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ORIGINAL ARTICLE

Seasonal distribution and epidemiological characteristics of human metapneumovirus infections in pediatric inpatients in Southeast China

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Abstract Human metapneumovirus (hMPV) is an important respiratory pathogen in young children whose seasonal activity varies substantially from year to year among different populations. This study was conducted to investigate if there was a seasonal variation in the incidence of hMPV infection in young children and possible associations between hMPV infection and local meteorological parameters in Suzhou, China. A total of 6,655 children with acute respiratory tract infection (ARTI) admitted to the Children's Hospital affiliated to Soochow University, Suzhou, were tested from January 2006 to December 2009 for the presence of hMPV using reverse-transcription polymerase chain reaction. The relationship between the presence of the virus and regional meteorological conditions was analyzed by linear and multivariate regression analysis. The overall hMPV infection incidence over the four-year study was 8.2 %, 8.1 %, 12.7 % and 7.4 % per year, respectively. Four hundred eighty-eight hMPV-positive children (78.2 %) were younger than 3 years of age. hMPV infections appear to have a seasonal distribution in Suzhou. In 2006, 2007 and 2009, the peak seasons were in December to January, while in 2008, the peak of hMPV activity occurred in May. The incidence of hMPV infection was negatively correlated with the average monthly temperature and rainfall. hMPV was one of the most common viral pathogens after respiratory syncytial virus that was associated with acute respiratory tract infection in children in Suzhou. hMPV infection occurred throughout the year with peaks during late winter and early spring. Climatic factors, especially monthly average temperature, may affect the prevalence of hMPV in Suzhou.

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Introduction

Acute respiratory tract infection (ARTI) is a major cause of morbidity and mortality worldwide, especially for infants and young children [1]. A variety of viruses, including influenza viruses (Inf), respiratory syncytial virus (RSV), parainfluenza virus (PIV), adenovirus (ADV), rhinovirus, and coronavirus have all been associated with ARTI in children. In 2001, a novel respiratory virus called human metapneumovirus (hMPV) was first identified in the Netherlands and later in other European countries, North America and Asia [2–5]. hMPV is a negative single-stranded RNA virus and a major cause of upper (URI) and lower respiratory illness (LRI) in all age groups including children, immunocompromised individuals, and the elderly. Children under 3 years of age are particularly susceptible to infections caused by hMPV [6–8]. Global hMPV activity varies substantially from year to year among different populations and regions, with the majority of hMPV infections occurring in either winter or spring [9]. A study conducted in the Netherlands demonstrated that the peak of an hMPV epidemic occurred in February, with the season beginning in November and ending in May [10]. Falsey and colleagues found that the incidence of hMPV infection in New York was highest from December to March [11]. In contrast, hMPV infection in Hong Kong, a subtropical region, is quite high during spring and early

summer [12]. Another study performed in Italy showed that the epidemic peak occurred in February during one season, and in March during a second [13].

Meteorological conditions such as temperature and relative humidity have recently been associated with the prevalence of respiratory syncytial virus (RSV) [14]. Despite this finding, the relationship between hMPV annual epidemics and meteorological factors is still unclear. Suzhou, located in the temperate southeast of China, has a subtropical maritime monsoon climate with a high prevalence of respiratory infections during the winter and spring seasons. In this study, we determined the prevalence and clinical characteristics of hMPV infection in infants and young children hospitalized with ARTI from January 2006 to December 2009 in order to investigate hMPV epidemic characteristics and correlate them with meteorological factors in a tropical environment.

Materials and methods

Study group

From January 2006 to December 2009, a total of 6,655 inpatients diagnosed with acute respiratory illness at the Department of Respiratory Medicine at the Children's Hospital Affiliated to Soochow University, were enrolled in this study. Children from infancy to 10 years of age were eligible for this study. On admission, the medical history and physical characteristics of the children were recorded systematically. Among the subjects, 4,059 patients (61 %) were male and 2,596 (39 %) patients were female, with a male-to-female ratio of 1.56:1. This study was approved by the Ethical Committee of Soochow University. Written informed consent was obtained from the parents of all participating children.

