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Clinical Characteristics and Viral Load of Respiratory Syncytial Virus and Human Metapneumovirus in Children Hospitaled for Acute Lower Respiratory Tract Infection

Xiao-li Yan,[1,2] Yu-ning Li,[1] Yi-jie Tang,[3] Zhi-ping Xie,[2] Han-chun Gao,[2] Xue-mei Yang,[1] Yu-mei Li,[1] Li-jun Liu,[1] and Zhao-jun Duan[2]*

[1]*Department of Pediatrics, The First Hospital of Lanzhou University, Lanzhou, Gansu, China*
[2]*Key Laboratory for Medical Virology, Ministry of Health, National Institute for Viral Disease Control and Prevention, China Center for Disease Control, Beijing, China*
[3]*Department of Respiration, Beijing Electric Power Hospital of The North China Grid Company Limited, Beijing, China*

Respiratory syncytial virus (RSV) and human metapneumovirus (HMPV) are two common viral pathogens in acute lower respiratory tract infections (ALRTI). However, the association of viral load with clinical characteristics is not well-defined in ALRTI. To explore the correlation between viral load and clinical characteristics of RSV and HMPV in children hospitalized for ALRTI in Lanzhou, China. Three hundred and eighty-seven children hospitalized for ALRTI were enrolled. Nasopharyngeal aspirates (NPAs) were sampled from each children. Real-time PCR was used to screen RSV, HMPV, and twelve additional respiratory viruses. Bronchiolitis was the leading diagnoses both in RSV and HMPV positive patients. A significantly greater frequency of wheezing (52% vs. 33.52%, $P = 0.000$) was noted in RSV positive and negative patients. The RSV viral load was significant higher in children aged <1 year ($P = 0.003$), children without fever and wheezing ($P = 0.015$ and $P = 0.000$), days of illness <14 days ($P = 0.002$), children with bronchiolitis ($P = 0.012$) and children with RSV single infections ($P = 0.000$). No difference was found in the clinical features of HMPV positive and negative patients. The HMPV viral load had no correlation with any clinical characteristics. The incidences of severe disease were similar between single infection and coinfection for the two viruses (RSV, $P = 0.221$; HMPV, $P = 0.764$) and there has no statistical significance between severity and viral load ($P = 0.166$ and $P = 0.721$). Bronchiolitis is the most common disease caused by RSV and HMPV. High viral load or co-infection may be associated with some symptoms but neither has a significant impact on disease severity for the two viruses. ***J. Med. Virol. 89:589–597, 2017***.

KEY WORDS: respiratory syncytial virus; human metapneumovirus; epidemiology

INTRODUCTION

Acute lower respiratory tract infections (ALRTI) are one of the most common childhood diseases and is a leading cause of morbidity and mortality in children aged <5 years worldwide [Rudan et al., 2008; Walker et al., 2013]. There are 120 million new cases occur per year; 14 million develop into severe disease [Walker et al., 2013]. The World Health Organization (WHO) estimated that globally in 2010, approximately 7.6 million children died and pneumonia caused 1.4 million deaths in children aged <5 years worldwide [Liu et al., 2012].

A number of pathogens are capable of causing ALRTI including bacteria, viruses, and fungi. Viruses are the most frequent cause of respiratory infection

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X-l. Y, Y-n. L and Y-j. T contributed equally to this work.

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*Correspondence to: Zhao-jun Duan, Department of Viral Diarrhea, National Institute for Viral Disease Control and Prevention, China Center for Disease Control, 100 Ying-Xin St, Xuan-Wu District, Beijing 100052, China.
E-mail: zhaojund@126.com

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[Williams et al., 2002]. Respiratory syncytial virus (RSV) and human metapneumovirus (HMPV) are two common causes of ALRTI; these two viruses are negative sense single-stranded RNA viruses belonging to the pneumovirinae subfamily of the family *Paramyxoviridae*. RSV belongs to genus pneumovirus and is the leading cause of ALRTI [Rudan et al., 2013]; it is estimated that 68.8% of children aged <1 year and 82.6% of children aged <2 years are infected with RSV [Glezen et al., 1986]. Globally, RSV results in 33.8 million new episodes ALRTI in children aged <5 years and results in at least 3.4 million cases of severe disease [Nair et al., 2010]. HMPV belongs to the genus metapneumovirus and was first found in 2001, retrospective serological studies have found that HMPV antibodies in humans have existed more than 50 years [van den Hoogen et al., 2001]. In children aged <5 years, the hospitalization rate associated with HMPV is one per 1,000 children [Edwards et al., 2013].

