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Assessment of Herd Immunity to SARS-CoV-2 Virus among the Population of the Kyrgyz Republic during COVID-19 Pandemic

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Abstract. This paper presents the results of a three round seroepidemiological study of the population for SARS-CoV-2 virus antibodies presence. **The aim** of the study was to assess herd immunity in the population of the Kyrgyz Republic stratified by age, gender and region. **Materials and methods.** The material for the study was blood samples and personal data (questionnaire) of individuals, who agreed to participate in the study. **Results and discussion.** The results of the three rounds study indicated that the proportion of seroprevalent persons at the 1st round was 30.8% (95% CI 29.5-32.1), at the 2nd round - 71.2% (95% CI 69.9-72.5), at the 3rd round - 92.3% (95% CI 91.5-93.1). High herd immunity in the population was defined by short-term and low incidence in the fourth wave of the pandemic compared to the global indicators of the COVID-19 fourth wave. Uneven territorial distribution of seroprevalence was observed through all rounds of the study. All three rounds of the study were characterized by the lack of coronavirus infection symptoms in some of the seropositive individuals. Symptoms presence in seropositive individuals in the second and third rounds tended to decrease compared to the 1st round results in general. The percentage of SARS-CoV-2 virus seropositive individuals among children was lower versus the adult population. The share of seropositive samples was less for men compared to women. Paired sera examination, collected after 8-20 months, showed the decreased level of antibodies by 64.6% compared to those collected 1-2 months after the disease. At the same time, an increased level of antibodies was observed in 35.4% of individuals.

Key words: population immunity, pandemic, seroprevalence, coronavirus, seroepidemiological study, incidence.

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Оценка коллективного иммунитета к вирусу SARS-CoV-2 среди населения Кыргызской Республики в период пандемии COVID-19

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В статье представлены результаты трех этапов сероэпидемиологического исследования населения на наличие антител к вирусу SARS-CoV-2. **Целью** исследования была оценка коллективного иммунитета у населения Кыргызской Республики, стратифицированного по возрасту, полу и региону. **Материалы и методы.** Материалом для исследования послужили образцы крови и персональные данные (анкеты) лиц, давших согласие на участие в исследовании. **Результаты и обсуждение.** Результаты трех раундов исследования показали, что доля серопревалентных лиц на 1-м этапе составила 30,8 % (95 % ДИ 29,5–32,1), на 2-м этапе – 71,2 % (95 % ДИ 69,9–72,5), на 3-м – 92,3 % (95 % ДИ 91,5–93,1). Высокий уровень коллективного иммунитета в популяции определялся коротким периодом и низким уровнем заболеваемости в четвертую волну пандемии по сравнению с мировыми показателями четвертой волны COVID-19. Неравномерное территориальное распределение серопревалентности наблюдалось на протяжении всех раундов исследования. Для всех трех фаз исследования характерно отсутствие симптомов коронавирусной инфекции у части серопозитивных лиц. Показатели наличия симптомов у серопозитивных лиц на втором и третьем этапах имели тенденцию к снижению. Процент серопозитивных к вирусу SARS-CoV-2 лиц среди детей был ниже, чем среди взрослого населения. Доля серопозитивных лиц ниже у мужчин по сравнению с женщинами. Анализ результатов сравнения парных сывороток, собранных через 8–20 месяцев, показал снижение уровня антител на 64,6 % по сравнению с результатом первой пробы, полученной через 1–2 месяца после болезни, в то же время повышенный уровень антител наблюдался у 35,4 % лиц.

Ключевые слова: популяционный иммунитет, пандемия, серопревалентность, коронавирус, сероэпидемиологическое исследование, заболеваемость.

Конфликт интересов. Авторы подтверждают отсутствие конфликта финансовых/нефинансовых интересов, связанных с написанием статьи.

Финансирование. Авторы заявляют об отсутствии дополнительного финансирования при проведении данного исследования.

Биоэтика. Перед проведением всех процедур было получено информированное согласие пациента.

