included in this study. Chest radiography was conducted according to the clinical situation of the patients. Enrolled patients with vomiting, poor appetite, stomachache or diarrhea were categorized as having GP. Enrolled patients without any of these four GP symptoms were categorized as the "without GP" group. The patients who identified with acute gastroenteritis were excluded in this study.

Throat swab samples were collected and refrigerated at 2°C-

8°C in viral transport medium, transported on ice to the State Key Laboratory of Respiratory Diseases, and analyzed immediately or stored at -80°C before testing.

2.3 Real-time PCR for respiratory tract virus detection

Fifteen respiratory tract viruses were tested simultaneously for each sample: influenza A and B virus (infA, infB), respiratory syncytial virus (RSV), human metapneumovirus (HMPV), four types of parainfluenza (PIV1, PIV2, PIV3, PIV4), enterovirus (EV), four strains of human coronavirus (HCoV-229E, OC43, NL63 and HKU1), adenovirus (ADV) and human bocavirus (HBov). The test procedures have been described in previous reports according to the manufacturer's protocols (Guangzhou HuYanSuo Medical Technology Co., Ltd, Guangzhou, China).^[10,14] In brief, 50 μ l RNA/DNA were extracted from a 200 μ l sample, and real-time PCR was conducted using 25 μ l reaction mix, containing M-MLV RT, Taq polymerase and 5 μ l extracted RNA/DNA. Cycling conditions included an initial reverse transcription at 55°C for 10 min, incubation at 94°C for 2 min, followed by 40 cycles of 94°C for 10 sec and 55°C for 35 sec (ABI-7500 real-time PCR instrument, Life Technologies, Singapore).

2.4 Statistical analysis

Statistical analyses were performed using SPSS statistical software (version 19.0; SPSS Inc., Chicago, IL, USA). To compare categorical data, χ^2 test and Fisher's exact tests were used, as appropriate. All tests were two-tailed, and $p < .05$ was considered statistically significant.

3. RESULTS

A total of 4,242 pediatric patients, ranging from 1 day to 14 years old, were enrolled in this study. The male to female ratio was 1.9:1. The median age was 1.5 years (interquartile range, 0.7 to 3.0). Of these patients, 1,223 (28.8%) patients had GP and 3,019 (71.2%) patients were without GP. The male to female ratios in patients with and without GP were 1.9:1 and 2.0:1 ($p = .165$), and the median ages were 1.3 years (interquartile range, 0.6 to 3.0) and 1.7 years (interquartile range, 0.7 to 3.5), respectively. 90.6% (1,108/1,223) of patients with GP were under the age of 5 years.

Throat swab samples of the 4,242 patients were collected and 15 common respiratory viruses were tested simultaneously. The virus positivity rate for the total ARI-patients was 51.3% (2,178/4,242). No statistical difference was found in the virus positivity rates or co-pathogen detection rates ($p > .05$) between the patients with and without GP (see Table 1). The most frequently detected viruses in patients with GP were RSV (21.1%, 258/1,223), EV (10.1%, 124/1,223), infA (7.8%, 95/1,223), ADV (5%, 61/1,223), HMPV (4.1%,

50/1,223), and HBoV (3.5%, 43/1,223), followed by HCoV-OC43, infB, PIV3, PIV1, PIV2, HCoV-NL63, HCoV-229E, HCoV-HKU1, and PIV4 with positivity rates under 3.5% (see Table 1, Figure 1). Statistically significant differences

were found in the positivity rates of RSV ($p = .001$), EV ($p < .001$) and infA ($p = .002$) between patients with and without GP (see Table 1). A comparison of the virus positivity rates in patients is shown in Figure 1.

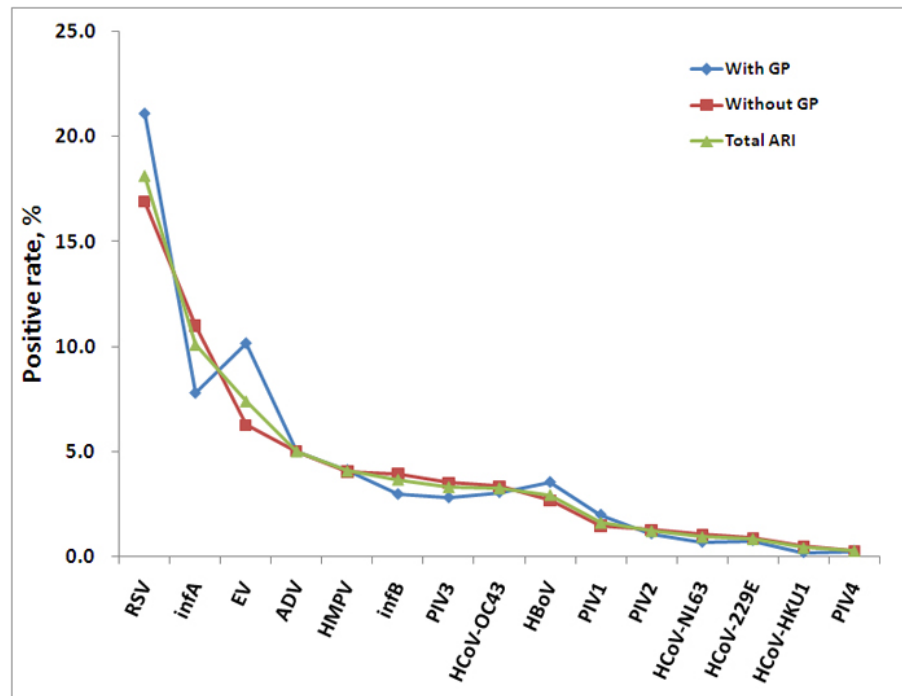


Figure 1. Detection rates for the 15 respiratory viruses tested in different patient groups in Guangzhou. Throat swabs were collected and tested by real-time PCR for 15 respiratory viruses from pediatric patients (≤ 14 years old) with ARI.

