

Original Article

Prevalence of Common Human Coronaviruses (NL63, 229E, and OC43) in Adults before the COVID-19 Pandemic: a Single-Center Study from Turkey, 2015–2020

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ABSTRACT: Common Human Coronaviruses (HCoVs), such as NL63, HKU1, 229E, and OC43, induce respiratory tract infections worldwide. Epidemiological studies of HCoVs are of paramount importance because the disease burden and trajectory (in years) have not been well addressed in adults. Here, we aimed to describe the burden of HCoVs in a hospital setting over five years before the coronavirus disease 2019 pandemic. This was a retrospective study of patients (>18 years) between January 1, 2015, and January 1, 2020, whose respiratory specimens were tested by multiplex real-time polymerase chain reaction. In total, 7,861 respiratory samples (4,540 patients) were included, 38% of which tested positive for any respiratory virus. Of these, 212 (12.2%) samples were positive for HCoVs, and their co-infection with other respiratory viruses was 30.6%. Rhinovirus (27.6%) was the most common co-infection among all three HCoVs. The overall prevalence of HCoVs tended to be the highest in the winter (40.9%). Patients aged ≥ 60 years had the highest prevalence of overall HCoVs (39.7%). Given the duration and large sample size, this study from Turkey is one of the largest to date among adults in the literature. These epidemiological data and molecular surveillance of HCoVs have important implications for the control and prevention of respiratory infections.

INTRODUCTION

Human coronaviruses (HCoVs) are common acute respiratory infections of different species. They are called "coronavirus," meaning "crowned virus," because of their rod-shaped extensions on their surface. The first member of 229E was detected 50 years ago in the United Kingdom. OC43 was the second described at the National Institutes of Health, and HKU1 was the third member described in Hong Kong. NL63, the last family member, was described in 2002 in Amsterdam. These four HCoVs have long been circulating in human populations. HCoVs impact almost all age groups and are prevalent throughout the year regardless of clinical symptoms, although seasonal variations in the number of HCoVs detected have been observed (1–3).

In the last two decades, three different coronaviruses

have passed the species barrier and spilled over to humans, infecting thousands. The severe acute respiratory syndrome-related coronavirus (SARS-CoV) emerged in China in 2003 and claimed the lives of 774 people (4). The Middle East respiratory syndrome-related coronavirus (MERS-CoV) has been a challenge since its first detection in Saudi Arabia in 2012 (1). Finally, and most deadly, SARS-CoV-2 was detected in December 2019 in Wuhan and later spread worldwide. SARS-CoV-2 belongs to the same genus of beta coronaviruses as HKU1 and OC43, whereas 229E and NL63 belong to alphacoronavirus. Unlike HCoVs, SARS-CoV and MERS-CoV do not show endemic seasonality. However, it is still too early to predict whether the novel coronavirus SARS-CoV-2 will have an endemic seasonal occurrence (5–7).

HCoVs may be detected in clinical specimens from mild to moderate upper respiratory tract infections or asymptomatic individuals, are widespread globally, and prevalent in autumn and winter (8). However, SARS-CoV, MERS-CoV, and SARS-CoV-2 cause severe lower respiratory tract infections with substantial mortality. The fatality rate is 11% for SARS-CoV and 35–50% for MERS-CoV; the actual fatality rate of SARS-CoV-2 is still debated (1,9,10).

Epidemiological studies on HCoVs in adults have been conducted, though most studies focused on

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children (6,11). Therefore, the current understanding of the epidemiology of 229E, HKU1, NL63, and OC43 in adults is limited. A large study from multiple cohorts reported that infection rates with 229E and OC43 ranged from 2.8% to 26% in immunocompetent adults, with marked variation in the predominant circulating types (12). Co-infections with other respiratory viruses are approximately 30–50% (6,13–15). Additionally, respiratory virus co-infection is significantly more common with 229E than with OC43 (12,14). Recently, Jo et al. found no significant differences in the need for hospital admission or clinical severity between single infections and viral co-infections with HCoVs in pediatric patients (16). In another study with adults, the most common co-infection pattern with HCoVs was influenza, and co-infection with other pathogens was more likely to be found in lower respiratory tract specimens. Whether coronaviruses contribute to disease severity in such co-infections is currently unclear. Further research is needed to determine the possible contribution of co-infections with HCoVs to comorbidity and mortality, the pathogenesis of influenza-like illnesses, or severe acute respiratory infection (17).