Most previous studies have focused on epidemiological characteristics and the viral genetics of RSV and HMPV. With the development of real-time PCR, it is now possible to clarify the correlation between viral load and clinical characteristics resulting from these viral pathogens. In this study, the correlation between clinical features and viral load of RSV and HMPV in 387 children hospitalized for ALRTI in Lanzhou, China was explored.

METHODS

Study Design and Subjects

From December 2011 to November 2012, 387 children hospitalized for ALRTI in the Department of Pediatrics at The First Hospital of Lanzhou University were enrolled. Children were diagnosed in three groups: bronchitis, pneumonia, and bronchiolitis. The diagnostic criteria of pneumonia was based on Defining pneumonia in critically ill infants and children [Langley and Bradley, 2005], and the diagnostic criteria of bronchiolitis was based on diagnosis and management of bronchiolitis [American Academy of Pediatrics Subcommittee on Diagnosis and Management of Bronchiolitis, 2006]. There has no definite and strict diagnostic criteria for bronchitis, the main signs are cough, expectoration. Bronchitis is not associated with constitutional symptoms and other signs such as tachypnea, hypoxemia, and so on. Auscultatory findings of bronchitis consist of coarse crackles, medium crackles, and slight wheezes. The definition of disease severity was based on strategy to enhance influenza surveillance worldwide [Ortiz et al., 2009]. For children >5 years old, all of the following four items must be required: (i) Sudden onset of fever >38°C, (ii) Cough or sore throat, (iii) Shortness of breath (breathing faster than 25 breaths/min) or difficult breathing, (iv) Requires hospitalization. For children ≤5 years old, with cough or difficult breathing and at least one sign or symptom of follows: (i) Shortness of breath: breathing faster than 60 breaths/min (infants <2 months), breathing faster than 50 breaths/min (2–12 months), breathing faster than 40 breaths/min (1–5 years), (ii) Unable to drink or breastfeed, (iii) Vomits everything, (iv) Convulsions, (v) Lethargic or unconscious, (vi) Chest indrawing or stridor in a calm child.

NPAs were collected from each patient within 3 days of admission to hospital. Two milliliter virus preservation solution (200 U/ml penicillin, 200 U/ml streptomycin, 200 U/ml amphotericin B, and 0.25% BSA) was added to each NPA immediately after collection. All specimens were transported on dry ice to the National Institute for Viral Disease Control and Prevention, China CDC and stored at −80°C. The demographic characteristics and medical history of each child was obtained from their parents or hospital record. The process obtained families informed consent and the study protocol was approved by the hospital ethics committee.

Extraction of Viral Nucleic Acid

Viral nucleic acid were extracted from 200 μl NPA using a Qiamp Minelute Virus Spin Kit(QIAGEN, Germany) according to the manufacturer's instruction. This kit is useful for extracting of both viral RNA and DNA simultaneously.

Real-time PCR for RSV and HMPV. Real-time PCR assays targeted the M gene of RSV (84 bp) and the F gene of HMPV (80 bp) [Weinberg et al., 2013]. The primers and probes used were as follows (all primers and probes are written from $5' \rightarrow 3'$);, RSV-F: GGC AAA TAT GGA AAC ATA CGT GAA; RSV-R: TCT TTT TCT AGG ACA TTG TAY TGA ACA G; RSV-Pb: FAM-CTG TGT ATG TGG AGC CTT CGT GAA GCT-BHQ. HMPV-F: CAA GTG TGA CAT TGC TGA YCT RAA; HMPV-R: ACT GCC GCA CAA CAT TTA GRA A; HMPV-Pb: FAM-TGG CYG TYA GCT TCA GTC AAT TCA ACA GA-BHQ. A one Step RT-PCT Kit (Applied Biosystems) was used to amplify viral RNA. Each 20 μl reaction mixture contained 2 × RT-PCR Buffer, 10 μl; each forward and reverse premier(20 μM), 0.4 μl; probe (10 μM), 0.4μl; 25 × RT-PCR enzyme, 0.8 μl; template RNA, 4 μl; ddH_2O, 4 μl. Real-time PCR conditions were 50°C for 30 min, 95°C for 10 min, 40 cycles of 95°C for 15 sec, and 60°C for 30 sec. In order to standardize the quantification of RSV and HMPV, known numbers of RNA transcripts containing the primer targets were used in 10-fold serial dilutions (10^0 to 10^7 copies/μl). Any amplification detected before cycle 40 cycle was considered to be a positive amplification. Quantification of $>10^0$ copies/μl (10^3 copies/ml) was considered as a positive result.