Note. GP and ARI stand for gastrointestinal presentation and acute respiratory illness, respectively.

In the 1,223 patients with GP, 60.0% (734/1,223) of patients presented with “Poor appetite”, followed by 39.2% (480/1,223) with “Vomiting”, 24.6% (301/1,223) with “Diarrhea” and 6.0% (73/1,223) with “Stomachache”. The virus positivity rates in patients with these four symptoms were 54.5% (400/734) for “Poor appetite”, 52.5% (158/301) for “Diarrhea”, 49.2% (236/480) for “Vomiting” and 39.7% (29/73) for “Stomachache” ($p = .051$). The distribution of the virus detection rates in the patients with different gastrointestinal symptoms is shown in Table 2. Significant differences in the virus positivity rates among patients with each of the four symptoms were found for RSV, EV and PIV3 ($p < .05$) (see Table 2, Figure 2A). InfA, infB, ADV and HMPV appeared to have higher positivity rates with “Stomachache”, but this difference did not reach statistical significance (see Table 2, Figure 2B). The remaining viruses that had enough positive samples to compare, PIV1 and HBoV, like RSV, EV and PIV3, had the lowest positivity rates in “Stomachache”; however, no significant differences were found for these two viruses (see Table 2, Figure 2C). The features of the other six viruses were not clear because too few positive samples

were obtained to run statistical tests.

The 1,223 patients with GP were divided by age into seven groups. Statistically significant differences were found in the virus positivity rate ($p = .044$), and incidence of infA ($p = .005$), infB ($p = .006$), RSV ($p < .001$), PIV3 ($p = .019$), ADV ($p < .001$), and HBoV ($p = .009$) among the age groups (see Table 3). RSV was predominantly detected in patients under 2 years old (90.3%, 233/258) and its frequency declined with age, while infA and infB incidence trended in the opposite direction, increasing with age (see Figure 3A). ADV, HBoV, and PIV3 reached their highest peaks in patients among the age groups of 6-10 years old (11%), 7-12 months (8%) and 4-6 months (5.8%), respectively (see Figure 3B). There were no significant differences in the positivity rates for EV, PIV1, HCoV-OC43 or HMPV among the age groups, and the distributions of these four viruses are illustrated in Figure 3C. The age distributions of the remaining five viruses, PIV2, PIV4, HCoV-229E, HCoV-NL63, and HCoV-HKU1, are included in Table 3, but are inconclusive because of the low number of positive samples obtained.