Epidemiological studies of HCoVs are of paramount importance because the disease burden and its trajectory in years have not been well addressed in adults. Additionally, because SARS-CoV-2 may emerge as an endemic seasonal virus, knowledge of the periodicity of the prevalent endemic HCoV subtypes (HCoV-OC43, -229E, and -NL63) may help improve public health strategies for the development of early and effective countermeasures to combat coronavirus disease 2019 (COVID-19) (6). Therefore, we sought to understand the burden of these infections among adults by examining five years of data before the onset of the COVID-19 pandemic. Additionally, we aimed to expand the existing evidence base and provide new insights into the epidemiology of HCoVs infections in adults.

MATERIALS AND METHODS

This study was performed as a retrospective study in adult patients (>18 years) at Hacettepe University Adult Hospital in Turkey between January 1, 2015, and January 1, 2020 (before the COVID-19 pandemic). The patients were divided into three age groups (18–40, 41–59, and ≥60 years). Adult patients with acute respiratory symptoms (cough, sore throat, increased or new sputum, dyspnea, and wheezing with or without fever) were included in the study. Clinical examinations were performed after the patients were identified as eligible for analysis using multiplex real-time polymerase chain reaction (RT-PCR) at the Molecular Microbiology Laboratory. Respiratory specimens (nasopharyngeal swabs, sputum, and other upper respiratory samples) were also collected. The detected HCoVs (NL63, 229E, and OC43) were reported by year, season, patient age, and co-infection with other respiratory viruses. The study did not include specimens obtained from the same patient within 30 days ($n = 3,321$) to avoid misinterpretation and duplications because prolonged coronavirus shedding was found in follow-up testing in previous studies (18). Respiratory samples were

sent to the laboratory in sterile transport containers. Following nucleic acid isolation, a multiplex one-step RT-PCR kit (Allplex Respiratory Panel Assay 1/2/3; Seegene Inc. [Seoul, South Korea]) was used. The multiplex one-step RT-PCR kit was used according to the manufacturer's protocols. The Allplex Respiratory Panel 1/2/3 is a multiplex PCR assay for detecting 16 respiratory viruses (influenza A virus [IAV], influenza B virus [IBV]), respiratory syncytial virus A (RSV-A), respiratory syncytial virus B (RSV-B), adenovirus (AdV), enterovirus (EV), metapneumovirus (MPV), parainfluenza virus 1 (PIV-1), parainfluenza virus 2 (PIV-2), parainfluenza virus 3 (PIV-3), parainfluenza virus 4 (PIV-4), bocavirus (BoV), coronavirus 229E (229E), coronavirus NL63 (NL63), coronavirus OC43 (OC43), and rhinovirus (RV)), and the first clinical assay based on multiple detection temperatures. The kit could not detect coronavirus HKU-1 (HKU-1) (19). Age is described using the median and interquartile range (IQR). Viral prevalence was compared using the chi-squared test for categorical variables. IBM SPSS Statistics (version 23.0; IBM Corp., Armonk, NY, USA) was used to perform statistical analyses. Results were considered statistically significant when the P -value was <0.05.

This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethics committee approval was obtained from the Hacettepe University Observational Research Ethics Committee (Ethics Committee Approval No: 066-2017).

RESULTS

Respiratory samples ($N = 7,861$) were obtained from patients with acute respiratory symptoms and sent for analysis during the study period. Samples ($n = 3,321$) obtained from the same patient within the last 30 days were excluded. After exclusion, 4,540 respiratory samples from patients with acute respiratory symptoms were screened and analyzed. A total of 38% (1,727/4,540) samples were positive for any respiratory viruses. Of these, in 12.3% (212/1,727) of samples, HCoVs were detected, while other respiratory viruses were detected in the remaining 87.7% (1,515). Figure 1 shows the sample selection process for HCoVs (NL63, OC43, and 229E). The median age of the HCoV-positive patients was 49 years (IQR:18–94), and the female-to-male ratio was 1.12 (112:100). Hypertension (91 patients [42.9%]) was the most common comorbidity followed by coronary artery disease (90 patients [42.5%]), malignancy (82 patients [38.7%]), and diabetes (70 patients [33%]) (Table 1). Just over half of the HCoV-positive patients were receiving immunosuppressive therapy (53.3%).