Sample collection

Nasal aspirate samples (2 ml) were obtained from each patient (all patients had respiratory symptoms) within 24 h of admission using a sterile disposable catheter, which was introduced into the lower part of the pharynx via the nasal cavity passage. Nasal aspirate samples were centrifuged and were stored at −80 °C until tested. Collected samples were divided into two aliquots for the detection of seven common viruses (RSV, adenovirus, influenza viruses A and B, and parainfluenza viruses 1, 2 and 3) and hMPV, respectively. Blood samples were collected for the counting of leukocytes and the C-reactive protein (CRP) test. The clinical characteristics of enrolled patients were obtained from their hospital files.

Detection of seven common viruses by direct immunofluorescence assay

Nasopharyngeal aspirates from patients were tested for seven common respiratory viruses (RSV, adenovirus, influenza viruses A and B, and parainfluenza viruses 1, 2 and 3) using a direct immunofluorescence assay as described previously [15]. Briefly, antigen detection was carried out using a Light Diagnostics Respiratory Panel I Viral Screening and Identification Kit (Chemicon International Inc., Temecula, CA, USA) according to the manufacturer's instructions. Slides were examined by fluorescence microscopy (Leica 020-518.500, Germany).

Detection of the hMPV gene by RT-PCR

Primer design

Primers were designed using sequence information from the N gene sequence available from the GenBank database (accession number AF371337). To detect the hMPV gene, primer pairs were designed to specifically amplify a 213-bp fragment of the N gene (nucleotide position 562-774). Primers were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., using the following sequences: sense, 5'-AACCGTGTACTAAGTG ATGCACTC-3'; antisense, 5'-CATTGTTTGACCGGCC CCATAA-3'.

RT-PCR of hMPV RNA

Viral RNA was extracted from the nasal aspirate samples using the TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. Reverse transcription (RT) reactions were performed with M-MLV reverse transcriptase (Promega, USA) and random hexamers at 37 °C for 60 min for cDNA synthesis according to the manufacturer's specifications. PCRs were performed in a volume of 25 µl containing 6.5 µl of ddH_2O, 10 µl of cDNA, 2.5 µl of 10x reaction buffer, 3 µl of 25 mM $MgSO_4$, 0.5 µl of dNTP (10 mM), 0.5 µl of Taq polymerase (5 U/µl) and 1 µl of each primer (10 µM). The PCR conditions were as follows: denaturation at 95 °C for 5 min, then 45 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 68 °C for 30 s, followed by a final extension at 68 °C for 7 min.

Sequencing

The PCR products were separated by electrophoresis on 1.5% (w/v) agarose gels and visualized by ethidium bromide staining. hMPV-positive samples resulted in the production of a 213-bp amplicon. Ten positive amplicons

were selected randomly and purified from the agarose gels and then sequenced directly. Gene sequencing was performed by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., China, using an ABI PRISM 310 Genetic Analyzer and the sense and antisense primers as described above. The sequences obtained were then compared with hMPV sequences available from GenBank using the Chromas software (http://www.technelysium.com.au/chromas.html).

Meteorological data collection

Meteorological data, including average monthly temperature (°C), average monthly humidity (%), total rainfall (mm) and total bright sunshine (h), were provided by the Meteorological Bureau of Suzhou. The weather observation station is located at longitude 120°.6′ east, latitude 31°.3′ north.

Statistical analysis

Statistical analysis was performed using SPSS version 10.0 software (SPSS, Inc., Chicago, IL, USA). The enumeration data were compared using the chi-square test, and a normality and homogeneity of variance test was performed on the measurement data. Normally distributed data were compared by the Pearson correlation analysis, and other data were compared by the Spearman non-parametric tests. Univariate regression analysis with the Pearson correlation coefficient was used to analyse the relationship between hMPV incidence and meteorological factors, whereas independent associations were analysed by multiple linear regression using the forward stepwise method. All tests were two-tailed, and p-values less than 0.05 were considered statistically significant.

