International Journal of Infectious Diseases 120 (2022) 205–209

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Original Article

Human metapneumovirus prevalence during 2019-2021 in Israel is influenced by the COVID-19 pandemic

Michal Stein[1,2,*], Hodaya Cohen[3,*], Ital Nemet[3], Nofar Atari[3], Limor Kliker[3], Ilana S. Fratty[3], Efrat Bucris[3], Miranda Geva[3], Ella Mendelson[2,3], Neta Zuckerman[3,#], Michal Mandelboim[2,3,#,**]

[1] Pediatric Infectious Disease Unit, Sheba Medical Center, Tel-Hashomer, Israel
[2] Sackler Faculty of Medicine, Tel-Aviv University, Israel
[3] Central Virology Laboratory, Public Health Services, Ministry of Health and Sheba Medical Center, Tel-Hashomer, Israel

ARTICLE INFO

Article history:
Received 20 October 2021
Revised 3 April 2022
Accepted 20 April 2022

Keywords:
Respiratory system
Viruses
⟨13:italic ⟩Pneumoviridae⟨/13:italic⟩
Phylogeny

ABSTRACT

Objectives: To compare infection rates and circulating subtypes of human metapneumovirus (hMPV) before (2019-2020) and after the emergence of coronavirus disease 2019 (COVID-19) (2021) in Israel.
Methods: In total, 12,718 respiratory samples were collected from hospitalized patients of all ages during the years 2019 to 2021 at the Sheba Medical Center in Israel and subjected to reverse transcription-polymerase chain reaction analysis. In addition, whole-genome sequencing was performed to characterize the subtypes of hMPV circulating in Israel between 2019 and 2021.
Results: A total of 481 samples were found positive for hMPV**.** Before the emergence of COVID-19, hMPV peaked in winter months and declined thereafter. In sharp contrast, during the COVID-19 pandemic, we observed a delayed peak in hMPV infection cases and higher infection of young children. Viral sequencing showed a shift in the most prevalent circulating hMPV strain from A2b to B1 during the years 2019, 2020, and 2021.
Conclusion: Compared with the years before the COVID-19 pandemic, in 2021, hMPV mostly affected young children, and the most prevalent circulating subtype shifted from A2b in 2019 to B1.

Introduction

Current Coronavirus Disease 2019 (COVID-19) pandemic is affecting the circulation and epidemiology of other seasonal respiratory viruses (AgcaAgca et al., 2021; Olsen et al., 2020; Weinberger Opek et al., 2021). COVID-19 symptoms include fever, cough, fatigue and other respiratory tract symptoms which may end up lethal (Shahgolzari et al., 2021). Furthermore it is possible that symptoms such as anosmia, ageusia, gingival inflammation and other symptoms attached to the oral cavity may appear or sustain after the disease disappear (Khodavirdipour et al., 2021a). Before the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the main circulating winter viruses were influenza viruses, respiratory syncytial virus (RSV), and human metapneumovirus (hMPV) (Hedberg et al., 2022). hMPV, a member of the *Pneumoviridae* family, which accounts for approximately 5-15% of all respiratory tract infections (RTIs), infects children, the elderly, and adults and is especially dangerous for infants aged 6-12 months (PERCH Study Group., 2019; Jain et al., 2015; Van den Hoogen et al., 2001; Williams et al., 2004). hMPV, similarly to RSV, has a genomic organization that contains 8 genes encoding eight open reading frames. Although the viruses are similar, RSV has nonstructural genes that hMPV lacks (Collins et al., 2013; Groen et al., 2021). hMPV has two major lineages (A and B) subdivided into four sublineages (A1, A2, B1, B2), which are further divided into A2a, A2b, B2a, and B2b (Groen et al., 2021).