Благодарности. Мы выражаем глубокую благодарность страновому офису ВОЗ в Кыргызстане и ЕВРО ВОЗ за техническую помощь в проведении сероэпидемиологического исследования на наличие антител к SARS-CoV-2 в Кыргызской Республике. Также мы хотели бы поблагодарить сотрудников медицинских организаций Кыргызской Республики за их участие и поддержку, оказанную в ходе этого исследования.

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In December 2019, a highly pathogenic SARS-CoV-2 β-coronavirus emerged on the maritime market in the Chinese city of Wuhan, which became the etiological factor of the 2019 novel coronavirus disease (COVID-19) [1]. In the next few days, the virus quickly spread across various territories of China, and to other countries due to the active population migration. In March 2020, the World Health Organization assigned the status of a pandemic to this event [2].

The first case of COVID-19 was registered in the Kyrgyz Republic on March 18, 2020 in the southern regions of the country. At the beginning, isolated cases were registered, which were imported by pilgrims who visited Saudi Arabia and labor migrants who returned from the Russian Federation and other countries. Then it quickly spread throughout the republic among the contact persons and general population. In this connection, by Decree of the Kyrgyz Republic Government No. 93-p, the emergency state regime was introduced throughout the country from March 22, 2020. Since March 24, the state of emergency, quarantine and curfew, completely restricting the movement of the population at night, was introduced in the southern regions and in the city of Bishkek. On May 11, 2020 to the effect of supporting the economic situation, the state of emergency was canceled, followed by population movements and visiting public places, which in combination with insufficient compliance with anti-epidemic measures led to the increased incidence in the Republic. The mid June 2020 intensive incidence rate in the Republic was at the level of 32,4 per 100,000 population, and mortality rate - 1,2% (26 out of 2093 patients died) [3]. Due to rapid spread of coronavirus infection, the Republican Headquarters for Coronavirus Infection Control raised the issue of the necessity to study population immunity to SARS-CoV-2 virus in order to assess the dynamics of post-infection humoral immunity formation and to predict development of the epidemiological situation in the country [4-6]. The initiative of the Republican Headquarters and the Ministry of Health to conduct the study was supported by the WHO. At the end of June 2020, the 1st round of COVID-19 seroepidemiological study was launched to inform public health response. In total, three rounds of the study were carried out from June 2020 through December 2021, covering all three periods of the pandemic incidence rise in the Republic. At the second and third stages, the humoral response duration in individuals who had previously had COVID-19 was also assessed.

Materials and methods

The material for the study was blood serum samples collected from the population stratified by age categories and regions of the Kyrgyz Republic, as well as questionnaires necessary to obtain information from the patients on age, gender, symptoms of the disease, healthcare seeking behavior, hospital admission and frequency of disease symptoms manifestation.

Study design: seroepidemiological cross-sectional, prospective population study aimed at obtaining the data on the main serological and epidemiological patterns of SARS-CoV-2 coronavirus infection spread.

The population sampling was carried out in two stages: at the first stage, the sample size was stratified by 9 geographic regions, and at the second stage the sample size was stratified by five age groups for each region: 0-9 years old, 10-19 years old, 20-44 years old, 45-64 years old, and 65 and above.

Health care organizations (HCO) of primary health care (PHC) were selected by random sampling in all 9 regions of the country. Individuals were randomly recruited to participate in the study from the list of the population, enrolled to the selected PHC HCOs.

The sample size was calculated using the online formula http://www.openepi.com/Menu/OE_Menu.htm [7].

Due to the lack of data on the true SARS-CoV-2 virus prevalence during each new wave of the epidemic in the regions, the expected incidence rate was equated to 50%. The estimated population size for this antibody serological test is 384 individuals:

$n = [DEFF \cdot N \cdot p \cdot (1–p)] / [(d^2 / Z^2_{1–\alpha/2} \cdot (N–1) + p \cdot (1–p)]$,

where n = sample size; N = population = 6,456.515; p = hypothetical % factor outcome frequency in population = 50% ± 5; d = confidence limits as % of 100 (absolute ± %) = 5%; D = sampling/design effect (for cluster surveys) = 1.

