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ORIGINAL ARTICLE

Diagnostic Implications of Long-Term Polymerase Chain Reaction Data for Human Metapneumovirus

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ABSTRACT

Background: **The aim of this study was to systematically examine the annual, seasonal, age-specific, and gender-specific epidemiological trends of human metapneumovirus (hMPV) infections, utilizing extensive real-world data collected over 18 years through a consistent testing methodology and analytical protocol.**
Methods: **Real-time reverse transcription polymerase chain reaction assay results of patients admitted to Dankook University Hospital (2007 - 2024) were retrospectively analyzed. Descriptive statistics were used to assess annual, age-specific, and seasonal trends in hMPV positivity rates. We further evaluated the feasibility of using these data to establish robust epidemiological surveillance systems and standardized monitoring metrics.**
Results: **Among 23,284 specimens, 991 (4.2%) were positive for hMPV. The highest annual positivity rates were recorded in 2007 (7.7%) and 2011 (8.1%). Children aged 2 - 11 years exhibited the highest positivity rate (6.0%), with positivity rates declining with increasing age, except for a slight increase in individuals aged ≥ 65 years. Seasonally, infections peaked in spring (11.1%), followed by summer (2.8%), winter (1.4%), and autumn (0.8%). Since the onset of the coronavirus disease 2019 (COVID-19) pandemic (2020 - 2024), the overall positivity rate dropped to 1.6% from 4.7% before the pandemic, accompanied by notable changes in seasonal patterns.**
Conclusions: **This study revealed significant shifts in hMPV epidemiology following the COVID-19 pandemic, including reduced overall circulation, disrupted spring seasonality, and persistent impacts on high-risk pediatric populations. These findings provide evidence to optimize diagnostic testing for hMPV while providing actionable evidence to adjust optimal diagnostic testing windows, enhance infection prevention strategies, and implement focused monitoring of high-risk populations. Specifically, hMPV testing should be prioritized for pediatric patients during the typical peak months (March - May) and expanded to include atypical winter peaks (December - February) in the post-pandemic era. For older adults (≥ 65 years), targeted testing during winter months may enhance diagnostic yield and support timely clinical management.**
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KEYWORDS

human metapneumovirus, infection dynamics, respiratory tract infections, seasonal trend, surveillance, viral epidemiology

INTRODUCTION

Human metapneumovirus (hMPV) is a negative-sense, single-stranded RNA virus in the Pneumoviridae family, first identified in the Netherlands in 2001 [1]. Since its discovery, hMPV has become recognized as a significant respiratory pathogen affecting individuals of all

ages, including children, older adults, and immunocompromised patients [2]. In pediatric populations, hMPV is commonly associated with pneumonia, bronchiolitis, and asthma exacerbations [3]. Among elderly individuals, individuals with chronic medical conditions, and immunocompromised patients, hMPV is a leading cause of severe respiratory complications [4]. The clinical presentation of hMPV infection often resembles that of respiratory syncytial virus (RSV); however, there are currently no approved vaccines or targeted antiviral treatments available, so supportive care remains the standard approach to manage the disease [5]. Detecting hMPV in patients with respiratory symptoms can significantly impact clinical decision-making, including hospitalization, isolation protocols, and antibiotic stewardship. Despite this, there remains a lack of long-term epidemiological data to inform and support these clinical practices [6].

Globally, hMPV infections most commonly occur in the winter and spring, exhibiting both annual and multi-year epidemic cycles that are shaped by factors such as climate, population density, public health interventions, and viral evolution [7,8]. The coronavirus disease 2019 (COVID-19) pandemic brought about stricter infection control measures and widespread social distancing, resulting in significant changes to the transmission patterns of respiratory viruses, including hMPV [9,10]. These changes have led to shifts and delays in the typical seasonal circulation of hMPV. Long-term laboratory-based studies are needed to clarify how these interventions have altered hMPV seasonality and to refine diagnostic strategies applied in clinical laboratories [11].

However, most prior research has been limited to short-term outbreaks or confined to specific pediatric populartions, with few studies comprehensively analyzing long-term hMPV incidence trends across multiple years, age groups, seasons, and pre- versus post-pandemic periods [12]. Furthermore, inconsistencies in testing methods and analytical approaches between studies have limited direct comparisons across institutions, underscoring the need for standardized laboratory-based data. While hMPV is an important cause of both hospital-acquired and community-acquired respiratory infections, few investigations have differentiated between or linked nosocomial and community outbreaks. This lack of comprehensive integrated data limits early outbreak detection and impedes effective use of healthcare resources. Additionally, recent studies have identified a variety of hMPV genotypes - specifically A1, A2, B1, and B2 - among which the A2 subgroup within the A-lineage has been associated with more severe clinical outcomes, including increased rates of hospitalization and lower respiratory tract involvement [13]. This shift in genotype distribution may affect viral transmission and immune escape, underscoring the importance of implementing genotype-based surveillance systems in the future.