Several works have shown that the current coronavirus disease 2019 (COVID-19) pandemic is affecting the circulation and epidemiology of other seasonal respiratory viruses (Agca et al., 2021; Olsen et al., 2020; Weinberger Opek et al., 2021). Several methods have been suggested for the detection of RTIs, especially during the winter season, when multiple viruses co-emerge. Detection

** Corresponding author: Michal Mandelboim, Central Virology Laboratory, Tel-Hashomer Hospital, Ramat-Gan 11, Ramat Gan, Israel
E-mail address: michalman@sheba.health.gov.il (M. Mandelboim).
* These authors contributed equally to this work.
# These authors contributed equally to this work.

https://doi.org/10.1016/j.ijid.2022.04.037

of multiple viruses is usually performed using a multiplex reverse transcription-polymerase chain reaction (RT-PCR) assay. Detection of SARS-CoV-2 and other respiratory viruses can also be performed using the specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) method. This novel technique uses CRISPR/Cas13 that can show ribonuclease activity with the activation of Cas13 nucleases and thus detect multiple sequences (Khodavirdipour et al., 2021b). However, the SHERLOCK technique has not been commercialized yet.

This study aimed to apply multiplex RT-PCR to genetically and epidemiologically characterize the late seasonal hMPV outbreak in 2021 alongside the COVID-19 pandemic, and compare it with that of the 2019-2020 season.

Materials and Methods

Patients and samples

Respiratory clinical samples (nasopharyngeal swabs or aspirates) were collected from 12,718 patients hospitalized at the Sheba Medical Center, Israel, because of respiratory illnesses and influenza-like symptoms. All samples were sent for routine clinical analysis to identify the presence of various respiratory viruses. Retrospective analysis was performed on samples collected during the years 2019 to 2021, before and during the SARS-CoV-2 pandemic. Raw data of the details provided in this paper are presented in the Supplementary material (Table S1).

Nucleic acid extraction and real-time PCR analysis

All viral samples were extracted using the MagNA Pure 96 and the High Pure Viral RNA extraction kits (Roche Diagnostics, Mannheim, Germany). From 2020 onward, the STARMag Viral DNA/RNA 200C kit (Seegene, Seoul, South Korea) was used for viral nucleic acid extraction. The presence of hMPV was tested using the Allplex^{TM} RV multiplex real-time RT-PCR kit (Seegene). The viruses detected by this kit are influenza A, influenza B, hMPV, RSV, parainfluenza, rhinovirus, and adenovirus (Folgueira et al., 2019). The primers used to identify the viruses are not disclosed by the manufacturer. Extraction and RT-PCR procedures were conducted in accordance with the manufacturers' instructions.

Phylogenetic analysis

Whole-genome sequencing was performed on RNA from hMPV-positive samples, using Illumina RNA Prep with Enrichment (L) Tagmentation and Respiratory Virus Oligos Panel V2 kits (Illumina, San Diego) in accordance with the manufacturer's instructions. Library validation and mean fragment size were determined using the 4200 TapeStation via DNA HS D1000 kit (Agilent Technologies, Santa Clara, United States). Libraries were pooled, denatured, and diluted to 1 nM and sequenced on NovaSeq using the SP kit of 150 × 2 cycles (Illumina) following manufacturer's instructions. Sequences were mapped to hMPV reference genomes (NC_039199.1) with Burrows-Wheeler Aligner-MEM (Li and Durbin, 2009). Multiple alignment of sequences with reference sequences and phylogenetic tree construction were performed using the Nextstrain Augur pipeline and visualized with Auspice (Hadfield et al., 2018).

Statistical analysis

A chi-squared test was performed to evaluate the differences between the age groups of 0-2 and >60 years from 2019-2021. A P-value <0.05 was considered statistically significant ($P<0.05$).

Results

Circulation of hMPV before and during the SARS-CoV-2 pandemic

In total, 12,718 samples of hospitalized patients were collected and tested during the years 2019-2021, before and during the COVID-19 pandemic. Of these, 481 were found positive for hMPV. The SARS-CoV-2 data presented in Figure 1 summarizes all SARS-CoV-2 infections detected in Israel, as was updated daily by the Ministry of Health (IMoH, 2022).