With a non-response rate of 25%, the sample size was 384 + 25% = 480 participants.

The three-round study was conducted at intervals of 6-8 months and included the determination of antibodies to SARS-CoV-2 virus and examination of individuals involved in the study.

The 1st round of the study was conducted in July, 2020 and included 4691 individuals (1480 men and 3211 women), the 2nd round - in April, 2021 included 4735 persons (1469 men and 3266 women) and the 3rd one - in December, 2021, involving 4713 people (1244 men and 3469 women). Total of 14,139 people

were studied. The men to women ratio was 29.7% and 70.3%, respectively.

To study the dynamics of changes in the level of antibody response in 285 study participants tested positive in the first round of the study, repeated blood sampling was carried out in the second or third rounds, that is, two sera samples from each participant were used: a positive sample from the first round and the sample from the same person, collected 8 to 14 months following the first test.

The serological population study was carried out using ELISA "SARS-CoV-2-Ab ELISA WANTAI" test system for qualitative determination of antibodies to SARS-CoV-2.

Quantitative measurements of the level of SARS-CoV-2 antibodies were carried out in the ELISA test system "COVID-SeroKlir", Kantaro Semi - Quantitative SARS-CoV-2 Antidody Kit". The quantitative measurements of the antibody response level were performed using the SoftMaxPro software. The established analytical measurement range (AMR) was 3.2-125 conventional units per milliliter (Au/ml) for SARS-CoV-2 spike protein. The result was considered negative if the value of the test sample was below 3.2 Au/ml and positive if the value was equal to or higher than 3.2 Au/ml.

Statistical analysis of the data was carried out using the methods of variation statistics in the Excel program. Calculation of the prevalence of SARS-CoV-2 antibodies with a 95% confidence interval (CI) was conducted among the studied population taking into account the age, gender, region and manifestation or lack of disease symptoms, seeking healthcare behavior, hospitalization, frequency of manifestation of disease symptoms throughout all rounds of the study.

Ethical issues. All respondents were included in the study on the basis of their prior informed consent. Respondents were provided with an information notice about the processing of their personal data within the survey in accordance with national requirements. Prior to the start of the study, an ethical committee resolution was obtained that the study is not in conflict with international ethical practices. The registration number of the ethical committee at the IORG Research and Production Association "Preventive Medicine" at the Department of Health and Human Services (USA) is IORG0008909.

Results and discussion

In total, 206,876 cases of COVID-19 were registered in the Kyrgyz Republic as of May 2, 2023, with the intensive incidence rate of 3065 per 100 thousand population [8]. All in all, four pandemic waves were recorded. The collection and testing of blood samples were carried out at intervals of 6-8 months during the pandemic evolution periods, from the first through the third wave. The fourth wave was low and did not last long. The COVID-19 pandemic virtually ended in the Kyrgyz Republic in May, 2022, with sporadic cases reported later (Fig. 1).

The proportion of seropositive individuals in Kyrgyzstan at the 1st round of the study was 30.8% (95% CI 29.5-32.1), 2nd round - 71.2% (95% CI 69.9-72.5) and 3rd round - 92.3% (95% CI 91.5-93.1). An uneven territorial distribution of seroprevalence was observed through all rounds of the study: during the 1st round, the seropositivity rate varied from 13.0% to 62.7% across regions of Kyrgyzstan (Batken, Jalal-Abad and Naryn regions), during the 2nd round - from 63.9 to 77.8% (Bishkek and Talas region) and the 3rd one - from 88.1 to 95.6% in Talas and Chui regions (Fig. 2).