Therefore, the aim of this study was to systematically examine the annual, seasonal, age-specific, and gender-specific epidemiological trends of hMPV infections, utilizing extensive real-world data collected over 18 years through a consistent testing methodology and analytical protocol. By leveraging this standardized, long-term dataset, our analysis facilitated reliable comparisons of hMPV epidemiology across different institutions and countries and aimed to provide practical insights for optimizing hMPV diagnostic testing and improving laboratory efficiency and patient care. Drawing on 23,284 real-time reverse transcription polymerase chain reaction (RT-PCR) test results from a single institution between 2007 and 2024, we comprehensively assessed the temporal and demographic dynamics of hMPV infections. These insights are expected to supply critical evidence for strengthening laboratory-based surveillance of hMPV, supporting the implementation of standardized diagnostic indicators and improving efficiency in clinical laboratory workflows. By clarifying long-term epidemic shifts, particularly before and after the COVID-19 pandemic, our findings provide practical guidance for diagnostic preparedness and resource optimization in laboratory medicine, thereby supporting effective clinical and public health decision-making.

MATERIALS AND METHODS

Study design

This retrospective observational study was conducted at Dankook University Hospital in Cheonan-si, Republic of Korea. This study was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki, and the protocol was reviewed and approved by the Institutional Review Board of Dankook University (IRB no. DKU 2025-02-004-003, approval date: April 23, 2025). All personally identifiable information was excluded from the analysis, and data were anonymized prior to processing. The requirement for informed consent was waived, because the research utilized data from routine diagnostic tests and did not involve identifiable patient information. The analysis utilized molecular testing data for hMPV derived from 23,284 nasopharyngeal swab specimens, collected between January 2007 and December 2024. The study population comprised 13,961 male and 9,323 female patients whose samples were submitted to the hospital laboratory during this 18-year period.

Data collection

Data for this study were obtained from the comprehensive database of multiplex real-time PCR tests performed on hospitalized and outpatient individuals who presented with respiratory symptoms. In instances of indeterminate results, repeat testing was conducted using the original specimen to ensure accuracy. Records lacking essential information - such as gender, age, or date of testing - were excluded from the analysis.

Laboratory procedures

Nasopharyngeal swab specimens were processed immediately after collection and stored at 4°C until analysis. Nucleic acids were extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The purified nucleic acids were then tested with the AdvanSure^{Tm} RV real-time PCR assay (LG Life Sciences, Changwon-si, Republic of Korea), which utilizes primers and probes specific for a range of respiratory viruses, including hMPV. PCR amplification was performed on the SLAN real-time PCR system (LG Life Sciences, Changwon-si, Republic of Korea), adhering to the cycling conditions provided by the manufacturer. All results were interpreted strictly in line with the manufacturer's guidelines to ensure accurate detection of viral targets.

Data preprocessing

Data on patients' age, gender, testing date, and hMPV test results were collected in an anonymized format from the hospital's laboratory information system. To ensure data integrity and analytical reproducibility, records lacking essential demographic or temporal information were excluded. Only specimens with complete data on age, gender, and date of testing were retained for analysis. Age was categorized into five groups based on the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) E11 guideline: infants (0 - 1 years), children (2 - 11 years), adolescents (12 - 18 years), adults (19 - 64 years), and older adults (≥ 65 years). These age categories are widely recognized in clinical and epidemiological research, reflecting critical differences in immunity and infection susceptibility between pediatric and adult populations. Descriptive statistical analyses were conducted to assess annual positivity rates, seasonal distribution - defined as spring (March - May), summer (June - August), autumn (September - November), and winter (December - February) - as well as distribution by gender and age group.

Data analysis

Statistical analyses were conducted to evaluate annual variations in hMPV positivity rates and differences according to age group, gender, and season. Annual variations and group differences were assessed using Pearson's chi-squared test (χ^2). When significant differences were detected, Bonferroni-adjusted post-hoc pairwise comparisons (two-proportion z-tests; pairwise.prop.test) were applied to identify specific pairs with statistically significant differences. In addition, univariate logistic regression analysis was performed to examine the association between gender (male as the reference group) and hMPV positivity, with calculation of odds ratios (ORs) and 95% confidence intervals (CIs). All statistical analyses were performed using R software (version 4.5.1), and statistical significance was defined as p-value < 0.05.

