Vital Surveillances

Seasonal and Genetic Characteristics of Human Metapneumovirus Circulating — Henan Province, China, 2017–2023

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ABSTRACT

Introduction: This study examines the seasonal and genetic characteristics of human metapneumovirus (HMPV) in Henan from 2017 to 2023.

Methods: Samples from patients with acute respiratory infection (ARI) testing positive for HMPV were subjected to real-time reverse transcription polymerase chain reaction The G gene was amplified and sequenced from these samples for epidemiological and phylogenetic analysis.

Results: We enrolled 2,707 ARI patients from October 2017 to March 2023, finding an HMPV positivity rate of 6.17% (167/2,707). Children under five exhibited the highest infection rate at 7.78% (138/1,774). The 2018 and 2019 HMPV outbreaks predominantly occurred in spring (March to May), with peak positivity rates of 31.11% in May 2018 and 19.57% in May 2019. A notable increase occurred in November 2020, when positivity reached a historic high of 42.11%, continuing until January 2021. From February 2021 through March 2023, no significant seasonal peaks were observed, with rates ranging from 0% to 8.70%. Out of 81 G gene sequences analyzed, 46.91% (38/81) were identified as subtype A (A2c: 45.67%, 37/81; A2b: 1.23%, 1/81) and 53.09% (43/81) as subtype B (B1: 9.88%, 8/81; B2: 43.21%, 35/81). Notably, an AAABBA switch pattern was observed in HMPV subtypes. The dominant strains were $A2c_{111nt\text{-}dup}$ in subtype A and B2 in subtype B.

Conclusions: Six years of surveillance in Henan Province has detailed the seasonal and genetic dynamics of HMPV, contributing valuable insights for the control and prevention of HMPV infections in China. These findings support the development of targeted HMPV vaccines and immunization strategies.

Human metapneumovirus (HMPV) is a prevalent pathogen responsible for acute respiratory infections (ARIs) across various demographic groups, including children, adults, the elderly, and immunocompromised individuals (*1*). In 2018, it was estimated that around 11.1 million cases of acute lower respiratory infections globally were attributable to HMPV, leading to approximately 502,000 hospitalizations and 113,000 fatalities (*2*). The most frequently observed clinical manifestations of HMPV infection entail infections of both the upper and lower respiratory tracts (*3*).

HMPV is a single-stranded, negative-sense, nonsegmented RNA virus (*4*). In 2016, HMPV was reclassified into the *Metapneumovirus* genus within the family *Pneumoviridae* (*5*). Its genome comprises eight genes that encode nine proteins. F gene and G gene code for the fusion protein (F protein) and the attachment glycoprotein (G protein), respectively, which are primary targets for the molecular typing of HMPV. Based on the antigenic properties of the G or F proteins, HMPV is categorized into subtypes A and B, further divided into six genotypes: A1, A2a, A2b, A2c, B1, and B2 (*6*). Notably, HMPV strains exhibiting 180 and 111 nucleotide duplications (nt-dup) in the G gene have been identified in Japan since 2014 and in Spain since 2017, respectively (*7*–*8*). Phylogenetic analyses have placed these variants within the A2c genotype, designating them as $A2c_{180nt\text{-}dup}$ and $A2c_{111nt\text{-}dup}$ strains. Given that the G gene is the most variable region of the HMPV genome and prone to large nt-dup, it is particularly useful for genotyping efforts. In China, the $A2c_{111nt\text{-}dup}$ strains have been detected in Guangdong, Beijing, and Shandong provinces, suggesting their potential emergence as dominant strains (*9*).

Following the COVID-19 pandemic, global reports have indicated seasonal variations in the epidemiology of influenza virus and human respiratory syncytial virus (HRSV) infections (*10*). In the United States, the early 2023 detection rates for HMPV rose from 7% to between 10% and 19% (*11*). However, the epidemiological understanding and genetic profiling of

HMPV in China remain underexplored, particularly post-COVID-19. Henan, a populous province, has maintained ongoing surveillance for influenza-like illness (ILI) since 2010 and severe acute respiratory infection (SARI) since 2015. These surveillance efforts provide a unique opportunity to examine the prevalence and genetic attributes of HMPV. Consequently, this study leveraged the ILI and SARI surveillance data from Henan to assess the epidemiological and genetic trends of HMPV, aiming to enhance HMPV infection control measures in China and support the development of relevant vaccines and immunization policies.

