


BMJ Open Investigation of SARS-CoV-2 seroprevalence in relation to natural infection and vaccination between October 2020 and September 2021 in the Czech Republic: a prospective national cohort study

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ABSTRACT

Objective Examine changes in SARS-CoV-2 seropositivity before and during the national vaccination campaign in the Czech Republic.

Design Prospective national population-based cohort study.

Setting Masaryk University, RECETOX, Brno.

Participants 22 130 persons provided blood samples at two time points approximately 5–7 months apart, between October 2020 and March 2021 (phase I, before vaccination), and between April and September 2021 (during vaccination campaign).

Outcome measures Antigen-specific humoral immune response was analysed by detection of IgG antibodies against the SARS-CoV-2 spike protein by commercial chemiluminescent immunoassays. Participants completed a questionnaire that included personal information, anthropometric data, self-reported results of previous RT-PCR tests (if performed), history of symptoms compatible with COVID-19 and records of COVID-19 vaccination. Seroprevalence was compared between calendar periods, previous RT-PCR results, vaccination and other individual characteristics.

Results Before vaccination (phase I), seroprevalence increased from 15% in October 2020 to 56% in March 2021. By the end of phase II, in September 2021, prevalence increased to 91%; the highest seroprevalence was seen among vaccinated persons with and without previous SARS-CoV-2 infection (99.7% and 97.2%, respectively), while the lowest seroprevalence was found among unvaccinated persons with no signs of disease (26%). Vaccination rates were lower in persons who were seropositive in phase I but increased with age and body mass index. Only 9% of unvaccinated subjects who were seropositive in phase I became seronegative by phase II.

Conclusions The rapid increase in seropositivity during the second wave of the COVID-19 epidemic (covered by phase I of this study) was followed by a similarly steep rise in seroprevalence during the national vaccination campaign, reaching seropositivity rates of over 97% among vaccinated persons.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ The prospective seroconversion coronavirus study (PROSECO) provides nationwide data from the Central European region heavily affected by COVID-19.
- ⇒ The levels of anti-SARS-CoV-2 antibodies and the dynamics of seroconversion were assessed using a harmonised network of accredited clinical laboratories.
- ⇒ Major strengths of the study are its size, coverage, start before vaccination period, evaluation of natural SARS-CoV-2 infection and ongoing longitudinal follow-up inclusive of vaccination.
- ⇒ The duration of anti-SARS-CoV-2 antibodies after infection in unvaccinated subjects is assessed.
- ⇒ The main limitation relates to the fact that study subjects were volunteers at the baseline, and this may affect the representativeness of the cohort.

INTRODUCTION

During the COVID-19 pandemic, monitoring of the seroprevalence of antibodies in the population is an important tool to design and adjust preventive strategies. As a part of this process, it is essential to assess the contribution of natural infections and vaccination to the immune response to SARS-CoV-2. The Serotracker platform has recorded hundreds of SARS-CoV-2 serological studies worldwide (serotracker.com).¹ Most national seroprevalence studies were performed before the start of massive vaccination programme in Europe,² but there are only few published European seroprevalence studies covering both prevaccination and after vaccination campaign periods. Overall, these studies, mainly based in Western Europe, reported rising seroprevalence after the national

vaccination programmes.^{3–7} However, very few published studies have been conducted in Central and Eastern Europe, where the dynamics of both the epidemics and vaccine uptake differed from the Western European countries.

We have previously reported findings from a national cross-sectional survey of 30 000 persons in the Czech Republic who were examined between October 2020 and March 2021, a period covering the second wave of the epidemic, which was also the period before the start of national vaccination campaign. We found that by March 2021, 53% of participants had measurable antibodies against SARS-CoV-2.⁸ This was consistent with governmental data using cumulative PCR testing data. These rates were considerably higher than those reported in Western Europe,^{2 9–11} due to a strong second wave of natural infection in the Czech Republic in autumn 2020.⁸

In this report, we report longitudinal data on repeated assessment of the same population sample in the period April 2021–September 2021, a period coinciding with the rollout of the national vaccination programme. The objectives of this analysis were to (1) examine the trends in seropositivity before and during the national vaccination campaign, (2) assess the contributions of natural infections and vaccination to the seropositivity, (3) to assess seroconversion rates in previously seronegative persons, (4) to assess duration of seropositivity after natural infection and (5) to estimate the rate ratio of seroconversion and vaccination associated with sociodemographic indicators.

