

HERD IMMUNITY TO SARS-CoV-2 AMONG THE POPULATION OF THE REPUBLIC OF BELARUS AMID THE COVID-19 PANDEMIC

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Abstract. Objective was to investigate the SARS-CoV-2 collective immunity status of the population of Belarus within the context of the COVID-19 pandemic. *Materials and methods.* The work was carried out according to the methodology for assessing SARS-CoV-2 population immunity, developed by Rospotrebnadzor Russia and the Ministry of Health of Belarus with the participation of the St. Petersburg Pasteur Institute, taking into account WHO recommendations.

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The Bioethics Committee of Belarus and the local ethics committee of the St. Petersburg Pasteur Institute approved the study. Selection of participants was carried out using a questionnaire method and online technology (internet, cloud server). Volunteers were randomized into seven age groups (years of age): 1–17; 18–29; 30–39; 40–49; 50–59; 60–69; and 70+. Regional randomization ensured proportional representation of volunteers from each region, and no more than 30 people were included from one enterprise. In accordance with manufacturer instructions, blood plasma samples were analyzed for: IgG antibodies (Abs) to the SARS-CoV-2 nucleocapsid (Nc) using a quantitative ELISA test system; and IgG Abs to the receptor binding domain (RBD) of the SARS-CoV-2 S (spike) surface glycoprotein using a qualitative ELISA test system. Statistical processing was carried out using Excel 2010 and other software. Statistical differences were designated as significant when $p < 0.05$, unless otherwise indicated. **Results.** The level of seroprevalence, in terms of Abs to Nc among the Belarusian population, was 38.4% (95% CI 37.6–45.4). The highest Ab levels were found among individuals in older age groups (50–70+ years old). The lowest were found in children 1–17 years old and in young people 18–39 years old. The distribution of seroprevalence across Belarusian regions was relatively homogeneous, with the exception of the Minsk Region, where a statistically significant decrease in the indicator was noted. In terms of profession, the largest share of seropositive individuals was found among transportation workers; the smallest was found in business. The moderate COVID-19 incidence has not led to a dramatic increase in the number of contacts. The base reproduction number (R_0) was 1.3. In the Republic of Belarus, there was a moderate level of asymptomatic COVID-19 among seropositive individuals (45.3% [95% CI 44.0–46.7]). This form of infection was observed most often among children aged 1–17 years old (65.0% [95% CI 61.3–68.6]). In parallel with seroprevalence assessment, SARS-CoV-2 vaccination was carried out. We used two vaccines: Gam-COVID-Vac (also known as Sputnik V, developed by Gamaleya National Center for Epidemiology and Microbiology, Russia); and BBIBP-CorV (Sinopharm, PRC). Vaccination against SARS-CoV-2 was accompanied by an increase in the level of anti-RBD Abs (95% [95% CI 94.7–96.7]). Taking into account the vaccination of a subset of the population with BBIBP-CorV, the overall herd immunity, inferred from the analyzed indicators (presence of anti-Nc or anti-RBD Abs), was 47.1% (95% CI 46.3–48.0). **Conclusion.** COVID-19 in Belarus was characterized by a moderately pronounced course of the epidemic process. The threshold level of herd immunity to SARS-CoV-2 has not yet been reached, as a result of which the conditions for progression of the epidemic remain.

Key words: coronaviruses, SARS-CoV-2, COVID-19, morbidity, seroprevalence, asymptomatic course, Republic of Belarus, population, vaccination, anti-nucleocapsid antibodies, anti-RBD antibodies, Gam-COVID-Vac vaccine (Sputnik V), BBIBP-CorV vaccine.

КОЛЛЕКТИВНЫЙ ИММУНИТЕТ К SARS-CoV-2 НАСЕЛЕНИЯ РЕСПУБЛИКИ БЕЛАРУСЬ В УСЛОВИЯХ ПАНДЕМИИ COVID-19

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Резюме. Задача — изучить коллективный иммунный статус населения Беларуси против SARS-CoV-2 в контексте пандемии COVID-19. *Материалы и методы.* Работа проводилась по методике оценки иммунитета населения SARS-CoV-2, разработанной Роспотребнадзором России и Минздравом Беларуси при участии Санкт-Петербургского института Пастера с учетом рекомендаций ВОЗ. Комитет по биоэтике Беларуси и локальный этический комитет Санкт-Петербургского института Пастера одобрили исследование. Отбор участников проводился анкетным методом и онлайн-технологиями (Интернет, облачный сервер).

Добровольцы были рандомизированы на семь возрастных групп (лет): 1–17 лет, 18–29, 30–39, 40–49, 50–59, 60–69 и 70+. Региональная рандомизация обеспечила пропорциональное представительство волонтеров от каждого региона, и от одного предприятия было включено не более 30 человек. В соответствии с инструкциями производителя образцы плазмы крови были проанализированы на IgG-антитела (Abs) к нуклеокапсиду SARS-CoV-2 (Nc) с использованием количественной тест-системы ELISA и на IgG-антитела к рецептор-связывающему домену (RBD) поверхностного гликопротеина SARS-CoV-2 S (шип) с использованием качественной тест-системы ELISA. Статистическая обработка проводилась в программе Excel 2010 и в ряде других. Статистические различия считались значимыми при $p < 0,05$, если не указано иное.

Результаты. Уровень распространенности серотипов по отношению Abs к Nc среди населения Беларуси составил 38,4% (95% ДИ 37,6–45,4), самые высокие уровни Ab были зарегистрированы среди лиц в старших возрастных группах (50–70+ лет), самые низкие – у детей 1–17 лет и у молодежи 18–39 лет. Распределение серопревалентности по регионам Беларуси было относительно однородным, за исключением Минской области, где было отмечено статистически значимое снижение показателя. В профессиональном отношении наибольшая доля серопозитивных лиц была обнаружена среди транспортных работников, наименьшая – в сфере предпринимательства. Умеренная заболеваемость COVID-19 не привела к резкому увеличению числа контактов. Базовый номер репродукции (R_0) равнялся 1,3. В Республике Беларусь среди серопозитивных лиц отмечен средний уровень бессимптомного COVID-19 (45,3% [95% ДИ 44,0–46,7]). Эта форма заражения чаще всего наблюдалась у детей в возрасте от 1 до 17 лет (65,0% [95% ДИ 61,3–68,6]). Параллельно с оценкой серологической распространенности проводилась вакцинация против SARS-CoV-2. Мы использовали две вакцины: Gam-COVID-Vac (также известный как «Спутник V», разработанный Национальным исследовательским центром эпидемиологии и микробиологии им. Н.Ф. Гамалеи, Россия) и BBIBP-CorV (Sinopharm, КНР). Вакцинация против SARS-CoV-2 сопровождалась повышением уровня антител к RBD (95% [95% ДИ 94,7–96,7]). Принимая во внимание вакцинацию подгруппы населения BBIBP-CorV, общий коллективный иммунитет, выведенный из анализируемых показателей (наличие антител против Nc или против RBD), составил 47,1% (95% ДИ 46,3–48,0).