In 2019, before the emergence of COVID-19, hMPV infections peaked at the end of February-March (week 13) and declined at the beginning of June (week 26), followed by a low infection rate throughout the summer (Figure 1A and Table S1). In 2020, hMPV infections were first detected in February (week 5) and peaked in March, similar to 2019. In parallel, SARS-CoV-2 infections were first detected in Israel in February 2020 (Last, 2020). Between March 2020 and December 2020, three lockdowns were declared by the Israeli government, and during this period, only sporadic cases of hMPV infection were detected. The first lockdown, which began on March 17, 2020 (week 13), resulted in decreased numbers of SARS-CoV-2 infection cases after 4 weeks (Figure 1). The second lockdown (September 18, 2020; week 40) led to a decline in SARS-CoV-2 infection cases after 3 weeks, and the third lockdown (December 27, 2020; week 52) also decreased SARS-CoV-2 infection cases alongside vaccination efforts from December 2020 to January 2021. hMPV infection cases were again reported at the end of March 2021 (week 14) and peaked unexpectedly from June to July 2021—months in which hMPV is not prevalent, as reported in previous years.

Age-related hMPV incidence in the years 2019-2021

Analysis of the age distribution of hMPV-infected patients found that viral prevalence was highest in young children and adults older than 60 years, with lower levels found in other age groups. Increased rates of hMPV infection were observed in 2021 compared with previous years, mainly in the 0-2 years and 3-5 years age groups. In contrast, significantly fewer individuals younger than 60 years were infected in 2021 than in 2020 and 2019 (Figure 2).

Human metapneumovirus phylogenetic analysis by year

The most prevalent subtype of hMPV detected in hospitalized patients varied from 2019 to 2021 (Figure 3). In this period, three known subtypes were detected: A2b, B1, and B2. In 2019, the most frequently detected hMPV subtype was A2b, whereas in 2020, the three subtypes were equally prevalent. Interestingly, in 2021 all sequenced samples were of the B1 subtype. These results show that although 2019 and 2021 had a dominant subtype, there was no dominant circulating subtype in 2020. Importantly, the COVID-19 pandemic in 2020 led to a decrease in hospitalized patients with respiratory symptoms who were found positive for hMPV (Figure 1 and S1) (Agca et al., 2021).

Discussion

The respiratory virus hMPV was first detected in 2001. hMPV infection results in a mostly symptomatic disease in both upper and lower respiratory tracts and is one of the most common causative pathogens of bronchiolitis, pneumonia, and otitis media in both children and adults. It is still debated whether it leads to asthma in young children (Van den Hoogen et al., 2001; Zhou et al., 2020; Hedberg et al., 2022). Because of the increasing incidence in Israel, it is important to characterize the circulating