Results

Virus presence in clinical specimens

Between January 2006 and December 2009, a total of 6,655 specimens were collected, 2,142 (32.2 %) of which were positive for at least one virus (18 specimens were positive for two viruses). Positive rates of respiratory viruses by year were 28.8 % (475/1646) in 2006, 38.4 % (647/1682) in 2007, 35.7 % (516/1444) in 2008 and 26.7 % (504/1883) in 2009, respectively. Among 6,655 clinical specimens the most commonly detected virus was RSV (15.7 %, 1048/6655), followed by hMPV (8.9 %, 596/6655), Pinf-3 (2.7 %, 183/6655), Inf-A (2.4 %, 161/6655), ADV (1.3 %, 89/6655), Pinf-1 (0.4 %, 29/6655), Inf-B (0.37 %, 25/6655) and Pinf-2 (0.16 %, 11/6655). Positive rates of hMPV detection were 8.2 % (135/1646) in 2006, 8.1 % (137/1682) in 2007, 12.7 % (184/1444) in 2008, and 7.4 % (140/1883) in 2009, and there was a statistically significant difference among the four years, i.e., the detection rate in 2008 was higher than the other three years ($\chi^2 = 33.23$, $p < 0.05$).

Of the 596 hMPV-positive patients, 18 were co-infected with other respiratory viruses, of which RSV was the most common at 61.1 % (11/18), followed by Pinf-1 11.1 % (2/18), Pinf-3 11.1 % (2/18), InfA 11.1 % (2/18) and adenovirus 5.6 % (1/18).

Gender and age distribution of hMPV-infected children

The hMPV infection incidence in male and female patients was 9.4 % (370/3939) and 8.9 % (219/2456), respectively. No gender difference was observed in the incidence of hMPV infection ($p > 0.05$). Of the 596 hMPV-positive patients, the average age was 22.34 ± 10.21 months, with the youngest being 30 days old and the oldest 10 years of age. A total of 466 hMPV-positive children, approximately 78.2 %, were younger than 3 years of age. To analyze the age distribution of hMPV-positive patients precisely, patients were grouped by age as follows: <6 months, 6-12 months, 12–36 months, 36–60 months, and ≥60 months (Table 1). The hMPV infection rate was highest among the 12–36 months group (33.6 %) and lowest among the ≥60 months group (6.4 %). This observed difference in hMPV prevalence by age was statistically significant ($p < 0.001$).

Clinical characteristics of hMPV infection

The clinical findings of hMPV infection were analyzed in 596 children. Thirty (5 %) were diagnosed with upper respiratory tract infection, 15 (2.5 %) with bronchitis, 121 (20.3 %) with bronchiolitis, 359 (60.2 %) with pneumonia, and 71 (11.9 %) with asthma exacerbation. Common symptoms included cough (97.7 %), rhinitis (54 %), fever (50.7 %), nasal congestion/runny nose (26 %) and dyspnea (10.9 %). The average leukocyte count in peripheral blood in hMPV-positive children was 8.67 ± 3.12×10^9/L. The mean CRP value was 5.35 ± 2.12 mg/l. Abnormalities identified by chest radiography were found in 566 cases (95.0 %), including 207 (36.6 %) cases with increased and blurred bilateral lung markings, 333 cases (58.8 %) with patchy shadows, and 131 cases (23.2 %) with emphysema. The average hospitalization time was 7.25 ± 1.69 days.

Seasonal distribution of hMPV infection

To determine the seasonal distribution of hMPV, we assessed prevalence for a four-year period from January 2006 to December 2009. hMPV infection was observed in each year of the four-year study. The analysis of

Table 1 Distribution of hMPV infections in children by age

Age (months)	No. children	No. children with hMPV (%)	Percentage of total cases
0-6	1750	123 (7.0 %)	20.6 %
6-12	1530	143 (9.3 %)	24.0 %
12-36	1863	200 (10.7 %)	33.6 %
36-60	866	92 (10.6 %)	15.4 %
≥60	646	38 (5.8 %)	6.4 %
Total	6655	596 (8.9 %)	100 %

seasonality of infection showed a pattern with peaks in the winter and spring (Fig. 1) However, variations in this trend were observed from one year to another. In 2006, two peaks of hMPV activity occurred, one in January (15.6 %) and the other in December (23.8 %). In 2007, hMPV infection was observed from January to March with a peak in January. The highest peak over the four-year study was observed in May 2008 (31.6 %), followed by a lower peak in January 2009 (18.4 %). The hMPV-positive rate for the four seasons (spring, summer, fall, winter) was 11.6 %, 7.6 %, 4.7 % and 11.7 %, respectively, and the incidence rates of hMPV infection in winter and spring was significantly higher than those in summer and autumn ($\chi^2 = 74.67$, $p < 0.001$).