Detection of Other Viruses

The presence of 12 additional viruses were also tested by real-time PCR; these included human

rhinovirus (HRV), enterovirus (EV), adenovirus (ADV), human bocavirus (HBoV), influenza virus A and B (IFVA, IFVB), parainfluenza virus 1–4 (PIV1-4), and coronaviruses HKU1 and NL63 [Esposito et al., 2006; Kantola et al., 2010; Jansen et al., 2011; McLeish et al., 2012; Weinberg et al., 2013]. Real-time PCR reagent concentrationss and conditions for the detection of all RNA viruses were the same as for RSV and HMPV. Taqman Universal Master Mix II with UNG (Applied Biosystems) were used to detect ADV and HBoV; real-time PCR conditions were 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 sec and 60°C for 30 sec. The criteria for a positive result were the same as RSV and HMPV.

Statistical Analysis

Viral load was expressed as the initial copy number per real-time PCR reaction, all quantitative results were transformed as the $\log_{10}$ of viral copy number/ml. Continuous variables were given as mean values ± standard deviation (SD) and compared using the Independent-Samples *t*-test, One-Way ANOVA or Binary logistic. Categorical variables were described using frequency or percentage, comparisons were made using the chi-squared or Fisher's exact test. Statistical analysis were performed using SPSS17.0 software (Chinese version). The specific statistical results were not shown. $P < 0.05$ was considered to be statistically significant.

RESULTS

Patient Characteristics

During the study period, 387 children diagnosed with ALRTI were enrolled. The majority of children (86.30%) were aged less than 3 years, the median age was 12 months. The oldest patient was aged 15 years and the youngest was just 8 days. The majority (67.7%) of patients were males. Pneumonia was diagnosed in 189 cases (48.84%), followed by bronchiolitis in 160 cases (41.34%), and bronchitis in 38 cases (9.82%). The mean duration of hospitalization (days ± SD) was 8.35 ± 4.49. An underlying condition was observed in 19.12% (74/387) cases and 48 children were diagnosed with severe disease.

Virus Identification and Epidemiological Characteristics

At least one potential viral pathogen was detected in 366 (94.57%) of the study children. Most virus positive cases were children aged less than 3 years and the detection rate was significantly higher than children older than 3 years old ($P = 0.000$). Two hundred and five children (52.97%) were positive for RSV and 47 children (12.14%) were positive for HMPV. Furthermore, 24.55% is positive for HRV, 16.80% for AdV, 14.57% for PIV3, 10.08% for HBoV, 8.27% for NL63, 4.65% for IFVA, 4.65% for IFVB, 3.36% for PIV4, 2.84% for PIV1, 2.58% for EV, 1.55% for PIV2, and 0.26% for HKU1. There was no difference in the detection rate for RSV and HMPV and gender (RSV: 51.53% vs. 56.00%, $P = 0.410$; HMPV: 12.60% vs. 11.20%, $P = 0.694$).

RSV was detected throughout the year, although the detection rate was highest in the winter months (February, November, and January), followed by spring, autumn, and summer ($P = 0.000$). HMPV was detected every month except for October; the detection rate was highest in June and July, but there was no significant difference between four seasons ($P = 0.843$; Fig. 1).

Patients who tested positive for RSV ranged in age from 8 days to 12 years, the median age was 9 months. The HMPV positive patients ranged in age from 1 month to 5 years, with the median age 12 months. Patients were devided into 5 age groups (≤6 m, ~12 m, ~36 m, ~60 m, >60 m). We found different distribution in RSV positive patients and HMPV positive ones. RSV was detected in all age groups although 93.66% (192/205) of positive cases were children aged less than 3 years. The highest RSV positive rate was observed in age group under 6 months, followed by age group 7–12 months and 13–36 months. Children aged <6 months were particularly vulnerable ($P = 0.000$). HMPV was not detected in children older than 5 years. The highest HMPV rate was detected in age group 3–5years old. ($P = 0.045$; Fig. 2).