Заключение. COVID-19 в Беларуси характеризовался умеренно выраженным течением эпидемического процесса. Пороговый уровень коллективного иммунитета к SARS-CoV-2 еще не достигнут, вследствие чего сохраняются условия для развития эпидемии.

Ключевые слова: коронавирусы, SARS-CoV-2, COVID-19, заболеваемость, серологическая распространенность, бессимптомное течение, Республика Беларусь, численность населения, вакцинация, антинуклеокапсидные антитела, антитела против RBD, вакцина Gam-COVID-Vac (Sputnik V), вакцина BBIBP-CorV.

Introduction

The Republic of Belarus is a country located practically at the center of Europe. In fact, the Republic's territory includes Europe's geographical center (55°30'N, 28°48'E). It is quite natural that the novel coronavirus infection (COVID-19), which began in the Chinese city of Wuhan and almost instantly spread throughout the world, could not bypass an entire Eastern European country like Belarus. The first COVID-19 case was detected on February 28, 2020 in a student who arrived from Iran [1]. In the following month, predominantly sporadic cases were recorded. Only on March 30, 2020, were 58 infections detected in a single day for the first time [3].

Two days later, the first wave of the disease started. The peak incidence, of 969 primarily infected people per day, fell on May 17, 2020 (Fig. 1). Following that, there was a gradual decrease in the number of infected people, reaching a plateau from August 5 to 21, when the number of new cases did not exceed 100–127 people per day. Starting from August 21, 2020, an uptrend formed. Incidence grew rapidly and reached a maximum (1,972 people/day) on January 13, 2021. This figure was 2-fold higher than the maximum of the first rise. A subsequent, uneven decrease was observed in the period until July 15, 2021 (the ob-

servation period). However, at the end of this period, the number of new cases per day on different days ranged from 600 to 1,300 people. In aggregate, as of July 22, 2021: the incidence rate in the Republic was 4.7‰; and the number of deaths was 0.8% of the total number of infected [3].

Arrows mark the sampling period for determining the level of Ab to SARS-CoV-2 Nc (14.05.2021–19.05. 2021, the 19th–20th week of the year). The black solid line shows the trend curve described by the 6th degree polynomial equation. The regression equation and coefficient of determination (R^2) are shown on the graph.

A feature of Belarus is the tactics aimed at minimizing restrictive government measures [20, 39]. In the country, a 14-day self-isolation was introduced for persons arriving from abroad or having contact with COVID-19 patients. In addition, the use of protective masks and observance of social distancing in public places (public transport, trade establishments, etc.) were strongly recommended. Distance learning and schedule changes in schools and universities were also used [39, 51]. No other restrictive government measures were applied in the Republic. As the official statistics show, the refusal to introduce global restrictions did not cause a significant increase in the number of cases, the number of which remains

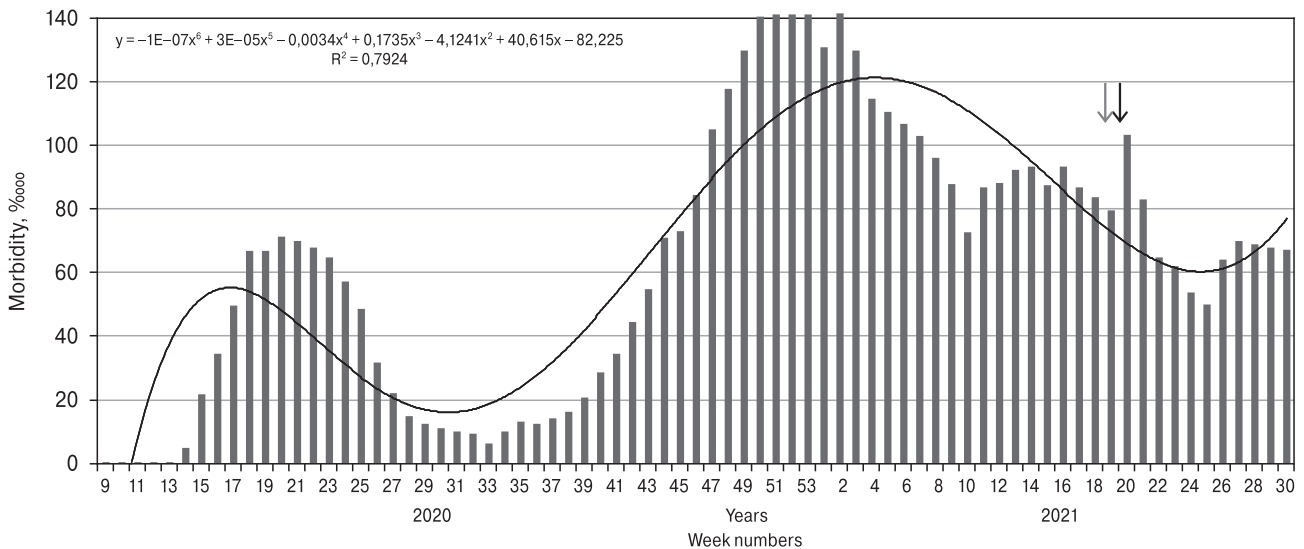


Figure 1. COVID-19 morbidity dynamics of the Belarusian population during the pandemic, 2020–2021

at about 50.00‰‰‰. In the global ranking of countries, Belarus ranks 51st in terms of morbidity.

A likely reason for the relatively low COVID-19 incidence may be low population density (45.5 per km²). In neighboring countries, this level was lower only in Latvia and the Smolensk Region of Russia (29.0 and 18.5 per km², respectively). Ukraine (75.8 per km²), Poland (121.1 per km²), and the Czech Republic (135.7 per km²) feature much higher population densities [5]; their incidence values were from 1.1 to 3.3-fold higher than that of Belarus. It is logical to assume that, in the context of relatively low morbidity, seroprevalence may also be low. A significant decrease in the intensity of contacts with residents of foreign countries, as well as the initiation of vaccination against SARS-CoV-2, may have positively affected the epidemic situation. Unfortunately, we could not find any published data specifically on this issue.

The objective of the study was to investigate the (SARS-CoV-2) population immunity structure in Belarus at the 15th month of the COVID-19 pandemic.