hMPV infection and correlation with meteorological factors

The Suzhou area, with its typical temperate climate, has a monthly average temperature of 16.7 ± 8.7 °C, humidity of 68.9 ± 4.8 %, monthly total rainfall of 85.7 ± 57.7 mm, and monthly total bright sunshine duration of 142.2 ± 51.9 h (Table 2). Regression analysis was performed from the meteorological parameters collected over the 4 years (i.e., 2006 to 2009) in which this study took place. Univariate analysis showed that the incidence of hMPV infection was negatively correlated with monthly average temperature ($r = -0.406$, $p = 0.004$), monthly total rainfall ($r = -0.367$, $p = 0.01$), monthly average relative humidity ($r = -0.249$, $p = 0.08$) and monthly total bright sunshine duration ($r = -0.072$, $p = 0.628$). When the associations were further analysed by multivariate analysis, temperature was found to be the only independent associating factor ($p = 0.019$) (Table 3).

Discussion

In this present study, more than 6,655 specimens were collected from pediatric patients with ARTI and tested for hMPV by RT-PCR over a four-year period. hMPV was detected in 8.9 % of the patients and was the second-most common viral pathogen after RSV, confirming that hMPV is also an important viral pathogen causing respiratory infections in children in Suzhou, China. The prevalence of hMPV infection is similar to that found in studies reported from Western European countries, the United States, Canada, South America, Australia and Asia [4, 6, 10, 16–20].

In the present study, pneumonia and bronchiolitis were the most frequent diagnoses associated with hMPV-infected children, which is consistent with previous reports [10, 12, 21]. The main clinical manifestations in the 596 cases with hMPV infection included cough, rhinitis, fever, nasal congestion/runny nose, dyspnea and abnormalities on chest radiographs, including increased and blurred bilateral lung markings and patchy shadows. The clinical symptoms of hMPV infection did not differ very much from those of other respiratory viruses such as RSV, influenza virus and parainfluenza virus [22, 23].

Most studies have shown that the incidence of infection with hMPV is highest among young infants under 2 years of age [24–26]. In our study, the incidence of infection with hMPV among children reached the highest level in infants less than 3 years of age and accounted for 78.2 % of the total cases. Moreover, the incidence peaked at the age of 12-36 months. Our study was therefore consistent with other studies that indicated that young infants are more

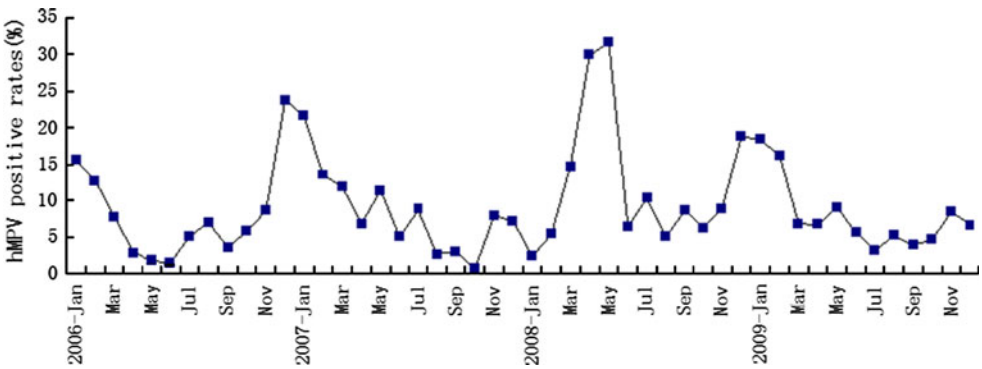

Fig. 1 Monthly distribution of hMPV-positivity rates among children with acute respiratory tract infections from January 2006 to December 2009

Table 2 hMPV incidence and meteorological parameters in Suzhou from January 2006 to December 2009