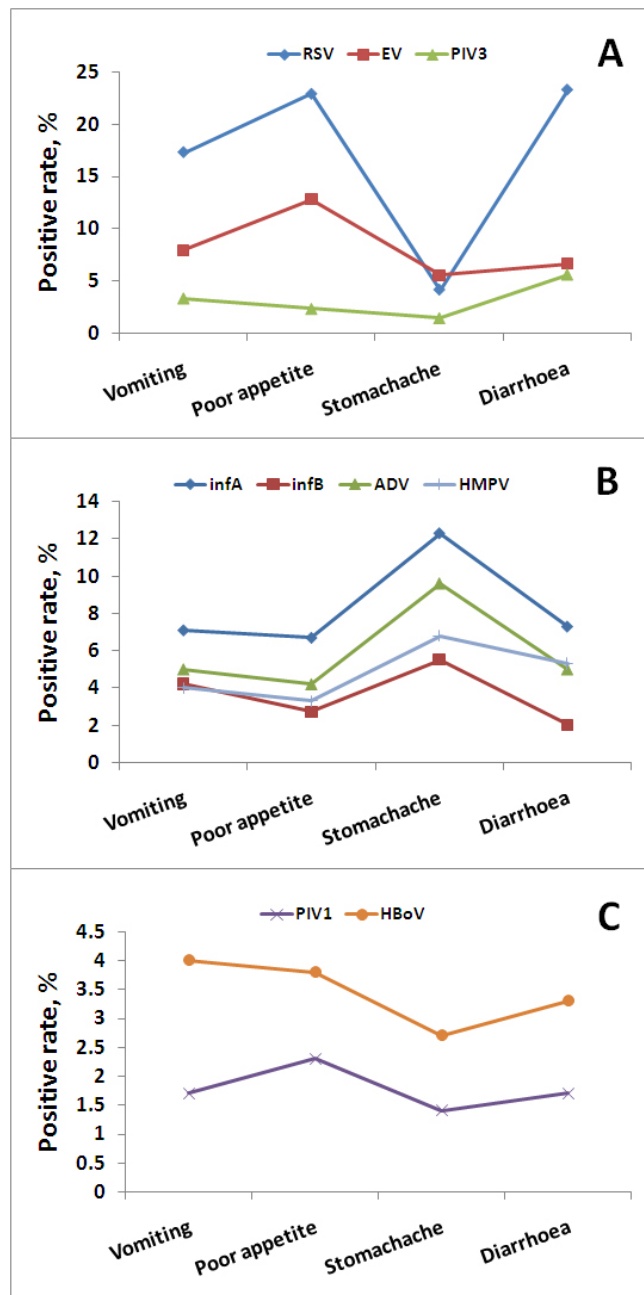


Figure 2. Respiratory virus distribution in the four gastrointestinal presentation groups. Subjects reporting GP were categorized based on the following four symptoms: Vomiting, Poor Appetite, Stomachache, and Diarrhea. For each virus, the rate of detection in patients with each of these symptoms is shown.

In general, the sample positivity rates in patients with GP increased when seasons changed, and RSV, EV, infA, ADV, HMPV, and HBoV formed the bulk of the positive samples (see Figure 4). The small numbers of positive samples available for the remaining nine viruses found in this study

prevented the determination of their seasonal patterns. RSV and infA infection mainly occurred at the change from winter to spring and summer to autumn. EV and HBoV occurred mostly in summer, and HBoV was prevalent in winter. ADV mainly occurred in summer and autumn. HMPV occurred at the change from spring to summer and winter to spring (see Figure 4).

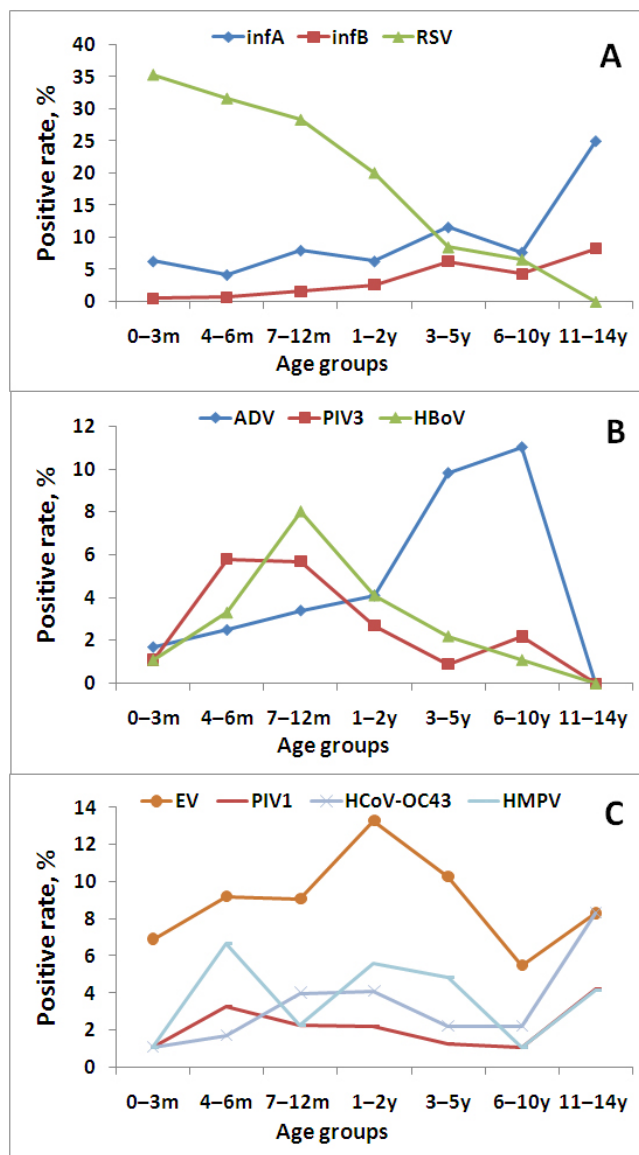


Figure 3. Respiratory virus distribution in the seven different age groups of patients with gastrointestinal presentations. Subjects reporting GP were categorized into seven age-group categories: 0-3 m, 4-6 m, 7-12 m, 1-2 y, 3-5 y, 6-10 y, and 11-14 y. For each virus, the rate of detection in patients in each of these age groups is shown.

Note. m: months; y: year(s).