Materials and methods

Formation and randomization of the cohort of volunteers

The study was organized and carried out within a framework for scientific cooperation between countries of Eastern Europe, Transcaucasia, and Central Asia. The framework is in accordance with a Russian Government Order (dated 06.18.2021, No. 1658-p.) and decisions by: the head of the Russian Federal Service for Supervision of Consumer Rights Protection and Human Welfare (Rospotrebnadzor); and the Deputy Minister of Health, Chief State Sanitary Doctor, of Belarus. A cross-sectional cohort randomized study of SARS-CoV-2 herd immunity was carried out according to a program developed by Rospotrebnadzor with the participation of the St. Petersburg Pasteur Institute, taking into account WHO recommendations [52]. The study was conducted on May 14–19, 2021 (weeks 19–20 of the year). In all stages of the study (organizing, collecting, and analyzing results), cloud (Internet) technologies were used [15].

Table 1. Distribution of volunteers by region

Region	Population	Number of individuals studied	Representation % (95% CI)
Brest Region	1,347,000	1,690	0.13 (0.12–0.13)
Vitebsk Region	1,133,000	1,648	0.15 (0.14–0.15)
Grodno Region	1,025,000	1,685	0.16 (0.15–0.17)
Gomel Region	1,386,000	1,606	0.12 (0.11–0.12)
Mogilev Region	1,023,000	1,718	0.17 (0.16–0.18)
Minsk Region	147,2000	1,602	0.11 (0.10–0.11)
Minsk	2,020,000	2,977	0.14 (0.14–0.15)
Total	9,408,440	12,926	0.14 (0.14–0.14)

In accordance with the chosen methodology, the first step was the formation and subsequent randomization of a volunteer cohort by means of questionnaires. Each volunteer, or their legal representative, was familiarized with the goals and conditions of the upcoming study and signed an informed consent. The study was organized in accordance with the provisions of the Declaration of Helsinki and approved by the Bioethics Committee of Belarus (protocol No. 2, dated 13.05.2021) and the local ethics committee of the St. Petersburg Pasteur Institute (protocol No. 64, dated 26.05.2020). In accordance with the specific equation of the De Moivre–Laplace Theorem [16], 12,926 people, from all regions of Belarus, were selected to participate in the study. Volunteers were randomized according to their place of residence, taking into account proportional representation from each region of the Republic (Table 1).

In total, 0.14% (95% CI 0.13–0.14) of the total population was selected for the cohort of volunteers throughout Belarus. In regions of the Republic, the representation varied from 0.11 to 0.17%. Although the differences in SARS-CoV-2 seroprevalence between regions turned out to be statistically significant, it is unlikely that the range of mean values (0.06%) in the subpopulations could have a significant impact on the state of seroprevalence in the Republic with a population of 9,349 million people. The cohort of volunteers consisted of 4,375 men and 8,551 women (a ratio of approximately 1:2). In addition to regional randomization, the cohort was stratified into seven age groups (Table 2).

Vaccination against SARS-CoV-2

Some of the volunteers took part in coronavirus vaccination launched in Belarus. The program used two vaccines. The first, Gam-COVID-Vac (Sputnik V), was developed by the Gamaleya National Center for Epidemiology and Microbiology (Moscow, Russia) [35, 56]. It is a vector, heterologous 2-component vaccine containing: recombinant adenovirus type 26 (rAd26); and a vector of recombinant adenovirus type 5 (rAd5). Both components carry the SARS-CoV-2 spike glycoprotein gene (rAd26-S, rAd5-S). The second vaccine was an inactivated design, BBIBP-CorV, manufactured by Sinopharm Group Co., Ltd., (Shanghai, PRC) [50, 54]. Vaccinations were carried out in accordance with their instructions for use. As a consequence of their compositions, immunity resulting from vaccination with Sputnik V is mainly aimed at binding the RBD of the spike protein [32], while the BBIBP-CorV vaccine (inactivated) induces Abs against all viral antigens [37].

Analysis of volunteers for the presence of SARS-CoV-2 antibodies

All volunteers in the formed cohort underwent blood sampling from the cubital vein (3 ml into va-

Table 2. Age structure of the surveyed volunteer cohort

Age group, in years	Surveyed	
	absolute number	% of total surveyed
1–17	1,727	13.4
Including	1–6	199
	7–13	803
	14–17	725
18–29	1,761	13.6
30–39	1,862	14.4
40–49	1,900	14.7
50–59	1,958	15.2
60–69	1,911	14.8
70+	1,807	13.9
Total	12,926	100.0

Note. Since children feature different stages of general immune development 1–17 years old children group was divided into three subgroups to refine analysis: 1–6; 7–13; and 14–17 years old * — pediatric subgroup values are percentage of the overall group (1–17 years old).

cutainers containing EDTA). Blood plasma samples, after separation from the cellular component, were used for the quantitative determination of IgG Abs to the Nc (SARS-CoV-2) antigen by enzyme immunoassay, using a reagent kit manufactured by the St. Petersburg Pasteur Institute. In addition, vaccinated volunteers were qualitatively analyzed for the presence of anti-RBD Abs using a reagent kit developed by the Gamaleya National Center for Epidemiology and Microbiology.

Statistical analysis

Data processing was performed using Excel 2010. Confidence intervals (95% CI) were calculated by the method of A. Wald and J. Wolfowitz [49], with correction by the method of A. Wald and J. Wolfowitz [49], with correction by A. Agresti and B.A. Coull [18]. Correlation analysis was performed according to Spearman's method. The statistical significance of differences was calculated by the z-test, using an online calculator [2].

Results

Age distribution of Nc antigen seroprevalence in the Belarusian population

The share of residents with Abs to SARS-CoV-2 Nc (seroprevalence), in Belarus as a whole at 15 months after epidemic onset, was 38.4% (95% CI 37.6–39.3) (Table 3). Seroprevalence among men (39.7% [95% CI 38.3–41.2]) was higher than that among women (37.8% [95% CI 36.8–38.8]). Statistical differences are significant at $p < 0.05$.

The largest shares of seropositive persons were noted among volunteers in 3 older groups: 50–59;

Table 3. Seroprevalence of anti-Nc antibodies in different volunteer age groups

Age group, years	Total studied (N)	Seropositive (n)	Seroprevalence, % (95% CI)
1–17	1,727	677	39.2 (33.3–41.6)
Including	1–6	199	29.6 (23.4–36.5)
	7–13	803	42.2 (38.8–45.7)
	14–17	725	38.5 (34.9–42.1)
18–29	1,761	490	27.8 (21.7–30.0)*
30–39	1,862	546	29.4 (25.6–31.4)*
40–49	1,900	699	36.8 (34.4–39.0)
50–59	1,958	922	47.1 (44.6–51.3)*
60–69	1,911	853	44.6 (41.7–46.9)*
70+	1,807	778	43.1 (38.4–45.4)*
Overall	12,926	4,965	38.4 (37.6–45.4)

Note. * — statistically significant differences with the final value of seroprevalence in the cohort, upward or downward. In all comparison pairs, the level of statistical significance by the z-test was $p < 0.001$.