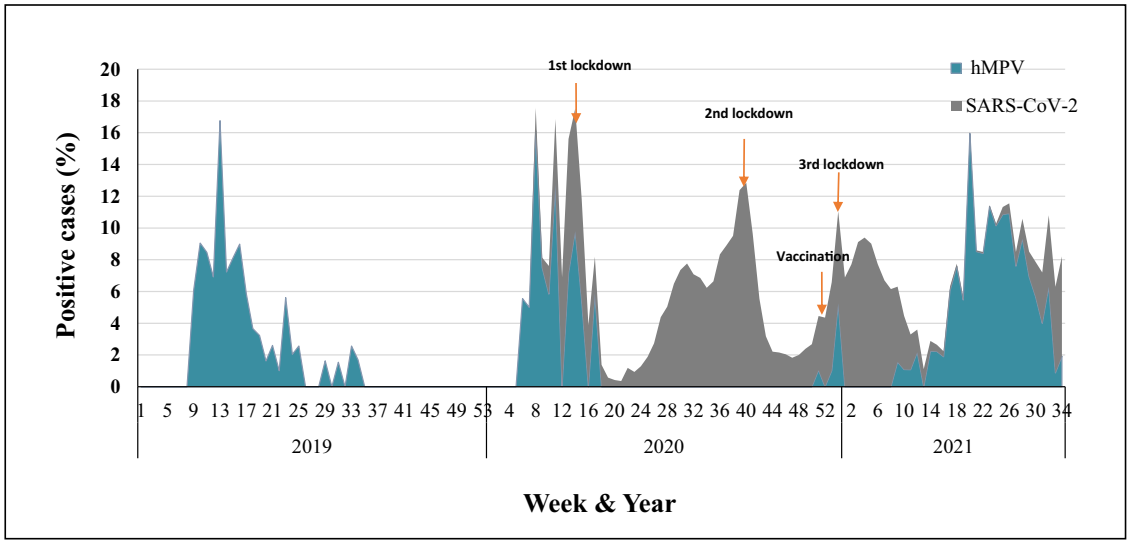

Figure 1. hMPV and SARS-CoV-2 infection rates in Israel, 2019-2021. The graph summarizes percentages of positive cases of hMPV and SARS-CoV-2 infection between 2019 and 2021 among hospitalized patients. The x-axis shows the week number in each year. The *arrows* indicate start of lockdown or vaccination.
hMPV, human metapneumovirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

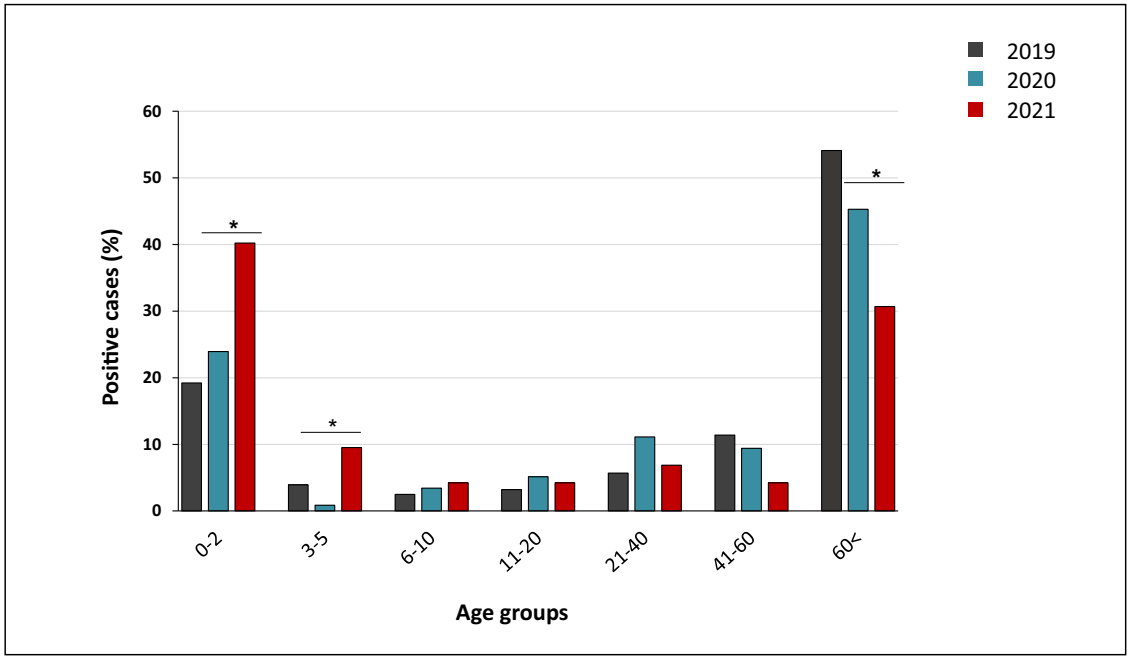

Figure 2. Age distribution of patients infected with hMPV in Israel, 2019-2021. Calculation of percentage was based on the sum of positive hMPV cases per year (*$P<0.05$).
hMPV, human metapneumovirus.

subtypes infecting young children in tandem with the COVID-19 pandemic.

