ORIGINAL RESEARCH

© Yatsyshina S.B., Mamoshina M.V., Shipulina O.Yu., Podkolzin A.T., Akimkin V.G., 2020

Analysis of human coronaviruses circulation

Svetlana B. Yatsyshina, Marina V. Mamoshina, Olga Yu. Shipulina, Alexandr T. Podkolzin, Vasiliy G. Akimkin

Central Research Institute for Epidemiology of the Federal Service for Supervision of Consumer Rights Protection and Human Welfare, Moscow, 111123, Russia

Introduction. The novel SARS-CoV-2 coronavirus, which emerged at the end of 2019 and caused a worldwide pandemic, triggered numerous questions about the epidemiology of the novel COVID-19 disease and about well-known coronavirus infections, which used to be given little attention due to their mild symptoms.
The purpose: The routine screening-based multiyear retrospective observational study of prevalence and circulation patterns of epidemic-prone human coronaviruses in Moscow.
Material and methods. The real-time polymerase chain reaction was used to detect RNA of human coronaviruses (HCoVs) in nasal and throat swabs from 16,511 patients with an acute respiratory infection (ARI), aged 1 month to 95 years (children accounted for 58.3%) from January 2016 to March 2020, and swabs from 505 relatively healthy children in 2008, 2010 and 2011.
Results. HCoVs were yearly found in 2.6–6.1% of the examined patients; the detection frequency was statistically higher in adults than in children, regardless of sex. At the height of the disease incidence in December 2019, HCoVs were detected in 13.7% of the examined, demonstrating a two-fold increase as compared to the multi-year average for that month. The statistical frequency of HCoV detection in ARI pediatric patients under 6 years was significantly higher than in their healthy peers (3.7 vs 0.7%, $p = 0.008$).
Conclusion. HCoVs circulate annually, demonstrating a winter-spring seasonal activity pattern in the Moscow Region and reaching peak levels in December. Over the years of observation, the HCoV epidemic activity reached maximum levels in December 2019–February 2020 and decreased in March to the multi-year average. Amid a growing number of SARS-CoV-2 cases imported to Moscow in March 2020, the HCoV detection frequency dropped sharply, which can be explained by the competition between different coronaviruses and by the specificity of HCoV detection with the diagnostic test kit used in this study.

Keywords: *coronaviruses; polymerase chain reaction; seasonality; epidemic activity.*

For citation: Yatsyshina S.B., Mamoshina M.V., Shipulina O.Yu., Podkolzin A.T., Akimkin V.G. Analysis of human coronaviruses circulation. *Problems of Virology (Voprosy Virusologii)*. 2020; 65(5): 267-274. (In Russ., in Engl.). DOI: https://doi.org/10.36233/0507-4088-2020-65-5-3

For correspondence: Svetlana B. Yatsyshina, PhD. Sci. (Biol.), Head of the Scientific Group on the Development of New Diagnostic Methods of ARI diagnostics, Central Research Institute of Epidemiology, Moscow, 111123, Russia. E-mail: svetlana.yatsyshina@pcr.ms

Information about the authors:
Yatsyshina S.B., https://orcid.org/0000-0003-4737-941X
Mamoshina M.V., https://orcid.org/0000-0002-1419-7807
Shipulina O.Yu., https://orcid.org/0000-0003-4679-6772
Podkolzin A.T., https://orcid.org/0000-0002-0044-3341
Akimkin V.G., https://orcid.org/0000-0003-4228-9044

Contribution: Yatsyshina S.B – research design, analysis of the results and writing of the text; Mamoshina M.V. – statistical data processing, drawing up the list of references; Shipulina O.Yu. – organization and conduct of laboratory research; Podkolzin A.T. – research concept and design; Akimkin V.G. – organization of research.
Acknowledgments. The study had no sponsorship.
Conflict of interest. The authors declare no conflict of interest.

Received 2 June 2020
Accepted 31 August 2020

Анализ циркуляции коронавирусов человека

Яцышина С.Б., Мамошина М.В., Шипулина О.Ю., Подколзин А.Т., Акимкин В.Г.

ФБУН «Центральный научно-исследовательский институт эпидемиологии» Роспотребнадзора, 111123, Москва, Россия

Введение. Появление в конце 2019 г. нового коронавируса SARS-CoV-2, ставшего причиной пандемии, породило массу вопросов относительно эпидемиологии нового заболевания COVID-19 и известных ранее

инфекций, вызываемых коронавирусами, которым по причине более лёгкого течения заболеваний уделяли мало внимания.

Цель данной работы – многолетнее ретроспективное исследование распространённости и особенностей циркуляции эпидемических коронавирусов человека в Москве при проведении рутинного скрининга.

Материал и методы. Методом полимеразной цепной реакции с детекцией в режиме реального времени исследовали на РНК эпидемических коронавирусов человека (HCoVs) мазки из носо- и ротоглотки 16 511 больных острой респираторной инфекцией (ОРИ) в возрасте от 1 мес до 95 лет (58,3% составили дети), собранные с января 2016 г. по март 2020 г., и мазки 505 условно-здоровых детей, собранные в 2008, 2010 и 2011 гг.

Результаты. HCoVs обнаруживали у 2,6–6,1% обследованных больных в год, статистически значимо чаще у взрослых по сравнению с детьми, без различий по полу. На пике заболеваемости в декабре 2019 г. HCoVs обнаружены у 13,7% обследованных, что в 2 раза выше среднемноголетнего уровня данного месяца. У больных ОРИ детей до 6 лет HCoVs выявляли статистически значимо чаще, чем у здоровых (3,7 vs 0,7%, $p = 0{,}008$).

