HK J Paediatr (new series) 2013;**18**:6-11

Original Articles

Human Metapneumovirus Infection in Hospitalised Children with Acute Lower Respiratory Tract Infection in Hangzhou, China

TL ***Wang,*** GM ***Zheng,*** ZY ***Jiang,*** LF ***Tang,*** HF ***Tang,*** ZM ***Chen***

Abstract

Objectives: To investigate human metapneumovirus (hMPV) pathogens in children with acute lower respiratory tract infection (ALRIs) in Hangzhou, China. ***Methods:*** A total of 2911 paediatric patients with ALRIs hospitalised in our unit from January 2011 to December 2011 were enrolled. Their clinical characteristics were recorded and hMPV was detected by direct immunofluorescence. ***Results:*** hMPV was found in 70 (2.40%) samples. The positive rate was highest in children aged >1 to 3 years (3.32%), and lowest in children over 3 years (0.63%) with a significant difference among 3 different age groups (χ^2=11.795, P=0.003). The positive rate were higher in bronchiolitis and asthma with infection, and lower in pneumonia and bronchitis with a significant difference among 4 different groups (χ^2=9.270, P=0.026). The positive rates of hMPV in children with bronchiolitis and asthma with infection were 2.706 (95%CI: 1.057-6.924) and 2.806 (95%CI: 1.095-7.187) folds of that in children with pneumonia. Moreover, the positive rate was highest in spring with a rate of 5.87%, followed by winter (3.30%), autumn (0.44%) and summer (0.36%). It dramatically increased in spring, and peaked on March with a rate of 7.47% while no hMPV positive sample was found from July to September. ***Conclusions:*** hMPV is an important cause of ALRIs. hMPV infection commonly occurs in young children, and spring is the peak infection season in Hangzhou, China

Key words Chinese; Children; Direct immunofluorescence assay; Human metapneumovirus; Lower respiratory tract infection

The Children's Hospital of Zhejiang University School of Medicine, Hangzhou, China

TL **Wang** (汪天林) *MD*
GM **Zheng** (鄭桂梅) *Postgraduate*
ZY **Jiang** (姜仔彥) *Postgraduate*
LF **Tang** (唐蘭芳) *PhD, MD*
HF **Tang** (湯宏峰) *MD*
ZM **Chen** (陳志敏) *PhD*

The Key Laboratory of Reproductive Genetics (Zhejiang University), Ministry of Education, Hangzhou, China

LF **Tang** (唐蘭芳) *PhD, MD*

Correspondence to: Dr LF **Tang**

Received May 16, 2012

Introduction

Acute lower respiratory tract infections (ALRIs) are the main cause of morbidity and mortality in younger children. It accounts for 1/3-1/2 of deaths in children less than 5 years, and most of them in underdeveloped countries.[1] In developed countries, viruses, including respiratory syncytial virus (RSV), adenovirus (ADV), influenza virus (Flu) and parainfluenza virus (PIV), are the major cause of lower respiratory tract infection,[2,3] although they with lower mortality. The distribution of viral pathogens was some different depending on the population, geographic locales, climate and the economic levels.[3-6] ALRIs are one of the main reasons for consultation in paediatrics services in primary and secondary health care units in

China. However, about 14%-35% of ALRIs pathogens remain unidentified.[7-9]

Human metapneumovirus (hMPV), first isolated from children with respiratory tract disease in the Netherlands, is classified in the family *Paramyxoviridae, subfamily Pneumovirinae,* and *metapneumovirus* genus based on gene installation and phylogenetic analysis.[10] hMPV infections have been documented in a variety of regions, which suggests a world-wide distribution.[11-14] Also, it indicates that the virus is an important viral pathogen accounting for respiratory infection in children. Recently, several papers about the epidemiology of hMPV in children in the mainland of China were reported. However, most data were from Beijing, Chongqing, Guangzhou, Tianjin and Suzhou,[15-19] no similar study about the information from Zhejiang province was reported until now. Herein, we investigate the epidemiology of hMPV in 2911 hospitalised paediatric patients at Hangzhou district, which is the capital of Zhejiang province and located in the east of China.