Clinical Characteristics of RSV and HMPV Positive Patients

The viral detection rate was highest in bronchiolitis patients (98.75%, 158/160), followed by bronchitis (92.11%, 35/38) and pneumonia (91.53%, 173/189) patients; significant differences were observed in viral etiologies associated with each diagnoses ($P = 0.010$). HRV, ADV, and PIV3 were the most common viruses in children with bronchitis. For children with pneumonia, RSV was the most common virus followed by HRV, ADV, PIV3, and HMPV. For children with bronchiolitis, the most common virus

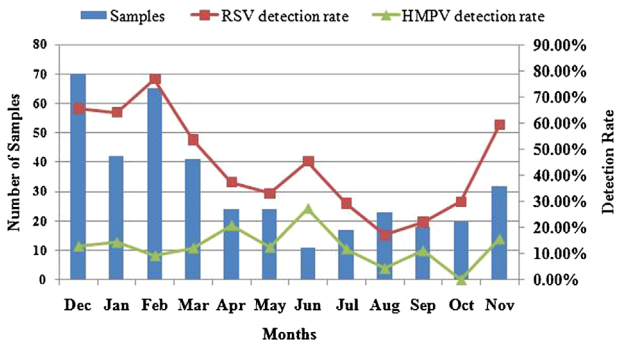


Fig. 1. Seasonal distribution of RSV and HMPV.

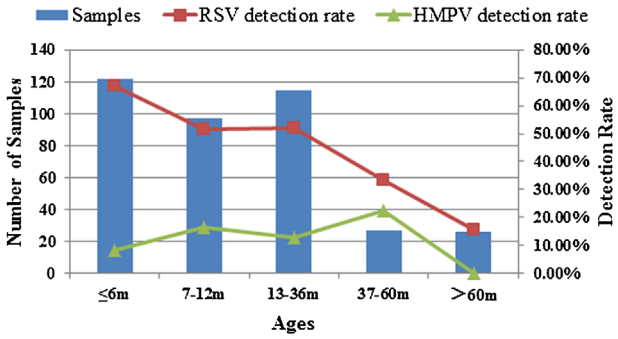


Fig. 2. Detection rate of RSV and HMPV in different age groups.

was RSV, followed by HRV, ADV, HMPV, and HBoV. Bronchiolitis was the leading diagnoses in RSV and HMPV positive patients, followed by pneumonia and bronchitis. Patients were divided into two groups: those with RSV or HMPV infection and those without RSV or HMPV infection (Table I). For RSV and HMPV positive patients, the most common symptoms were cough, wheezing, and fever. A high frequency of wheezing (52% vs. 33.52%, $P = 0.000$) was noted as significant in RSV positive patients in comparison with RSV negative patients. However there was no significant difference with respect to clinical features between HMPV positive and HMPV negative patients.

Viral Load and Clinical Features

Among the 205 RSV positive samples, the log copies of RSV-RNA per ml ranged from 3.03 to 11.32; the median viral load value was 8.07, the mean viral load (mean ± SD) was 7.40 ± 2.02. For the 47 children that were HMPV positive, the log copies of HMPV-RNA per ml ranged from 3.10 to 9.24, with the median viral load of 7.24 and the mean viral load of 6.36 ± 2.13.

The relationship between viral load and clinical characteristics including age, gender, respiratory rate, temperature, wheezing, cyanosis, diagnosis, hospitalization, days of illness, white blood cells (WBC), and C reactive protein (CRP) were evaluated (Table II). For RSV, the viral load was significantly higher among children aged <1 year. Without fever and wheezing were associated with a significantly higher RSV viral load. The RSV viral load was significantly different between three diagnostic groups; children with bronchiolitis had higher RSV viral loads than those with pneumonia, however most other clinical characteristics had no significant correlation with the RSV load. There was no correlation between HMPV viral load and any clinical characteristic (Table II and Fig. 3). Thirty of 205 RSV positive children and 9 of 47 HMPV positive children had an underlying chronic illness; there was no significant difference between children with or without underlying illness and viral load (RSV: $P = 0.070$; HMPV: $P = 0.634$).