The results indicated different level of seroprevalence across the age groups. Among children under 9 years of age, seroprevalence rates were lower during all study rounds compared to other age groups. Seroprevalence rate among children aged 0-9 years

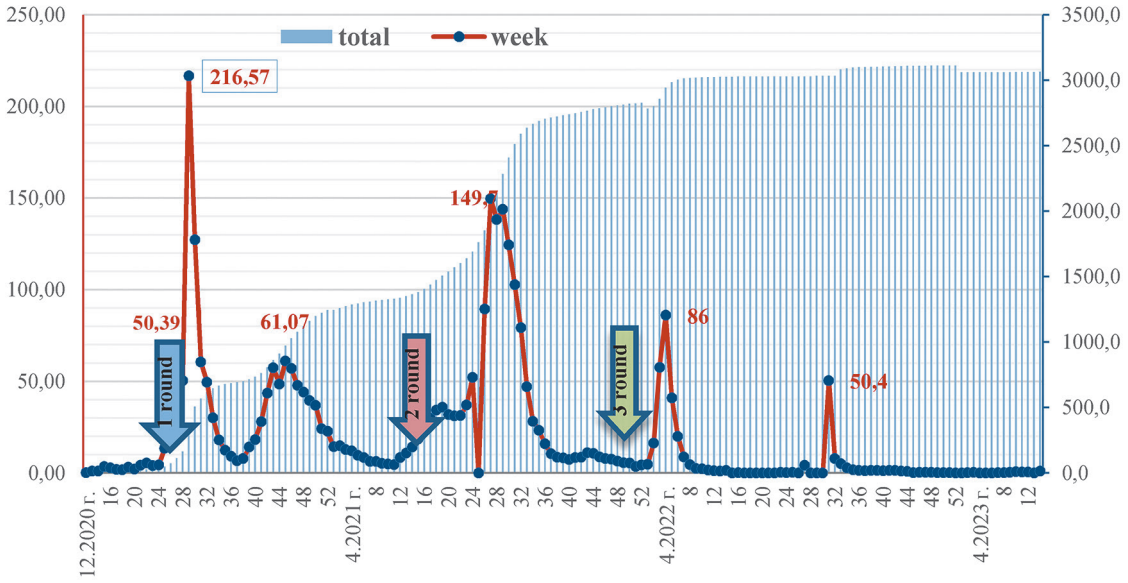

Fig. 1. Weekly incidence of COVID-19 in the Kyrgyz Republic, 2020-2023

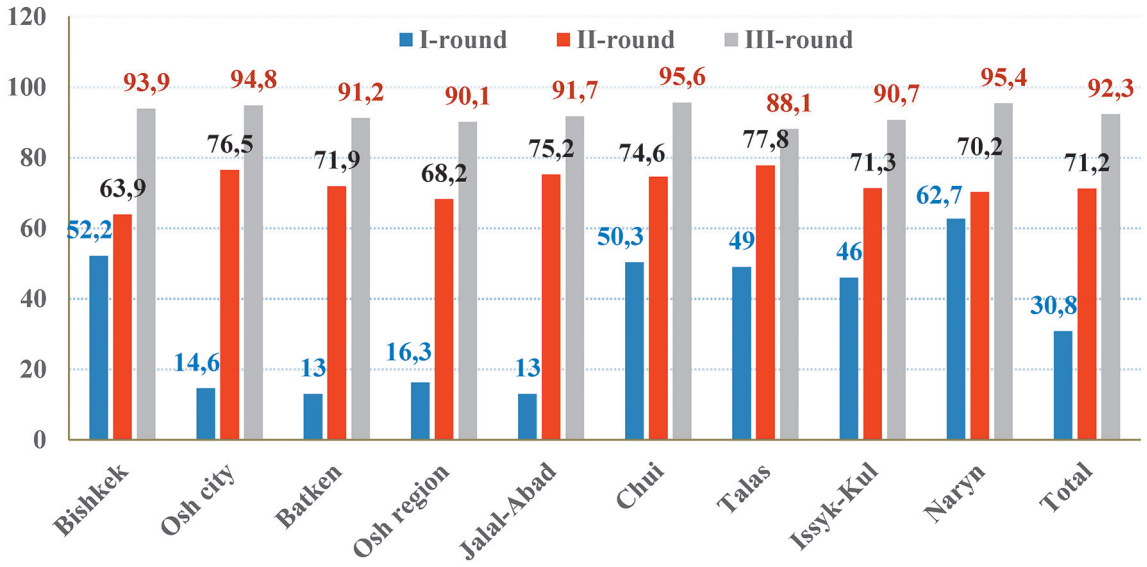

Fig. 2. Seroprevalence to SARS-CoV-2 virus by regions of the Kyrgyz Republic (July 2020, April 2021, and December 2021)