Of the 212 specimens in which HCoVs were detected, NL63 had the highest detection rate (4.16%, 72/212), followed by OC43 (3.7%, 64/212), and 229E (3.64%, 63/212). Also, 65 (30.7%) had a co-infection with other respiratory viruses. Among HCoV detected patients, 13 had HCoV infections mixed with other HCoV strains: 229E/NL63 ($n = 11$) and 229E/OC43 ($n = 2$). Co-infection was more common in patients infected with 229E than in those infected with other HCoVs; however, there was no significance in terms of

Prevalence of Common Human Coronaviruses in Adults

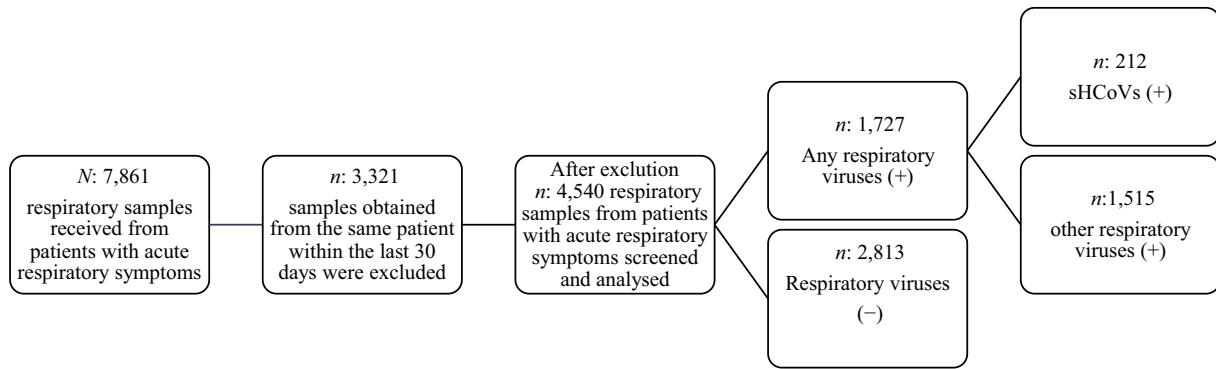


Fig. 1. The flow chart shows the samples' selection process with seasonal human coronaviruses (HCoVs) (NL63, OC43, and 229E).

Table 1. Demographics of patients with documented HCoVs (n: 212)

Age, median (IQR), years	49 (18–94)
Male/female - n (%)	112 (52.8)/100 (47.2)
Common comorbidities- n (%)	
Diabetes mellitus	70 (33)
Hypertension	91 (42.9)
Coronary artery disease	90 (42.5)
Chronic obstructive pulmonary disease	42 (19.8)
Malignancy	82 (38.6)
Renal disease	42 (19.8)
Immunosuppressive therapy	113 (53.3)
Other comorbidities- n (%)	
Hypothyroidism	10 (4.7)
Autoimmune diseases	12 (5.6)
Anemia	10 (4.7)
Cystic fibrosis	8 (3.7)
Benign prostatic hypertrophy	7 (3.3)
Alzheimer's disease & dementia	3 (1.4)
Number of comorbidities- n (%)	
0	12 (5.7)
1	41 (19.3)
≥ 2	159 (75)
Level of clinical care- n (%)	
Inpatient	
Intensive care unit	29 (13.7)
Other inpatient units	50 (23.6)
Outpatient	133 (62.7)

frequency. The prevalence of HCoV co-infection was higher with RV (27.6%), IAV (13.8%), RSV-B (12.4%), BoV/IBV (7.7%), and IAV/IAB (6.2%). OC43 was associated with co-infections with RV and IAV, whereas NL63 and 229E were associated with RV and IBV. Figure 2 demonstrates the co-infection prevalence of HCoVs and other respiratory viruses.