Correlation between viral load and severity of disease was also estimated. To refer to previously publications, we consider age, sex, and underlying diseases may be the confounding factors of the disease severity and viral load. We conducted a Binary logistic to analyze the association between disease severity and viral load. For RSV and HMPV, there has no statistical significance between severity and viral load when excluding these three factors ($P = 0.166$ and 0.721).

Co-Infection With Other Viruses

Of the 205 RSV positive specimens, at least one additional virus was detected in 117 (57.07%); the most common viruses observed in co-infections with RSV were HRV (n = 37), AdV (n = 26), HMPV (n = 20) and PIV3 (n = 19). For HMPV, the co-infection rate was even higher (77.47%; 35/47); the most common viruses associated with HMPV detection were RSV (n = 20), AdV (n = 13), HRV (n = 10), and HBoV (n = 7).

TABLE I. Clinical Features of RSV- or HMPV-Positive Patients

	RSV			HMPV		
Clinical data	Positive	Negative	*P*	Positive	Negative	*P*
Cough[a]	200 (97.56%)	180 (98.90%)	0.455	47 (100.00%)	333 (97.94%)	1.000
Wheezing[a]	108 (52.68%)	61 (33.52%)	**0.000**	25 (53.19%)	144 (42.35%)	0.160
Fever[a]	112 (54.63%)	103 (56.59%)	0.699	30 (63.83%)	185 (54.41%)	0.223
Rhinorrhoea[a]	45 (21.95%)	28 (15.38%)	0.099	4 (8.51%)	65 (19.12%)	0.075
Tachypnea[a]	33 (16.10%)	20 (10.99%)	0.145	7 (14.89%)	46 (13.53%)	0.799
Cyanosis[a]	15 (7.32%)	13 (7.14%)	0.947	2 (4.26%)	26 (7.65%)	0.555
WBC ($\times 10^9$ cell/L) (mean ± SD)[b]	8.26 ± 3.84 (n = 202)	8.38 ± 4.99 (n = 179)	0.782	9.42 ± 5.73 (n = 46)	8.17 ± 4.18 (n = 335)	0.156
Hospitalization (mean ± SD)[b]	8.13 ± 3.53	8.60 ± 5.37	0.314	7.53 ± 3.25	8.46 ± 4.63	0.183

Statistical differences value is indicated in bold.
[a]Calculated using the chi-squared test or Fisher's exact test.
[b]Calculated using the Independent-Samples *t*-test.

TABLE II. Correlation Between Clinical Features and Viral Load

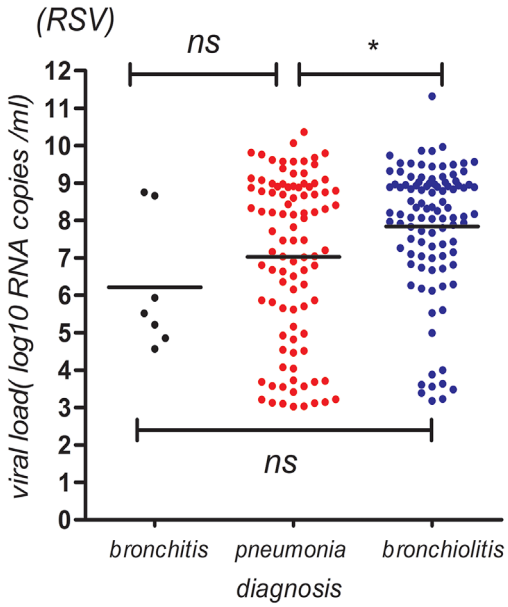
	RSV			HMPV		
Clinical data	n	Mean viral load	*P*	n	Mean viral load	*P*
Age[a]						
<1 y	114	7.77 ± 1.82	**0.003**	19	6.21 ± 1.98	0.694
≥1 y	61	6.93 ± 2.17		28	6.46 ± 2.25	
Gender[a]						
Male	135	7.42 ± 2.12	0.838	33	6.36 ± 2.09	0.982
Female	70	7.36 ± 1.83		14	6.34 ± 2.29	
Respiratory rate[a]						
Normal	172	7.32 ± 2.02	0.227	40	6.27 ± 2.16	0.493
Higher	33	7.78 ± 1.99		7	6.87 ± 2.00	
Temperature[a]						
Normal	93	7.77 ± 1.87	**0.015**	17	6.11 ± 1.96	0.561
Fever	112	7.09 ± 2.09		30	6.49 ± 2.23	
Wheezing[b]						
No	97	6.87 ± 2.21	**0.000**	22	6.55 ± 2.10	0.565
Yes	108	7.88 ± 1.71		25	6.19 ± 2.18	
Cyanosis[a]						
No	190	7.36 ± 2.02	0.296	45	6.27 ± 2.12	0.183
Yes	15	7.92 ± 2.01		2	8.33 ± 1.17	
Diagnosis[b]						
Bronchitis	7	6.21 ± 1.76	**0.012**	3	6.50 ± 2.76	0.980
Pneumonia	97	7.02 ± 2.21		18	6.28 ± 2.24	
Bronchiolitis	101	7.83 ± 1.74		26		
Hospitalization[a]						
<7 days	72	7.09 ± 1.88	0.106	21	5.95 ± 2.14	0.250
≥7 days	133	7.57 ± 2.08		26	6.68 ± 2.10	
Days of illness						
<14 days	98	7.85 ± 1.71	**0.002**	20	6.84 ± 2.06	0.185
≥14 days	107	6.98 ± 2.20		27	6.00 ± 2.14	
WBC[a]						
≤10 ($\times 10^9$ cell/L)	165	7.48 ± 1.91	0.342	34	6.53 ± 1.99	0.573
>10 ($\times 10^9$ cell/L)	37	7.06 ± 2.48		12	6.12 ± 2.48	
CRP[a]						
<8 (mg/L)	122	7.56 ± 2.00	0.251	22	6.71 ± 1.91	0.472
≥8 (mg/L)	67	7.20 ± 2.05		20	6.23 ± 2.31	