RESULTS

Annual trends in hMPV detection rates

Between 2007 and 2024, a total of 991 cases of human metapneumovirus (hMPV) infection were confirmed among patients presenting with respiratory symptoms. The annual distribution revealed relatively high positivity rates during the early years of surveillance, with 82 cases (7.7%) in 2007, 46 cases (3.0%) in 2008, 64 cases (5.0%) in 2009, 55 cases (3.3%) in 2010, and a peak of 128 cases (8.1%) in 2011. From 2012 through 2015, the number of positive cases fluctuated, with 79 cases (5.8%) in 2012, 74 cases (4.7%) in 2013, 117 cases (6.9%) in 2014, and 42 cases (3.0%) in 2015.

In the following years (2016 - 2019) positivity rates remained relatively stable: 51 cases (3.1%) in 2016, 66 cases (4.5%) in 2017, 69 cases (3.7%) in 2018, and 53 cases (3.7%) in 2019. A marked decline was observed in 2020 (11 cases; 1.3%), and notably, no hMPV-positive cases were detected in 2021. The average positivity rate during the pre-pandemic period (2007 - 2019) was 4.7%, which dropped to 1.6% in the post-pandemic period (2020 - 2024). While there was a modest increase in positive cases in 2022 (16 cases; 1.8%), 2023 (25 cases; 2.4%), and 2024 (13 cases; 1.9%), these rates remained well below pre-pandemic levels (Figure 1).

Overall, the annual variation in hMPV positivity rates was statistically significant ($\chi^2 = 225.0$, df = 17, $p < 0.001$; Supplemental Table S1). Post-hoc pairwise comparisons revealed that 2011 and 2014 had significantly higher positivity rates than did multiple post-pandemic years. Specifically, 2011 vs. 2020 ($p < 0.001$) and 2014 vs. 2021 ($p < 0.001$) showed large and statistically significant differences.

Seasonal patterns of hMPV circulation

Seasonal analysis of hMPV revealed that spring had the highest positivity rate at 11.1%, followed by summer at 2.8%, winter at 1.4%, and autumn at 0.8%. The distribution of hMPV cases across seasons was statistically significant ($\chi^2 = 1{,}048.5$, df = 3, $p < 0.001$; Table 1).

On a monthly scale, the majority of hMPV cases occurred during the spring months (March through May), with marked peaks in April (312 cases) and May (276 cases). The number of positive cases then declined sharply through the summer, with 78 cases in June, 37 in July, and 20 in August. In autumn, monthly case counts generally remained below 20. Although winter positivity was lower compared to spring, there was a slight increase in positive cases in December (21 cases), January (25 cases), and February (48 cases) (Figure 2).

Examining trends over time, the pre-pandemic period (2007 - 2019) consistently showed spring as the primary season for hMPV circulation. Notably high case numbers were recorded in spring 2011 (100 cases), spring 2014 (85 cases), and spring 2012 - 2013 (65 cases). An exception occurred in 2017, when winter cases (16) were relatively elevated compared to other years. In the post-pandemic period (2020 - 2024), overall case num-

Table 1. Seasonal distribution of total tests and human metapneumovirus-positive and human metapneumovirus-negative cases from 2007 through 2024.

Season	Total	Positive (observed)	Positive (expected)	Negative (observed)	Negative (expected)	Positivity rate (%)	χ^2	p
Spring	6,391	712	272.0	5,679	6,118.9	11.1	1,048.5	< 0.001
Summer	4,810	135	204.7	4,675	4,605.2	2.8		
Autumn	5,607	50	238.6	5,557	5,368.3	0.8		
Winter	6,476	94	275.6	6,382	6,200.3	1.4		

Overall chi-squared (χ^2) and p-values are presented.

Table 2. Gender-specific distribution of total tests, observed and expected human metapneumovirus-positive and metapneumovirus-negative cases, and positivity rates.

Gender	Total	Positive (observed)	Positive (expected)	Negative (observed)	Negative (expected)	Positivity rate (%)	χ^2	p
Male	13,961	563	594.1	13,398	13,366.8	4.0	4.1	0.038
Female	9,323	428	396.8	8,895	8,926.2	4.5		

Overall chi-squared (χ^2) and p-values are presented.

Table 3. Age-specific distribution of total tests, observed and expected human metapneumovirus-positive and metapneumovirus-negative cases, and positivity rates.