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2006												
hMPV incidence	15.6	12.7	7.8	2.9	1.8	1.5	5.1	7.0	3.6	5.8	8.6	23.8
Temperature (°C)	5.3	5.5	11.6	16.9	21.2	26.2	29.6	30.3	23.4	21.8	14.8	5.2
Rainfall (mm)	150.9	75.7	37.9	115	100.8	66.8	142.4	86.7	81.8	4.4	132.7	15.1
Relative humidity (%)	73	69	64	67	69	74	75	70	74	72	73	71
Sunshine (h)	90.1	88	172.7	162.8	157.8	153.2	178.5	263	130.4	147.8	102.8	140.8
2007												
hMPV incidence	21.6	13.5	11.8	6.8	11.4	5.0	8.8	2.7	3.1	0.7	7.9	7.2
Temperature (°C)	5	9.4	11.9	16	23.2	25.1	30.3	29.8	24.9	19.9	13.1	8.4
Rainfall (mm)	55.9	64.6	92.7	88.2	61	79	104.1	69.3	169	146.2	17.3	48.7
Relative humidity (%)	72	73	70	63	63	76	70	69	73	68	66	74
Sunshine (h)	101.8	152.3	144.2	187.9	216.4	93	170.6	232.6	127.4	150.9	142.1	83
2008												
hMPV incidence	2.4	5.4	14.8	29.9	31.6	6.5	10.3	5.0	8.7	6.3	8.9	18.8
Temperature (°C)	3.2	3.4	11.5	15.9	21.9	24	30.4	28.3	25.6	20.5	12.7	7.3
Rainfall (mm)	65.3	145.	29.3	19.5	18.5	326.1	61.4	81.2	110.1	65	61.2	27.5
Relative humidity (%)	73	66	63	67	63	76	67	71	71	70	67	59
Sunshine (h)	61	171	179.8	108.4	243.1	76.6	240.7	185.8	153.1	128.1	120.2	153.4
2009												
hMPV incidence	18.4	16.2	6.8	6.7	9.1	5.6	3.2	5.2	3.9	4.7	8.5	6.6
Temperature (°C)	3.5	8.6	10.5	16.6	22.4	26.4	29.1	28.2	25	21.2	10.9	6.0
Rainfall (mm)	51.9	123.7	76.7	79.3	51.9	156.1	210.9	145.3	66.7	4.6	116.2	67.6
Relative humidity (%)	64	76	67	63	56	70	69	76	73	63	73	68
Sunshine (h)	119.1	48.7	119.1	196.6	224.7	159.8	204	133.6	124.8	205.5	94.3	138.5

Table 3 Correlation between hMPV incidence and meteorological factors

Meteorological factors	Univariate regression analysis*		Multivariate regression analysis*	
	Pearson coefficient	*p*-value	Standardized beta coefficient	*p*-value
Temperature (°C)[#]	−0.406	0.004[※]	−0.330	0.019[※]
Rainfall (mm)[&]	−0.367	0.01[※]	−0.288	0.059
Relative humidity (%)[#]	−0.249	0.08	−0.094	0.532
Sunshine (h)[&]	−0.072	0.628	0.078	0.656

* hMPV monthly incidence as dependent variable

[#] Mean daily measurement of the month

[&] Total measurement of the month

[※] $p<0.05$

susceptible to hMPV [24, 25, 27]. A less well developed and efficient immune response, the small size of the conducting airways and the incomplete development of the lung structure may explain why infants have a higher incidence of hMPV infection [28]. Several studies have shown a strong association between HMPV infection and wheezing illness [4, 29, 30]. Conversely, Rawlinson et al. took the opposite view, suggesting that asthma attacks in children are related to human rhinovirus rather than to hMPV [31]. In the present study, 47.9 % of cases of hMPV infection were accompanied by wheezing, and the majority of wheezing patients were infants, indicating that hMPV infection may play a part in childhood asthma or, more likely, that wheezing may be the early manifestation of an asthma attack.