Viral load described as mean ± standard deviation.
Statistical differences values are indicated in bold.
[a]Calculated using Independent-Samples *t*-test.
[b]Calculated using one-way ANOVA.

Children positive for RSV alone had a higher viral load than children who were co-infected with other viruses (mean ± SD: 8.18 ± 1.35 vs. 6.81 ± 2.23, $P = 0.000$). However, the viral load was not significantly different between HMPV alone and HMPV associated with other viruses (mean ± SD: 6.75 ± 1.99 vs. 6.22 ± 2.18, $P = 0.465$) (Fig. 4). The severe lower respiratory tract infection rates were 17.05% (15/88) in RSV single infections and 11.11% (13/117) in RSV associated with other viruses. The incidence of severe disease was not significantly different between these two groups ($P = 0.221$); the same result was observed with HMPV (8.33% vs. 11.43%, $P = 0.764$).

DISCUSSION

In this study, real-time PCR was used to explore the disease etiology and examine correlation between viral load and clinical features of pediatric RSV and HMPV infections in Lanzhou, China. This study found that 94.57% patient samples had at least one detectable potential viral pathogen; this detection rate was significantly higher than previous studies (48.1–90%) that used traditional PCR or direct immunofluorescence [Singleton et al., 2010; Ahmed et al., 2012; Bicer et al., 2013; Bukhari and Elhazmi 2013] and was higher than a German study that used real-time PCR (detecting 14 viruses with a result of 78% positive) [Franz et al., 2010]. It is possible that the detection rate in this study could be due to the increased sensitivity of real-time PCR compared to conventional PCR and viral culture methods. This study also detected 14 different viruses, the viral pathogen spectrum was more comprehensive than many previous studies (7–12 viruses) [Singleton et al., 2010; Ahmed et al., 2012; Bicer et al., 2013; Bukhari and Elhazmi, 2013]. Additionally patients in this study all were diagnosed with ALRTI, and in many instances, the patients were suffering from severe disease. Meerhoff et al. [2010] indicated that using NPA resulted in a higher detection rate than using a nasal swab as a sample, it is possible that the use of NPA might also be associated with the higher rate of detection seen in



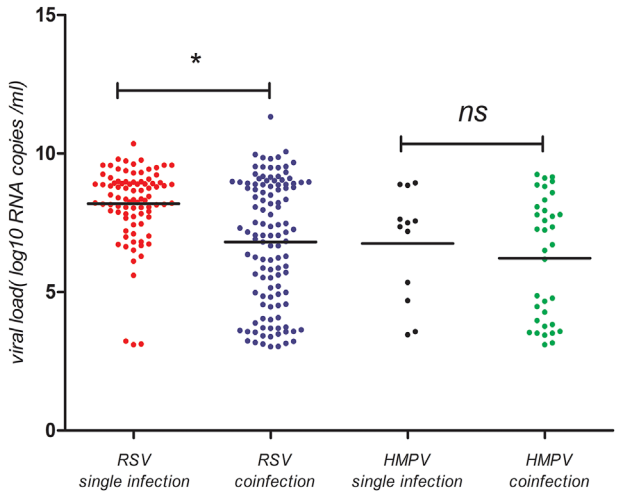


Fig. 4. Viral load of RSV and HMPV in single infections and other viral coinfections. *Represents $P < 0.05$; ns represents no significant difference.

