



Epidemiological studies have shown that respiratory virus outbreaks have a seasonal distribution. Influenza, human coronavirus, and respiratory syncytial virus (RSV) increase significantly in the winter months; they are also called winter viruses. Human bocavirus, human metapneumovirus, and rhinovirus are monitored throughout the year. The detection frequency and number of cases of enterovirus infections increase in the summer months. They increase in spring and autumn, depending on the type of parainfluenza virus. Although rhinovirus infection occurs all year round, severe infections occur in the winter months (Moriyama et al. 2020).

There is compelling evidence that the use of masks can effectively prevent respiratory virus outbreaks. Studies have consistently shown that masks provide protection for droplet sizes in the range of 2–3  $\mu\text{m}$ , regardless of the droplet amount and exposure time (Djeghdar et al.).

Currently, two types of masks are in use: the surgical face mask and the high barrier N95, FFP2 or FFP3 mask. While surgical face masks with standard protection are suitable for general use, masks with high protection are recommended for healthcare workers involved in patient treatment (Azap et al. 2020).

The main objective of this study was to assess the effect of mask use on the diversity and transmission of seasonal flu viruses. To achieve this, we compared the seasonal flu agents of SARS-CoV-2 negative patients during 2021–2022, when all restrictions were eased except for the mask mandate, and 2022–2023, when the mask mandate was also lifted.

## Experimental

### Materials and Methods

In our study, we retrospectively analyzed viral panel PCR test results obtained from respiratory tract samples of 1,335 patients (483 female and 852 male,  $66.4 \pm 13.6$  years), with negative SARS-CoV-2 PCR test who were hospitalized with the diagnoses of asthma attack, chronic obstructive pulmonary disease (COPD) exacerbation and pneumonia and subsequently were treated at the Training and Research Hospital Pulmonary Disease Clinic between October 2021 and May 2023. PCR test results were accessed by using the Public Health Management Information System of the Ministry of Health.

The following inclusion criteria were used: i) age  $\geq 18$  years, ii) all patients hospitalized with a diagnosis of COPD exacerbation, pneumonia, and asthma attacks. Exclusion criteria were as follows: i) SARS-CoV-2 PCR positive ( $n = 103$ ), ii) patients with negative respiratory virus panel PCR results ( $n = 80$ ).

In Türkiye, all pandemic restrictions were eased except for the mask mandate (schools, cafes, bars, and other restaurants were allowed to open, and public transportation and intercity travel were resumed) on July 1, 2021. In contrast, on April 27, 2022, the requirement to wear masks in indoor areas, excluding health-care facilities, was lifted.

Our results were evaluated by dividing the study interval into two sub-periods and three seasons (fall, winter, and spring). The first period included data from October 2021 to May 2022, when all restrictions were eased except for the mask mandate; the second period included data from October 2022 to May 2023, when the mask mandate was also lifted.

Respiratory samples were investigated using the Bio-speedy® Respiratory Tract RT-qPCR MX-24 Panel (Bioeksan R&D Technologies Inc., Türkiye).

The viruses investigated in the clinical samples through PCR method were influenza A, influenza A (H1N1), influenza A (H3N2), influenza B, adenovirus, coronavirus hku1, coronavirus 229e, coronavirus nl63, coronavirus oc43, entero-rhinovirus, human bocavirus, human metapneumovirus, respiratory syncytial virus (RSV/AB), parechovirus, and one bacterial species – *Mycoplasma* spp.

An ethical approval was granted by the Non-Interventional Clinical Research Ethics Committee (Decision Number: 2023/4/20, Date: 03/01/2023).

All the data collected were statistically analyzed with IBM SPSS Statistics for Windows v23.0 (IBM Corp., USA). We calculated frequency and percentage values for the categorical variables and mean and standard deviation values for the quantitative variables. The statistical evaluation of categorical variables was performed by the chi-square test.

## Results

The data of 1,335 patients, 483 female and 852 male, who were hospitalized at our pulmonary disease clinic between October 2021 and May 2023 with the diagnoses of asthma attack, COPD exacerbation, and pneumonia were examined retrospectively. The patients' mean age was found to be  $66.4 \pm 13.6$  years (females:  $67 \pm 15.3$ , males:  $66.1 \pm 12.6$ ). The most frequent comorbidities were hypertension (59%), COPD (53.2%), and ischemic heart disease (35%). The demographic features of the patients are exhibited in Table I.