60–69; and 70+ years old (Table 3). Differences from the overall cohort mean were statistically significant at $p < 0.001$. The lowest seroprevalence indicators were found in the groups 18–29 and 30–39 years old. Differences from the cohort mean were statistically significant at $p < 0.001$. Unlike most regions of Russia [6], in Belarus, there was no predominant seroprevalence among children. There was a slightly higher level of seropositivity among children aged 7–13 years. However, the differences were statistically insignificant in comparison with the overall data ($0.05 > p > 0.1$). At the same time, a statistically significant increase in the proportion of seropositive volunteers in older groups may indicate a greater infection in this category of people with coronavi-

rus against the background of a limited set of non-pharmacological interventions in the context of an increase in COVID-19 incidence [39]. For a more accurate assessment of the age structure of seropositivity, the distribution of volunteers by level of Ab to SARS-CoV-2 Nc was determined (Table 4, Fig. 2).

The proportion of volunteers with an Ab level of 100–186 U/ml was 12.2% (95% CI 10.7–13.8) among children 1–17 years old and decreased to 6.2% (95% CI 5.1–7.4) among people aged 70+ years. The differences were statistically significant at $p < 0.0001$. The age dependence had a negative trend and was described by the regression equation $y = 0.0712x^4 - 1.2894x^3 + 7.9167x^2 - 19.373x + 25$, with a coefficient of determination of $R^2 = 0.90$. The ma-

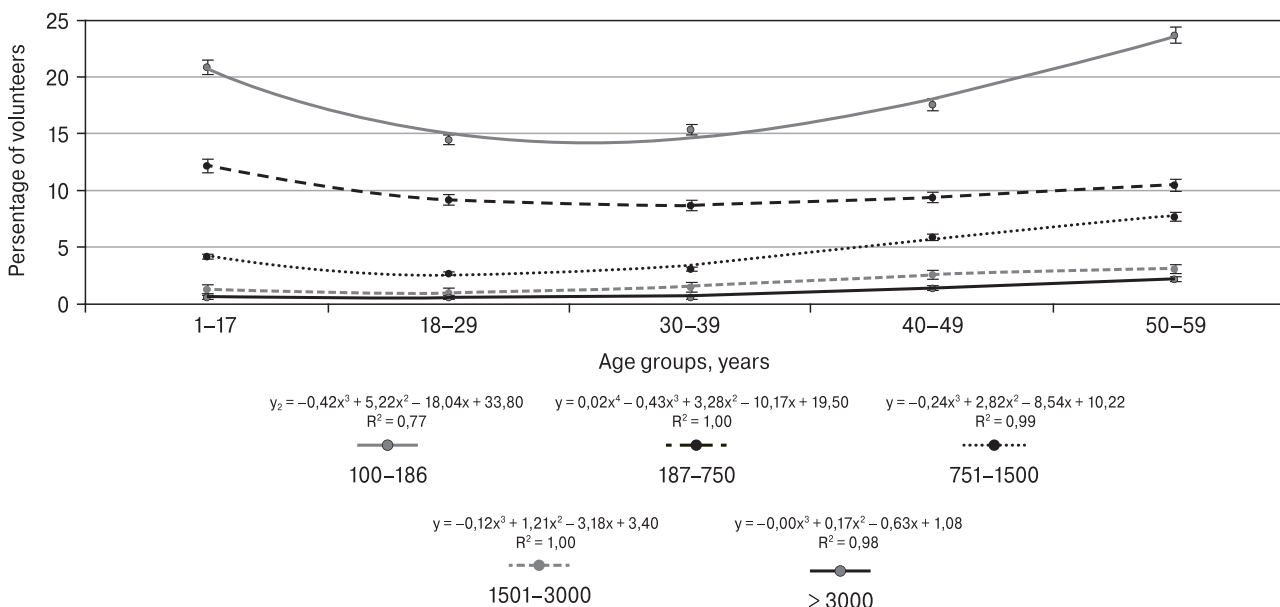


Figure 2. Distribution of the shares of volunteers in different age groups by antibodies to Nc antigens level

Legend: Ab sero-interval groups; colored dots with black vertical lines (confidence intervals) — the proportion of seropositive volunteers who have Abs to SARS-CoV-2 Nc in their blood in the corresponding interval; solid colored lines — forecast trends related to the corresponding sero-interval group. In the upper part of the diagram, the regression equations and R² determination coefficients are presented (in colors matching those of the corresponding sero-interval group). The numerical values of the points are given in Table 4.

jority of seropositive volunteers had low Ab levels in the range 187–750 U/ml. Among individuals in this group, there were: elevations in children 1–17 years old (20.9% (95% CI 19.0–22.8) $p < 0.05$) and those 50–59 years old (23.7% (95% CI 20.1–25.6) $p < 0.0001$); and decreased Ab levels in the age groups of 18–19 years old (14.5% (96% CI 12.8–16.2) $p < 0.0001$) and 30–39 years old (15.4% (95% CI 13.8–17.1) $p < 0.0001$).

An attempt to build a linear trend turned out to be unsatisfactory, since R^2 was -0.11 . Use of a 5th order polynomial was more successful ($y = 0.0808x^5 - 1.517x^4 + 9.9769x^3 - 26.621x^2 + 24.404x + 14.5$), with R^2 of 0.91. Interestingly, in this group, the regression also had a negative direction, although not as pronounced as in the first group (Fig. 2). Two processes described were observed in individuals with very low or low Abs to SARS-CoV-2 Nc.

A change in trend was noted in volunteers with average Ab values ranging from 750 to 1500 U/ml. When approximated by the least squares method, the slope coefficient ($tg\alpha$) was: -0.8 in the Ab sero-interval group 100–186 U/ml; and $tg\alpha$ was 0.5 in the Ab sero-interval group 186–150 U/ml. This indicates that the lowest Ab levels are inherent in children and individuals aged 18–29 years. As age increases, Ab levels increase. This increase started at the 186–750 U/ml Ab level and reached its maximum in the group with Abs in the 751–1500 U/ml range ($tg\alpha = 1.0$). In addition, the minimum level was noted among 18–29 year olds (2.7% [95% CI 2.0–3.5] $p < 0.0001$) (Fig. 2, green dots). Consistent with a linear prognosis, Ab levels increased with age (Fig. 2). Among persons 40–49 years old, there were 5.9% (95% CI 4.8–7.0). In the group of 60–69 years old, their share increased to 9.6% (95% CI 8.3–11.0) ($p < 0.0001$). Finally, among volunteers aged 70+ years, there was a statistically insignificant decrease (8.3% [95% CI 7.0–9.6]).