METHODS

Case Definition and Sources

ILI and SARI cases were recruited from Luohe Central Hospital, a national ILI and SARI surveillance sentinel hospital in Henan, from October 2017 to March 2023. In accordance with WHO guidelines, ILI cases were defined as "an acute respiratory illness with a measured temperature of ≥38 °C and cough, with onset within the last 10 days," while SARI cases were defined as "an acute respiratory illness with a history of fever or measured fever of ≥38 °C and cough, with onset within the last 10 days that necessitates hospitalization" (*12*). Standardized forms were utilized to gather epidemiological and clinical data from enrolled patients, and throat swab samples were collected for analysis.

HMPV Identification and G Gene Sequencing

After extracting nucleic acids from patients diagnosed with ARI, we carried out viral detection using multiplex real-time reverse transcription polymerase chain reaction (qRT−PCR) (Kinghawk, Beijing, China). This method successfully identified nine viruses, including HMPV (*13*). We then detailed the epidemiological and clinical features of patients who tested positive for HMPV. Subsequently, the full-length G genes of HMPV (ranging from 654 to 867 base pairs) were amplified using RT-PCR, following methodologies previously described (*13*). Amplicons that tested positive were subjected to Sanger sequencing. The sequences were then assembled using Sequencher software version 5.4.5 (GeneCode, Ann Arbor, Michigan, USA) to derive the complete sequences of the HMPV G genes.

Bioinformatics Analysis

The phylogenetic analysis of the HMPV G gene was conducted using MEGA software version 7.0 (Mega Ltd., Auckland, New Zealand) (*14*). This analysis incorporated sequences derived from this study and representative HMPV sequences downloaded from GenBank. The maximum likelihood method was utilized to construct the phylogenetic tree. To test the reliability of tree topologies, bootstrapping with 1000 replicates was performed. Evolutionary distances were calculated using the Kimura 2-parameter method. Additionally, the Chiplot online tool was employed for phylogenetic tree construction (*15*). Genetic distances within and between groups were also computed using MEGA software.

Statistical Analysis

The IBM SPSS software, version 22.0 (IBM Corp., Armonk, N.Y., USA), was employed for statistical analysis. The Chi-square test was utilized to assess the differences in positive rates across various cases, sexes, age groups, and months. Two-tailed p-values less than 0.05 were considered statistically significant.

RESULTS

Clinical Characteristics of HMPV Patients

In this study, a total of 2,707 patients with ARIs were included, consisting of 2,201 with SARIs and 506 with ILIs. Through qRT-PCR analysis, 167 samples (6.17%) tested positive for HMPV. The prevalence of HMPV was higher in patients with ILIs (9.09%, 46/506) compared to those with SARIs (5.50%, 121/2,201) (χ^2=9.177, P=0.002). The infection rate was also greater in females (7.24%, 84/1,160) than in males (5.37%, 83/1,547) (χ^2=4.031, P=0.045) (Table 1). The age of participants ranged from 5 months to 17 years, with the highest percentage of HMPV positivity (7.78%, 138/1,774) observed in children under 5 years old (χ^2=26.742, P<0.001). Among this age group, the lowest positivity rate (3.19%, 6/188) was found in children under 1 year old (χ^2=9.54, P=0.049) (Table 1).

Among the HMPV-positive patients, 72.46% (121/167) had single infections, while 27.54% (46/167) were co-infected with one to three other respiratory viruses, such as HRSV (4.79%, 8/167), rhinovirus (4.79%, 8/167), and influenza virus (4.19%, 7/167) (Supplementary Table S1, available at https://weekly.chinacdc.cn/). The predominant clinical

TABLE 1. Characteristics of the population comprising enrolled patients with ARIs and those testing positive for HMPV.