Заключение. HCoVs циркулируют ежегодно, демонстрируя в Московском регионе зимне-весеннюю сезонность с пиком в декабре. За годы наблюдения эпидемическая активность HCoVs росла до максимальных значений в декабре 2019 г. – феврале 2020 г., снизившись в марте до среднемноголетнего уровня. На фоне растущего количества случаев завоза SARS-CoV-2 в Москву в марте 2020 г. частота выявления HCoVs резко понизилась, что, по-видимому, отражает наличие конкуренции между разными коронавирусами и подтверждает специфичность выявления HCoVs использованным в данной работе диагностическим набором.

Ключевые слова: *коронавирусы; полимеразная цепная реакция; сезонность; эпидемическая активность.*

Для цитирования: Яцышина С.Б., Мамошина М.В., Шипулина О.Ю., Подколзин А.Т., Акимкин В.Г. Анализ циркуляции коронавирусов человека. *Вопросы вирусологии.* 2020; 65(5): 267-274.
DOI: https://doi.org/10.36233/0507-4088-2020-65-5-3

Для корреспонденции: Яцышина Светлана Борисовна, канд. биол. наук, руководитель Научной группы по разработке новых методов диагностики ОРЗ, ФБУН «Центральный научно-исследовательский институт эпидемиологии» Роспотребнадзора, 111123, Москва. E-mail: svetlana.yatsyshina@pcr.ms

Участие авторов: Яцышина С.Б. – дизайн исследования, анализ результатов и написание текста; Мамошина М.В. – статистическая обработка данных, оформление списка литературы; Шипулина О.Ю. – организация и проведение лабораторных исследований; Подколзин А.Т. – концепция и дизайн исследования; Акимкин В.Г. – организация выполнения исследования.

Финансирование. Исследование не имело спонсорской поддержки.

Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов.

Поступила 02.06.2020
Принята в печать 31.08.2020

Introduction

Coronaviruses causing infectious diseases in animals and humans are common throughout the world. They belong to the family *Coronaviridae*, the subfamily *Orthocoronavirinae*, which is further divided into four genera: *Alphacoronavirus, Betacoronavirus, Deltacoronavirus* and *Gammacoronavirus* [1]. Representatives of *Gammacoronavirus* and *Deltacoronavirus* genera primarily infect birds. Viruses belonging to *Alphacoronavirus* and *Betacoronavirus* genera are found in mammals.

Human coronaviruses (HCoVs) circulating year-round in the human population, i.e. epidemic-prone coronaviruses, cause acute respiratory infections (ARI) [2] generally affecting an upper respiratory tract [3–6]. In rare cases, they are associated with a lower respiratory tract disease [7]; there were cases reported when human coronaviruses were isolated from patients with pneumonia [8].

Currently, there are four types of epidemic-prone human coronaviruses (HCoVs) that circulate continuously in the human population.

Human coronavirus 229E (the genus *Alphacoronavirus*, the subgenus *Duvinacovirus*) and *Betacoronavirus 1* (formerly referred to as HCoV-OC43; the genus *Betacoronavirus*, the subgenus *Embecovirus*) have been known since the mid-1960s. *Human coronavirus NL63* (the genus *Alphacoronavirus,* the subgenus *Setracovirus*) and *Human coronavirus HKU1* (the genus *Betacoronavirus*, the subgenus *Embecovirus)* were discovered in 2004 and 2005, respectively.

Coronavirus virions are spherical particles with diameters of 120 nm; they contain helical nucleocapsid (genomic RNA bound to nucleoprotein (N)) coated with a lipid membrane with integral proteins: spike glycoprotein (S) forming a monolayer of club-shaped protrusions; hemagglutinin-esterase (HE); membrane protein (M) and envelope small membrane protein (E) [9]. Viruses enter cells of mucous membranes by using their spike proteins that bind to specific receptors; moreover, coronaviruses of animals and different types of HCoVs target different receptors [10].

The coronavirus genome, the largest genome among all RNA-containing viruses, consists of a linear, positive-sense, single-stranded RNA molecule, 27–32 kb in size.

RNA recombination of different types of coronaviruses can generate new viral variants characterized by altered tissue tropism, increased virulence and the ability to overcome the interspecies barrier [11–13]. When occurred between coronavirus genomes of bats and other animals, such recombination events, which are established by natural selection, triggered the emergence of coronaviruses highly virulent for humans: SARS-CoV, the virus caus-