Age (years)	Total	Positive (observed)	Positive (expected)	Negative (observed)	Negative (expected)	Positivity rate (%)	χ^2	p
0 - 1	9,421	488	400.9	8,933	9,020.0	5.1	168.7	< 0.001
2 - 11	5,535	336	235.5	5,199	5,299.4	6.0		
12 - 18	701	10	29.8	691	671.1	1.4		
19 - 64	2,935	57	124.9	2,878	2,810.0	1.9		
≥ 65	4,692	100	199.6	4,592	4,492.3	2.1		

Overall chi-squared (χ^2) and p-values are presented.

bers declined across all seasons, with no hMPV detections reported in spring from 2020 through 2022. Although a small resurgence was observed in 2023, positivity rates remained below those documented prior to the pandemic (Supplemental Table S2).

Gender-based differences in hMPV positivity

In the gender-specific analysis, hMPV was detected in 4.0% of samples from male patients (563 out of 13,961) and in 4.5% of samples from female patients (428 out of 9,323). This difference was statistically significant (χ^2 = 4.1, df = 1, p = 0.038; Table 2); however, the effect size was small. Univariate logistic regression analysis showed that female gender was associated with slightly higher odds of hMPV positivity than was male gender (OR = 1.14, 95% CI: 1.01 - 1.30, p = 0.039).

When stratified by age group, the highest positivity rate among male patients was observed in the 0 - 1 year group (5.6%), followed by 5.0% in the 2 - 11 years group. Among female patients, the highest positivity was in the 2 - 11 years group (7.3%), followed by 4.5% in the 0 - 1 year group. In all age groups 12 years and older, female patients generally had higher positivity rates than did male patients (Figure 3; Supplemental Table S3).

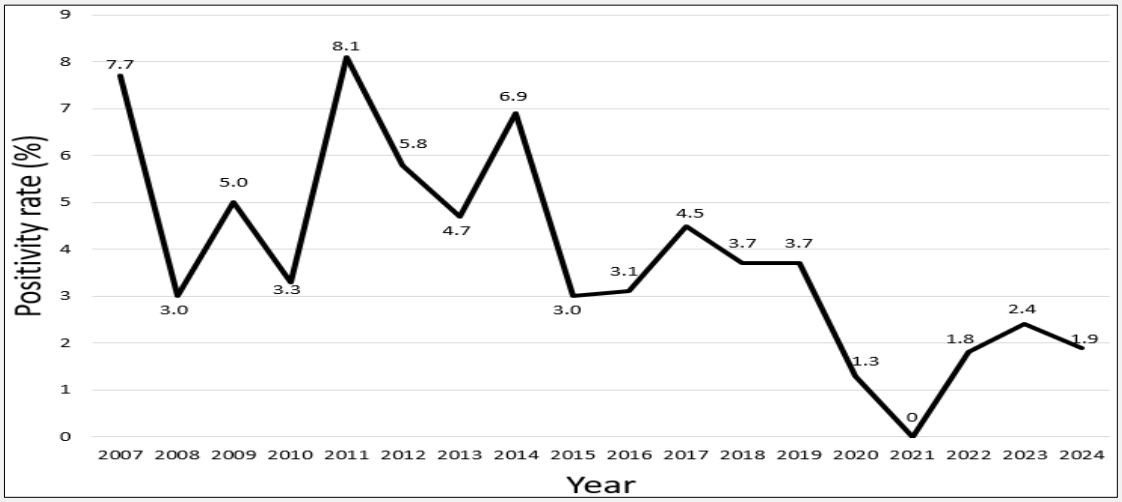

Figure 1. Annual positivity rates of human metapneumovirus (hMPV) infection from 2007 through 2024 among patients whose samples underwent multiplex real-time reverse transcription polymerase chain reaction testing following respiratory symptoms.

Positivity rates were calculated by dividing the number of hMPV-positive cases by the total number of individuals tested each year. Significant differences in yearly positivity rates were evaluated using Pearson's chi-squared test ($p < 0.001$), followed by Bonferroni-adjusted post-hoc pairwise comparisons.