Group	Frequency of enrolled patients (*n*=2,707)	Frequency of HMPV-positive patients (%) (*n*=167)	χ^2	*P* value
Case group			9.177	0.002
ILI	506	46 (9.09)		
SARI	2,201	121 (5.05)		
Sex			4.031	0.045
Male	1,547	83 (5.37)		
Female	1,160	84 (7.24)		
Age group			26.742	<0.001
0–4 years	1,774	138 (7.78)*		
5–17 years	634	26 (4.15)		
17–59 years	173	1 (0.58)		
60–96 years	134	2 (1.49)		
Age (<5 years old)			9.540	0.049
<1 year	188	6 (3.19)†		
1 year	634	54 (8.52)		
2 years	278	16 (5.76)		
3 years	433	39 (9.01)		
4 years	241	23 (9.54)		

Abbreviation: HMPV=human metapneumovirus; ILI=influenza-like illness; SARI=severe acute respiratory infection.
* Pairwise comparisons across various age groups revealed that the highest HMPV positivity rate was observed in children aged 0 to 4 years.
† Among children under 5 years old, pairwise comparisons indicated that the HMPV positivity rate was lowest in children younger than 1 year.

symptoms included fever (42.51%, 71/167), cough (41.32%, 69/167), and expectoration (14.37%, 24/167). Furthermore, 50.90% (85/167) of the HMPV-positive patients were diagnosed with pneumonia, and 9.59% (16/167) with bronchitis. Patients with only HMPV infection exhibited a lower incidence of fever (χ^2=5.901, *P*=0.015) and cough (χ^2=7.908, *P*=0.005) compared to those with co-infections (Supplementary Table S2, available at https://weekly.chinacdc.cn/).

Temporal Distribution of HMPV Infection

During the 2018-2019 period, the typical HMPV outbreak season was identified as spring, spanning from March to May (Figure 1). The peak incidence rates for these years were recorded in May, with 31.11% (14/45) in 2018 and 19.57% (9/46) in 2019. However, the pattern shifted significantly beginning in 2020. An atypical surge in HMPV cases occurred from November 2020 to January 2021, peaking in November 2020 with a positivity rate of 42.11% (32/76) (Figure 1). In contrast, HMPV was undetectable from April to December 2021. Furthermore, from January 2022 through March 2023, the HMPV positivity rate remained low, ranging from 0% to 8.11%, and did not exhibit any distinct seasonal peak.

Genetic Characteristics of HMPV

Using HMPV-specific RT-PCR and Sanger sequencing, we successfully extracted 81 G gene sequences of HMPV from 167 HMPV-positive samples. Phylogenetic analysis (Figure 2) revealed that 46.91% (38/81) of these sequences corresponded to subtype HMPV-A, while 53.09% (43/81) were classified as HMPV-B.

According to the phylogenetic analysis, the sequences identified in this study were categorized into four genotypes: A2c (45.67%, 37/81), A2b (1.23%, 1/81), B1 (9.88%, 8/81), and B2 (43.21%, 35/81). Within each genotype, the sequences displayed high homology, exhibiting nucleotide identities ranging from 90.48% to 100%.

Among the A2c strains, 94.59% (35 out of 37) sequences exhibited nucleotide duplications (nt-dup) in the G gene. These were categorized into $A2c_{180nt\text{-}dup}$ (17.14%, 6 out of 35) and $A2c_{111nt\text{-}dup}$ (82.86%, 29 out of 35) variants. Nucleotide identity within the