When the viral agents were examined according to the hospitalization diagnoses of the patients, in the patients with COPD exacerbation, influenza A with 11.2% (H1N1: 9.5%, H3N2: 8.1%) was found to be the most frequent viral agent, followed by enterovirus/rhinovirus with 5.3%, and human coronavirus with 4%.

Table I  
Demographic features of the patients.

Age (Mean ± SD)	66.4 ± 13.6
Gender (n, %)	
Male	852 (63.8%)
Female	483 (36.2%)
Comorbidity (n, %)	
Hypertension	787 (59%)
COPD	714 (53.2%)
IHD	467 (35%)
Asthma	323 (24.2%)
CVD	173 (13%)
Malignancy	87 (6.5%)
Bronchiectasis	66 (4.9%)
CRF	49 (3.7%)
Alzheimer	33 (2.5%)
Diabetes	28 (2.1%)
DILD	18 (1.3%)
Hospitalization diagnoses (n, %)	
COPD exacerbation	645 (48.3%)
Pneumonia	435 (32.6%)
Asthma attack	255 (19.1%)

COPD – chronic obstructive pulmonary disease,  
IHD – ischemic heart disease, CVD – cerebrovascular disease,  
CRF – chronic renal failure, DILD – diffuse interstitial lung disease

For those hospitalized with pneumonia, influenza A was detected at a rate of 10.8% (H1N1: 9%, H3N2: 5.7%), followed by enterovirus/rhinovirus with 6%, and human coronavirus with 3.9%. For asthma attack patients, influenza A was 12.2% (H1N1: 9.8%, H3N2: 8.6%), enterovirus/rhinovirus was 7.5%, and human coronavirus was 5.5% (Table II).

The comparison of the viral agents between the first period with mandatory mask use and the second period when the mask mandate was lifted revealed that the viral agents detected in the second period (41.6%) increased significantly compared to the first period (23.4%) ( $p < 0.001$ ). During the period after the mask mandate was lifted, a significant increase was detected in influenza A, H1N1, and RSV/AB ( $p = 0.019$ ,  $p = 0.003$ ,  $p < 0.001$ ), whereas coronavirus HKU1, coronavirus 229E, human metapneumovirus, and parainfluenzavirus 2 and 4 decreased. Parechovirus and the bacteria – *Mycoplasma* spp. were not detected in both periods (Table III, Fig. 1).

In the seasonal comparison of the viral agents detected in the first and second periods, it was seen that during the first period, there was a marked increase in H1N1 and RSV/AB during the winter and in Influenza A and B during the spring. In the second period, when mandatory mask use was lifted, there was

Table II  
Viral and *Mycoplasma* panel PCR results  
by hospitalization diagnoses.

	COPD n (%)	Pneumonia n (%)	Asthma n (%)
<b>Influenza A</b>	72 (11.2)	47 (10.8)	31 (12.2)
H1N1	61 (9.5)	39 (9)	25 (9.8)
H3N2	52 (8.1)	25 (5.7)	22 (8.6)
<b>Influenza B</b>	3 (0.5)	4 (0.9)	3 (1.2)
<b>Other Respiratory Viruses</b>	98 (15.2)	68 (15.6)	56 (22)
Adenovirus	5 (0.8)	4 (0.9)	1 (0.4)
Human coronavirus	26 (4)	17 (3.9)	14 (5.5)
Parainfluenzavirus 1–4	8 (1.2)	2 (0.5)	7 (2.7)
Enterovirus/Rhinovirus	34 (5.3)	26 (6)	19 (7.5)
Human bocavirus	1 (0.2)	0	2 (0.8)
Human metapneumovirus	3 (0.5)	3 (0.7)	5 (2)
RSV/EU	22 (3.4)	16 (3.7)	9 (3.5)
Parechovirus	0	0	0
Bacteria	0	0	0
<i>Mycoplasma</i> spp.	0	0	0

COPD – chronic obstructive pulmonary disease,  
RSV – respiratory syncytial virus

a marked decrease in parainfluenzavirus 1–4, human metapneumovirus during winter, and enterovirus/rhinovirus during spring. Co-infection was found in six patients in the first and 15 patients in the second periods ( $p > 0.05$ ).