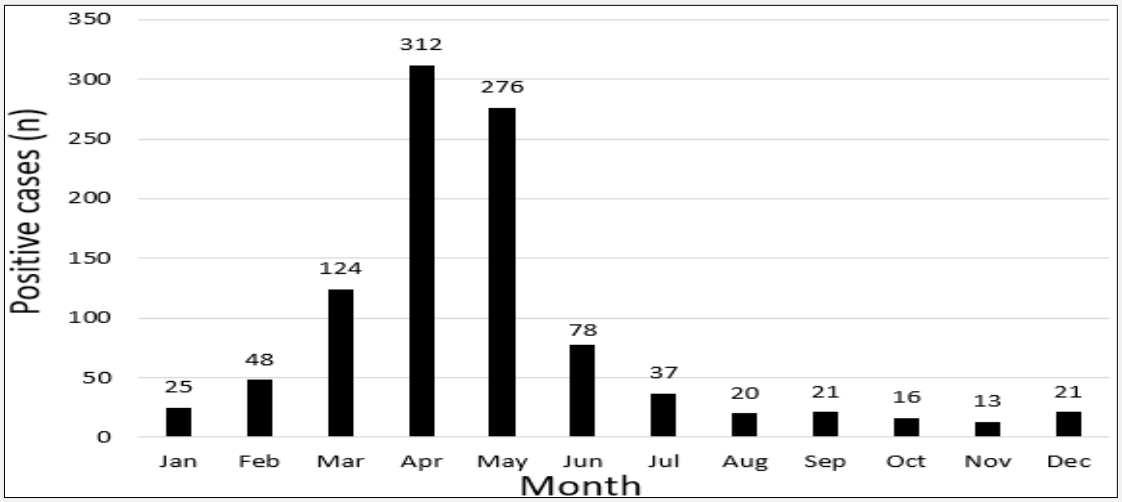

Figure 2. Bar chart of monthly distribution of human metapneumovirus (hMPV)-positive cases from 2007 through 2024 over an 18-year period.

In the chart, the bars corresponding to winter (December - February), spring (March - May), summer (June - August), and autumn (September - November) illustrate the seasonal distribution of positive cases. The highest concentration was observed in spring, with pronounced peaks in April and May. Notably, during the coronavirus disease 2019 (COVID-19) pandemic years (2020 - 2022), this typical seasonal pattern was disrupted, and no spring peaks were detected.

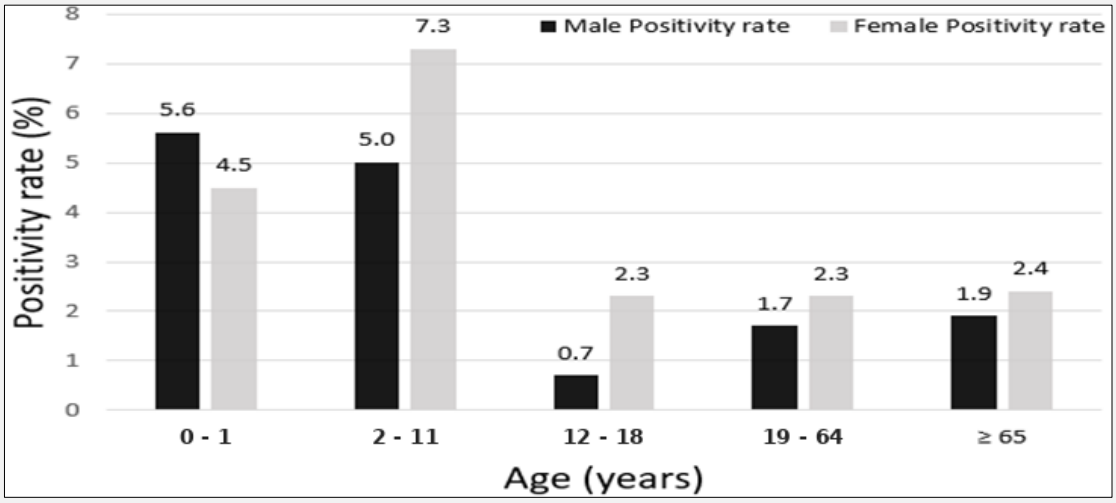

Figure 3. Bar chart of age-specific positivity rates of human metapneumovirus (hMPV) infection by gender.

Male (black bars) and female (gray bars) patients across five age groups: 0 - 1 year, 2 - 11 years, 12 - 18 years, 19 - 64 years, and ≥ 65 years. The highest positivity rate was observed among females aged 2 - 11 years (7.3%), whereas the largest number of overall cases occurred in males aged 0 - 1 year. This analysis revealed a statistically significant difference in positivity rates between gender (p = 0.038, Pearson's chi-squared test).