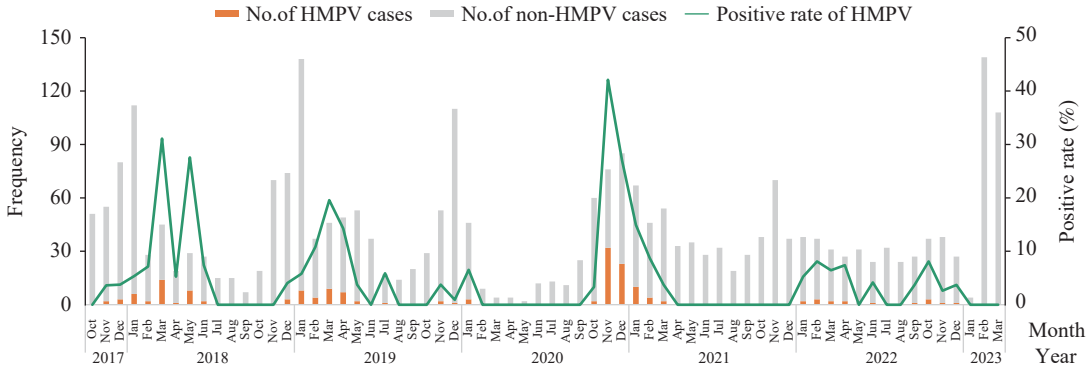

FIGURE 1. Temporal distribution of HMPV cases in Henan Province, China.
Note: "No." represents the number of cases.
Abbreviation: HMPV=human metapneumovirus.

$A2c_{111nt\text{-}dup}$ sequences ranged from 92.61% to 100%, while it spanned from 99.07% to 100% among the $A2c_{180nt\text{-}dup}$ variants.

Circulation Patterns of HMPV Subtypes and Genotypes

HMPV-A represented a significant proportion of cases in 2017 (100%, 1/1), 2018 (80%, 16/20), 2019 (70%, 14/20), and 2022 (87.5%, 7/8). Conversely, HMPV-B dominated in 2020 (100%, 23/23) and 2021 (100%, 9/9) (Figure 3A). These findings suggest that either HMPV-A or HMPV-B, or both, were prevalent in different years, exhibiting a possible subtype switching pattern "AAABBA" from 2017 to 2022 in Henan Province (Figure 3A).

In addition to subtype variations, the genotypes of HMPV also fluctuated over the years (Figure 3B). Two A2c strains, lacking both the 111nt-dup and 180nt-dup variations, were identified exclusively in 2018. Meanwhile, the $A2c_{180nt\text{-}dup}$ variant was observed during 2017–2018. The $A2c_{111nt\text{-}dup}$ variant was first identified in 2018 and subsequently emerged as the prevalent strain of HMPV-A in Henan during 2019 and 2022. The B2 genotype became the predominant strain of HMPV-B in 2020 and 2022 (Figure 3B).

DISCUSSION

To better understand the seasonal and genetic characteristics of HMPV, a retrospective study was carried out in Henan, China, from October 2017 to March 2023. The overall positivity rate for HMPV was 6.17%. The majority of HMPV cases occurred in children under five years of age, aligning with previously documented age distributions and positivity rates (*16*). Among children younger than five, the lowest positivity rate for HMPV was observed in infants under one year of age. However, previous research indicates that this age group exhibits the highest positivity rate for HRSV (*17*). Consequently, the demographic most susceptible to HMPV appears to be slightly older than that for HRSV.

In the Northern Hemisphere, HMPV typically reaches its peak prevalence during the winter and spring months. Similarly, in China, the highest incidence of HMPV occurs from March to May (*18*). However, this study documents an atypical surge of HMPV during the autumn and winter seasons, with the positivity rate escalating to an unprecedented 42.11% in November 2020, compared to previous peaks of 31.11% in May 2018 and 19.57% in May 2019. Similar deviations in HMPV activity and seasonal patterns have been observed in various countries (*19*–*21*). For instance, following an initial decline in cases after the COVID-19 outbreak in 2020, South Korea experienced an off-season epidemic in the autumn of 2022, where HMPV prevalence increased by 2.5 times (*19*). The COVID-19 pandemic led to reduced exposure and transmission of HMPV, weaker maternal antibodies, and subsequently an increased pool of susceptible individuals, potentially contributing to higher than usual HMPV infection rates following the easing of nonpharmaceutical interventions (NPIs). More serological research is essential to determine the underlying factors for these shifts in HMPV prevalence. Additionally, the incidence of ARIs caused by various pathogens, including Mycoplasma pneumoniae, influenza, and HRSV, has also risen in China during the autumn and winter of 2023 (*22*). Continued surveillance of HMPV is critical to further understand these seasonal changes and their