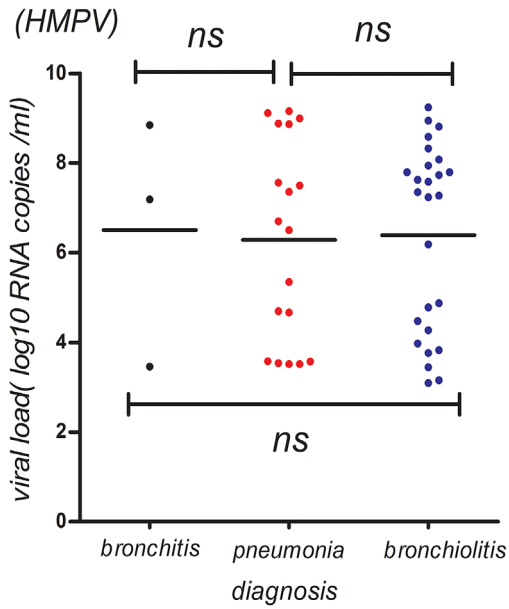


Fig. 3. Viral load of RSV and HMPV in three diagnostic groups. *Represents $P < 0.05$; ns represent no significant difference.

this study. Finally, other factors such as race and geographical conditions may have some influence on the viral burden in the population, and thus the detection rate.

RSV was detected in 52.79% of the children and it was the most common virus among the 14 viruses detected as part of this study. The detection rate was similar to that reported by Franz et al. [2010] who also used a real-time PCR approach. Only 12.14% of children were found HMPV positive, although this detection rate was higher than other studies that used the same detection method such as in Italy (2.9%), Germany (less than 9%), and the United States (9%) [Bosis et al., 2008; Martin et al., 2008; Franz et al., 2010]. Similar to other studies [Franz et al., 2010; Singleton et al., 2010; Bicer et al., 2013; Bukhari and Elhazmi, 2013], RSV was the most common virus in children with ALRTI [Franz et al., 2010; Singleton et al., 2010; Bicer et al., 2013; Bukhari and Elhazmi, 2013]. RSV and HMPV were associated with more severe illness [Singleton et al., 2010]. It is apparent that RSV and HMPV play an important role in children with ALRTI in different regions globally.

Many respiratory viruses have significant seasonality. Previous studies have demonstrated that cold exposure may increase the possibility of bronchoconstriction, airway congestion, secretion, and decrease innate immune mechanisms such as mucociliary clearance [Giesbrecht, 1995]. This indicates that cold seasons may increase the risk of respiratory infections. In this study, RSV showed a clear seasonal distribution in winter-spring. In contrast, the maximum prevalence was observed in March to August in Singapore and Brazil, but in September to November in Malaysia which also located in tropical zone [Chew et al., 1998; Pecchini et al., 2008; Khor et al., 2012]. There was no significant seasonality of HMPV observed in this study, but in studies performed in Korea and Turkey, the peak season was winter-spring [Kim et al., 2010; Bicer et al., 2013]. These observations suggest that not all respiratory virus infection is common in cold seasons. The seasonality of viruses was different in different regions; this may correlate with local geographical and climatic features.

Pathogens associated with respiratory tract infections can be very complex and there is no

specific symptom to distinguish one infection from another. Research about the viral etiology of bronchiolitis among children in northern Taiwan indicated that RSV and HMPV were the main virus for children with bronchiolitis, with RSV observed as the most common cause [Chen et al., 2012]. In this study, bronchiolitis patients were most likely to result in the identification of a potential viral pathogen among the three diagnoses; bronchiolitis was most often associated with RSV and HMPV positive patients. The main symptoms in RSV and HMPV positive patients were cough, wheezing, and fever.