METHODS

Study design and participants

Data for these analyses were derived from the first and second wave of the PROSECO study. The PROSECO study design and population recruitment has been described elsewhere.⁸ Briefly, phase I of the study recruited 30 054 unvaccinated adult volunteers from persons registered with the second largest health insurance company in the Czech Republic. Participants provided blood sample between October 2020 and March 2021, during the second epidemic wave in the Czech Republic. Of those, 22 130 participants were re-examined during the national vaccination programme between April 2021 and September 2021. Participants were invited for phase II in the same order as they participated in phase I, so most subjects were re-examined 5–7 months after the first visit. Comparison of the persons participating in both phases with those who only attended phase I is shown in online supplemental table S1. Those who participated in both assessments were older, more likely to be female, seropositive at phase I, more obese and more likely to have history of chronic non-communicable diseases.

In phase II, participants provided a second blood sample for detection of IgG antibodies against SARS-CoV-2 and completed a questionnaire on personal information, including educational level, weight and height

(to calculate body mass index (BMI)) and smoking status. Self-reported data about common non-communicable disorders (diabetes, hypertension, asthma and chronic obstructive pulmonary disease) were also collected together with self-reported results of RT-PCR tests (if performed) and records of COVID-19 vaccination. The second visit was organised at least 14 days after any vaccination (if completed).

Laboratory analyses

CE-marked serological tests were performed in accredited clinical laboratories. Antigen-specific humoral immune response was analysed by detection of IgG antibodies against the spike protein using commercial immunoassays LIAISON SARS-CoV-2 S1/S2 IgG (DiaSorin, Saluggia, Italy) and SARS-CoV-2 IgG II Quant (Abbott, Sligo, Ireland). Testing was conducted on the LIAISON XL (DiaSorin) and on the Alinity (Abbott, Lake Forest, Illinois, USA), respectively. Samples were tested individually and reported according to the manufacturers' criteria.

Statistical analysis

The primary aim of this study was to estimate seropositivity rates of the adult Czech population. We estimated seroprevalence rates and 95% CIs, we also standardised the seroprevalence rates by age and sex, using the Czech population as a standard. We used a multivariate Poisson regression model with a robust error variance to estimate the ratio of seroconversion and vaccination associated with sociodemographic indicators. Differences in prevalence were expressed as prevalence rate ratios (PRRs). We used standard descriptive statistics to characterise the study data set.

We adjusted the estimated values of seroprevalence for the sensitivity and specificity of serological tests used in this study, employing a standard correction formula based on Bayesian approach: $\text{seroprevalence} = (\text{proportion positive} + \text{specificity} - 1) / (\text{sensitivity} + \text{specificity} - 1)$.¹² As serological tests were performed using chemiluminescent immunoassay methods, the range of standardised seroprevalence values given by the 95% CI was adjusted based on the range of sensitivity and specificity values given by their 95% CIs declared by the manufacturers: DiaSorin LIAISON 95% CI for sensitivity 86.8% to 99.5%; 95% CI for specificity 97.5% to 99.2%, Abbott Alinity 95% CI for sensitivity 96.5% to 100%; 95% CI for specificity 99.2% to 99.8%. Combination of the most likely values of standardised seroprevalence, sensitivity and specificity yielded a range of values where the test-adjusted seroprevalence is likely to occur (online supplemental table S2).

Population data on COVID-19 were obtained from the Czech Central Information System of Infectious Diseases (ISID), which includes records of all consecutive patients with COVID-19 in the Czech Republic identified and confirmed by laboratory testing. ISID data are routinely collected in compliance with Act No. 258/2000 Coll. on the Protection of Public Health and are publicly available in aggregated and anonymised form of open or

Table 1 Characteristics of the study sample and proportions and prevalence rate ratios of seropositivity and vaccination