during the 1st round was 16.5% (95% CI 13.8-19.2), 2nd round - 51.4% (95% CI 47.5-55.2) and the 3rd round - 79.7% (95% CI 77.3-82.5). The highest level of seroprevalence was noted in the age group of 45-64 years, which at the 1st round of the study amounted to 36.1% (95% CI 33.5-38.7), 2nd round - 79.4% (95% CI 77.2-81.6), and the 3rd round - 97.8% (95% CI 96.9-98.7) (Fig. 3).

The results of the analysis show that the frequency of coronavirus infection symptoms among seropositive individuals decreased with each stage of the study. Manifestation of coronavirus infection symptoms in children aged 0-9 years was less pronounced than in adults. Also, with each round of the study, the proportion of those seeking healthcare and those hospitalized decreased in all age groups, regardless of gender of the studied persons.

With each study round, the proportion of people with coronavirus infection symptoms among the seropositive persons decreased from 64.0% to 47.1%. At the same time, decrease in the number of symptoms, characterizing the disease severity was observed. Thus, the presence of headaches in seropositive individuals decreased from 44.1 to 24.2%, while during the 1st round of study, headache ranked the first among the symptoms, and during the 3rd round was in the third place. The presence of rhinorrhea in the 1st round was registered in 38.6% of the cases, and dropped to 25.8% in the 3rd round. At the same time, the presence of rhinorrhea at the 3rd round ranked first among the symptoms versus the third place in the 1st round of the study. Cough was noted in 35.0% of the patients in the 1st round and ranked fifth among the symptoms, and during the 3rd round it was noted in 25.2% of the cases and came to the second place. Specific coronavirus infection symptoms, such as loss of taste and smell remained in the 10-12th place during the study, the frequency of their occurrence in different rounds ranged from 21.4 to 13.7% and from 20.9 to 14.5%, respectively.

To study the duration of the antibody response to SARS-CoV-2 in 285 individuals who received positive

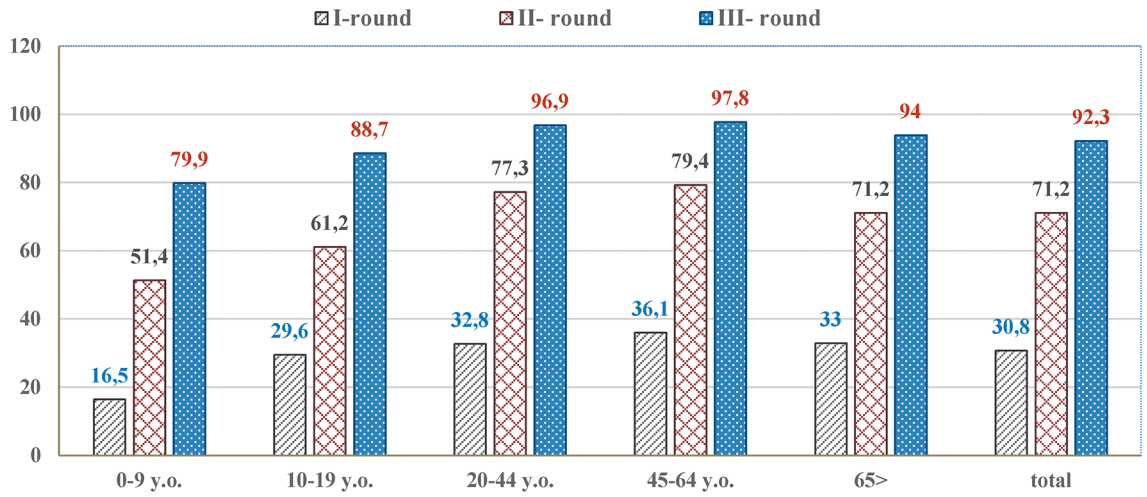

Fig. 3. SARS-CoV-2 seroprevalence by age groups in the Kyrgyz Republic (July 2020, April 2021, and December 2021)