Scagnolari et al. [2012] indicated that RSV viral load was positively associated with the clinical severity of bronchiolitis hospitalization. Fodha et al. [2007] also found that nasopharyngeal RSV loads were higher in patients with severe disease. In contrast, clinical aspects observed in patients with serious LRTIs, like respiratory rate, cyanosis, and hospitalization were not associated with high viral loads. We also analyzed the relationship between severity and viral load using multiple linear regression, the viral load showed no apparent significant difference between severe and not severe disease groups in this study. These findings are in agreement with previous studies indicating that RSV viral load may have no correlation with the disease severity [Wright et al., 2002; Jansen et al., 2010]. However, in this study, RSV viral load was significantly different between three diagnostic groups and a high RSV viral load may be a risk factor of developing bronchiolitis. Excluding severity and underlying diseases, Children aged <1 year had a higher viral load than other children. It is possible that this is due to the children aged <1 year having an immature immune system resulting in an inappropriate shift toward a Th2 immune response to RSV infection [Gerna et al., 2008]. In our study, days of illness <14 days had a higher viral load compared with ≥ 14 days for RSV, this was because the long days of illness may associated with co-infecting, this also consistent with the result in our study that children positive for RSV alone had a higher viral load than co-infected with other viruses.

Some previous studies have showed that high HMPV viral load may be associated with an increase in the presence of fever, bronchodilator use, obtaining chest radiograph, and hospitalization; viral load was significantly higher in children with LRTI and hospitalized children [Bosis et al., 2008; Martin et al., 2008]. These studies also showed a significant correlation between HMPV viral load and disease severity. In contrast, our study showed no correlation between viral load and disease severity, this was consistent with a previous study conducted in Chongqing, China [Peng et al., 2010], which also indicated that HMPV viral load was significant correlated with the course of illness; high viral load occurred between 6 and 11 days post-infection.

Co-infection of RSV was detected in 57.07% and HMPV was detected in 77.47% of the cases, these rates were higher than reported in previous studies [Franz et al., 2010; Martin et al., 2012]. However, this is consistent with the overall higher detection rate in observed this study as mentioned above. In this study, children infected with RSV alone had a higher viral load than in RSV patients co-infected with other viruses; this observation was not seen with HMPV and reflects in part an observation made by Franz et al. [2010]. Martin et al. [2012] showed that the correlation between viral load and viral co-infection was virus specific and in their study, both RSV and HMPV were present at consistently high viral loads regardless of whether the patient was co-infected with another virus. The effect of co-infection on disease RSV or HMPV disease severity remains unclear and controversial. Semple et al. [2005] found dual infection by HMPV and RSV was associated with severe bronchiolitis, while other studies have found no association of co-infection with disease severity [Peng et al., 2009] or co-infection was correlated with less severe disease [Martin et al., 2012]. In our study, the incidence of severe disease was similar between single infections and co-infections, indicating that co-infection was not associated with more severe disease. An immune response to the first virus infection might alter the disease severity of a subsequently virus by the induction of interferon and other anti-viral response modifiers [Martin et al., 2012]. The impact of these immunomodulators could be to restrain colonization and pathogenicity of the co-infecting virus. However, more research is needed to determine what the relationship between co-infecting viruses and disease development is.

There are some limitations in our study. It was difficult to determine at which stage of disease development patients are when they received the diagnosis of ALRTI. NPAs were not able to be collected at the same time point, although they were collected within 3 days of diagnosis of ALRTI; this may have influenced viral detection rate and viral load results. Furthermore, viral loads of many viruses in healthy children remain unknown; a control group of healthy children should be established in order to determine the basal viral load level to help determine loads associated with increased disease risk. In addition, follow-up examinations should be performed, to more accurately reflect the correlation between viral load and changing disease progression and severity.

In conclusion, the findings of the present study indicated that RSV and HMPV are very important viral pathogens in children with ALRTI in China. Bronchiolitis is the most common disease associated with RSV and HMPV detection. High viral loads or co-infection with other respiratory viruses may be associate with some symptoms but do not appear to have a significant impact on disease severity. We believe a series of global, multi-center, standard

case–control studies are necessary to further clarify the correlation between viral infections and the clinical course of them in ALRTI.

ETHICAL APPROVAL

The process obtained families informed consent and the study protocol was approved by the Ethics Committee of the First Hospital of Lanzhou University.

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