In the last two groups (Ab levels 1,501–3,000 and > 3000 U/ml), a similar tendency was observed even with a decrease in the arithmetic value of the proportion of seropositivity (Table 4). Thus, the interage distribution, of the proportion of seropositive individuals within the same serological interval, showed: a decrease in Ab levels among young and middle-aged people; and a statistically significant increase among older volunteers. This relationship between age and the level of anti-Nc Abs can be considered one of the features of SARS-CoV-2, which has been established in other studies [47, 55]. One of the probable reasons for this phenomenon may be more severe COVID-19 courses due to the widespread prevalence of premorbid pathology and decreased immune responsiveness in elderly and senile people [4, 34].

Regional seroprevalence structure of the Belarusian population

The study of population seroprevalence was conducted in all seven administrative regions of the Republic (Table 5).

Table 4. Age group distribution of antibodies to SARS-CoV-2 Nc, by titer range

Age group, in years	anti-Nc IgG Ab range, U/ml												
	100–186		187–750		751–1,500		1,501–3,000		> 3,000		0–99 (absence)		Total
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	
1–17	210	12.2 (10.6–13.8)	361	20.9 (19.0–22.8)	73	4.2 (3.3–5.3)	23	1.3 (0.8–2.0)	10	0.6 (0.3–1.1)	1,050	60.8 (58.5–63.1)	1,727
18–29	163	9.3 (7.9–10.7)	253	14.4 (12.8–16.1)	47	2.7 (2.0–3.5)	17	1.0 (0.6–1.5)	10	0.6 (0.3–1.0)	1,271	72.2 (70.0–78.0)	1,761
30–39	162	8.7 (7.5–10.1)	287	15.4 (13.8–17.1)	57	3.1 (2.3–3.9)	28	1.5 (1.0–2.2)	12	0.6 (0.3–1.1)	1,316	70.7 (69.0–74.5)	1,862
40–49	179	9.4 (8.1–10.8)	332	17.5 (15.8–19.3)	112	5.9 (4.9–7.0)	50	2.6 (2.0–3.4)	26	1.4 (0.9–2.0)	1,201	63.2 (60.9–65.6)	1,900
50–59	206	10.5 (9.2–12.0)	463	23.6 (21.8–25.5)	149	7.6 (6.5–8.9)	60	3.1 (2.3–3.9)	44	2.2 (1.6–3.0)	1,036	52.9 (49.0–55.4)	1,958
60–69	129	6.8 (5.7–8.0)	375	19.6 (17.8–21.4)	184	9.6 (8.3–11.0)	84	4.4 (3.6–5.4)	81	4.2 (3.3–5.2)	1,058	55.4 (53.1–58.2)	1,911
70+	112	6.2 (5.1–7.4)	346	19.2 (17.3–21.0)	149	8.2 (7.0–9.6)	88	4.9 (3.9–6.0)	83	4.6 (3.7–5.7)	1,029	56.9 (54.6–59.2)	1,807
Overall	1,161	9.0 (8.5–9.5)	2,417	18.7 (18.0–19.4)	772	6.0 (5.5–6.4)	350	2.7 (2.4–3.0)	266	2.1 (1.8–2.3)	7,961	61.6 (60.7–62.4)	12,926

Note. n — number of volunteers. A negative result was designated as 0–99 U/ml.

Table 5. Morbidity and SARS-CoV-2 seroprevalence in volunteers living in different regions of Belarus

Location	Population density, per km ²	Studied volunteers		Seroprevalence, % (95% CI)	Morbidity, ‰
		Total	Seropositive		
Brest Region	41	1,690	698	41.3 (34.4–43.7)	61.0
Vitebsk Region	28	1,648	587	35.6 (33.3–38.0)	86.8
Grodno Region	40	1,685	709	42.1 (35.0–44.5)	61.7
Gomel Region	34	1,606	635	39.5 (37.1–42.0)	48.5
Mogilev Region	35	1,718	685	39.9 (33.7–42.2)	77.8
Minsk Region	37	1,602	544	34.0 (31.6–36.3)*	93.5
Minsk	5761	2,977	1,107	37.2 (35.5–38.9)	130.4
Overall	45	12,926	4,965	38.4 (37.6–39.3)	80.9

Note. * — statistically significant difference with the overall value.

Among the surveyed Belarusian regions, higher seroprevalence (relative to the average for the Republic) was noted in the Grodno and Brest regions. They were, however, statistically insignificant. The lowest seroprevalence was found among volunteers in the Minsk Region (differences were statistically significant at $p < 0.0001$). A slightly higher proportion of seropositive volunteers was found in Minsk, while in other regions there was a significant morbidity. At first glance, there is no statistically significant relationship between morbidity and seroprevalence, which somewhat contradicts previously obtained results in other territories [9, 11, 12].

We tried to assess the presence of any relationship between morbidity and population density, assuming that these two indicators may be correlated. Such a dependence does exist with: a correlation coefficient of $r = 0.64$ ($0.05 > p < 0.1$); a descending straight trend line ($y = -0.292 \ln(x) + 1.7687$); and a determination coefficient of $R^2 = 0.41$. The connection turned out to be weak ($0.05 > p < 0.1$) (Fig. 3).

Based on these data, it can be concluded that an increase in seroprevalence is accompanied by a decrease

in the incidence rate per 100,000 population. This conclusion is quite consistent with published ideas that: as the level of herd immunity grows in the population, the rate of spread of the disease decreases until its complete cessation [23, 42, 43]. The relationship between population density and seroprevalence turned out to be slightly more significant. The trend line is an ascending straight line: $y = 0.2904 \ln(x) + 1.7182$; correlation coefficient 0.70 ($0.05 > p < 0.1$); and a determination coefficient of $R^2 = 0.47$ (Fig. 4).

In this case, the regression line was ascending. This reflects the well known fact in epidemiology that there is a direct relationship between population density and morbidity. Demographically, Belarus is a territory with a relatively evenly distributed population. As noted earlier, the average population density in the country is 45 per km². An exception to the trend is the capital of the Republic, Minsk, where the population density is 5819 per km² (126.5-fold higher than the national average). Interestingly, even with such a density, the seroprevalence level (37.2% [95% CI 35.5–38.9]) does not differ from the national average (38.4% [95% CI 37.6–39.3]).

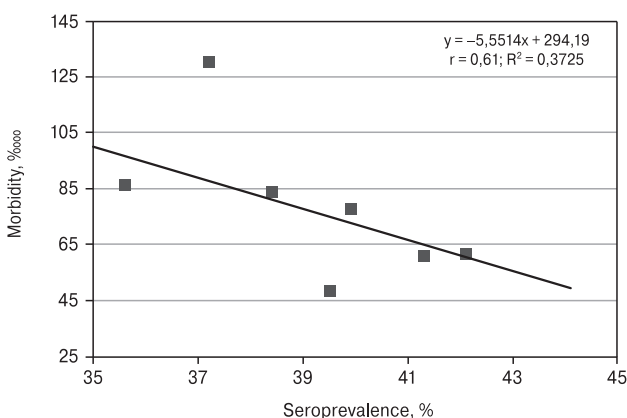


Figure 3. Correlation between morbidity and seroprevalence

Note. On the x and y axes, morbidity and seroprevalence are expressed as log₁₀. The correlation coefficient (r), the determination coefficient R², and the regression equation are shown.