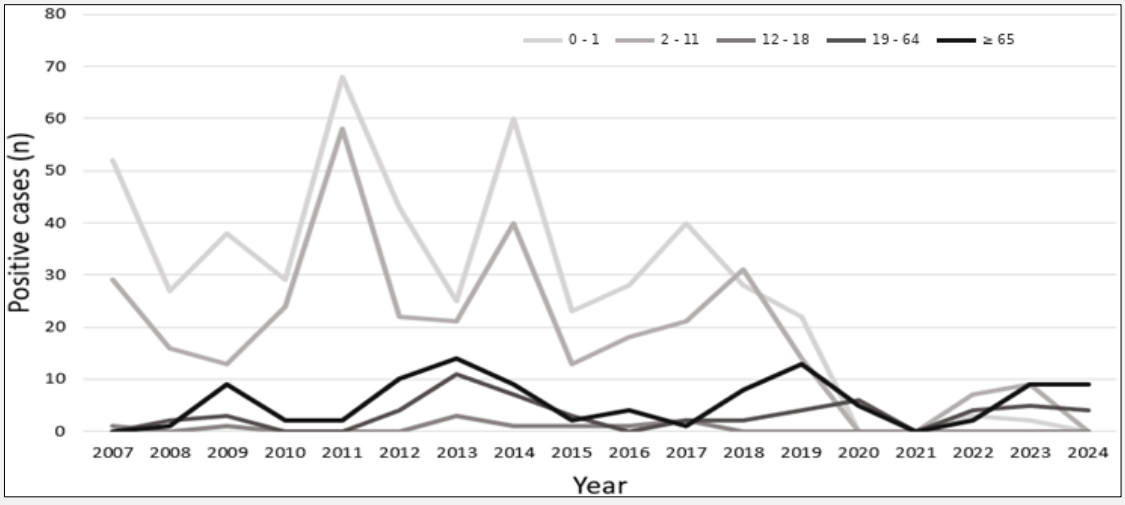

Figure 4. Annual number of human metapneumovirus (hMPV)-positive cases from 2007 through 2024, stratified by age group.

The line plots display the annual number of hMPV-positive cases for each of the five age groups: 0 - 1 year (light gray), 2 - 11 years (gray), 12 - 18 years (dark gray), 19 - 64 years (charcoal), and ≥ 65 years (black). Between 2007 and 2017, infants aged 0 - 1 year consistently recorded the highest annual case counts. However, during the coronavirus disease 2019 (COVID-19) pandemic years (2020 - 2022), hMPV detections dropped sharply across all age groups. Only a modest resurgence was observed in 2023 and 2024, with most new cases occurring among adults and older adults.

Age-specific positivity rates for hMPV

Analysis of the 23,284 specimens stratified by age group revealed that hMPV positivity was highest among children aged 2 - 11 years, with a rate of 6.0% (336 out of 5,535), followed by infants aged 0 - 1 years at 5.1% (488 out of 9,421). Despite this, infants in the 0 - 1 year group accounted for the greatest absolute number of positive cases (488). Among adolescents aged 12 - 18 years, the positivity rate was lower at 1.4% (10 out of 701), while adults aged 19 - 64 years and those aged 65 years and older had rates of 1.9% (57 out of 2,935) and 2.1% (100 out of 4,692), respectively. The distribution of positivity rates across age groups was statistically significant ($\chi^2 = 168.7$, df = 4, $p < 0.001$; Table 3).

When examining annual trends in hMPV-positive cases by age group, infants aged 0 - 1 year consistently represented the largest proportion of cases each year from 2007 through 2017 (Figure 4, Supplemental Table S4). However, in 2018 and 2023, the highest number of positive cases was observed in the 2 - 11 years age group. In both 2013 and 2019, notable increases in positive cases were also seen among adults aged 19 - 64 years and those 65 years and older. From 2020 through 2024, the number of positive cases dropped sharply - or was entirely absent - among both the 0 - 1 year and 2 - 11 years age groups, with sporadic detections limited mainly to adults aged 19 - 64 years and those aged 65 and above. Notably, in 2021, no hMPV-positive cases were reported in any age group.

DISCUSSION

This study aimed to systematically elucidate the epidemiological patterns of hMPV and the impact of the COVID-19 pandemic on viral transmission by epidemiological single-center data accumulated over 18 years. Notably, before the pandemic, hMPV consistently exhibited distinct seasonal epidemics each year; however, its transmission significantly declined during the pandemic, and positivity rates have remained below the pre-pandemic average of 4.7% despite its partial resume [14,15]. For instance, positivity rates in 2023 and 2024 were approximately 2.4% and 1.9%, respectively. These findings suggest that factors beyond nonpharmaceutical interventions (NPIs) may have contributed to the observed reduction in hMPV activity, consistent with previous reports of multifactorial influences on respiratory virus circulation. Furthermore, measures such as mask-wearing, enhanced hand hygiene, social distancing, school and workplace closures, and border controls have been shown to substantially suppress transmission of various respiratory viruses, including severe acute respiratory syndrome coronavirus-2, RSV, influenza, and hMPV, thereby substantially altering global viral circulation patterns [16,17]. Similar trends have been reported in Europe and North America, showing the structural reshaping of the ecosystem of respiratory viruses due to the pandemic [18].