The prevalence of HCoVs differed by year; it was highest in the 2019–2020 season and lowest in the 2015–2016 season. Overall, from 2015 to 2020, NL63 infections had the highest prevalence (16%). The prevalence of HCoVs infections is shown in Fig. 3. The monthly cumulative results and distribution across the 2015–2020 period are shown in Fig. 4. The overall

prevalence of HCoV infection tended to be the highest in the winter (40.9%), followed by spring (27.3%). The lowest activity was observed in the summer (11.7%). While the highest positivity rates were observed in December and March (15.5% and 13.2%), the lowest rates were observed in May and September (1.9% and 2.8%) ($P < 0.05$).

There were statistically significant differences in the prevalence of HCoVs and NL63 among the three age groups ($P < 0.001$). Patients aged ≥ 60 years had the highest prevalence of overall HCoVs (39.7%, 89/212) and NL63 (11.3%, 34/212) than the other age groups. In addition, 229E was the most common in patients under 40 years, whereas NL63 was the most common in those

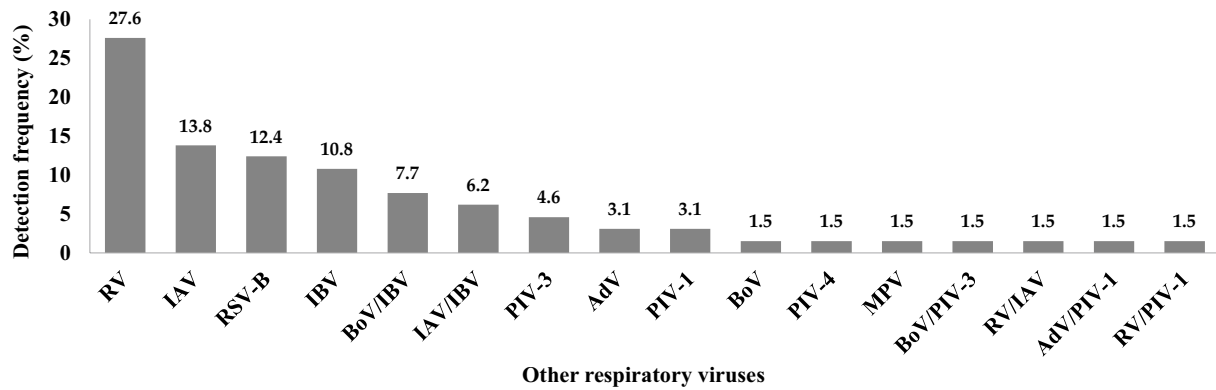


Fig. 2. Co-infection prevalence of HCoVs with other respiratory viruses IAV, influenza A virus; IBV, influenza B virus; RSV-A, respiratory syncytial virus A; RSV-B, respiratory syncytial virus B; AdV, adenovirus; MPV, metapneumovirus; PIV 1, parainfluenza virus 1; PIV3, parainfluenza virus 3; PIV 4, parainfluenza virus 4; BoV, bocavirus; RV, rhinovirus.

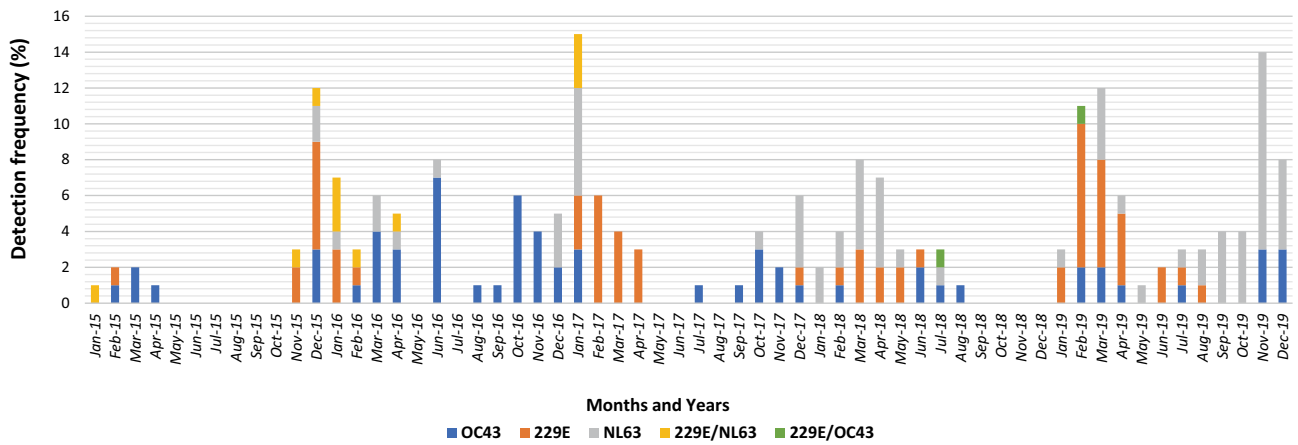


Fig. 3. (Color online) The prevalence of HCoVs by year.