DOI: https://doi.org/10.36233/0507-4088-2020-65-5-3

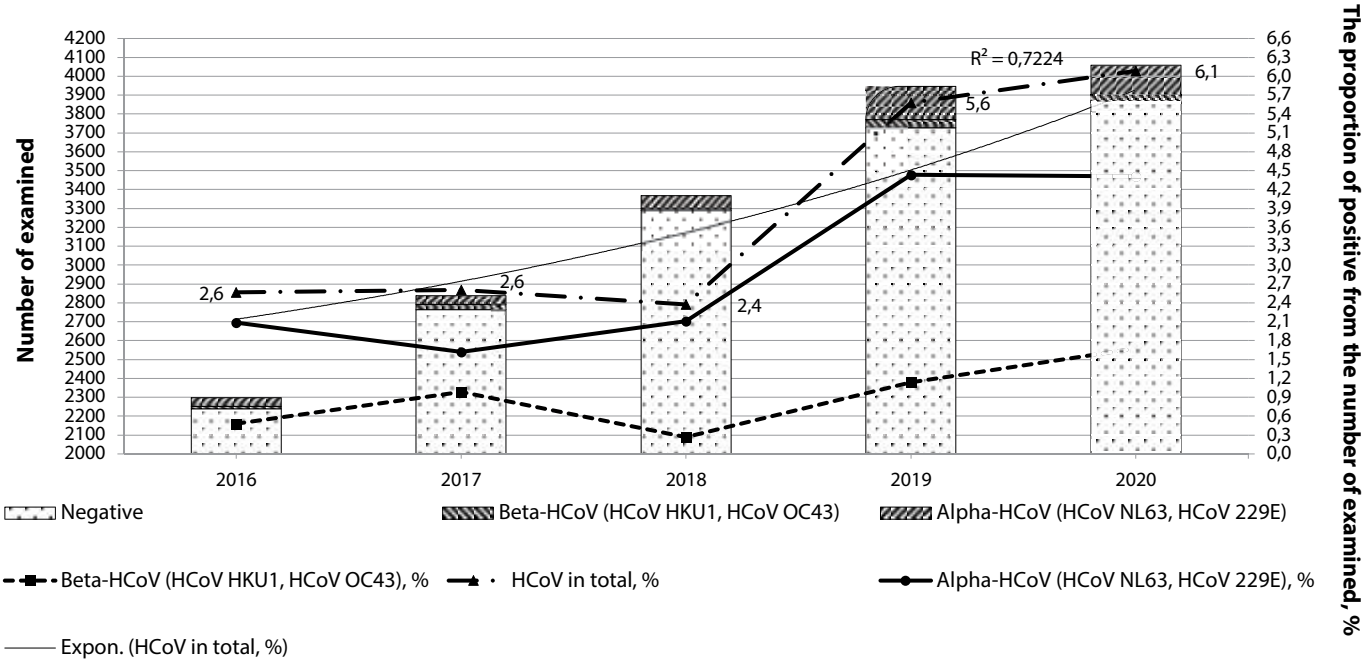

Fig. 1. Prevalence of epidemic-prone coronaviruses over five years.

The horizontal line – years of observation (from January 2016 to March 2020); the vertical line, on the left – the absolute number of the observed and positive cases; on the right – the proportion of positive cases.

ing severe acute respiratory syndrome (SARS) [14, 15], MERS-CoV, the virus causing Middle East respiratory syndrome [16], and SARS-CoV-2, the virus causing COVID-19 [17]; the viruses emerged in 2002, 2012 and 2019, respectively.

Apparently, HCoVs also emerged as a result of recombination events involving different types of coronaviruses of mammals [18, 19] with whom they share a common ancestor that existed millions of years ago [20].

The data about the epidemiology of infection caused by HCoVs are fragmentary: having been obtained in groups of patients in different years, they do not give decisive answers to questions regarding seasonality of coronavirus infection and HCoVs prevalence in different age groups of ARI patients. Different researchers inform about winter, spring or summer incidence peaks [21–27].

No long-term studies of human coronaviruses circulation have been performed in Russia.

The purpose of this work is to present the multiyear retrospective observational study of the prevalence of epidemic-prone human coronaviruses in Moscow. The study is based on the routine screening by using real-time polymerase chain reaction (PCR) method.

Materials and methods

Nasal and throat swabs from 16,511 patients with ARI symptoms were examined. The swabs were collected from January 2016 to March 2020 during the routine screening aimed at the identification of ARI etiology in Moscow. There are data on the age of 16,385 individuals and on the sex of 16,404 individuals. The age of the patients ranged from 1 month to 95 years; children aged 1 month through 18 years accounted for 58.3% of the examined.

The analysis also included the test findings for nasal and throat swabs collected from 505 relatively healthy children aged 1 month to 18 years (children under 6 years accounted for 56.2%); the swabs were collected in 2008, 2010 and 2011; the children had no symptoms of respiratory infection at the time of examination [28].

The RNA HCoVs detection based on real-time PCR test included differentiation by genera: *Alphacoronavirus* (HCoV NL63 and HCoV 229E) and *Betacoronavirus* (HCoV HKUI, HCoV OC43) and was performed with an AmpliSens ARVI-screen-FL kit (The Central Research Institute of Epidemiology of Rospotrebnadzor) according to the manufacturer's instructions on the real-time PCR instruments: Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN GmbH, Germany), and DT-96 (OOO NPO DNA-Technology, Russia).

Nasal and throat swabs were taken in accordance with the instructional guidelines «Laboratory Diagnostics of Influenza and Other ARVI by Using Polymerase Chain Reaction» MR 3.1.0117-17 and the clinical guidelines «Laboratory Diagnostics of Influenza and Other ARVI using Polymerase Chain Reaction» (2016)[1]. The collected swabs were placed in tubes with 0.5 ml of transport medium and stored at the temperature ranging from +4 to +8 C for a maximum of 3 days.

The statistical analysis included verification of the distributions for normality and calculation of the Pearson chi-square (χ^2) statistic by using the SPSS Statistics v. 18 software package; the 95% confidence interval (CI) was calculated by the Wald method [29].

[1]Clinical guidelines. https://fedlab.ru/upload/medialibrary/b71/_-_-_-_-_06122016.pdf (reference date: 23/6/2020).

Table 1. Coronavirus detection frequency in female and male patients with an acute respiratory infection