Notably, before the COVID-19 pandemic, hMPV infections were primarily concentrated in the spring season, a pattern consistently reported across temperate regions [19,20]. This seasonal predominance may be explained by several factors, including increased indoor activities during winter, reduced sunlight exposure, and temperature fluctuations that weaken mucosal immune defenses in the upper respiratory tract, with reduced vitamin D levels further contributing to diminished immunity [21-23]. In addition, group activities in schools and child-care facilities facilitate asymptomatic or mild infections among children, thereby promoting outbreaks in spring [24]. Following the onset of the pandemic, the typical spring dominance of hMPV circulation was completely lost. While infections were concentrated in the spring during 2007 - 2019, with positivity rates peaking at 11.1%, no hMPV cases were detected in spring from 2020 through 2022. In certain years during and after the pandemic, notably 2020 and 2024, peak activity occurred in the winter months, although these peaks were smaller than pre-pandemic spring peaks and absent in other years. A modest resurgence occurred in 2023, although seasonal positivity remained well below pre-pandemic levels. These shifts are unlikely to be explained by climatic factors alone and may reflect complex mechanisms such as reduced population immunity, viral genetic changes, and inter-viral interactions. The complete absence of circulation in the spring during 2020 - 2022 further suggests that NPIs profoundly disrupted inherent seasonal dynamics, with similar temporal shifts observed for RSV and influenza [25]. Such changes underscore the need for flexibility in diagnostic algorithms and infection control protocols. In particular, laboratories should periodically update testing criteria, expand testing windows, and adjust diagnostic workflows to ensure that hMPV testing is considered even during atypical peak months, thereby preventing missed diagnoses and delayed interventions. Further studies incorporating additional clinical and laboratory parameters will help clarify the mechanisms underlying these seasonal shifts.

When stratified by age, this gender difference was most pronounced in young children, particularly in the primary school-aged group, where female patients exhibited higher positivity than male patients. This suggests that the observed difference may be partially attributable to age-related distribution patterns.

Despite statistical significance, the clinical relevance of this finding remains uncertain and may reflect a statistical artifact due to unequal age composition between gender. However, biological mechanisms cannot be ruled out. Prior studies have shown that sex hormones, especially estrogens, enhance immune cell activation and cytokine production, which may increase susceptibility to respiratory viruses in female patients [26,27]. In addition, other confounding factors - such as differences in healthcare-seeking behavior, access to testing, underlying comorbidities, and lifestyle - could also contribute to the observed gender differences. Therefore, future studies should incorporate multivariate regression

models that adjust for these potential confounders to more accurately elucidate the role of gender in hMPV infection dynamics.

These findings underscore the need for further multivariate analyses adjusting not only for age but also for additional confounders such as underlying comorbidities, healthcare access, and exposure patterns. Further immunological investigations are warranted to elucidate the mechanistic basis of gender-specific differences in hMPV infection. Herein, the age classification used followed the ICH guidelines, which are widely recognized in clinical trials and epidemiological research for reflecting the key immunological and infectious disease characteristics distinguishing pediatric from adult populations. Notably, the 0 - 1 year and 2 - 11 years age groups presented the highest positivity rates, and these rates declined sharply in those aged ≥ 12 years [28,29]. Although the 2 - 11 years group exhibited the highest positivity rate, the total number of infections was greater in the 0 - 1 year group. This may be attributed to the higher frequency and severity of respiratory symptoms in infants, leading to a greater number of test orders in this age group [30]. These results suggest that a higher number of infections may not necessarily imply a higher infection rate, as discrepancies between test frequency and testing criteria may not appropriately reflect positivity rates and case numbers. Additionally, the low positivity rates observed among adolescents (12 - 18 years) and adults (19 - 64 years) may be attributed to the matured immune system and partial immunity due to previous hMPV exposures, which often result in milder or non-specific symptoms and lower testing rates [31,32]. Conversely, the increased positivity rate in individuals aged ≥ 65 years possibly reflected immunosenescence [33]. Overall, the data analysis revealed that the 0 - 1 year and 2 - 11 years age groups showed the highest observed positivity rates for hMPV infections in our dataset, underscoring the need for targeted surveillance and preventive strategies for these age groups. From a clinical perspective, these findings suggest that infants aged 0 - 1 year and children aged 2 - 11 years should be prioritized for hMPV testing during peak circulation periods to facilitate early diagnosis, avoid unnecessary antibiotic use, and reduce the risk of nosocomial transmission. Seasonal shifts after the COVID-19 pandemic indicate that diagnostic laboratories should anticipate potential winter peaks when planning testing schedules and reagent supplies. Furthermore, during the years when traditional spring circulation is absent, clinicians should broaden differential diagnoses to include hMPV for patients presenting with respiratory symptoms outside the usual peak season. Our findings suggest that diagnostic testing for hMPV should be prioritized for pediatric patients during spring and for older adults during atypical winter peaks. Laboratory resource allocation, including diagnostic kits and staffing, can be optimized according to seasonal and age-specific positivity patterns. Specifically, concentration of hMPV testing for children with respiratory symptoms between March and May can improve diagnostic efficiency, while testing of older adults (≥ 65 years) should be increased during winter months, when detection rates are higher. Furthermore, as external factors such as the COVID-19 pandemic can disrupt typical seasonality, laboratories are advised to review and update diagnostic algorithms at least annually to maintain flexibility in testing criteria.