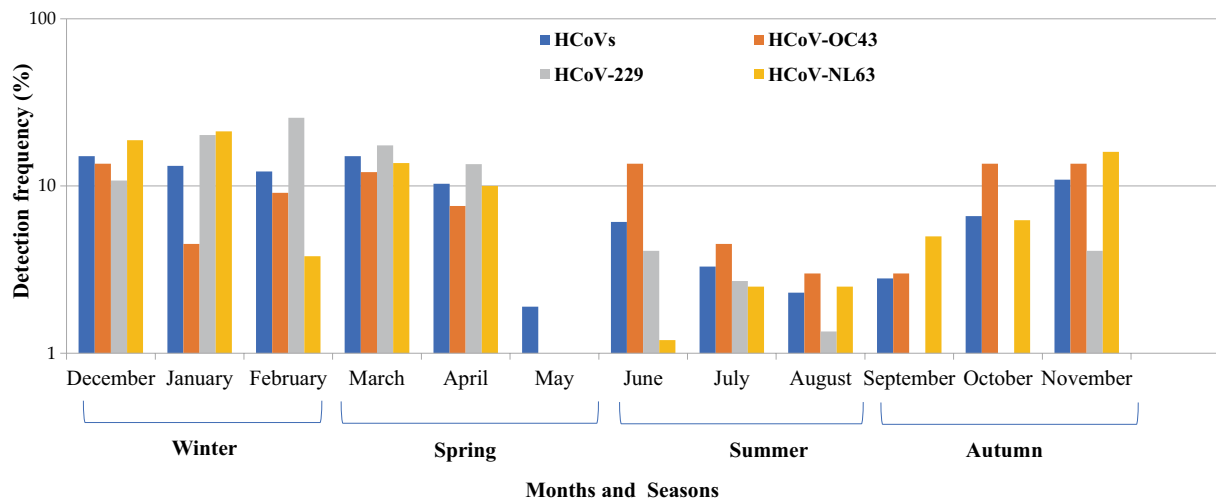


Fig. 4. (Color online) The monthly cumulative results and HCoVs distribution across the study period.

older than 40 years. The prevalence of specific HCoV types according to age is shown in Fig. 5.

DISCUSSION

Many people were exposed to common HCoVs (229E, NL63, OC43, and HKU1) before the COVID-19