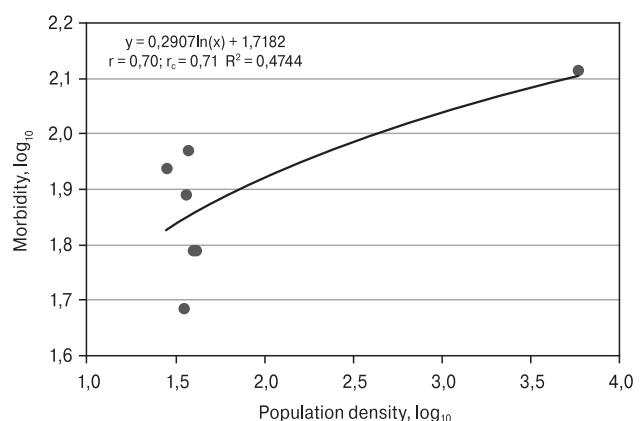


Figure 4. Correlation between morbidity rate and population density

Note. On the x and y axes, incidence and population density are expressed as log₁₀. The correlation coefficient (r), determination coefficient R², and the regression equation are shown.

Thus, the performed correlation analysis confirmed the presence of a statistically significant relationship between population density and morbidity, as well as between population density and seroprevalence. The latter phenomenon was revealed in other territories as well [13]. The desire to break this connection is precisely what caused the widespread use of self-isolation regimes in Belarus [22, 24, 26]. It can be assumed that the nature of the relationship between population density and morbidity is, to some extent, due to the late introduction of restrictive anti-epidemic measures to protect the population from SARS-CoV-2 [39].

The structure of (SARS-CoV-2 Nc) seroprevalence by profession

A factor such as profession can have a significant impact on the level of seroprevalence. There is an extensive list of professions that involve constant, broad contact with the surrounding population. Visual, and often tactile, contact with those around them are typical for workers in: healthcare; education; trade; transportation; catering; consumer services (hair salons, massage parlors, spas); and a number of other professions, such as manufacturing, were working remotely or in self-isolation isn't feasible [21, 30, 39, 46]. In Belarus, a survey of SARS-CoV-2 Ab seroprevalence was carried out among representatives of 12 professional groups (Table 6).

The distribution of seroprevalence, among 8,241 working-age volunteers in 12 professional groups, was generally quite homogeneous (Table 5). Unlike other researchers, we were unable to identify predominant

seroprevalence values in healthcare [8, 19] or education [14] professional groups. A higher SARS-CoV-2 seroprevalence was found among transport workers: 45.2% (95% CI 41.6–52.5; $p < 0.0001$). It can be assumed that this group of volunteers was dominated by employees of public transport enterprises who have frequent contact with passengers. The lowest seroprevalence was noted among business professionals: 32.0% (95% CI 28.1–36.2; $p < 0.0001$). In the remaining groups, statistically significant differences from the group mean were not found (Table 5). Thus, analysis of seroprevalence by professional group did not find substantial differences in Belarus as a whole.

Seroprevalence among COVID-19 convalescents and their contacts

Among the volunteers who participated in the study, 4,056 people indicated in the questionnaire that they had suffered from COVID-19 (31.4% [95% CI 30.6–32.2]). In the entire group of convalescents, the number of seropositive was 2,611 (64.4% [95% CI 62.6–65.8]). Correlated with the cumulative number of infections in Belarus as a whole (as of May 19, 2021), the total number of convalescents in the cohort was 1.07% (95% CI 1.03–1.10).

Keeping in mind that each patient in the latent and/or manifest period is highly likely to be a spreader of infection, it is reasonable to assume that a certain number of people have been in contact with patients [42, 43]. The share of such persons may vary and largely depends on the effectiveness of restrictive administrative measures. During the survey process (questionnaire), 4,967 people (38.4% [95% CI 37.6–39.3]) were identified with verified patient and/or convalescent contact. Among them, the number of seropositive persons was 2,043 (41.3% [95% CI 39.7–42.5]).

Table 6. SARS-CoV-2 seroprevalence among different professions groups

Professions	Number of individuals studied	Seropositive individuals	Seroprevalence, % (95% CI)
Healthcare	1,504	570	37.9 (35.4–40.4)
Science	91	33	36.3 (26.4–47.0)
Business	540	173	32.0 (28.1–36.2)*
Education	1,126	440	39.1 (36.2–42.0)
The Arts	148	56	37.8 (30.0–46.2)
Manufacturing	1,201	457	38.1 (35.3–40.9)
Transportation	328	151	46.0 (40.5–51.6)*
Armed Forces	99	39	39.4 (29.7–49.7)
Civil Service	365	147	40.3 (35.2–45.5)
Office Work	1,675	614	36.7 (34.3–39.0)
Unemployed	340	116	34.1 (29.1–39.4)
Other	823	275	33.4 (30.2–36.7)
Overall	8,241	3,071	37.3 (36.2–38.3)

Note. * — statistically significant differences from the mean ($p < 0.0001$).

Table 7. Age distribution of SARS-CoV-2 seropositive volunteers with an asymptomatic COVID-19 course

Age group, years	Number of seropositive individuals		Asymptomatic seropositive individuals, % (95% CI)
	total	asymptomatic courses	
1–17	677	440	65.0 (61.3–68.6)*
16–29	490	248	50.6 (46.1–55.1)
30–39	546	258	47.2 (43.0–52.0)
40–49	699	265	37.9 (34.3–41.6)
50–59	922	349	37.8 (34.7–41.1)
60–69	853	308	36.1 (32.9–39.4)
70+	778	383	49.2 (45.7–52.8)
Overall	4,964	2,251	45.3 (44.0–46.7)

Note. * — statistically significant difference from the cohort mean.

Distribution of asymptomatic COVID-19 forms among seropositive volunteers

In the surveyed cohort, asymptomatic individuals with a positive PCR test result were not identified. Antibodies to (SARS-CoV-2) Nc were identified in 4,964 people (38.4% [95% CI 37.6–39.2]). Of them, 2,251 people (45.3% [95% CI 44.0–46.7]) had no symptoms or indications of COVID-19, except for the aforementioned antibodies. This group of individuals was classified as seropositive volunteers with an asymptomatic course of infection (Table 7).