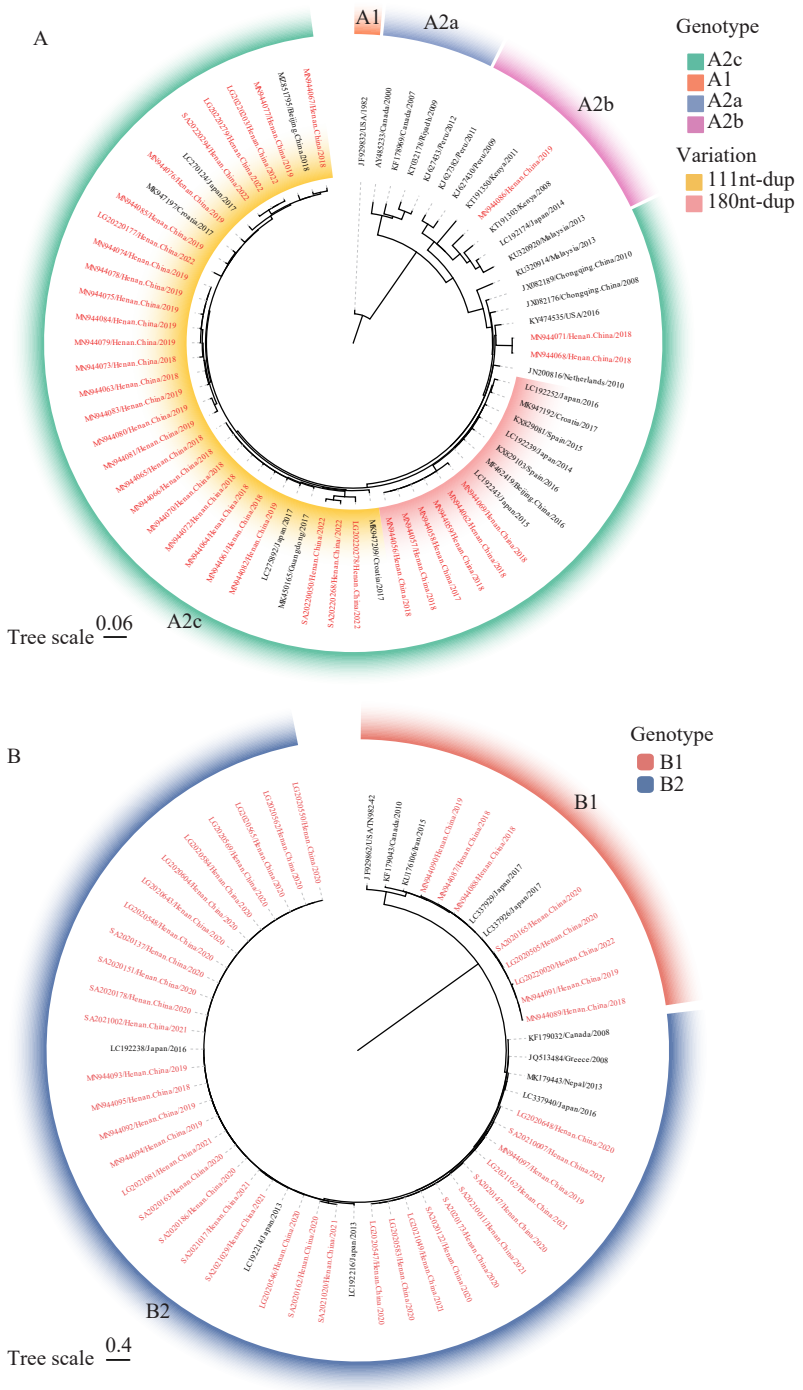

FIGURE 2. The phylogenetic tree of the HMPV G gene for (A) subtype A and (B) subtype B, constructed using the maximum likelihood method.
Note: Sequences from this study are highlighted in red, and the representative strains are shown in white.
Abbreviation: nt-dup=nucleotide duplication; HMPV=human metapneumovirus.

implications in China.