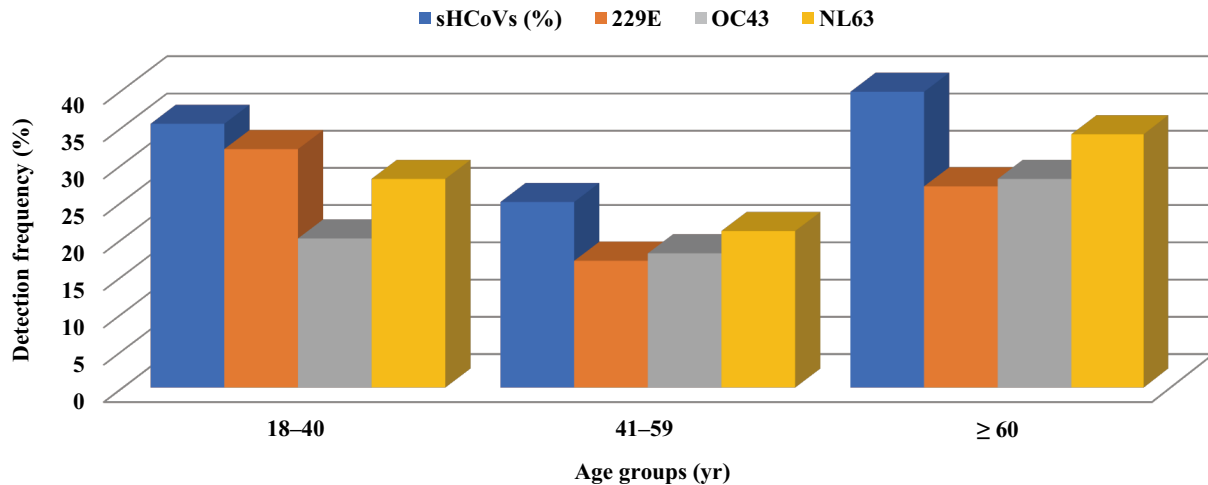


Fig. 5. (Color online) The prevalence of specific HCoV types according to age (yr: years).

pandemic. HCoV infection prevalence of respiratory viruses in adults greatly varies from 0.5 to 30% in previous studies (2,6,8,20–22). When data were visualized by census region, annual and seasonal patterns were similar to those seen nationally but higher than transnationally (23–29). Several factors, such as case definition, study setting, geographical settlement, climatic conditions, population structure, and differences in the host and pathogen can affect the study results; therefore, these factors should be considered upon interpretation. In our study, the prevalence of HCoVs in adults was 12.2%, with NL63 being the most detected type. Recently, a meta-analysis by Park et al. demonstrated that HCoV positivity rates were higher in trials that only tested children than in studies that included all age groups, consistent with results from studies on other respiratory viruses (6). Akagi et al. also found in a study in Japan that HCoVs were prevalent in up to 15–30% of common cold cases, which is consistent with our results (22). These findings imply that HCoVs cannot be neglected in adults and that they constitute a significant proportion of respiratory pathogens. Although recent studies have confirmed the critical role of HCoVs and their global circulation, pertinent data on the frequency and seasonality in the broader community context are still lacking (6,10,30). This study provides comprehensive data on the epidemiology of HCoVs in adults prior to the COVID-19 pandemic.

Here, the prevalence of HCoV infection differed each year between seasons and HCoV types. Looking at the overall prevalence from 2015 to 2020, NL63 (16%) showed the highest prevalence. Recent studies showed that OC43 was the most prevalent, while others showed that the prevalence of NL63 was similar to or even higher than that of OC43 (6,26). One study hypothesized that OC43 and NL63 might elicit an immune response that protects against subsequent infections caused by HKU1 and 229E, respectively, which may explain why OC43 and NL63 are the most frequently detected HCoV types (20). In addition, different HCoV types predominated in other years; NL63, OC43, and 229E showed more variability, with distinct peaks in one or two of the five years

examined. These results are consistent with previously published data indicating that individual HCoV types may only demonstrate peak activity every two to three years (6,17,31). Therefore, this suggests an effect of immunological interaction or interference. Most studies have highlighted the potential of interactions and co-infections between HCoVs and other respiratory viruses. In co-infections, the detection of HCoVs should not be interpreted as representing an incidental infection, and co-infection with another respiratory virus does not affect the HCoVs infectiousness. In addition, co-infection with other respiratory viruses and combination patterns were different in each study (2,5,17,20,29,32). In this study, 30.7% of patients (65/212) were co-infected with a second respiratory virus, similar to other studies. Additionally, co-infection was more common in patients infected with 229E than NL63 or OC43. Among children, RSV and influenza were the most common viruses causing co-infection with HCoVs (11,24,30). In a study reported by Graat et al. (33), RV (32%), followed by HCoVs (17%), were the two most common viruses associated with respiratory infections in adults. We found similar results: influenza and RV were the most common viruses co-infected adults with HCoVs. In addition, HCoVs-influenza co-infection rates were high in older patients, highlighting the need to increase vaccination rates in the elderly.