Years	Females				Males			
	number of the examined	number of individuals tested positive for Beta-HCoV, abs. (%)	number of individuals tested positive for Alpha-HCoV, abs. (%)	total number of individuals tested positive for HCoV, abs. (%)	number of the examined	number of individuals tested positive for Beta-HCoV, abs. (%)	number of individuals tested positive for Alpha-HCoV, abs. (%)	total number of individuals tested positive for HCoV, abs. (%)
2016	1115	7 (0.63; 95% CI* 0.25–1.29)	29 (2.60; 95% CI 1.75–3.71)	36 (3.23; 95% CI 2.27–4.44)	1167	4 (0.34; 95% CI 0.09–0.88)	18 (1.54; 95% CI 0.92–2.43)	22 (1.89; 95% CI 1.19–2.84)
2017	1352	10 (0.74; 95% CI 0.36–1.36)	21 (1.55; 95% CI 0.96–2.36)	31 (2.29; 95% CI 1.56–3.24)	1453	18 (1.24; 95% CI 0.74–1.95)	25 (1.72; 95% CI 1.12–2.53)	43 (2.96; 95% CI: 2.15–3.97)
2018	1687	5 (0.30; 95% CI 0.1–0.69)	45 (2.67; 95% CI: 1.95-3.55)	50 (2.96; 95% CI 2.21–3.89)	1671	4 (0.24; 95% CI 0.07–0.61)	26 (1.56; 95% CI 1.02–2.27)	30 (1.80; 95% CI 1.21–2.55)
2019	1966	29 (1.48; 95% CI 0.99–2.11)	104 (5.29; 95% CI 4.34–6.37)	133 (6.77; 95% CI 5.69–7.97)	1955	16 (0.82; 95% CI 0.47–1.33)	70 (3.58; 95% CI 2.8–4.5)	86 (4.40; 95% CI 3.53–5.4)
2020 (January–March)	2085	39 (1.87; 95% CI 1.33–2.55)	87 (4.17; 95% CI 3.36–5.12)	126 (6.04; 95% CI 5.06–7.15)	1953	29 (1.48; 95% CI 1.0–2.13)	91 (4.66; 95% CI 3.77–5.69)	120 (6.14; 95% CI 5.12–7.3)
Total	**8205**	**90 (**1.10; 95% CI 0.88–1.35)	**286 (**3.49; 95% CI 3.11–3.91)	**376 (**4.58; 95% CI 4.15–5.06)	**8199**	**71** (0.87; 95% CI 0.68–1.09)	**230** (2.81; 95% CI 2.47–3.19)	**301** (3.67; 95% CI 3.28–4.1)

Note. *Here and in table 2: CI – confidence interval.

Table 2. Coronavirus prevalence in different age groups of patients with an acute respiratory infection

Age, years	Number of the examined	Number of individuals tested positive for HCoV, abs. (%)	Number of individuals tested positive for Beta-HCoV, abs. (%)	Number of individuals tested positive for Alpha-HCoV, abs. (%)
< 1	1451	50 (3.45; 95% CI 2.57–4.52)	14 (0.96; 95% CI 0.53–1.61)	36 (2.48; 95% CI 1.74–3.42)
1–2	1259	47 (3.73; 95% CI 2.76–4.93)	10 (0.79; 95% CI 0.38–1.46)	37 (2.94; 95% CI 2.08–4.03)
3–5	4042	142 (3.51; 95% CI 2.97–4.13)	37 (0.92; 95% CI 0.65–1.26)	105 (2.60; 95% CI 2.13–3.14)
6–17	2803	66 (2.35; 95% CI 1.83–2.99)	16 (0.57; 95% CI 0.33–0.93)	50 (1.78; 95% CI 1.33–2.35)
18–44	5003	253 (5.06; 95% CI 5.26–5.7)	58 (1.16; 95% CI 0.88–1.5)	195 (3.90; 95% CI 2.19–4.47)
45–59	1213	83 (6.84; 95% CI 5.49–8.41)	20 (1.65; 95% CI 1.01–2.54)	63 (5.19; 95% CI 4.01–6.6)
> 60	614	35 (5.70; 95% CI 4.0–7.84)	4 (0.65; 95% CI 0.18–1.66)	31 (5.05; 95% CI 3.46–7.09)

Results

HCoVs were detected annually; the percentage of positive cases demonstrated exponential growth by 2020, from 2.6% to 6.1% of the total number of the examined patients. The annual occurrence of viruses belonging to the genus *Alphacoronavirus* (Alpha-HCoV) exceeded that of *Betacoronavirus* (Beta-HCoV) by 1.5–3 times (**Fig. 1**).

The HCoVs circulation demonstrated a distinct winter-spring seasonal pattern (**Fig. 2**). The incidence surge was observed from November to May when HCoVs were detected in more than 3% of the ARI patients; the peak levels were reached in December–February (7.4–5.5%). In summer months, the proportion of the HCoV-infected did not exceed 0.5% (**Fig. 2**). The Alpha-HCoV monthly detection frequency was several times as high as that of Beta-HCoV.

From November 2019 to February 2020, there was an increase in the HCoV epidemic activity: The detection frequency doubled as compared to the multi-year average (MYA) in each month of observation. In December 2019, at the height of the surge (**Fig. 3**), the frequency of HCoV detection was 13.7% (7.0% MYA); in January–February – 8% (3.1 and 4.2% MYA); in March it dropped sharply to 4.1% (4.2% MYA).

Infection prevalence among patients of different sex and age deserves attention.

The cumulative proportion of coronavirus infection among ARI female patients was higher than the proportion of coronavirus-infected ARI males (4.6 vs 3.7; $p = 0.013$), though the proportions varied across years (**Table 1**), thus giving no proof of statistically significant differences in HCoV prevalence among females and males.

The prevalence of coronavirus infection in ARI patients of different age is shown in **Table 2**. Despite the absence of a distinct age peak, HCoVs were detected more often among adults than among children (5.43%; 95% CI 4.92–6.0 vs 3.19%, 95% CI 2.86–3.56; $p < 0.01$) (**Fig. 4**).

During the examination of a group of children without ARI symptoms, HCoVs were detected in 12 (2.1%; 95% CI 1.23–4.11%) out of 505 cases; positive test results were reported