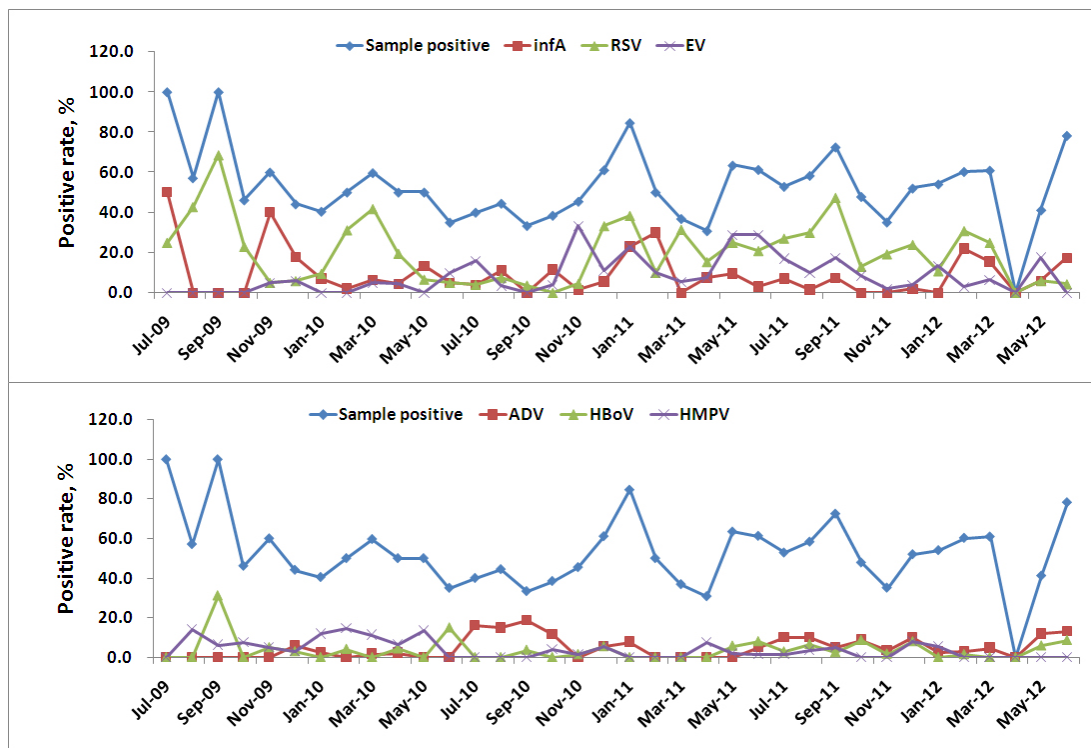


Figure 4. Seasonal distribution of virus-positive samples in patients with gastrointestinal presentations. To access the seasonal distribution of virus-positive samples, the month in which each positive sample of virus was collected was plotted in relation to the positive rates for each virus tested.

4. DISCUSSION

Respiratory and enteric viral infections cause significant morbidity and mortality worldwide and represent a major socioeconomic burden.^[15] RSV, infA, infB, PIV1-3, and ADV are the most important causative viruses for ARI, especially for lower respiratory tract illness.^[16-18] Rotavirus, Norwalk virus, Human astroviruses, EV and enteric ADV are important etiologic agents of acute gastroenteritis. Other viruses, such as toroviruses, coronaviruses, picobirnaviruses, Aichi virus, and HBoV, are increasingly being identified as causative agents of diarrhea.^[19-21] The respiratory tract and gastrointestinal tract are closely related, and viral infection often causes complex and diverse diseases, so it is often difficult to identify the virus from clinical manifestations alone. Respiratory virus infection frequently causes not only ARI but also gastrointestinal illness. However, only a few studies have reported the association of respiratory virus infection with gastrointestinal illness.^[8-13] In this work, we analyzed the relationship between infection with one or more 15 respiratory viruses and GP in children with ARI in the hopes of providing more useful information for clinical diagnosis and a better understanding of respiratory virus infection.

In this work, 4,242 patients with ARI were studied, of which 28.2% had GP. 52.9% of patients with GP were positive for

one or more of the 15 respiratory viruses studied. The results indicated that GP was common in patients with ARI and respiratory virus infection might be important as a pathogenic factor.

Table 1. Detection of respiratory viruses in children with gastrointestinal presentations by real-time PCR

Pathogens	Patients With GP * (n = 1,223)	Patients Without GP (n = 3,019)	Total (n = 4,242)	p
Positive samples	647 (52.9)	1,531 (50.7)	2,178 (51.3)	.196
Co-pathogens	127 (10.4)	296 (9.8)	423 (10)	.568
infA	95 (7.8)	332 (11)	427 (10.1)	.002
infB	36 (2.9)	119 (3.9)	155 (3.7)	.117
RSV	258 (21.1)	510 (16.9)	768 (18.1)	.001
HMPV	50 (4.1)	122 (4)	172 (4.1)	.944
PIV1	24 (2)	44 (1.5)	68 (1.6)	.236
PIV2	13 (1.1)	38 (1.3)	51 (1.2)	.596
PIV3	34 (2.8)	106 (3.5)	140 (3.3)	.227
PIV4	3 (0.2)	8 (0.3)	11 (0.3)	.909
EV	124 (10.1)	189 (6.3)	313 (7.4)	<.001
HCoV-229E	9 (0.7)	26 (0.9)	35 (0.8)	.683
HCoV-OC43	37 (3)	101 (3.3)	138 (3.3)	.594
HCoV-NL63	8 (0.7)	31 (1)	39 (0.9)	.249
HCoV-HKU1	2 (0.2)	15 (0.5)	17 (0.4)	.12
ADV	61 (5)	151 (5)	212 (5)	.985
HBoV	43 (3.5)	81 (2.7)	124 (2.9)	.145

Note. No. (%) of each group except where specifically stated. *GP: gastrointestinal presentation.