In a number of Russian regions, the share of asymptomatic, seropositive individuals ranged from 90 to 100% [7]. The share of such persons in Belarus, however, was about 2.2-fold lower (Table 6). The largest number of asymptomatic volunteers was found in the group 1–17 years old ($p < 0.0001$). Some higher seropositivity values were noted in the groups 16–29 years old and 70+ years old, but they were statistically insignificant ($p = 0.1$).

Seroprevalence among vaccinated individuals

Among the volunteer cohort who participated in the program assessing SARS-CoV-2 seroprevalence in the population, 1,735 people received SARS-CoV-2 vaccines. Twenty six volunteer samples were invalid. Therefore, further analysis was carried out of 1709 vaccinated volunteers (1,542 immunized with Sputnik V, 167 immunized with BBIBP-CorV).

The BBIBP-CorV vaccine induces Abs to all antigens of the SARS-CoV-2 complex. In response to Sputnik V vaccination, only Abs against the RBD are produced (not against Nc) [35, 54]. Among Sputnik V-vaccinated volunteers, however, about 20% were found to have anti-Nc Abs. Presumably, they were formed as a result of a transmitted asymptomatic

infection. This assumption is in satisfactory agreement with the data on Nc seropositivity in the entire cohort, which amounted to 38.4% (95% CI 37.6–45.4) (Table 1). Although the proportion of seropositive individuals is, in general, statistically significantly higher than those vaccinated (Table 8), it includes unvaccinated children and convalescents (who are recommended to observe a 3–6 month delay in vaccination). Considering that the Sputnik V vaccine does not induce anti-Nc Abs by nature of its design, we interpreted their presence in vaccinated volunteers as a sign of post-infectious immunity. Accordingly, anti-RBD Abs were attributed to post-vaccination immunity.

BBIBP-CorV vaccine, being polyvalent, naturally induced production of Abs against the entire viral antigen range. The proportion of Nc seropositive following BBIBP-CorV vaccination was 2.8-fold higher than that with Sputnik V. In this regard, it is logical to assume that the real contribution of the Chinese-made vaccine to the proportion of those seropositive for Nc Abs in immunized volunteers may be about 42%. Of course, this is a purely speculative conclusion that requires additional verification. In addition, this 42% refers to only 167 individuals vaccinated BBIBP-CorV. When recalculated for the entire vaccinated cohort, the additional contribution to the total proportion of individuals with anti-Nc plasma Abs will be only 4.1% (95% CI 3.2–5.1). This does not statistically differ from the proportion of individuals in which immunity from both Ab classes is absent.

As for Abs to the RBD, no surprises were noted. Both vaccines created comparable immunity with seroprevalence levels from 94.0% (95% CI 89.3–97.1) to 96.0% (95% CI 94.9–96.9); differences between the indicators were not statistically significant (Table 8). Thus, these vaccines used in the Republic

Table 8. Seroprevalence in the surveyed volunteers vaccinated against SARS-CoV-2

Vaccine	Total vaccinated	Post-infectious immunity is present (anti-Nc IgG antibodies)		Post-vaccination immunity is present (anti-RBD IgG antibodies)		Absence of immunity*	
		n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Sputnik V	1542	357	23.2 (21.1–25.3)	1,480	96.0 (94.9–96.9)	56	3.6 (2.8–4.7)
BBIBP-CorV	167	109	65.3 (57.5–72.5)**	157	94.0 (89.3–97.1)	8	4.8 (2.1–9.2)
Overall	1,709	466	27.3 (25.2–29.5)	1,637	95.8 (94.7–96.7)	64	3.7 (2.9–4.8)

Note. * — there is neither post-infectious nor post-vaccination immunity, ** — the presence of anti-Nc Abs in those vaccinated with BBIBP-CorV cannot be unambiguously interpreted as a consequence of prior infection.

Table 9. Presence of IgG antibodies to various SARS-CoV-2 proteins in volunteers vaccinated with Sputnik V (N = 1,542)

Sputnik V vaccine		anti-Nc IgG antibodies	
		present (n = 357)	absent (n = 1,185)
IgG antibodies to the RBD domain (S protein), number of volunteers	present (n = 1480)	351 22.8% (95% CI 20.7–25.0)	1,129 73.2% (95% CI 70.9–75.4)
	absent (n = 62)	6 0.4% (95% CI 0.1–0.8)	56 3.5% (95% CI 2.8–4.7)

Table 10. Presence of IgG antibodies to various SARS-CoV-2 proteins in volunteers vaccinated with BBIBP-CorV (N = 167)

BBIBP-CorV vaccine		anti-Nc IgG antibodies	
		present (n = 109)	absent (n = 58)
IgG antibodies to the RBD domain (S protein), number of volunteers	present (n = 157)	107 64.1% (95% CI 56.3–71.3)	50 29.9% (95% CI 23.5–37.3)
	absent (n = 10)	2 1.2% (95% CI 0.2–4.3)	8 4.8% (95% CI 2.1–9.2)

of Belarus have shown high efficiency in terms of Ab production to the RBD of SARS-CoV-2.

Further analysis was carried out only in relation to those immunized with Sputnik V, the composition of which allows: the most complete assessment of the contribution of vaccination to the structure of herd immunity (Table 9); and to compare these data with BBIBP-CorV vaccination results (Table 10). The number of persons with post-vaccination SARS-CoV-2 immunity induced by Sputnik V was 1,129.

Similar calculations for the BBIBP-CorV vaccine showed that its contribution to post-vaccination immunity was 0.4% (95% CI 0.3–0.5). The contribution of the BBIBP-CorV vaccine to Nc Ag immunity, calculated according to the principle above, was 0.9% (95% CI 0.7–1.9). The total contribution of Abs to the BBIBP-CorV vaccine to SARS-CoV-2 herd immunity was: $0.4 + 0.9 = 1.3\%$.

Taking into account the sum of post vaccination immunity from Sputnik V (1129 people) and the number of seropositive individuals with post infectious immunity (4,965 people), relative to the total cohort (12,926), the final indicator of SARS-CoV-2 herd immunity was 47.1% (95% CI 46.3–48.0). This is the average level of seroprevalence in the population of the Republic of Belarus.

Discussion

Based on the overall results of the study, it can be concluded that the epidemic process, of novel coronavirus infection in Belarus, can be characterized as moderately intense. This is manifested as a high proportion of convalescents, although it is worth noting that a noticeable morbidity did not lead to the activation of transmission or the appearance of a significant number of contact persons. The age structure of seroprevalence, in contrast to a number of Russian regions [10, 11, 13, 41], was characterized by a statistically significant increase in the indicator among persons in older age groups (50–70+ years; $p < 0.0001$) (Table 3). When quantifying Abs SARS-CoV-2 Nc, it was shown that: the minimum Ab levels (100 to 750 U/ml) prevailed among volunteers in the age groups 1–17 and 18–29 years old; and the maximum levels (751 to 3000+ U/ml) were identified among persons aged 50 to 70+ years. This is probably, to a certain extent, associated with a more severe disease course in elderly and senile people [4, 28, 44].