DOI: https://doi.org/10.36233/0507-4088-2020-65-5-3

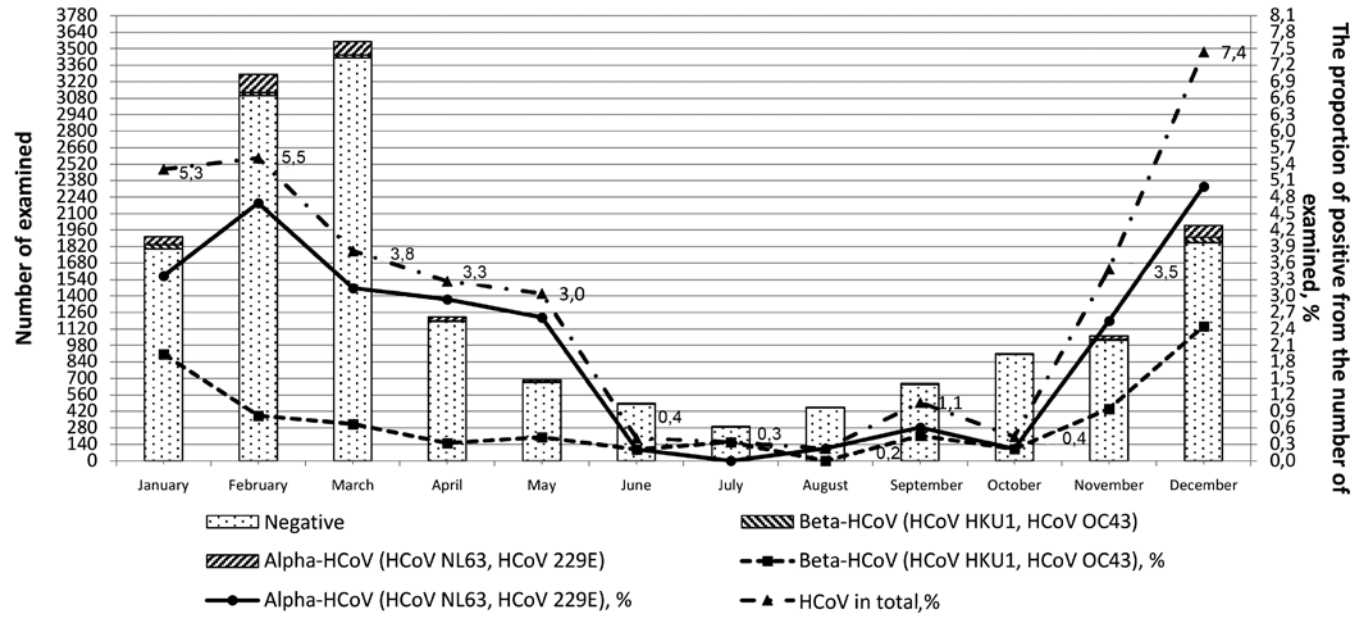

Fig. 2. Seasonal prevalence of epidemic-prone coronaviruses over 5 years of observation (from January 2016 to March 2020).
The horizontal line – months of observation. The vertical line, on the left – the total absolute number of the observed and positive cases; on the right – the proportion of positive cases per month for the entire observation period.

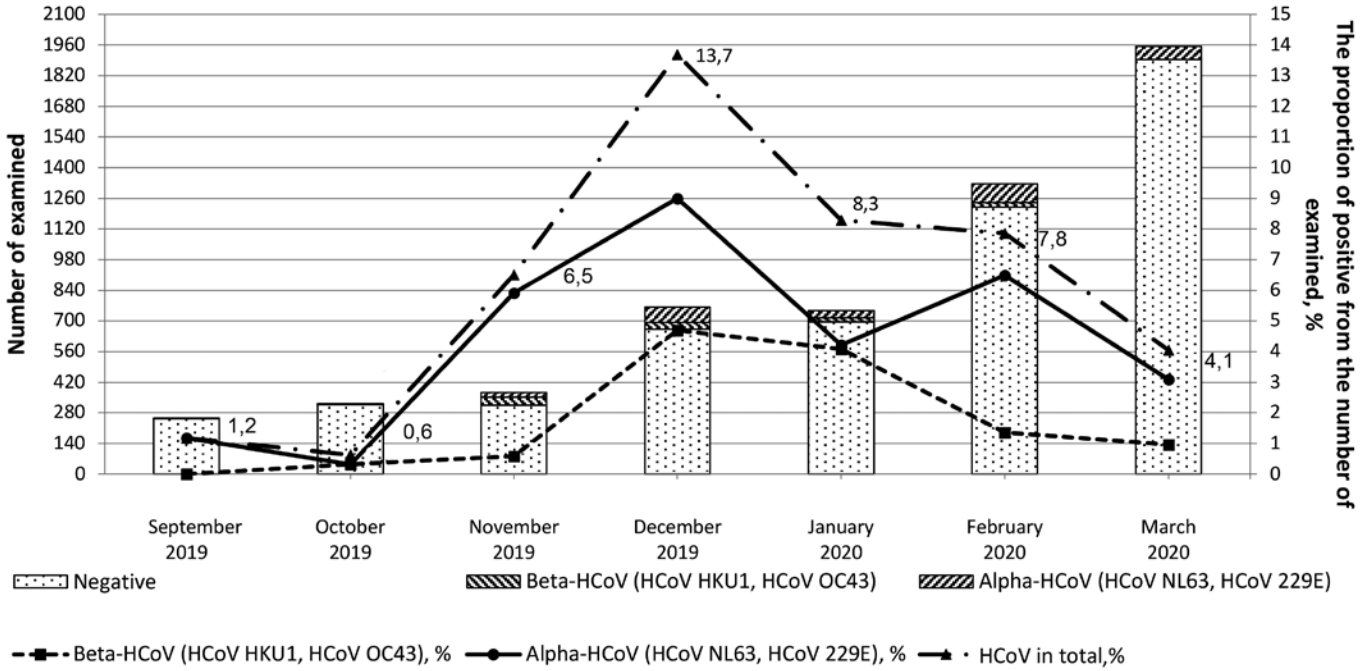

Fig. 3. Prevalence of epidemic-prone coronaviruses from September 2019 to March 2020.

throughout the year, though more than half of them (seven cases) were identified in spring.

In relatively healthy children under 6 years, HCoVs were detected significantly more rarely than in children of the same age, but with ARI symptoms (0.7% vs 3.7%; $p = 0.008$). The prevalence of HCoVs in pediatric ARI patients older than 6 years and in relatively healthy children of the same age did not have any statistically significant differences: 66 (2.4%) out of 2,803 vs 10 (4.5%) out of 221 ($p = 0.047$).