## Discussion

Recent literature has shown that restrictions such as travel bans, curfews, closure of schools, bars, restaurants, and cafes, prohibition of public transportation, and mandatory mask-wearing, which are called non-pharmacological measures taken by countries due to the pandemic, mitigate the spread and transmission of COVID-19. Furthermore, it has been reported that these non-pharmacological measures reduce the burden of virus exposure (Doshi 2020). Among the studies evaluating respiratory tract viruses before and during the pandemic period, those conducted by Olsen et al. (2020), Lei et al. (2020), and Feng et al. (2021) indicated that non-pharmacological measures reduced influenza activity during the pandemic by 98%, 64%, and 79.2%, respectively. Sullivan et al. (2020) observed a significant decrease in hospitalizations and deaths related to influenza and RSV, along with increased rhinovirus cases. Similarly, Redlberger-Fritz et al. (2021) reported a marked decrease in influenza, RSV, human metapneumovirus, and rhinoviruses during the quarantine period; however, rhinovirus cases significantly

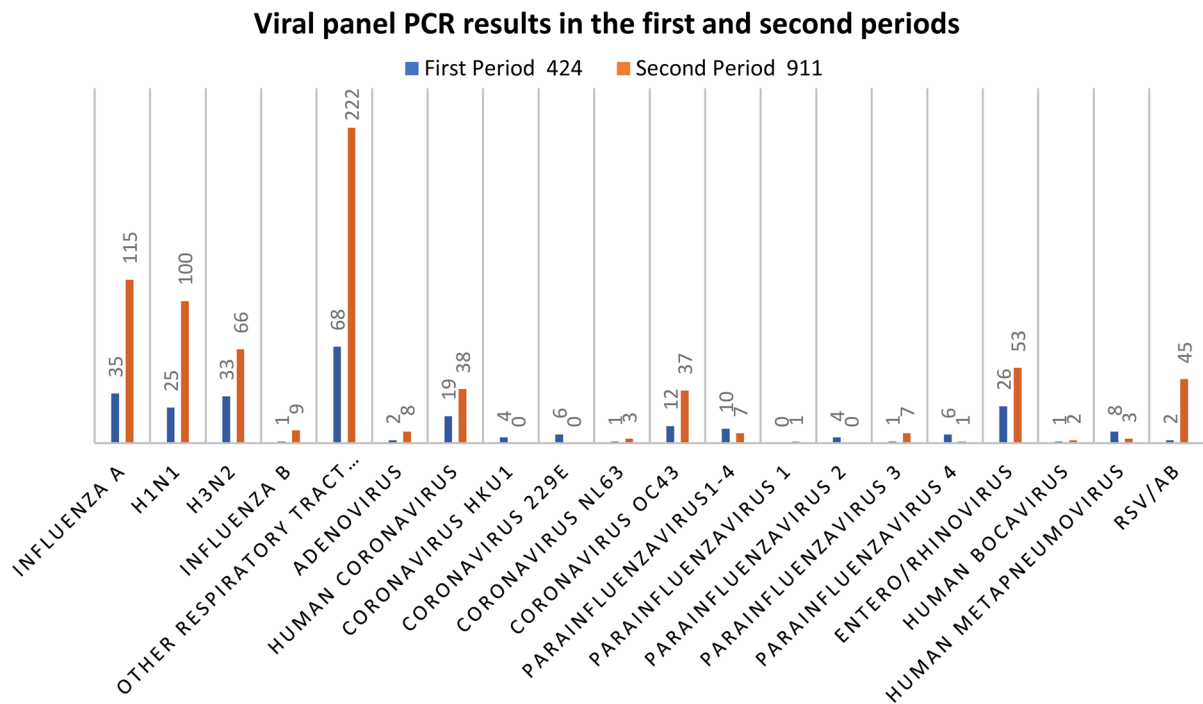


Fig. 1. Viral panel PCR results in the first and second periods.

Table III  
Viral and *Mycoplasma* panel PCR results in the first and second periods.

	First period (n = 424) n (%)	Second period (n = 911) n (%)	p-value
<b>Influenza A</b>	35 (8.3)	115 (12.6)	0.019
H1N1	25 (5.9)	100 (11)	0.003
H3N2	33 (7.8)	66 (7.2)	0.72
<b>Influenza B</b>	1 (0.2)	9 (1)	0.13
<b>Other Respiratory Tract Viruses</b>	68 (16)	222 (116.6)	0.69
<b>Adenovirus</b>	2 (0.5)	8 (0.9)	0.42
<b>Human Coronavirus</b>	19 (4.5)	38 (4.2)	0.78
Coronavirus HKU1	4 (0.9)	0	0.003
Coronavirus 229E	6 (1.4)	0	0.000
Coronavirus NL63	1 (0.2)	3 (0.3)	0.77
Coronavirus OC43	12 (2.8)	37 (4.1)	0.26
<b>Parainfluenzavirus1-4</b>	10 (2.4)	7 (0.7)	0.016
Parainfluenzavirus 1	0	1 (0.1)	0.49
Parainfluenzavirus 2	4 (0.9)	0	0.03
Parainfluenzavirus 3	1 (0.2)	7 (0.8)	0.24
Parainfluenzavirus 4	6 (1.4)	1 (0.1)	0.02
<b>Entero/Rhinovirus</b>	26 (6.1)	53 (5.8)	0.82
<b>Human Bocavirus</b>	1 (0.1)	2 (0.2)	0.95
<b>Human Metapneumovirus</b>	8 (1.9)	3 (0.3)	0.003
<b>RSV/AB</b>	2 (0.5)	45 (4.9)	0.000
<b>Parecoivirus</b>	0	0	0
<b>Bacteria</b>	0	0	0
<i>Mycoplasma</i> spp.	0	0	0
	116 (23.4)	379 (41.6)	0.000