Our study examined the circulation of hMPV during the COVID-19 pandemic, in the period that involved three lockdowns and SARS-CoV-2 vaccination efforts. No cases of hMPV infection were detected among hospitalized patients during the first lockdown in March 2020 and even later in May when the education system returned to frontal teaching. The absence of hMPV infections may have been due to the lockdown policy and the spring-summer season, during which incidence of hMPV is usually not high (Aberle et al., 2010). Likewise, during the second lockdown, which began in September and continued to mid-October 2020, no hMPV infections were detected. As COVID-19 incidence rose again in December 2020, the third lockdown was announced on December 27, 2020, concomitantly with the vaccination campaign that began on December 20, 2020, leading to a waning of SARS-CoV-2 circulation. Although sporadic cases of hMPV were detected during December and January, the waning of SARS-CoV-2 circulation was followed with a sudden off-season rise in numbers of hMPV infections registered in May 2021.

Circulation of hMPV occurs each year in February and March, as shown in other studies (Regev et al., 2012 Meningher et al., 2014). However, in our study, we observed a delayed increase in hMPV infection cases, which occurred in May and June 2021. We suggest that the lack of exposure to other respiratory viruses during 2020 and the rise in SARS-CoV-2 in January 2021 led to the atten-

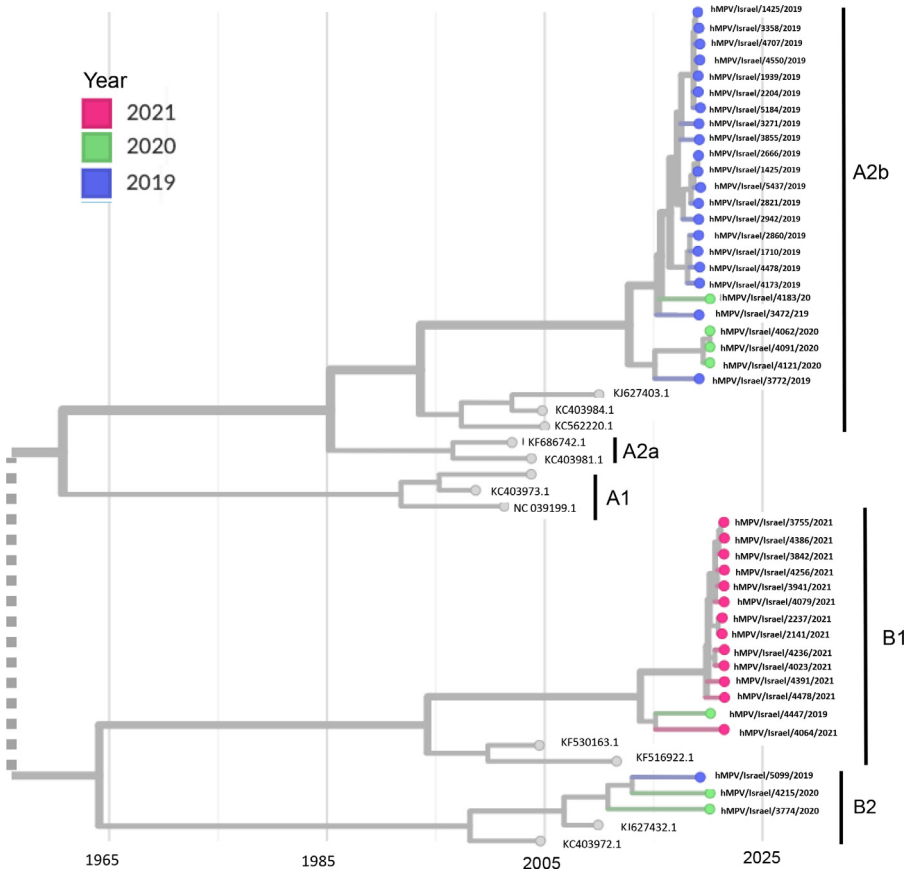

Figure 3. Phylogenetic tree of hMPV infections in Israel, 2019-2021. Phylogenetic tree representing complete hMPV genomes sequenced from 41 patients in Israel in 2019 (*blue*), 2020 (*green*) and 2021 (*pink*) and 12 reference sequences of different hMPV lineages (*gray*). The tree was constructed using the Nextstrain Augur pipeline and visualized with Auspice.
hMPV, human metapneumovirus.