In patients with GP, six of 15 viruses showed positivity rates greater than 3.5%, demonstrated the wide diversity of respiratory viruses contributing to GP (see Figure 1, Table 1). The rank of virus positivity rates of patients with GP was different from the total patients with ARI and patients without GP (see Figure 1). The most frequently detected viruses were RSV, EV, infA, ADV, HMPV, and HBoV in patients with GP, in contrast to the patients without GP, in which the positivity rate of infA was higher than EV, and the rates of infB were higher than HBoV and HCoV-OC43 (see Figure 1, Table 1).

RSV and HMPV belong to the family Paramyxoviridae and are important pathogens of lower respiratory tract illness.^[22,23] Although there have been no prior reports about the relationship between RSV or HMPV infections with GP, RSV and HMPV were the first and fifth most frequently detected viruses in patients with GP, and more RSV ($p = .001$) infections were found in patients with GP than in patients without GP. This study further characterizes a feature of RSV

and HMPV infections, and provides useful information for the clinical diagnosis of these viruses.

It is known that EV is a pathogen in both the gastrointestinal and respiratory tract.^[11-13,24,25] In this study, EV was the second most frequently isolated virus in patients with GP, and more EV ($p < .001$) infections were found in patients with GP than in patients without GP. Fewer infA ($p = .002$) infections in patients with GP than in patients without GP might suggest that GP was not a key presentation in patients with infA infection. ADV and HBoV were the only two DNA viruses detected in this study, and they were the fourth and sixth most frequently isolated viruses in patients with GP. Enteric ADV (ADV type 40, 41) are important etiologic agents of acute gastroenteritis.^[26] In this study, 5.0% of patients with GP were found to be ADV-positive. Previous studies have also reported that HBoV is associated with ARI and gastrointestinal illness.^[10,27]

Table 2. Distribution of respiratory viruses in 1,223 patients with gastrointestinal presentations

Pathogens	Vomiting (n = 480) #	Poor appetite (n = 734)	Stomachache (n = 73)	Diarrhea (n = 301)	p value
Positive samples	236 (49.2)	400 (54.5)	29 (39.7)	158 (52.5)	.051
Co-pathogens	44 (9.2)	80 (10.9)	7 (9.6)	33 (11)	.769
infA	34 (7.1)	49 (6.7)	9 (12.3)	22 (7.3)	.362
infB	20 (4.2)	20 (2.7)	4 (5.5)	6 (2)	.197
RSV	83 (17.3)	168 (22.9)	3 (4.1)	70 (23.3)	<.001
HMPV	19 (4)	24 (3.3)	5 (6.8)	16 (5.3)	.274
PIV1	8 (1.7)	17 (2.3)	1 (1.4)	5 (1.7)	.807
PIV2*	6 (1.3)	8 (1.1)	0 (0)	2 (0.7)	--
PIV3	16 (3.3)	17 (2.3)	1 (1.4)	17 (5.6)	.037
PIV4*	1 (0.2)	3 (0.4)	0 (0)	0 (0)	--
EV	38 (7.9)	94 (12.8)	4 (5.5)	20 (6.6)	.002
HCoV-229E*	6 (1.3)	4 (0.5)	0 (0)	0 (0)	--
HCoV-OC43*	16 (3.3)	22 (3)	0 (0)	10 (3.3)	--
HCoV-NL63*	1 (0.2)	6 (0.8)	1 (1.4)	4 (1.3)	--
HCoV-HKU1*	1 (0.2)	1 (0.1)	0 (0)	0 (0)	--
ADV	24 (5)	31 (4.2)	7 (9.6)	15 (5)	.239
HBoV	19 (4)	28 (3.8)	2 (2.7)	10 (3.3)	.935

Note. No. (%) of each group except where specifically stated.

#Sum to over 1223 because some patients had more than one diagnosis.

*Not done because of the small number of positive samples obtained.

In the 1,223 patients with GP, four groups were established according to four typical symptoms of acute gastroenteritis: “Poor appetite”, “Vomiting”, “Diarrhea” and “Stomachache”. There were no significant differences in the overall virus positivity rate or co-pathogen detection rate ($p > .05$) among the four patient groups (see Table 2). However, statistical dif-

ferences were found in the positivity rates of RSV ($p < .001$), EV ($p = .002$), and PIV3 ($p = .037$) among the four patient groups (see Figure 2A, Table 2). The highest positivity rates of RSV were obtained in patients with “Diarrhea” (23.3%) and “Poor appetite” (22.9%), which were followed by “Vomiting” (17.3%) and “Stomachache” (4.1%). 12.8% of patients

with “Poor appetite” were found to be EV-positive, and 5.6% of patients with “Diarrhea” were positive for PIV3 (see Table 2, Figure 2A). High positivity rates were observed for infA, infB, ADV, and HMPV in patients with “Stomachache”, but no significant differences were found among the four patient groups (see Figure 2B). PIV3 and HBoV also failed to display any significant differences in positivity rates among the four groups (see Figure 2C). The number of samples were too small to analyze any differences in the six other viruses.