	Model of antibodies of any origin (n=22 130)			Model of propensity to vaccination (n=22 130)			Model of antibodies in unvaccinated participants (n=7647)						
	No. of participants	No. of seropositive participants	No. of vaccinated participants	% of seropositive	PRR (95% CI)	P value	% of vaccinated	PRR (95% CI)	P value	No. of participants	% of seropositive	PRR (95% CI)	P value
Sex													
Female	13 824	12 067	8844	87.29	1.00	-	63.98	1.00	-	4980	67.25	1.00	-
Male	8306	7282	5639	87.67	0.99	0.012	67.89	1.05	<0.001	2667	65.47	0.95	<0.001
Age groups (years)													
18-29	1491	1202	770	80.62	1.00	-	51.64	1.00	-	721	61.72	1.00	-
30-39	2774	2275	1534	82.01	1.02	0.215	55.30	1.03	0.420	1240	61.05	0.97	0.338
40-49	6700	5725	4177	85.45	1.01	0.194	62.34	1.17	<0.001	2523	64.05	0.97	0.226
50-59	6049	5405	4061	89.35	1.03	0.003	67.14	1.23	<0.001	1988	70.32	1.04	0.170
60+	5116	4742	3941	92.69	1.05	<0.001	77.03	1.37	<0.001	1175	74.81	1.09	0.001
Education													
Basic	1952	1744	1295	89.34	1.00	-	66.34	1.00	-	657	70.02	1.00	-
Medium	8024	7119	5348	88.72	1.00	0.972	66.65	1.02	0.275	2676	69.21	1.02	0.337
High	7544	6689	5223	88.67	1.00	0.890	69.23	1.08	<0.001	2321	65.75	1.02	0.394
Missing	4610	3797	2617	82.36	0.97	0.003	56.77	0.87	<0.001	1993	63.07	1.00	0.923
COVID-19 in history													
Seronegative	11 352	8935	7882	78.71	1.00	-	69.43	1.00	-	3470	36.54	1.00	-
Seropositive—no symptoms	5597	5374	3458	96.02	1.28	<0.001	61.78	0.75	<0.001	2139	90.04	3.45	<0.001
Seropositive—with symptoms	5181	5040	3143	97.28	1.32	<0.001	60.66	0.78	<0.001	2038	93.28	3.58	<0.001
BMI													
<18.5	256	197	134	76.95	1.00	-	52.34	1.00	-	122	52.46	1.00	-
18.5-24.9	8192	6964	5038	85.01	1.04	0.127	61.50	1.09	0.141	3154	63.44	1.17	0.009
25-29.9	8080	7167	5488	88.70	1.05	0.077	67.92	1.15	0.020	2592	68.36	1.18	0.006
30+	4802	4369	3312	90.98	1.06	0.046	68.97	1.16	0.013	1490	74.30	1.20	0.003
Missing	800	652	511	81.50	0.98	0.515	63.88	1.18	0.017	289	52.25	0.95	0.498
NCDs in history													
No	13 888	11 958	8688	86.10	1.00	-	62.56	1.00	-	5200	65.23	1.00	-
Yes	7152	6500	5161	90.88	1.00	0.818	72.16	1.06	<0.001	1991	71.97	1.00	0.813
Missing	1090	891	634	81.74	1.02	0.266	58.17	0.91	0.002	456	59.21	1.12	0.005
Vaccination													

Continued

Table 1 Continued

	Model of antibodies of any origin (n=22 130)		Model of propensity to vaccination (n=22 130)		Model of antibodies in unvaccinated participants (n=7647)	
	No. of participants	% of seropositive	PRR (95% CI)	P value	% of vaccinated	PRR (95% CI)
Vaccination—no	7647	66.63	1.00	—	0.00	—
Vaccination—yes	14 483	98.42	1.52	<0.001	100.00	—
Total	22 130					7647

Seronegative=participants who were seronegative in the first phase of the study; seropositive—no symptoms=participants who were seropositive in the first phase of the study and did not suffer from the selected symptoms (temperature >37.5°C, cough, shortness of breath, loss of taste or olfactory sense, faintness); seropositive—with symptoms=participants who were seropositive in the first phase of the study and suffer from the selected symptoms (temperature >37.5°C, cough, shortness of breath, loss of taste or olfactory sense, faintness); NCDs in history=participant indicated that suffer from one or more from the following disorders (diabetes, hypertension, lung diseases (asthma, chronic obstructive pulmonary disease); vaccination—no=participant was not vaccinated against SARS-CoV-2 regardless of vaccine type or dose; vaccination—yes=participant was vaccinated against SARS-CoV-2 regardless of vaccine type or dose.
 BMI, body mass index; NCD, non-communicable disease; PRR, prevalence rate ratio.

authenticated data sets. All analyses were conducted using Stata V.15.1 (StataCorp, College Station, Texas, USA).

RESULTS

This report is based on data from 22 130 subjects who participated in both phases of the study and therefore had repeated antibody measurements. Characteristics of the analytical sample are shown in [table 1](#). Just under 20% were under 40 years of age and 23% were older than 60 years, 62% were females and 43% of participants had tertiary educational level and 65% (14 483) subjects reported vaccination by one of the four vaccines Comirnaty (BioNTech Manufacturing, Mainz, Germany), Spikevax (previously COVID-19 Vaccine Moderna; Moderna Biotech Spain, Madrid, Spain), Vaxzevria (previously COVID-19 Vaccine AstraZeneca; AstraZeneca, Södertälje, Sweden), Jcovden (previously COVID-19 Vaccine Janssen; Janssen-Cilag International, Beerse, Belgium) available in the Czech Republic. The proportion of vaccinated persons increased with increasing age and increasing BMI while it was lower in previously seropositive subjects. On the other hand, there was little variation in seroprevalence by sex and among ages groups. Individuals with history of chronic diseases were more likely to be vaccinated. A higher age of 60+ years was associated with a higher percentage of seropositivity. This was observed in both vaccinated and unvaccinated persons. Higher education was associated with higher vaccination rates. Among unvaccinated persons, seroprevalence was similar across the age range 18–59 years. Those who were seronegative in phase I of the study were more likely to be vaccinated than those who were infected with SARS-CoV-2 virus. The latter developed a specific mucosal immune response, including positivity of IgG anti-SARS-CoV-2 antibodies as a marker of systemic immune response ([table 1](#)). The proportion of self-reported vaccination was similar to official figures for the general population in the Czech Republic for September 2021 ([figure 1](#)).