Genetic analysis from this study suggests that HMPV-A and HMPV-B either prevailed independently or concurrently over different years, displaying a potential subtype switch pattern described as "AAABBA" from 2017 to 2022 in Henan Province. A comparable switching pattern has been observed in HRSV (*23*). However, these findings could be impacted by sampling bias and the relatively low rate of sequence recovery from positive samples (48.50%). Additional data is needed to further investigate the subtype switching patterns and genotype evolution in

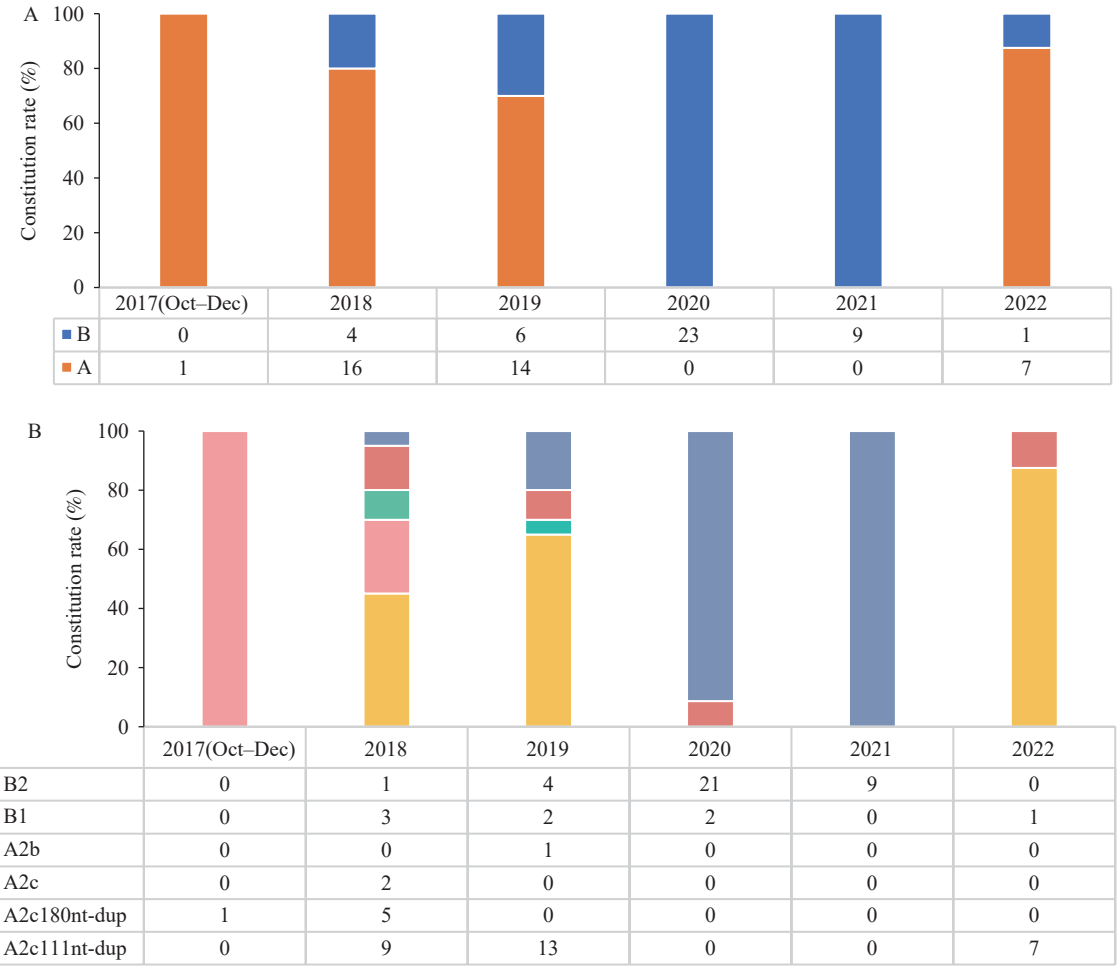

FIGURE 3. Proportions of HMPV (A) subtypes and (B) genotypes circulating in Henan Province, China, across various years.
Note: The accompanying table presents the count of sequences for each subtype or genotype.
Abbreviation: A2c=strains lacking duplications of 111 or 180 nucleotides in the G gene; nt-dup=nucleotide duplication.

HMPV.