uation of hMPV incidence in 2021 compared with previous years, possibly due to competition in the same ecological niche. To reinforce this assumption, we compared our results with those of the study by Regev et al., which showed an increase in hMPV infection cases after the influenza A (H1N1pdm) virus weakening in 2009. During 2009, with the presence of influenza A (H1N1pdm), there was a decrease in other respiratory viruses such as RSV, while the influenza A pandemic dominated (Meningher et al., 2014). In 2009, after the winter season, a significantly higher number of hMPV infection cases were registered in children below the age of 1 (Meningher et al., 2014). In our study, we observed a significantly higher number of hMPV infection cases among infants and toddlers in 2021 than in previous years. A possible explanation for this observation is that there was a longer-than-usual period of no hMPV circulation and consequently a larger-than-normal number of children exposed to the virus. In contrast, the number of cases in the elderly population decreased. Older population, especially adults aged >60 years, as a result of being at highest risk of COVID-19–related morbidity and complications, maintained respiratory protection guidelines and social distancing measures, even after the lockdown was eased, which might explain the relatively lower incidence among this age group during the hMPV 2021 outbreak.

The subtypes of hMPV found in our study between 2019 and 2021 were hMPV A2b, B1, and B2.

Generally, it is possible that one or more different hMPV subtypes may be detected each year, although only one subtype dominates (Mackay et al., 2006; Zhou et al., 2020). In most cases, the hMPV subtype switches from A to B and then regresses to A. This trend was observed in India, the United States, South Korea, and China (Agapov et al., 2006; Agrawal et al., 2011; Kim et al., 2010; Zhang et al., 2012).

Our study shows that most hMPV cases in 2019 were of the A2b subtype, whereas in 2020, during the COVID-19 pandemic, the dominant subtypes were A2b, B1, and B2. It is possible that the pandemic onset in 2020 influenced the heterogeneity of hMPV and should be further studied in the future.

Since the emergence of COVID-19, laboratories in Israel performed fewer hMPV tests than before the pandemic, and thus, there may have been more hMPV cases than identified. Therefore, it cannot be concluded with confidence that there were several dominant subtypes. In 2021, all samples tested were of the B1 subtype. Although the contributions of the A and B hMPV subtypes to disease severity are still under investigation, thus far, no evidence has been reported. Regardless, the variation in hMPV subtypes each year can be attributed to geographical and seasonal distribution changes, variations in immune system compatibility and viral evolutionary changes (Altizer et al., 2006; Falsey et al., 2010; Gaunt et al., 2011; Ishiguro et al., 2004).

In conclusion, the Israeli COVID-19 measures, which included three lockdowns aimed at suppressing the spread of COVID-19, most likely also halted the spread of other respiratory viruses, including hMPV, but later resulted in a higher-than-normal rate of children infected with hMPV. These combined results emphasize the need for the development of an hMPV vaccine, especially for the post–COVID-19 era and the young population.

Conflict of interest

The authors declare no conflict of interests.

Funding

None.

Ethical approval

The institutional review board of the Sheba Medical Center approved this study (Helsinki Number 7688-20-SMC). Nasopharyngeal swabs or aspirate samples were collected at the Chaim Sheba Medical Center and from other Israeli hospitals as part of the routine sampling performed in the clinical virology laboratory. The samples were tested and analyzed for the presence of various viruses as part of the routine tests performed in the Chaim Sheba Medical Center. The work described in this paper is an anonymous retrospective study; hence, informed consent (either written or verbal) was not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.04.037.

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