test results in the 1st round of the seroepidemiological study, repeated blood sampling was carried out during the 2nd or 3rd round of the study (Table). From each participant in this study, blood was collected 2 times: during the first and second rounds, or the first and third rounds of the study. 78% of repeated samples were collected 8-12 months after the disease and 22% - after 13-20 months.

The presence of antibodies at the level of 3.2 Au/ml and higher was registered in 97.2% (277/285) of the tested samples 1-2 months after the disease. There were no significant differences in the level of antibodies in the samples collected from the patients who had recovered after 8, 9-10, 11-12 and 18-20 months from the onset of the disease. In the samples, collected after 13-15 and 16-17 months, the level of antibodies above 3.2 Au/ml was identified in 100%.

The results of paired sera show a decreased antibody level in 64.6% (184/285) of samples, collected after 8-20 months compared with the result of the first sample, collected 1-2 months after the disease. An increased antibody level in samples collected from 8 to 20 months compared to the result of the first samples, collected 1-2 months after the disease, was observed in 35.4% (101/285) of cases.

To explore possible prerequisites for the increased level of antibodies in 101 participants, the results of laboratory testing were compared with the data of individual information files of these persons.

Confirmed cases of re-infection with COVID-19 and 12.9% of cases of vaccination against COVID-19 were supposed causes for the increase in the level of antibody response in 5.9%.

The results of the three-round herd seroepidemiological age-stratified study of coronavirus infection (COVID-19) in the Kyrgyz Republic indicate that seroprevalence among the individuals, tested for the presence of SARS-CoV-2 antibodies, increased gradually over the time since the pandemic onset in April, 2020, and amounted to 92.3% in December, 2021. The relatively small number of cases and short-term continuation of the fourth wave of the pandemic, which was registered at the end of January 2022 in comparison to the global indicators is attributed to high level of herd immunity of the population in the Kyrgyz Republic [9].

In the first round of the study (July 2020), the percentage of seropositive samples detection was low in the southern regions of the country (Osh, Batken, Jalal-Abad regions and Osh City) and varied from 13 to 16.3% and statistically significantly differed from the republican indicator of 30.8% (95% CI 29.5-32.1). This is due to the fact that these samples were collected at the end of June and early July of 2020, i.e. before the start of the first wave of the epidemic rise in the incidence in the country. In the northern regions (Chui, Naryn, Issyk-Kul, Talas regions and Bishkek City) of the country, seroprevalence rates were significantly higher at the 1st round of the study than in the south of the country and varied from 46.0 to 62.7%. The high level of seroprevalence can be attributed to the fact that samples were collected in the second half of July 2020, i.e. at the peak of COVID-19 incidence. It should also be noted that the rise in incidence in the northern regions began 2-3 weeks earlier than in the south of the country.

In the second round of the study (April 2021), SARS-CoV-2 virus seroprevalence was significantly higher and reached 71.2% versus 30.8% in the first round. Seroprevalence in the southern regions ranged from 68.2 to 76.5%, and from 63.9% to 77.8% in the northern regions. It should be noted that, in contrast to the result of the first round of the study, seroprevalence in many southern and northern regions under the second round did not statistically significantly differ (P>0,05) from the republican indicator (71.2±0.7), with slight differences in the city of Bishkek (63.9±1.7), Talas (77.8±3.0) and Jalal-Abad (75.2±1.4) regions.