Subjects and Methods

Subjects

All patients with ALRIs hospitalised in our unit from January 2011 to December 2011 were enrolled in this study. A total of 33 patients were excluded because their guardians are unwilling to perform the pathogens (including hMPV) detection and 7 were excluded due to underlying diseases (e.g. corticosteroids treatment, immunodeficiency disease). A total of 2911 paediatric patients with ALRIs who performed hMPV detection were included in this study. The diagnosis of ALRIs, including acute pneumonia, bronchitis, and bronchiolitis, was undertaken according to clinic feature and chest X-ray recommended in the Chinese textbook for paediatrician.[20] The diagnosis of asthma was undertaken according to the definition by Chinese National Paediatric Asthma Collaborative Group.[21] Asthma with infection was defined by asthma patients with worse cough and/or wheeze and 2 of the following conditions: (1) changes in sputum colour, including yellow sputum; (2) fever or other infection appearances; (3) increased acute reactants, including peripheral blood white cell (>12 x 10^9/L) or C-reactive protein (>8 mg/L); (4) patch shadow or other pneumonia features in chest X-ray; (5) positive finding of pathogens. All patients were diagnosed by more than 2 paediatricians.

Informed consent was obtained from the guardians of these patients and this study was approved by the Ethical Committee of our unit.

Viral Pathogen Detection

Nasopharyngeal aspirates were taken to collect epithelial cell from each patient on the first day of hospitalisation. Samples for virological study were diluted with equal volumes of saline, stored at 4°C and then processed within 24 h of collection. Exfoliated epithelial cells were collected for cell smear. Direct immunofluorescence assays following the manufacturer's instructions (Millipore Chemicon, USA) were used for the detection of the hMPV by special personnel. Positive and negative controls (uninfective and infective cells) provided in the kit were performed.

Statistical Analysis

Statistical analyses were conducted using SPSS software (11.5). Differences in frequencies between genders, age, primary disease and seasons were compared. Enumeration data were analysed by Chi-squared test. Odds ratio (OR) and 95% confidence interval (CI) were calculated. A P value less than 0.05 was considered to be significant.

Results

They were 1882 boys and 1029 girls. Their age ranged from 29 days to 14 years with a median age of 10 months, included 1578 cases younger than 1 year, 693 cases aged >1 to 3 years, 389 aged >3 to 6 years, and 251 over 6 years (Table 1).

Among 2911 samples, hMPV were found in 70 with an overall positive rate of 2.40%. Among these 70 hMPV positive patients, 5 had co-infection with other pathogens, including two of RSV, one of PIV 2, one of *Mycoplasma pneumoniae*, and one of *Klebsiella pneumoniae*. The positive rates in boys and girls were 2.55% (48/1882) and 2.14% (22/1029), respectively, without significant difference ($\chi^2 = 0.482$, P=0.487) (Table 1).

In this study, the patients were separated into three groups: (a) group 1: children aged from 2 months to one year (n=1578); (b) group 2: children aged >1 to 3 years (n=693); (c) group 3: older than 3 years (n=640). The total positive number was higher in group 1 with 43, followed by group 2 (23) and group 3 (4). However, the positive rate was highest in group 2 with a rate of 3.32%, followed by group 1 (2.72%) and group 3 (0.63%) with significant

difference among the three groups (χ^2=11.795, P=0.003). The positive rate of hMPV in children over 3 years had just 0.225 (95%CI: 0.080-0.628) folds of that in children younger than one year (Table 1).

The positive number was 57, 5, 5 and 3 in pneumonia, bronchiolitis, asthma with infection and bronchitis, respectively. However, the positive rate was higher in bronchiolitis and asthma with infection, and lower in pneumonia and bronchitis with a significant difference among these 4 groups (χ^2=9.270, P=0.026) as well. The positive rate of hMPV in children with bronchiolitis and asthma with infection had 2.706 (95%CI: 1.057-6.924) and 2.806 (95%CI: 1.095-7.187) folds of that in children with pneumonia (Table 1).

In order to analyse the seasonality of hMPV infection in children, records were maintained throughout one year. The positive rate was significantly different among 4 seasons (χ^2=64.209, P<0.001). It was highest in spring with a rate of 5.87%, followed by winter (3.30%), autumn (0.44%) and summer (0.36%) (Table 1). The hMPV infection increased from winter, dramatically increased in spring, and peaked on March with a rate of 7.47% (21/281). It is notable that no hMPV positive sample was found from July to September (Figure 1).