Discussion

The obtained data suggest that HCoVs circulate annually and their activity in the Moscow Region tends to increase in the winter-spring period, reaching the peak level in December. The seasonality pattern identified

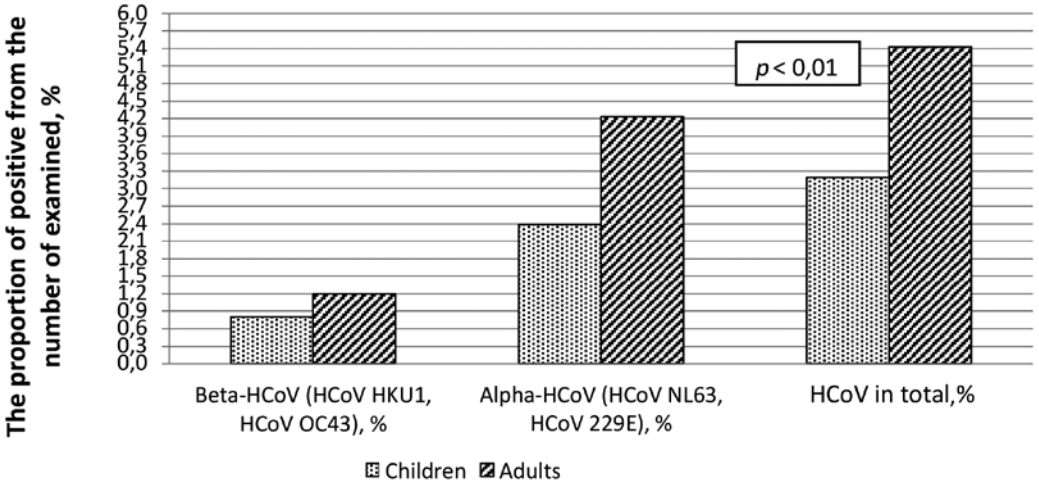

Fig. 4. Prevalence of epidemic-prone coronaviruses in children and adults.
The vertical line – the proportion of positive cases.

during our study corresponds to the data of the research performed in Norway [30], whereas studies performed in Israel and Hong Kong prove spring-and-summer activity of HCoVs [27, 31]. Apparently, it can be explained by region-specific climatic features affecting the circulation of HCoVs and other respiratory viruses [32].

The frequency of HCoV detection in our study is similar to the frequency in other countries [2, 27, 30, 33].

Unfortunately, most of the published studies focused on the child population. The only published study conducted in the United States among patients aged 0–96 years showed the average age of 22 years for the HCoV-infected patients [33]. In our study, the average age of HCoV-infected patients is 24 years and the median age is 23 years.

We detected HCoVs in 2.1% of the relatively healthy children. Similar results (1.9%) were obtained by researchers in Slovenia [25]. In the Netherlands, HCoVs were detected in children of the control group at higher frequency (10%) [30].

In our study, the HCoV detection frequency among children under 6 years and with ARI symptoms was significantly higher than among healthy children of the same age, thus corresponding to the data obtained by researchers in Slovenia [25]. As for children aged 6–18 years, the frequency of HCoV detection in the sick and healthy was almost identical, demonstrating similarity to the data obtained by Norwegian researchers [30].

These age-related differences can be explained by a milder and asymptomatic course of infection in older children due to the acquired anamnestic immune response. The prevalence of coronavirus infection in adult ARI patients was higher than in children, presumably, due to the age-related weakening of anamnestic immune response.

Both genera of HCoVs demonstrated the identical circulation seasonality, but seasonal surges of Beta-HCoV incidence were not as pronounced as those of Alpha-HCoV, while the prevalence of Beta-HCoV among children and adults was almost the same.

Data of seroepidemiological studies show that IgG antibodies to different types of HCoVs are detected fairly often, especially in adults. The study conducted in the USA shows that from 91 to 100% of the individuals older than 50 years had IgG antibodies to all the four HCoVs in blood serum and 8–30% of the cases had secretory IgA produced by the nasal mucosa, thus being indicative of high prevalence of the infection and the risk of becoming re-infected with coronaviruses of the same type [34].

Experiments demonstrated the absence of cross-reactivity of neutralizing antibodies to HCoVs of different genera and within one genus Beta-HCoV [35, 36].

Our data about the higher frequency of HCoV detection among adults than among children, together with the findings of immunology studies performed by our foreign colleagues suggest the existence of short-term immunological protection after infection and a probability of recurrence of infection with different types of HCoVs.

The seasonal sharp decrease in the HCoV detection frequency in March 2020 amid a growing number of SARS-CoV-2 cases imported to Moscow could result from the competition between different coronaviruses and decisively confirms the specificity of RNA HCoVs detection with the diagnostic kit used in this study.

Conclusion

The performed retrospective observational study made it possible to estimate the prevalence and to identify HCoV circulation patterns in Moscow over five years (2016–2020). The HCoV circulation featured a winter-spring seasonal pattern and dominance of Alpha-HCoVs both in females and males. The prevalence of HCoVs ranged from 2.6 to 6.1% of the total number of patients examined a year, and demonstrated a two-fold increase as compared to the multi-year average during the epidemic season in 2019–2020. The frequency of HCoV detection in ARI pediatric patients under 6 years was significantly higher than in their relatively healthy peers, thus proving the significance of pathogens for development of infection. The older children, possibly with a protective history of immunity, being infected whith HCoVs, appears to have mild

and asymptomatic disease, that may explain the similar frequency of detection of viruses in individuals of 6-18 years old with and without respiratory symptoms the similar frequency of detection of viruses in individuals with and without respiratory symptoms. The increased HCoV detection frequency in adults, which was revealed during this study, can be explained by the absent cross-reaction of neutralizing antibodies to different HCoVs and by the age-specific decrease in the level of antibodies.