In the third round of the study (December 2021), SARS-CoV-2 virus seroprevalence among the general population turned 92.3%. In many regions, seroprevalence did not statistically significantly differ (P>0.05) from the republican indicator (92.3±0.4). The analysis of seroprevalence ***among the age groups of the population*** shows that seroprevalence rate was increasing as the pandemic spread across regions over time. However, increase in seroprevalence was observed across all age groups, regardless of regions. Seroprevalence in all age groups in the 1st round of the study varied from 16.5 to 36.1%, 2nd round - from 51.4 to 79.4%, and during the 3rd round - from 79.9 to 97.8%.

The findings of the study indicate statistically significant differences of seroprevalence in different age groups (except the age 65+). The lowest seroprevalence rates at all study stages were noted in children under 9 years of age, amounting to 16,5% during the 1st round, 51,4% - in the 2nd round, and 79,9% - in the 3rd round. In all three rounds of the study, the highest rates of antibody prevalence were observed in socially active age groups at the ages of 20-44 (32.8%; 77.3% and 96.9%)

Data from personal information files for the study participants with increased level of SARS-CoV-2 antibody response

Period of sample collection	8 months	9-10 m.	11-12 m.	13-15 m.	16-17 m.	18-20 m.	Total
Confirmed cases of COVID-19 relapse	0/40	0/25	0/3	66.7% (2/3)	5.3% (1/19)	27.3% (3/11)	5.9% (6/101)
Vaccination against COVID-19	0/40	0/25	0/3	0/3	47.4% (9/19)	36.4% (4/11)	12.9% (13/101)
Total	0/40	0/25	0/3	66.7% (2/3)	42.6% (10/19)	63.6% (7/11)	18.8% (19/101)

and 45-64 years (36.1%; 79.4% and 97.8%) in almost all regions of the Kyrgyz Republic.

The results of seroepidemiological study show that from the beginning of the pandemic (March 18, 2020) the level of population immunity increased to 30.8% within three months (July 2020), then to 71.2% within nine months (April 2021). Eight months later (December 2021), the level of population immunity reached 92.3%. The highest increase in seroprevalence by 3.1 times (51.4 versus 16.5%) was observed in the age group 0-9 years in the 2nd round (Fig. 2). This indicates that the intensity of population immunity growth depends on the presence of a non-immune stratum of the population; with the decrease in the proportion of a non-immune population stratum, the intensity of the growth of herd immunity decreases in parallel, regardless of the presence of virus circulation and contact with sick people.

The analysis of COVID-19 incidence in the Kyrgyz Republic during the study of SARS-CoV-2 seroprevalence showed that the intensive incidence rates for the same period increased by 4.8 times; the incidence rate in the republic was 578.7 per 100 thousand population on August 1, 2020; April 1, 2021 - 1373.3; and December 1, 2021 - 2812.3. It indicated that the proportion of individuals with SARS-CoV-2 antibodies increases in parallel with the increase in the incidence of coronavirus infection. The higher the incidence of COVID-19 in cities/districts at the time of sampling, the higher the level of SARS-CoV-2 Ab seropositive results in the population and vice versa. Direct strong correlation was established (r=0.737) between the level of COVID-19 incidence rate in the regions and the level of SARS-CoV-2 seroprevalence among the population.

The results of all three rounds of the study showed a statistically significant difference in seroprevalence among men and women (P>0,001). Seroprevalence at different stages among men was 27.2%; 63.9% and 85.8±1.0; and 32.5%; 74.5% and 94.7±0.4 among women. Seropositive individuals experienced COVID-19 symptoms: at the 1st round 61.6% of men, at the 2nd round 40.2% and at the 3rd round 42.4%, and 64.9%; 54.2% and 48.7% of women respectively. Healthcare seeking was noted in 21.2%; 20.1% and 9.7% of cases for men and 22.0%; 27.3% and 15.6% for women; hospitalization indicators among men were 6.2%; 7.9% and 4.0%, and among women - 5.3%, 11.0%, and 7.2%.