increased after quarantine measures were lifted. Park et al. (2021) has found influenza, rhinovirus/enterovirus or parainfluenza virus test positivity to be 35% before and 7.2% during the pandemic period. Huang et al. (2020) detected that during the pandemic period, the circulation of the influenza virus decreased by 99.9%, RSV by 98%, human metapneumovirus by 92.2%, enterovirus by 82.2%, adenovirus by 81.4%, parainfluenza virus types 1–3 by 80.1% and rhinovirus by 74.6%, and rhinovirus levels (33%) increased first once the quarantine restrictions were eased. According to the study by Doroshenko et al. (2021), implementing pandemic measures resulted in significant reductions in the positivity rates of various respiratory viruses. The positivity rates of influenza A/B, RSV, seasonal coronaviruses (HKU1, 229E, OC43, NL63), human metapneumovirus, enterovirus/rhinovirus, and parainfluenza viruses (1–4) decreased from 17.98% to 0.98%, 6.99% to 0.56%, 3.79% to 1.21%, 4.2% to 1.07%, 15.81% to 3.98%, and 4.4% to 0.31%, respectively (Doroshenko et al. 2021). In a study comparing the period of complete lockdown with the period of eased lockdown measures in terms of viral agents, Hodjad et al. (2021) reported a decrease in all viral agents during the complete lockdown period. They also noted that the agents that first returned to pre-pandemic levels with gradual relaxation of measures were rhinovirus/enterovirus. There are reports in the literature indicating that the lifting of COVID-19 measures, including the mask mandate, led to the return of various respiratory viruses such as influenza, seasonal coronaviruses, RSV A/B, and parainfluenza virus to pre-pandemic levels (Hodjad et al. 2021). In relation to that, Marriot et al. (2021) found a significant decrease in test positivity for both COVID-19 and other respiratory viruses 11 weeks after the initiation of the lockdown period. In our study, a similar trend was observed in which seasonal influenza (influenza A and H1N1) and RSV A/B increased after the mask mandate was lifted. This suggested that wearing a mask is protective against the transmission of influenza and RSV A/B.

On the other hand, unlike other relevant studies, even though we found rhinovirus/enterovirus to be high in both periods, the difference between the periods was not significant. Thus, we considered that non-enveloped viruses are more resistant or that the effectiveness of masks in preventing the transmission of non-enveloped viruses may be lower. Besides, in our study, different from others, we also detected a decrease in coronavirus HKU1, coronavirus 229E, human metapneumovirus, and parainfluenzavirus 2 and 4. Although the exact reason for this different result cannot be fully explained, it could be attributed to the fact that the study excluded asymptomatic patients. Moreover, differing from other similar studies, we also

investigated the co-infection status of the viruses. We determined that despite not being statistically significant, there was a proportional increase in co-infection diversity after mask mandates were lifted. This finding suggested that masks can affect virus diversity.

Our study had certain limitations due to its retrospective nature and the inability to monitor individual behaviors despite the mask-wearing mandate continuously. Additionally, the study only included symptomatic patients who were hospitalized. Consequently, the results of asymptomatic individuals in the community were not included. Additionally, although travel restrictions have been lifted in 2021, this is only valid for some countries. For this reason, the decrease in our results in 2021 may be due to the decrease in the entry of viruses into the country and the obligation to use masks. We may have ignored the increase in our results by attributing the increase to the removal of the obligation to use masks without considering the increase in RSV and influenza in the world in 2022.

In conclusion, the results of our study are of significant importance, as they demonstrate the protective effect of mask use against the transmission of respiratory tract viruses during the pandemic. We believe that mask use can be beneficial, especially during influenza and RSV epidemics.

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#### Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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