When analyzing the relationship between seroprevalence and morbidity, an inverse correlation was found (rank correlation coefficient 0.61, $p < 0.05$; Fig. 3). Other features were revealed when assessing the influence of occupational factors on seroprevalence. Unlike other territories [27, 33, 38, 48], a predominant seroprevalence among medical workers was not established in Belarus. At the same time, there was a statistically significant increase in the proportion of seropositive people among transport workers.

The analysis of seroprevalence among convalescents and contact persons may be of some interest. First of all, attention is drawn to a rather large share of volunteers who indicated a COVID-19 illness, which amounted to 4,056 people. Of them, 2,611 people were seropositive for Abs SARS-CoV-2 Nc (64.4% [95% CI 62.6–65.8]). Naturally, a question arises about the reasons behind the lack of Abs among the remaining 35%. The answer likely lies in the work of Wu et al. [53], in which it was shown that about 30% of convalescents produce specific Abs in titers below detection thresholds. However, if physical protection measures are not followed, they are able to spread the virus through exhaled air, coughing, or sneezing [25, 45].

In the study, verified contacts with patients or convalescents were found in 4967 people. Of them, 2,043 had Abs to SARS-CoV-2 Nc (41.3% [95% CI 39.7–42.5]). Thus, light protection methods did not prevent SARS-CoV-2 transmission in the susceptible population; transmission was slowed, although probably not as effectively as would be expected according to mathematical models [23].

Among seropositive persons, a significant proportion are so-called asymptomatic cases, in which the disease proceeds without any clinical manifestations, leaving behind only a trace in the form of specific Abs [36]. This is most common among children [31]. There are at least two asymptomatic case types. Firstly, asymptomatic individuals can be defined as those without any signs of COVID-19 other than a positive PCR test. There were no such volunteers in the surveyed cohort. It is believed that such people are able to shed coronavirus longer than symptomatic COVID-19 patients, thus reducing the effectiveness of anti-epidemic measures [36]. The second asymptomatic type includes people without any signs of COVID-19, with the exception of Abs to SARS-CoV-2. Some of them may subsequently

develop a manifest infection, while others will remain asymptomatic (albeit with low level IgG Abs).

Among seropositive volunteers, the asymptomatic share was 45.3% (95% CI 44.0–46.7), which is approximately 2-fold lower than in Russia and other territories [7, 36]. It can be assumed that this is due to the low level of viral transmission among the population.

The implementation of a COVID-19 vaccination program in the Republic of Belarus started in December, 2020. Two vaccines were used: the Sputnik V vector, heterologous vaccine (Moscow, Russia); and the BBIBP-CorV inactivated vaccine (Sinopharm, PRC). As of May 19, 2021, 3.5% (95% CI 3.49–3.51) of the population were vaccinated in Belarus. In other words, no more than 57,000 people a month were vaccinated for six months. At the time of this writing, the proportion of vaccinated persons had increased 4.5-fold and amounted to 15.7% (95% CI 15.68–15.72). Among the volunteers, 1709 people were vaccinated (1542 with Sputnik V, 167 with BBIBP-CorV). Both vaccines showed almost equal efficacy in terms of anti-RBD Abs. A significantly higher response to Nc antigen was found in BBIBP-CorV vaccinated individuals. This is expected as the Chinese (polyvalent, inactivated) vaccine naturally induces a larger spectrum of Abs than the Russian Sputnik V (monovalent, vector) vaccine. Despite the small scale of vaccination within the surveyed cohort, it contributed to an increase in the level of herd immunity, on average, up to 47.1% (95% CI 46.3–48.0).

The achieved level of immunity is still far from the minimum threshold [42]. The base reproduction number (R_0) calculated for Belarus, a value characterizing the rate of spread of infection and the formation of herd immunity [44], was about 1.3. The value of R_0 among volunteers, at first glance, is mathematically small. However, one cannot fully rely on it, since it does not take into account: the real prevalence of infection among the population; the effectiveness of non-specific protective measures (distancing, protective masks, self-isolation, etc.); or vaccination.

Taking into account available mathematical modeling methods for COVID-19 morbidity, an R_0 of 3.0 to 3.5 is likely more realistic [17, 23]. This means that the minimum threshold for SARS-CoV-2 immunity cannot be less than 60–65%. Indeed, an extreme point of view has been expressed by A. Pollard, who believes that herd immunity is generally unattainable [29]. The truth, however, is probably somewhere in between. Even with vaccination of up to 80–90% of the country, COVID-19, if it does not completely disappear, will likely transform into another seasonal, acute respiratory viral infection. Achieving this limit is only possible with a significant increase in the rate of vaccination of the population.

Thus, at 15 months following the onset of the COVID-19 pandemic, the herd immunity of the Belar-

ussian population amounted to 38.4% (95% CI 37.6–45.4). Statistically significant differences between regions of the Republic were not noted, with the exception of a lower proportion of seropositive persons in the Minsk Region ($p < 0.0001$).

When assessing the age distribution of Nc seroprevalence (Abs to SARS-CoV-2 Nc), a significantly higher proportion of seropositive persons was noted among the older generation (50–70+ years). Further, low levels were found among younger volunteers (1–39 years old).

There was a statistically significant inverse relationship between morbidity and seroprevalence ($r = 0.61$; $p < 0.05$). The highest seroprevalence was observed in transportation workers (47.0% [95% CI 41.6–52.5]), and the lowest was noted in business (32.0% [95% CI 28.1–36.2]). The relatively high number of convalescents, 4,056 (31.4% [95% CI 30.6–32.2]), did not lead to a significant increase in the number of contact persons, amounting to 4,967 people (38.4% [95% CI 37.6–39.3]). A characteristic feature of Belarus is the low level of viral transmission from convalescents to healthy individuals. The base reproduction number (R_0) was 1.3, which is less than in most other countries and territories.

The Republic is characterized by a low proportion of asymptomatic forms of infection among seropositive volunteers (45.3% [95% CI 44.0–46.7]), with a statistically significant predominance of such forms among children 1–17 years old (65.0% [95% CI 61.3–68.6]). The obtained results show that the chosen tactics of combating the novel coronavirus have affected the epidemic process in certain ways, but have not led to the formation of an optimal level of herd immunity. The SARS-CoV-2 vaccination deployed in Belarus has contributed to the formation of a high level of anti-RBD immunity. However, the rate of vaccination clearly has not led to a rapid growth in herd immunity so far, which poses a threat of further COVID-19 epidemic growth.

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Conflict of interests

The authors declare that they have no conflicts of interest.

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