In this study, 90.6% patients with GP were under the age of 5, which is similar to the patients with ARI and acute gastroenteritis in previous reports.^[7, 19, 28] To study the age distributions of the 15 respiratory viruses in patients with GP, the patients were divided into seven age groups. A statistical difference was found in the virus positivity rate among age groups ($p = .044$). The high virus positivity rate

(> 54%) in the age groups of “4-6 months”, “7-12 months” and “1-2 years” (see Table 3) indicates that gastrointestinal illness from respiratory viruses is an important issue in children under 2 years old. In general, the prevalence of infA and B increased with age (see Figure 3A). The prevalence of RSV declined with age ($p \leq .006$). RSV was mostly found in patients under 2 years old (see Figure 3A), similar to findings from previous reports in patients with ARI.^[29–31] Significant differences among age groups were also seen for ADV, PIV3, and HBoV ($p \leq .019$), and the patterns were different from each other (see Figure 3B). EV, PIV1, HCoV-OC43, and HMPV positivity rates had no statistically significant differences among age groups (see Table 3, Figure 3C). This finding was consistent with previous serologic and epidemiologic reports of these pathogens in patients with ARI.^[11, 16, 32–35] Too few positive samples for the remaining five viruses were found to analyze (see Table 3).

Table 3. Distribution of respiratory virus-positive patients in different age groups

Pathogen	0-3 m [#] (n = 175)	4-6 m (n = 120)	7-12 m (n = 176)	1-2 y (n = 413)	3-5 y (n = 224)	6-10 y (n = 91)	11-14 y (n = 24)	<i>p</i>
Positive samples	88 (50.3)	70 (58.3)	103 (58.5)	226 (54.7)	113 (50.4)	35 (38.5)	12 (50)	.044
Co-pathogens	15 (8.6)	16 (13.3)	26 (14.8)	43 (10.4)	20 (8.9)	5 (5.5)	2 (8.3)	.214
infA	11 (6.3)	5 (4.2)	14 (8)	26 (6.3)	26 (11.6)	7 (7.7)	6 (25)	.005
infB	1 (0.6)	1 (0.8)	3 (1.7)	11 (2.7)	14 (6.3)	4 (4.4)	2 (8.3)	.006
RSV	62 (35.4)	38 (31.7)	50 (28.4)	83 (20.1)	19 (8.5)	6 (6.6)	0 (0)	<.001
HMPV	2 (1.1)	8 (6.7)	4 (2.3)	23 (5.6)	11 (4.9)	1 (1.1)	1 (4.2)	.059
PIV1	2 (1.1)	4 (3.3)	4 (2.3)	9 (2.2)	3 (1.3)	1 (1.1)	1 (4.2)	.759
PIV2*	4 (2.3)	3 (2.5)	1 (0.6)	3 (0.7)	2 (0.9)	0 (0)	0 (0)	--
PIV3	2 (1.1)	7 (5.8)	10 (5.7)	11 (2.7)	2 (0.9)	2 (2.2)	0 (0)	.019
PIV4*	1 (0.6)	0 (0)	0 (0)	1 (0.2)	0 (0)	1 (1.1)	0 (0)	--
EV	12 (6.9)	11 (9.2)	16 (9.1)	55 (13.3)	23 (10.3)	5 (5.5)	2 (8.3)	.161
HCoV-229E*	2 (1.1)	1 (0.8)	2 (1.1)	3 (0.7)	0 (0)	1 (1.1)	0 (0)	--
HCoV-OC43	2 (1.1)	2 (1.7)	7 (4)	17 (4.1)	5 (2.2)	2 (2.2)	2 (8.3)	.232
HCoV-NL63*	2 (1.1)	0 (0)	2 (1.1)	2 (0.5)	2 (0.9)	0 (0)	0 (0)	--
HCoV-HKU1*	0 (0)	0 (0)	1 (0.6)	1 (0.2)	0 (0)	0 (0)	0 (0)	--
ADV	3 (1.7)	3 (2.5)	6 (3.4)	17 (4.1)	22 (9.8)	10 (11)	0 (0)	<.001
HBoV	2 (1.1)	4 (3.3)	14 (8)	17 (4.1)	5 (2.2)	1 (1.1)	0 (0)	.009

Note. No. (%) of each group except where specifically stated.

[#]m: month(s); y: year(s).

*Not done because of the small number of positive samples obtained.

In general, the distribution of virus is closely related with the geographical location and the local climate. This study was conducted in Guangzhou, southern China, locating in a subtropical region. RSV, EV, infA, ADV, HMPV, and HBoV were the predominant pathogens; thus these viruses formed the main structure of our study (see Figure 4), and the seasonal distribution patterns were similar to previous reports in patients with ARI.^[1, 10, 36]

Many studies reporting the characteristics of respiratory virus infections in patients with upper and lower respiratory tract illness have improved our knowledge about these viruses. While virus infections often cause a wide spectrum of symptoms, some viruses, like EV, have previously been identified as the etiologic agents both in the respiratory tract and the gastrointestinal tract.^[11–13, 24, 25] In this study, the distribution of 15 respiratory viruses in children with ARI and GP were

studied. The study expands the information on respiratory virus infection and provides useful data for future research and clinical diagnosis.

5. CONCLUSIONS

The respiratory tract and gastrointestinal tract are closely related, and respiratory virus infections often cause both ARI and gastrointestinal illness. In this study, 52.9% of patients with GP were found to be positive for at least one of the 15 respiratory viruses we tested. An overview of the major features for infections from 15 respiratory viruses in patients with GP was analyzed in this study. We believe these results will provide useful information for clinical diagnosis and broaden the understanding of respiratory virus infection.