In the last few years, the epidemic activity of human coronaviruses steadily increased to reach its maximum in December 2019–February 2020, i.e. the time coincident with the emergence of the novel SARS-CoV-2 coronavirus (the genus *Betacoronavirus*) in China. This coincidence could not be accidental; rather, it was a natural consequence of active evolutionary processes in the population of mammals' coronaviruses, which should be further studied.

REFERENCES

1. de Groot R.J., Baker S.C., Baric R., Enjuanes L., Gorbalenya A.E., Holmes K.V., et al. Family Coronaviridae. In: King A.M., Lefkowitz E., Adams M.J., Carstens E.B. *Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses.* London, San Diego: Elsevier Academic Press; 2011.
2. Cabeça T.K., Granato C., Bellei N. Epidemiological and clinical features of human coronavirus infections among different subsets of patients. *Influenza Other Respir. Viruses*. 2013; 7(6): 1040-7. DOI: http://doi.org/10.1111/irv.12101
3. Bradburne A.F., Bynoe M.L., Tyrrell D.A. Effects of a "new" human respiratory virus in volunteers. *Br. Med. J.* 1967; 3(5568): 767-9. DOI: http://doi.org/10.1136/bmj.3.5568.767
4. Esposito S., Bosis S., Niesters H.G.M., Tremolati E., Begliatti E., Rognoni A., et al. Impact of human coronavirus infections in otherwise healthy children who attended an emergency department. *J. Med. Virol*. 2006; 78(12): 1609-15. DOI: http://doi.org/10.1002/jmv.20745
5. Dare R.K., Fry A.M., Chittaganpitch M., Sawanpanyalert P., Olsen S.J., Erdman D.D. Human coronavirus infections in rural Thailand: a comprehensive study using real-time reverse-transcription polymerase chain reaction assays. *J. Infect. Dis*. 2007; 196(9): 1321-8. DOI: http://doi.org/10.1086/521308
6. Nikolaeva S.V., Zvereva Z.A., Kanner E.V., Yatsyshina S.B., Usenko D.V., Gorelov A.V. A clinical-laboratory characteristic of coronavirus infection in children. *Infektsionnye bolezni.* 2018; 16(1): 35-9. DOI: http://doi.org/10.20953/1729-9225-2018-1-35-39 (in Russian)
7. Gaunt E.R., Hardie A., Claas E.C.J., Simmonds P., Templeton K.E. Epidemiology and clinical presentations of the four human coronaviruses 229E, HKU1, NL63, and OC43 detected over 3 years using a novel multiplex real-time PCR method. *J. Clin. Microbiol*. 2010; 48(8): 2940-7. DOI: http://doi.org/10.1128/JCM.00636-10
8. Woo P.C.Y., Lau S.K.P., Chu C., Chan K., Tsoi H., Huang Y., et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol*. 2005; 79(2): 884-95. DOI: http://doi.org/10.1128/JVI.79.2.884-895.2005
9. Dent S., Neuman B.W. Purification of coronavirus virions for Cryo-EM and proteomic analysis. *Methods Mol. Biol*. 2015; 1282: 99-108. DOI: https://doi.org/ 10.1007/978-1-4939-2438-7_10
10. Gralinski L.E., Baric R.S. Molecular pathology of emerging coronavirus infections. *J. Pathol*. 2015; 235(2): 185-95. DOI: http://doi.org/10.1002/path.4454
11. Poland A.M., Vennema H., Foley J.E., Pedersen N.C. Two related strains of feline infectious peritonitis virus isolated from immunocompromised cats infected with a feline enteric coronavirus. *J. Clin. Microbiol*. 1996; 34(12): 3180-4.
12. Woo P.C., Lau S.K., Huang Y., Yuen K.Y. Coronavirus diversity, phylogeny and interspecies jumping. *Exp. Biol. Med. (Maywood)*. 2009; 234(10): 1117-27. DOI: http://doi.org/10.3181/0903-MR-94/
13. Lu S., Wang Y., Chen Y., Wu B., Qin K., Zhao J., et al. Discovery of a novel canine respiratory coronavirus support genetic recombination among betacoronavirus1. *Virus Res.* 2017; 237: 7-13. DOI: http://doi.org/10.1016/j.virusres.2017.05.006
14. Poon L.L.M., Chu D.K.W., Chan K.H., Wong O.K., Ellis T.M., Leung Y.H.C., et al. Identification of a novel coronavirus in bats. *J. Virol.* 2005; 79(4): 2001-9. DOI: http://doi.org/10.1128/JVI.79.4.2001-2009.2005
15. Luk H.K.H., Li X., Fung J., Lau S.K.P., Woo P.C.Y. Molecular epidemiology, evolution and phylogeny of SARS coronavirus. *Infect. Genet. Evol*. 2019; 71: 21-30. DOI: http://doi.org/10.1016/j.meegid.2019.03.001
16. Abdel-Moneim A.S. Middle East respiratory syndrome coronavirus (MERS-CoV): evidence and speculations. *Arch. Virol.* 2014; 159(7): 1575-84. DOI: http://doi.org/10.1007/s00705-014-1995-5
17. Andersen K.G., Rambaut A., Lipkin W.I., Holmes E.C., Garry R.F. The proximal origin of SARS-CoV-2. *Nat. Med.* 2020; 26(4): 450-2. DOI: http://doi.org/10.1038/s41591-020-0820-9
18. Corman V.M., Muth D., Niemeyer D., Drosten C. Hosts and sources of endemic human coronaviruses. *Adv. Virus Res*. 2018; 100: 163-88. DOI: http://doi.org/10.1016/bs.aivir.2018.01.001
19. Ye Z.W., Yuan S., Yuen K.S., Fung S.Y., Chan C.P., Jin D.Y. Zoonotic origins of human coronaviruses. *Int. J. Biol. Sci.* 2020; 16(10): 1686-97. DOI: http://doi.org/10.7150/ijbs.45472
20. Wertheim J.O., Chu D.K., Peiris J.S., Kosakovsky Pond S.L., Poon L.L. A case for the ancient origin of coronaviruses. *J. Virol*. 2013; 87(12): 7039-45. DOI: http://doi.org/10.1128/JVI.03273-12
21. Jevšnik M., Uršič T., Zigon N., Lusa L., Krivec U., Petrovec M. Coronavirus infections in hospitalized pediatric patients with acute respiratory tract disease. *BMC Infect. Dis.* 2012; 12: 365. DOI: http://doi.org/10.1186/1471-2334-12-365
22. Varghese L., Zachariah P., Vargas C., LaRussa P., Demmer R.T., Furuya Y.E., et al. Epidemiology and clinical features of human coronaviruses in the pediatric population. *J. Pediatric. Infect. Dis. Soc*. 2018; 7(2): 151-8. DOI: http://doi.org/10.1093/jpids/pix027
23. Monto A.S., Cowling B.J., Peiris J.S.M. Coronaviruses. In: Kaslow R.A., Stanberry L.R., Le Duc J.W., eds. *Viral Infections of Humans: Epidemiology and Control.* Boston, MA: Springer US; 2014: 199-223. DOI: http://doi.org/10.1007/978-1-4899-7448-8_10
24. Dominguez S.R., Robinson C.C., Holmes K.V. Detection of four human coronaviruses in respiratory infections in children: a one-year study in Colorado. *J. Med. Virol*. 2009; 81(9): 1597-604. DOI: http://doi.org/10.1002/jmv.21541
25. Jevšnik M., Steyer A., Pokorn M., Mrvič T., Grosek Š., Strle F., et al. The role of human coronaviruses in children hospitalized for acute bronchiolitis, acute gastroenteritis, and febrile seizures: a 2-year prospective study. *PLoS One*. 2016; 11(5): e0155555. DOI: http://doi.org/10.1371/journal.pone.0155555
26. Vabret A., Dina J., Gouarin S., Petitjean J., Tripey V., Brouard J., et al. Human (non-severe acute respiratory syndrome) coronavirus infections in hospitalised children in France. *J. Paediatr. Child Health*. 2008; 44(4): 176-81. DOI: http://doi.org/10.1111/j.1440-1754.2007.01246.x.
27. Friedman N., Alter H., Hindiyeh M., Mendelson E., Shemer Avni Y., Mandelboim M. Human coronavirus infections in Israel: epidemiology, clinical symptoms and summer seasonality of HCoV-HKU1. *Viruses.* 2018; 10(10): 515. DOI: http://doi.org/10.3390/v10100515
28. Yatsyshina S.B., Spichak T.V., Kim S.S., Vorob'eva D.A., Ageeva M.R., Gorelov A.V., et al. Revealing of respiratory viruses and