Globally, HCoVs display marked seasonality spanning December and March, with the rate at which they are detected varying with time and location. In general, OC43, 229E, NL63, and HKU1 have been reported to exhibit winter seasonality (28,34,35); however, several studies have demonstrated differences between HCoV species seasonalities and winter preference. NL63 showed a peak during spring and summer in Hong Kong, indicating that the seasonality of NL63 infection may not be restricted to winter in tropical and subtropical regions (36). Cabeça et al. tested 1,137 samples in São Paulo, Brazil, from 2001 to 2010 and observed a peak prevalence of NL63 in July (winter); it was a unique HCoV species with cases also being detected in the summer (28). Differences in host susceptibility, environmental factors, and population behavior are potential determinants of seasonality

(2,5,6,23). The strength of our study was that it involved interactions throughout the calendar year, not solely during winter. Nevertheless, the limitation of this study was that it was a single-center study conducted in one geographic region. While the highest prevalence was observed in December and March (15.5% and 13.2%, respectively), the lowest rates were observed in May and September. In addition, the HCoV-OC-43 was most prevalent in winter, and it was the most frequently detected HCoV in summer. Overall, NL63 was the most frequently detected type, circulating in all seasons. This was associated with the cold climate of Ankara. Owing to its altitude and inland location, Ankara has cold, snowy winters and hot, dry summers. Rainfall occurs mainly during spring and autumn. Monthly mean temperatures range from 0.3°C in January to 23.5°C in July, with an annual mean of 12.02°C (37). A high proportion of co-circulating HCoVs during influenza and RSV seasons implies the possibility of a substantial increase in the demand for healthcare system resources during winter. In addition, unlike HCoVs, MERS-CoV and SARS-CoV have not become fixed in seasonality on a global scale (10,38,39).

In studies conducted on adults, the most common HCoV infections differed according to age groups. These studies suggest that HCoV infection prevalence varies depending on the population characteristics or geographic region (2,5,6,11,14,25,40). In our study, the most common HCoV infection under 40 was 229E, while that above 40 years was NL63. In addition, statistically significant differences were found in the prevalence of HCoVs and NL63 by age group ($P < 0.001$). Patients aged ≥ 60 years had the highest HCoV prevalence (39.7%, 89/212) and NL63 incidence (11.3%, 34/212) than the other age groups. This finding may be due to the weakened immunity of older adults, which causes vulnerability to respiratory infections (13,20,23,26). Our results provide insight into the epidemiology of HCoVs among different age groups of patients and support the notion that HCoV infections are prevalent in children and the elderly. Notably, the receptors for 229E and NL63 coronaviruses are aminopeptidase N and angiotensin-converting enzyme 2 (ACE2), respectively, whereas other HCoVs utilize sialic acid and heparan sulfate when infecting the target cell. NL-63 predominance among people over 40 years of age is also concordant with elevated ACE-receptor expression at 40 years of age (1). Therefore, extra vigilance is required for the elderly population.

Our findings should be interpreted with caution in light of these limitations. First, this was a single-center, retrospective study, and its findings could be biased by the attending clinician's RT-PCR order. However, all samples were collected from symptomatic patients, suggesting that the symptoms were severe enough for prompt sampling. In addition, information regarding the disease severity was not available. Another limitation is that our diagnostic tool remains suboptimal since a specific infectious agent, HKU-1, could not be detected. In a recent study, patients with respiratory illnesses showed a very low overall incidence of HKU-1. Consequently, testing for HKU-1 was discontinued in Scotland (40). Finally, our study was carried out on an enormous number of samples sent for molecular

testing over 5 years. Therefore, our results extend the knowledge gained from previous studies on the epidemiology of HCoV in adults. While SARS-CoV-2 may mimic the epidemiological pattern of SARS-CoV and fade with time, there is still a risk that it could become an endemic human respiratory coronavirus, such as OC43, 2299E, NL63, and HKU1. Therefore, even if the COVID-19 pandemic can be controlled, continuous monitoring is necessary, and these results might contribute to the understanding of the future endemic circulation of SARS-CoV-2 (18).

In conclusion, the significance and impact of HCoVs as respiratory viruses in adults are poorly characterized. Detection of pathogens in respiratory infections is crucial for diagnosis, patient management, and avoiding improper antibiotic treatment. Recent improvements in RT-PCR assays have resulted in better respiratory virus detection and have helped to understand the role of HCoVs in respiratory infections among adults. Therefore, HCoVs are better recognized and contribute to our study as essential respiratory pathogens in adults, particularly the elderly. Moreover, to our knowledge, our results provide the most comprehensive epidemiological data from Turkey and help us understand the trajectory of HCoVs over the years.

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Conflict of interest None to declare.

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