A number of previous studies have reported that respiratory syncytial virus is a principal cause of co-infection with hMPV, presumably because the seasonal distribution of hMPV and RSV appears to overlap [32]. Reported prevalences of co-infections with other respiratory viruses

vary from 5 % to 30 % [33–35]. In this study, the hMPV/RSV co-infection rate was at a lower level (3.9 %), which is inconsistent with these previous studies. In this four-year consecutive study, we found that hMPV infections occur throughout the year but that seasonal hMPV activity varied substantially from year to year. The overall hMPV infection incidence of the four years among hospitalized children with ARTI was 8.2 %, 8.1 %, 12.7 % and 7.4 % respectively, and the incidence rates of hMPV infection were statistically significant across the four-year period ($\chi^2 = 74.67$, $p < 0.001$). This result is consistent with a previous study in Italy in which the incidence of hMPV infection also varied over a three-year study period, with the rates of positivity for hMPV recorded at 7 % in 2001 but 37 % and 43 % in 2000 and 2002, respectively [13]. Moreover, Falsey also reported that the hMPV infection rate was 1.5 % in 2000 in the USA, significantly lower than the rate of 7 % in 2001 [36].

It has been described in earlier studies that hMPV infections seem to have a seasonal distribution. In Hong Kong, hMPV outbreaks occurred during the spring/summer seasons in 2001–2002 [12]. In contrast, Døllner and colleagues reported that the hMPV infection rate of children who were hospitalized in Norway was 21 % during the 2002 to 2003 winter season, with a rate of 57 % during November-December alone; this is the highest rate ever reported [37]. Other reports suggest that hMPV may also be circulating throughout the year [38]. Data from the present study show that hMPV infections seem to have a seasonal distribution in Suzhou. In 2006, 2007 and 2009, the peak seasons were in December-January, indicating that hMPV was prevalent in winter. However, in 2008, the peak of hMPV activity occurred in May, indicating that hMPV was prevalent in spring. Epidemiological observations in Austria, Croatia and Switzerland have suggested that hMPV activity appears to follow a cycle of variation similar to previous findings with respiratory syncytial virus (RSV) [19, 39, 40]. Aberle et al. reported that hMPV activity was characterized by a biennial pattern of alternating winter and spring activity, with spring activity occurring every second year over a 7-year study period in the temperate climatic zone of Austria [19]. We speculate that hMPV activity also followed a pattern of biannual variation in Suzhou, similar to that observed by Aberle et al [19]. However, surveillance over more years is required in order to provide further evidence that hMPV follows a biannual variation pattern in our region.

In our study, the monthly average rainfall during April and May 2008 was significantly reduced compared to the same period (with a similar monthly average temperature) in 2006, 2007 and 2009. Interestingly, hMPV infection during April and May 2008 was more prevalent (29.9 % and 31.6 %) compared to the same period of the other 3 years, indicating that monthly total rainfall may play an important role and affect transmission of hMPV among children. This is despite detection of low correlations between hMPV positive rates and total monthly rainfall. To our knowledge, this is the first study demonstrating a correlation between hMPV annual epidemics and meteorological factors. hMPV activity showed a negative correlation with the monthly average temperature, but no correlation with total rainfall, monthly mean relative humidity or total bright sunshine duration. The monthly average temperature was the factor most closely correlated with the number of cases of hMPV in Suzhou, suggesting that low temperature plays a more important role than average rainfall during these seasons. A limited amount of literature is available on hMPV transmission and the role of temperature [41]. However, more-thorough investigations on transmission and temperature associations have been undertaken for influenza, a typical winter disease; Yusuf et al. [42] found that respiratory syncytial virus (RSV) transmission is temperature dependent and provided direct experimental evidence to support the role of weather conditions in the dynamics of RSV transmission. They found increased viral shedding and enhanced transmission at 5 °C compared with 20 °C. This may be a result of the increased stability of the virus in secretions in the colder environment [43].

Finally, there are two areas in our study that require further investigation. Firstly, surveillance was only performed in patients with infections severe enough to be hospitalized, and so prospective surveillance is required to monitor the epidemiology of hMPV infections in the community as a whole. Secondly, year-long active surveillance studies in consecutive years and in different geographic regions are required in order to better define the correlation between annual epidemics of hMPV and meteorological factors.

In conclusion, our 4-year study has demonstrated that hMPV was one of the most common virus in respiratory infections associated with ARI in young children in Suzhou, China. hMPV infections occurred throughout the year, with infection peaks during the late winter and early spring. Children aged less than 3 years were more susceptible to hMPV. Our data strongly suggest that the monthly average temperature is an important predictor of hMPV activity in this region of China, and this should be investigated further.

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Conflict of interest The authors declare no conflict of interest associated with this article.

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