In this study, 94.59% (35/37) of the A2c strains were classified into two variants: $A2c_{180nt\text{-}dup}$, forming 17.14% (6/35), and $A2c_{111nt\text{-}dup}$, comprising 82.86% (29/35). Initially identified in 2014 and 2017, respectively, these strains have emerged as the predominant HMPV-A strains worldwide during the period 2016–2021 (*24*–*25*). Surveillance data from six sentinel hospitals in China, collected between 2017 and 2019, indicated that the $A2c_{111nt\text{-}dup}$ variant represented 44.44% (16/36) of HMPV-A cases (*16*). This study expands on these findings by providing genetic insights from ongoing surveillance of HMPV in Henan over the past six years. It was observed that the $A2c_{111nt\text{-}dup}$ became the dominant strain in 2019 and maintained this status until 2022. Notably, the substantial duplication in the dominant $A2c_{111nt\text{-}dup}$ variants augmented potential glycosylation sites and extended the extracellular domain of the G protein (*13*,*26*). The implications of these modifications on the functionality of the HMPV G protein warrant further investigation.

This study is subject to some limitations. First, the rigorous enforcement of NPIs during the early phase of the COVID-19 pandemic resulted in a lower enrollment of patients with ARIs, potentially skewing the analysis of their temporal distribution. Second, it was challenging to determine changes in the seasonal patterns of HMPV due to potential influences from various factors. These factors include changes in immune reserve capacities prompted by NPIs and interactions with other respiratory viruses. Additionally, since only 48.50% (81/167) of HMPV-positive cases had G gene sequences available, the conclusions regarding shifts in HMPV subtypes and genotypes might be subject to bias.

In conclusion, this study has presented six years of consecutive surveillance data on HMPV in Henan,

China, elucidating the epidemic patterns and genetic characteristics of the virus. This information could inform strategies for the prevention and control of HMPV infection in China and aid in the development of vaccines and immunization tactics.

Conflicts of interest: No conflicts of interest.

Funding: Supported by the National Health Commission Major Public Health Project (ZDGWXFTF23-04).

doi: 10.46234/ccdcw2024.087

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Submitted: April 08, 2024; Accepted: May 11, 2024

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. The simple or co-infected viruses detected in the HMPV positive cases.

Viral pathogen	Number of cases (%)
Simple infection of HMPV	121 (72.46)
Co-infected with two viruses	41 (24.55)
HMPV+HRSV	7 (4.19)
HMPV+RV	7 (4.19)
HMPV+IFV	6 (3.59)
HMPV+HBoV	6 (3.59)
HMPV+EV	5 (2.99)
HMPV+HAdV	4 (2.40)
HMPV+HCoV	4 (2.40)
HMPV+HPIV	2 (1.20)
Co-infected with three viruses	4 (2.40)
HMPV+HCoV+HAdV	1 (0.60)
HMPV+IFV+HPIV	1 (0.60)
HMPV+HPIV+EV	1 (0.60)
HMPV+HRSV+HPIV	1 (0.60)
Co-infected with four viruses	1 (0.60)
HMPV+RV+HPIV+HCoV	1 (0.60)
Total	167 (100.00)

Abbreviation: HMPV=human metapneumovirus; HRSV=human respiratory syncytial virus; RV=rhinovirus; IFV=influenza virus; HBoV=human bocavirus; EV=enterovirus; HAdV=human adenovirus; HCoV= human coronavirus; HPIV=human parainfluenza virus.

SUPPLEMENTARY TABLE S2. The clinical symptoms and diagnosis of simple or co-infection cases of HMPV.

Symptoms or diagnosis	Simple infection of HMPV (*n*=121)	Co-infection with HMPV (*n*=46)	χ^2	*P*	Total of HMPV positive cases (*n*=167)
Symptoms					
Fever	44 (36.36)	27 (58.70)	5.901	0.015	71 (42.51)
Cough	42 (34.71)	27 (58.70)	7.908	0.005	69 (41.32)
Expectoration	17 (14.05)	7 (15.22)	0.037	0.848	24 (14.37)
Running nose	7 (5.79)	1 (2.17)	0.326	0.568	8 (4.79)
Sore throat	1 (0.83)	3 (6.52)	2.509	0.113	4 (2.40)
Diagnosis					
Pneumonia	59 (48.76)	26 (56.52)	0.803	0.370	85 (50.90)
Bronchitis	9 (7.44)	7 (15.22)	1.517	0.218	16 (9.58)

Abbreviation: HMPV=human metapneumovirus.