atypical bacteria in children with pneumonia and healthy children for ten years of observation. *Pediatriya. Zhurnal im. G.N. Speranskogo.* 2016; 95(2): 43-50. (in Russian)

29. Sauro J., Lewis J. Estimating completion rates from small samples using binomial confidence intervals: comparisons and recommendations. *Proc. Hum. Factors Ergon. Soc. Annu. Meet.* 2005; 49(24): 2100-3. DOI: http://doi.org/10.1177/154193120504902407
30. Heimdal I., Moe N., Krokstad S., Christensen A., Skanke L.H., Nordbø S.A., et al. Human coronavirus in hospitalized children with respiratory tract infections: a 9-year population-based study from Norway. *J. Infect. Dis*. 2019; 219(8): 1198-206. DOI: http://doi.org/10.1093/infdis/jiy646
31. Chiu S.S., Chan K.H., Chu K.W., Kwan S.W., Guan Y., Poon L.L.M., et al. Human coronavirus NL63 infection and other coronavirus infections in children hospitalized with acute respiratory disease in Hong Kong, China. *Clin. Infect. Dis.* 2005; 40(12): 1721-9. DOI: http://doi.org/10.1086/430301
32. Li Y., Reeves R.M., Wang X., Bassat Q., Brooks W.A., Cohen C., et al. Global patterns in monthly activity of influenza virus, respiratory syncytial virus, parainfluenza virus, and metapneumovirus: a systematic analysis. *Lancet Glob. Health.* 2019; 7(8): e1031-45. DOI: http://doi.org/10.1016/S2214-109X(19)30264-5
33. Biggs H.M., Killerby M.E., Haynes A.K., Dahl R.M., Gerber S.I., Watson J.T. Human coronavirus circulation in the USA, 2014 – 2017. *Open. Forum Infect. Dis.* 2017; 4(Suppl. 1): S311-2. DOI: http://doi.org/10.1093/ofid/ofx163.727
34. Gorse G.J., Patel G.B., Vitale J.N., O'Connor T.Z. Prevalence of antibodies to four human coronaviruses is lower in nasal secretions than in serum. *Clin. Vaccine Immunol*. 2010; 17(12): 1875-80. DOI: http://doi.org/10.1128/CVI.00278-10
35. Chan C.M., Tse H., Wong S.S.Y., Woo P.C.Y., Lau S.K.P., Chen L., et al. Examination of seroprevalence of coronavirus HKU1 infection with S protein-based ELISA and neutralization assay against viral spike pseudotyped virus. *J. Clin. Virol*. 2009; 45(1): 54-60. DOI: http://doi.org/10.1016/j.jcv.2009.02.011
36. McIntosh K., Dees J.H., Becker W.B., Kapikian A.Z., Chanock R.M. Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. *Proc. Natl. Acad. Sci. USA*. 1967; 57(4): 933-40. DOI: http://doi.org/10.1073/pnas.57.4.933