Table 1 hMPV positive case and rate among patients with different groups

		Number	Positive (%)	OR (95%CI)	χ^2	P value
Total		2911	70 (2.40)			
Gender	Boys	1882	48 (2.55)	1		
	Girls	1029	22 (2.14)	0.835 (0.501-1.391)	0.482	0.487
Age	0-1 (year)	1578	43 (2.72)	1		
	>1-3 (year)	693	23 (3.32)	1.225 (0.733-2.050)		
	>3 (year)	640	4 (0.63)	0.225 (0.080-0.628)	11.795	0.003
Diagnosis	Pneumonia	2648	57 (2.15)	1	9.270	0.026
	Bronchiolitis	89	5 (5.61)	2.706 (1.057-6.924)		
	Asthma with infection	86	5 (5.81)	2.806 (1.095-7.187)		
	Bronchitis	88	3 (3.41)	1.604 (0.493-5.226)		
Season	Winter (December-February)	698	23 (3.30)	1	64.209	<0.001
	Spring (March-May)	698	41 (5.87)	1.831 (1.087-3.086)		
	Summer (June-August)	828	3 (0.36)	0.107 (0.032-0.357)		
	Autumn (September-November)	687	3 (0.44)	0.129 (0.038-0.431)		

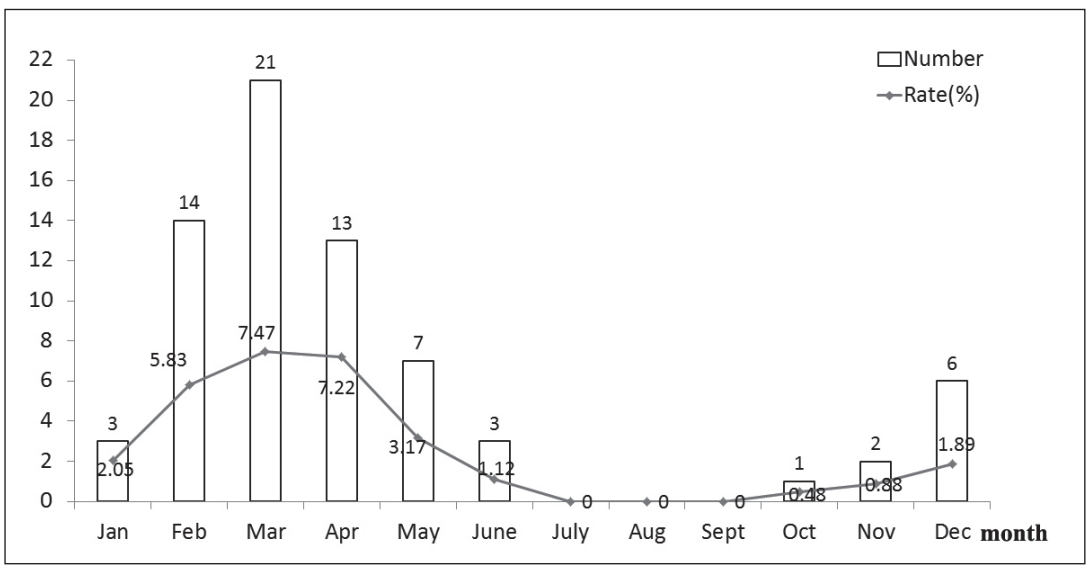

Figure 1 The positive cases and rate of hMPV in nasopharyngeal aspiration from children with acute lower respiratory tract infection among 12 months. Both of they increased from winter and peaked in spring.

Discussion

Since hMPV was first isolated from children with ALRIs in 2001, more and more researchers have focused on this virus. Genetic analysis has revealed two major groups (A and B) and four minor subgroups (A1, A2, B1 and B2), mainly based on the sequence variability of the attachment (G) and fusion (F) surface glycoproteins.[22] The clinical disease spectrum caused by hMPV varies from upper respiratory tract infection to pneumonia, even acute encephalitis in children and adults.[23] Although several studies investigated the prevalence and clinical features of hMPV in China,[15-18,24] most were not consecutive, and have geographic differences. Similar study from Zhejiang province was not reported. In this study, we focus on hMPV infection in hospitalised children with ALRIs in Hangzhou, the capital of Zhejiang province.

Among these 2911 paediatric patients, the positive rate of hMPV infection in ALRIs was 2.40%. It was lower than that of RSV (23.6%) and PIV 3 (4.3%), but higher than that of Flu A (2.0%), ADV (1.7%), PIV I (0.6%), Flu B (0.2%) and PIV 2 (0.1%) that we investigated from 2001 to 2006.[3] This high positive rate indicated that hMPV was an important pathogen in childhood ALRIs, especially in young children (<3 years) in our area. These positive rate of hMPV was similar with that reported in Beijing and Indian paediatric patients, which showed a positive rate of 3.46% and 3% in hospitalised children with acute respiratory infections.[19,25] Williams et al also reported a similar positive rate (3.8%) among children <5 years hospitalised with ARI or fever in two U.S. counties and estimated an overall annual rate of hMPV-associated hospitalisations of 1.2 per 1000 children <5 years.[26] However, higher positive rates were reported from Korean (7.1%),[12] Suzhou (6.6%),[18] and Tianjin (6.5%) in paediatric patients hospitalised for acute respiratory infections.[17] These difference might be associated the differences of population, age, samples size, season of the sample collection, geographic locales, sampling technique or even detection methods.