All three rounds of the study indicated that some seropositive persons did not experience symptoms of coronavirus infection. Symptom occurrence rates in seropositive individuals in the second and third rounds tended to decrease, the proportion of people with symptoms in all three rounds of the study was higher in older age groups, and lower in the age group of 0-9 years. Children accounted for 1-5% of all symptomatic cases of COVID-19 globally [10].

The most common symptoms in COVID-19 patients were fever, chills, headache, muscle pain, dry cough, fatigue, shortness of breath. Our study showed that the incidence of disease symptoms tended to decrease among the seropositive individuals. The results obtained correspond to the literature data, indicating that in the SARS-CoV-2 infected, the disease can have asymptomatic or mild and severe course [11].

According to the results of all study rounds, proportion of people seeking medical help increases in parallel with the increasing age.

In general, the rate of seeking medical help during the 1st round was 21.8%, in the 2nd round - 25.4%, and in the 3rd round - 14.2%. 1.17 times increased rate in the 2nd round compared to the first round of the study (25.4 vs 21.8%) indicates public awareness of preventive measures as regards coronavirus infection and increase in the capacity of healthcare system to manage patients with COVID-19 symptoms. The decrease in the number of visits in the 3rd round down to 14.2% versus 25.4% in the 2nd round is associated with the increased proportion of a mild clinical course; our study showed that in the third round of study, the share of people with symptoms among the seropositive individuals decreased by 28.4% (47.1% vs 64.0%) compared to the 1st round of the study.

The proportion of hospitalized people among the seropositive persons was 5.5%, 10.1%, and 6.5%. The increase in the proportion of hospitalized persons in the 2nd round by 83.6%, in our opinion, is associated with the Kyrgyz Republic healthcare system capacity to hospitalize. During the period of May 2020 - April 2021, more than 3500 additional beds were deployed for COVID-19 patients. The decrease in the proportion of hospitalized patients in the 3rd round is associated with an increase in the proportion of clinically mild coronovirus infection course.

The results of studying the dynamics of duration of SARS-CoV-2 antibody response revealed the decreased level of antibodies in 64.6% of samples and its increase in 35.4% of samples, collected after 8-20 months from the onset of the disease compared with the result of the first sample, collected after 1-2 months from the disease onset. The cause for the increase in antibody levels in 5.9% of the cases was confirmed cases of relapse and in 12.9% of cases - COVID-19 vaccination. The increase in the level of antibody response in 81.2% of samples could be associated with a “boosting” of the antibody response at all stages of repeated sampling, when there could have been a second encounter with SARS-CoV-2, which was asymptomatic. Also, it should be considered that blood sampling at the first stage of the seroepidemiological study was carried out at the peak of the first rise in COVID-19 incidence in July 2020, 1-2 months after the disease, when the antibody response to SARS-CoV-2 in some of the patients was still not built completely. It is possible that the increase in the level of antibodies in the samples, collected after 8 and > months could be due to the prolonged formation of a full-fledged antibody response after the infection. The impact of vaccination and reinfection on the rise in antibody response to SARS-CoV-2 plays an important role in groups of individuals, whose samples were col-

lected 13 months or more after the previous COVID-19 disease.

Thus the results of the three-stage seroepidemiological study show that during the period from July 2020 until December 2021, population immunity to SARS-CoV-2 virus increased by 3 times and reached 92.3% among the entire population of the Kyrgyz Republic. The percentage of SARS-CoV-2 seropositive children was lower than that in the adult population. The number of seropositive persons among men was lower than among women. During all three rounds of the study, frequency of symptom manifestations in children was statistically significantly lower than in adults, as well as the proportion of those seeking medical help and subject to hospitalization.

The study of the humoral response duration indicated a decrease in the level of antibodies in 64.6% of samples, collected after 8-20 months compared to the result of the first sample, collected 1-2 months after the disease, however, an increased level of antibodies was observed in 35.4%.

Conflict of interest. None to declare.

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Bioethics. Patient's informed consent was obtained before all procedures.

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