ACKNOWLEDGEMENTS

This study was supported by The State Major Infectious Disease Research Program (2017ZX10103011,

2018ZX10102001), National Natural Science Foundation of China (31500143), Guangzhou Science and Technology Program Key Project (201508020252). The sponsors of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

AUTHORS' CONTRIBUTIONS

RZ, WKL and QL designed the study. WKL, QL, SYQ, DX, and CL performed pathogen testing. DHC, WPT and SJG collected clinical data. All authors participated in the data analysis. WKL, QL and RZ drafted the manuscript. All authors read and approved the final version of this manuscript.

CONFLICTS OF INTEREST DISCLOSURE

The authors declare that they have no competing interests.

REFERENCES

- [1] Berman S. Epidemiology of acute respiratory infections in children of developing countries. *Rev Infect Dis.* 1991; 13 Suppl 6: S454-462. PMID:1862276. https://doi.org/10.1093/clinids/s/13.Supplement_6.S454
- [2] Monto AS. Acute respiratory infection in children of developing countries: challenge of the 1990s. *Rev Infect Dis.* 1989; 11(3): 498-505. PMID:2665005. <https://doi.org/10.1093/clinids/11.3.498>
- [3] Kirkwood BR, Gove S, Rogers S, et al. Potential interventions for the prevention of childhood pneumonia in developing countries: a systematic review. *Bull World Health Organ.* 1995; 73(6): 793-798. PMID:8907773.
- [4] Williams BG, Gouws E, Boschi-Pinto C, et al. Estimates of worldwide distribution of child deaths from acute respiratory infections. *Lancet Infect Dis.* 2002; 2(1): 25-32. [https://doi.org/10.1016/S1473-3099\(01\)00170-0](https://doi.org/10.1016/S1473-3099(01)00170-0)
- [5] Weber MW, Mulholland EK, Greenwood BM. Respiratory syncytial virus infection in tropical and developing countries. *Trop Med Int Health.* 1998; 3(4): 268-280. PMID:9623927. <https://doi.org/10.1046/j.1365-3156.1998.00213.x>
- [6] Glezen P, Denny FW. Epidemiology of acute lower respiratory disease in children. *N Engl J Med.* 1973; 288(10): 498-505. PMID:4346164. <https://doi.org/10.1056/NEJM197303082881005>
- [7] Henrickson KJ, Hoover S, Kehl KS, et al. National disease burden of respiratory viruses detected in children by polymerase chain reaction. *Pediatr Infect Dis J.* 2004; 23(1 Suppl): S11-18. PMID:14730265.
- [8] Watanabe A, Carraro E, Camargo C, et al. Human adenovirus detection among immunocompetent and immunocompromised patients presenting acute respiratory infection. *Rev Soc Bras Med Trop.* 2013; 46(2): 161-165. PMID:23666662. <https://doi.org/10.1590/0037-8682-1699-2013>
- [9] Allander T, Tammi MT, Eriksson M, et al. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci U S A.* 2005; 102(36): 12891-12896. PMID:16118271. <https://doi.org/10.1073/pnas.0504666102>
- [10] Liu WK, Chen DH, Liu Q, et al. Detection of human bocavirus from children and adults with acute respiratory tract illness in Guangzhou, southern China. *BMC Infect Dis.* 2011; 11: 345. PMID:22168387. <https://doi.org/10.1186/1471-2334-11-345>
- [11] Portes SA, Da Silva EE, Siqueira MM, et al. Enteroviruses isolated from patients with acute respiratory infections during seven years in Rio de Janeiro (1985-1991). *Rev Inst Med Trop Sao Paulo.* 1998; 40(6): 337-342. PMID:10436652. <https://doi.org/10.1590/S0036-46651998000600001>
- [12] Khetsuriani N, Lamonte-Fowlkes A, Oberst S, et al. Enterovirus surveillance—United States, 1970-2005. *MMWR Surveill Summ.* 2006; 55(8): 1-20.
- [13] Svraka S, Duizer E, Vennema H, et al. Etiological role of viruses in outbreaks of acute gastroenteritis in The Netherlands from 1994 through 2005. *J Clin Microbiol.* 2007; 45(5): 1389-1394. PMID:17360839. <https://doi.org/10.1128/JCM.02305-06>
- [14] Liu WK, Liu Q, Chen DH, et al. Epidemiology and clinical presentation of the four human parainfluenza virus types. *BMC Infect Dis.* 2013; 13: 28. PMID:23343342. <https://doi.org/10.1186/1471-2334-13-28>
- [15] Fitch PM, Henderson P, Schwarze J. Respiratory and gastrointestinal epithelial modulation of the immune response during viral infection. *Innate Immun.* 2012; 18(1): 179-189. PMID:21239454. <https://doi.org/10.1177/1753425910391826>
- [16] Broor S, Parveen S, Bharaj P, et al. A prospective three-year cohort study of the epidemiology and virology of acute respiratory infections of children in rural India. *PLoS One.* 2007; 2(6): e491. PMID:17551572. <https://doi.org/10.1371/journal.pone.0000491>
- [17] Mahony JB. Detection of respiratory viruses by molecular methods. *Clin Microbiol Rev.* 2008; 21(4): 716-747. PMID:18854489. <https://doi.org/10.1128/CMR.00037-07>