However, this study has some limitations. First, this study used retrospective data from a single institution; hence, the results may differ from nationwide or international epidemiological patterns. Second, the available clinical data were limited, which limited detailed analysis of potential confounding factors such as disease severity, underlying conditions, and co-infections. Third, although the same testing platform was maintained over 18 years, changes in diagnostic kits could have introduced potential bias. Lastly, the data analysis did not quantitatively adjust for external environmental factors, such as social distancing measures and public health policies, which might have influenced seasonality and age-specific patterns. Nevertheless, this study provides significant results owing to its systematic analysis of hMPV epidemiological characteristics based on a large-scale, standardized, real-world dataset spanning 18 years.

Although genotype information was not available in our dataset, recent studies have reported an increasing global predominance of the A2b2 genotype, particularly in China [19]. This emerging trend may influence the transmissibility, seasonality, and immune evasion characteristics of hMPV. Therefore, future research should incorporate genotype-specific analyses and immunological investigations to better understand immunity duration, cross-protection, and the molecular epidemiology of hMPV. Additionally, international comparisons of hMPV circulation patterns in countries with similar climates are warranted to identify global trends. Refinement of molecular surveillance systems will be crucial for the early detection of large-scale outbreaks, especially those driven by immunological gaps. It is equally important for clinical laboratories to establish standardized reporting indicators and adopt harmonized diagnostic workflows to enable interinstitutional comparisons and optimize resource allocation during seasonal shifts.

In conclusion, our 18-year retrospective analysis revealed significant shifts in hMPV epidemiology following the COVID-19 pandemic, including reduced overall circulation, disrupted spring seasonality, and persistent impacts on high-risk pediatric populations. These shifts in epidemic patterns were likely influenced by NPIs and broader immunological mechanisms, although their practical impact lies in the need to adjust laboratory testing strategies in response to disrupted seasonality. By identifying infants (0 - 1 year) and children (2 - 11 years) as the most vulnerable groups, our findings provide actionable evidence for optimizing diagnostic testing. Specifically, hMPV testing should be prioritized for

pediatric patients during the typical peak months (March - May) and expanded to include atypical winter peaks (December - February) in the post-pandemic era. For older adults (≥ 65 years), targeted testing during winter months may enhance diagnostic yield and support timely clinical management. From a laboratory perspective, seasonal and age-specific positivity patterns can inform the efficient allocation of diagnostic kits, personnel, and laboratory resources. Furthermore, refining diagnostic algorithms, updating testing criteria according to seasonal shifts, and harmonizing reporting indicators across institutions will be essential for improving diagnostic efficiency and ensuring interlaboratory comparability. Overall, these results underscore the diagnostic relevance of long-term hMPV monitoring and provide practical guidance for improving laboratory workflows, surveillance capacity, and patient care within clinical laboratory practice.

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Data Availability Statement:
The data supporting the findings of this study are derived from patient records at Dankook University Hospital and are subject to ethical and legal restrictions. Due to privacy and confidentiality concerns, the raw datasets cannot be shared publicly. Anonymized summary data are available from the corresponding author upon reasonable request, subject to Institutional Review Board approval.

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Declaration of Interest:
The authors declare that they have no conflicts of interest.

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