Several studies reported that males were more susceptible to hMPV infection.[17,27] However, no significant difference of prevalence was noted between different genders in our series, which was similar with Hara report.[28] Moreover, we noted that the hMPV infections seem to occur at a young age. More than half hMPV infection children (43/70) aged younger than one year while the positive rate was high in children aged from 0 to 3 years with a rate of about 3%. This characteristic of age distribution was similar to RSV infection in children,[3] and may be associated the fact that both hMPV and RSV belongs to the *Pneumovirinae* subfamily of the family *Paramyxoviridae*. It was also reported that hMPV infection was more common in older adults.[24] The high positive rate of hMPV in younger children and old adults might be caused by their immune status. Although the outcome was well in these paediatric patients, it was reported that the infection can turn out to be more severe in older or immunocompromised patients.

hMPV preferentially targets ciliated epithelial cells of the human respiratory tract. The clinical manifestations of these patients with hMPV infection were analysed in our series. Although most hMPV positive patients was found in children with acute pneumonia, the higher positive rate was found in patients with bronchiolitis and asthma with infection. This suggested that hMPV infection was related to wheezing, asthma exacerbations or bronchiolitis, as previous study described.[11] In younger children with wheezing, asthma exacerbations or bronchiolitis, especially on spring, hMPV infection should be considered.

Previous studies showed that some viral infections were associated with climate. It was reported that the outbreaks hMPV infections were common during winter-spring season in most areas of the world.[18,25,29] Hangzhou is a temperate region with four distinct seasons. In our series, the hMPV infections increased from winter, but peaked during the spring. It was similar with other research reports of the temperate zone,[10] but differ from several reported in some areas (e.g. no significant seasonal distribution in Tianjin, prominent from March through May in US.).[17,26] These differences might be associated with the factors of climate (e.g. temperature, moist, sunshine, demography) or some other factors (e.g. economy and genetic predisposition). Further study about these factors is required.

The limitation of this study was the fact that only inpatient in a single hospital with ALRTI was included. Therefore, the prevalence of hMPV in these children may underestimate its prevalence in the community. The present results cannot be extrapolated to the entire population of Chinese children. Moreover, we investigated only one year, and cannot find whether this virus outbreak each year or every several years. Despite this limitation, this study confirms the aetiologic role of hMPV in respiratory infections among children in our area. A widescale community-based study is recommended for further characterisation of its epidemiology, genotype, and risk factor for infections.

In summary, hMPV is an important viral agent for

ALRTI in children in Hangzhou area. Moreover, it is most common in young children and spring in Hangzhou area. This information may be helpful in the future for the development of prophylactic and control measures, and clinical therapy. A widescale community-based study is recommended for further characterisation of its epidemiology, genotype, and risk factor for infections.

Acknowledgments

We thank all the patients and their parents for participating in this research project. This work is supported, in part, by grants from the National Natural Science Foundation of China (81170016 and 81070008), Zhejiang Provincial Natural Science Foundation of China (Y2100337), and Zhejiang Health Department (2009B098).

Declaration of Conflicts of Interest

There are no competing interests.