- [18] Henrickson KJ. Lower respiratory viral infections in immunocompetent children. *Adv Pediatr Infect Dis*. 1994; 9: 59-96. PMID:8123226.
- [19] Wilhelmi de Cal I, Mohedano del Pozo RB, Sanchez-Fauquier A. [Rotavirus and other viruses causing acute childhood gastroenteritis]. *Enferm Infecc Microbiol Clin*. 2008; 26 Suppl 13: 61-65.
- [20] Ospino DU, Young G, Navarro OA. Viral gastroenteritis and diversity of Rotavirus strains in Colombian children: a systematic review. *J Infect Dev Ctries*. 2008; 2(2): 99-105. PMID:19738332. <https://doi.org/10.3855/T2.2.99>
- [21] Okitsu-Negishi S, Nguyen TA, Phan TG, et al. Molecular epidemiology of viral gastroenteritis in Asia. *Pediatr Int*. 2004; 46(2): 245-252. PMID:15056259. <https://doi.org/10.1046/j.1442-200x.2004.01896.x>
- [22] Pavia AT. Viral infections of the lower respiratory tract: old viruses, new viruses, and the role of diagnosis. *Clin Infect Dis*. 2011; 52 Suppl 4: S284-289. PMID:21460286. <https://doi.org/10.1093/cid/cir043>
- [23] Papenburg J, Boivin G. The distinguishing features of human metapneumovirus and respiratory syncytial virus. *Rev Med Virol*. 2010; 20(4): 245-260. PMID:20586081. <https://doi.org/10.1002/rmv.651>
- [24] Hable KA, O'Connell EJ, Herrmann EC, et al. Group B coxsackieviruses as respiratory viruses. *Mayo Clin Proc*. 1970; 45(3): 170-176. PMID:5435857.
- [25] Kepfer PD, Hable KA, Smith TF. Viral isolation rates during summer from children with acute upper respiratory tract disease and healthy children. *Am J Clin Pathol*. 1974; 61(1): 1-5. PMID:4358333. <https://doi.org/10.1093/ajcp/61.1.1>
- [26] Ece G, Samlioglu P, Ulker T, et al. Rotavirus and adenovirus prevalence at Tepecik education and research hospital (Turkey). *Infez Med*. 2012; 20(2): 100-104. PMID:22767308.
- [27] Kesebir D, Vazquez M, Weibel C, et al. Human bocavirus infection in young children in the United States: molecular epidemiological profile and clinical characteristics of a newly emerging respiratory virus. *J Infect Dis*. 2006; 194(9): 1276-1282. PMID:17041854. <https://doi.org/10.1086/508213>
- [28] Ruuskanen O, Lahti E, Jennings LC, et al. Viral pneumonia. *Lancet*. 2011; 377(9773): 1264-1275. [https://doi.org/10.1016/S0140-6736\(10\)61459-6](https://doi.org/10.1016/S0140-6736(10)61459-6)
- [29] Liu WK, Liu Q, Chen de H, et al. Epidemiology of acute respiratory infections in children in guangzhou: a three-year study. *PLoS One*. 2014; 9(5): e96674. PMID:24797911. <https://doi.org/10.1371/journal.pone.0096674>
- [30] Cox NJ, Subbarao K. Global epidemiology of influenza: past and present. *Annu Rev Med*. 2000; 51: 407-421. PMID:10774473. <https://doi.org/10.1146/annurev.med.51.1.407>
- [31] Palmer LJ, Rye PJ, Gibson NA, et al. Airway responsiveness in early infancy predicts asthma, lung function, and respiratory symptoms by school age. *Am J Respir Crit Care Med*. 2001; 163(1): 37-42. PMID:11208623. <https://doi.org/10.1164/ajrccm.163.1.2005013>
- [32] Lau SK, Yip CC, Que TL, et al. Clinical and molecular epidemiology of human bocavirus in respiratory and fecal samples from children in Hong Kong. *J Infect Dis*. 2007; 196(7): 986-993.
- [33] Henrickson KJ. Parainfluenza viruses. *Clin Microbiol Rev*. 2003; 16(2): 242-264. PMID:12692097. <https://doi.org/10.1128/CMR.16.2.242-264.2003>
- [34] Ebihara T, Endo R, Kikuta H, et al. Comparison of the seroprevalence of human metapneumovirus and human respiratory syncytial virus. *J Med Virol*. 2004; 72(2): 304-306. PMID:14695674. <https://doi.org/10.1002/jmv.10572>
- [35] Singleton RJ, Redding GJ, Lewis TC, et al. Sequelae of severe respiratory syncytial virus infection in infancy and early childhood among Alaska Native children. *Pediatrics*. 2003; 112(2): 285-290.
- [36] Mathisen M, Strand TA, Sharma BN, et al. RNA viruses in community-acquired childhood pneumonia in semi-urban Nepal: a cross-sectional study. *BMC Med*. 2009; 7: 35. PMID:19635124. <https://doi.org/10.1186/1741-7015-7-35>