References

1. Garbino J, Gerbase MW, Wunderli W, et al. Respiratory viruses and severe lower respiratory tract complications in hospitalized patients. Chest 2004;125:1033-9.
2. Kim MR, Lee HR, Lee GM. Epidemiology of acute viral respiratory tract infections in Korean children. J Infect 2000; 41:152-8.
3. Tang LF, Wang TL, Tang HF, Chen ZM. Viral pathogens of acute lower respiratory tract infection in China. Indian Pediatr 2008;45:971-5.
4. Yu ZS, Zou CC, Zheng JY, Zhao ZY. Cytomegalovirus gB genotype and clinical features in Chinese infants with congenital infections. Intervirology 2006;49:281-5.
5. Gao LW, Xie ZD, Liu YY, Wang Y, Shen KL. Epidemiologic and clinical characteristics of infectious mononucleosis associated with Epstein-Barr virus infection in children in Beijing, China. World J Pediatr 2011;7:45-9.
6. Zeng M, Zhu QR, Wang XH, Yu H, Shen J. Human bocavirus in children with respiratory tract infection in Shanghai: a retrospective study. World J Pediatr 2010;6:65-70.
7. Don M, Fasoli L, Paldanius M, et al. Aetiology of community-acquired pneumonia: serological results of a paediatric survey. Scand J Infect Dis 2005;37:806-12.
8. Cevey-Macherel M, Galetto-Lacour A, Gervaix A, et al. Etiology of community-acquired pneumonia in hospitalized children based on WHO clinical guidelines. Eur J Pediatr 2009; 168:1429-36.
9. Michelow IC, Olsen K, Lozano J, et al. Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children. Pediatrics 2004;113:701-7.
10. van den Hoogen BG, de Jong JC, Groen J, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med 2001;7:719-24.
11. Yahia S, Kandeel AY, Hammad E, El-Gilany AH. Human Metapneumovirus (hMPV) in Acute Respiratory Infection: A Clinic-Based Study in Egypt. Indian J Pediatr 2012. DOI: 10.1007/s12098-011-0677-5. [Epub ahead of print]
12. Kim HR, Cho AR, Lee MK, Yun SW, Kim TH. Genotype variability and clinical features of human metapneumovirus isolated from Korean children, 2007 to 2010. J Mol Diagn 2012; 14:61-4.
13. Banerjee S, Sullender WM, Ahuja RK, Broor S. Seroepidemiological study of human metapneumovirus in New Delhi, India. Indian J Med Microbiol 2011;29:363-7.
14. Wong CK, Lai V, Wong YC. Comparison of initial high resolution computed tomography features in viral pneumonia between metapneumovirus infection and severe acute respiratory syndrome. Eur J Radiol 2012; 81:1083-7.
15. Zhang C, Du LN, Zhang ZY, et al. Detection and Genetic Diversity of Human Metapneumovirus in Hospitalized Children with Acute Respiratory Infections in Southwest China. J Clin Microbiol 2012;50:2714-9.
16. Chen X, Zhang ZY, Zhao Y, Liu EM, Zhao XD. Acute lower respiratory tract infections by human metapneumovirus in children in Southwest China: a 2-year study. Pediatr Pulmonol 2010; 45:824-31.
17. Li XY, Chen JY, Kong M, et al. Prevalence of human metapneumovirus in hospitalized children with respiratory tract infections in Tianjin, China. Arch Virol 2009;154:1831-6.
18. Ji W, Wang Y, Chen Z, Shao X, Ji Z, Xu J. Human metapneumovirus in children with acute respiratory tract infections in Suzhou, China 2005-2006. Scand J Infect Dis 2009; 41:735-44.
19. Zhu RN, Qian Y, Zhao LQ, et al. Characterization of human metapneumovirus from pediatric patients with acute respiratory infections in a 4-year period in Beijing, China. Chin Med J (Engl) 2011;124:1623-8.
20. Xue XD, Du LZ (Eds.). Pediatrics. 1 edition. Beijing: People's Health Publishing House; 2005 [In Chinese].
21. National Pediatric Asthma Collaborative Group. [Children's Asthma Prevention Conventional (trial)]. Chin J Pediatr 1998; 36:747-51.
22. van den Hoogen BG, Herfst S, Sprong L, et al. Antigenic and genetic variability of human metapneumoviruses. Emerg Infect Dis 2004;10:658-66.
23. Sanchez Fernandez I, Rebollo Polo M, Munoz-Almagro C, Monfort Carretero L, Fernandez Urena S, Rueda Munoz A, Colome Roura R, Perez Duenas B: Human Metapneumovirus in the Cerebrospinal Fluid of a Patient With Acute Encephalitis. Arch Neurol 2012;69:649-52.
24. Li J, Wang Z, Gonzalez R, et al. Prevalence of human metapneumovirus in adults with acute respiratory tract infection in Beijing, China. J Infect 2012;64:96-103.
25. Banerjee S, Sullender WM, Choudekar A, et al. Detection and genetic diversity of human metapneumovirus in hospitalized children with acute respiratory infections in India. J Med Virol

2011; 83:1799-810.
26. Williams JV, Edwards KM, Weinberg GA, et al. Population-based incidence of human metapneumovirus infection among hospitalized children. J Infect Dis 2010;201:1890-8.
27. Samransamruajkit R, Thanasugarn W, Prapphal N, Theamboonlers A, Poovorawan Y. Human metapneumovirus in infants and young children in Thailand with lower respiratory tract infections; molecular characteristics and clinical presentations. J Infect 2006;52:254-63.
28. Hara M, Takao S, Fukuda S, Shimazu Y, Miyazaki K. Human metapneumovirus infection in febrile children with lower respiratory diseases in primary care settings in Hiroshima, Japan. Jpn J Infect Dis 2008;61:500-2.
29. Al-Turab M, Chehadeh W, Al-Mulla F, Al-Nakib W. Human metapneumovirus in patients with respiratory tract infection in Kuwait. J Med Virol 2011;83:1811-7.