[Figure 1](#) shows the temporal trends in outcomes related to COVID-19 over both phases of the study. From March 2021 (end of phase I), the seroprevalence increased from 56% to 91% in September 2021. While the rapid increase in seropositivity rates during phase I was due to natural infection, a substantial part of the increase during phase II was due to vaccination.

At phase I, 10 778 (49%) of participants were SARS-CoV-2 seropositive. Of the 11 352 seronegative subjects at phase I, 1009 reported positive PCR test between first and second blood sample ([table 2](#)). [Table 3](#) shows seroprevalence rates at phase II by SARS-CoV-2 infection status at phase I and vaccination status. After standardisation to the Czech national population, the seroprevalence of anti-SARS-CoV-2 IgG antibodies was 24% among those who were seronegative at phase I and unvaccinated in phase II; 90% among those who were seropositive at phase I or reported SARS-CoV-2 infection before phase II; 97% among infection free before but vaccinated at phase II,

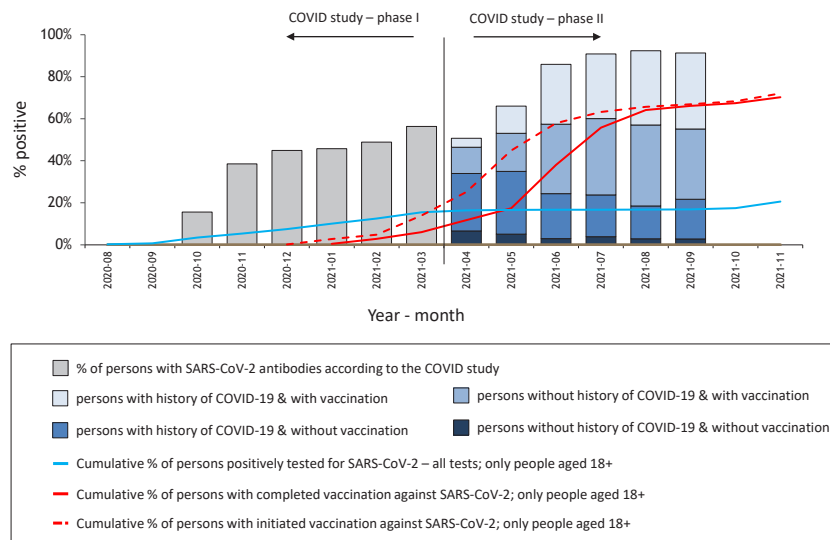


Figure 1 Temporal trends in indicators related to COVID-19 epidemic in the PROSECO study and in the Czech national statistics.

and almost 100% among those who both had SARS-CoV-2 infection before and were vaccinated at phase II. In addition, only 9% of 4367 unvaccinated subjects who were seropositive in phase I became seronegative over the 5–7 months until phase II. From 7495 SARS-CoV-2 immune-naïve persons, only 210 (2.8 %) did not produce detectable IgG antibodies with 4–6 weeks after vaccination.

DISCUSSION

In this prospective population-based study, we examined the changes in seroprevalence in a population-based sample with IgG antibodies measured twice, the second measurement being 5–7 months after the first on average. We found that after the rapid increase in seroprevalence during first phase (conducted in the second wave of the COVID-19 epidemic in the Czech Republic), there was further substantial increase in seroprevalence during the national vaccination campaign. By the end of phase II

of the study, 91% of examined individuals had IgG antibodies against SARS-CoV-2; among vaccinated persons this proportion was over 97%.

Strengths and limitations

The main methodological limitation of this study is the selection bias related to response rates. In phase I, the response rates could not be established, since the number of persons who were invited by their insurance companies to participate in the study was known, as only the first 30000 of those who attended were accepted in the study. These respondents were volunteers who were not entirely representative for the national population.⁸ In addition, only about 74% of those who participated in phase I also participated in phase II; as described in the ‘Methods’ section, the phase II sample included slightly more women (62%) than the phase I had (61%).

Notwithstanding this limitation, the availability of repeated antibody measurements on a large number of individuals with high-quality chemiluminescent immunoassay is a major strength, since the prospective design allows assessment of antibody response in different groups of people. Both sex groups showed comparable seropositivity in both phases of the PROSECO study; the male and female rates in phase I (October 2020 to March 2021) were 46.1% vs 47.2% due to natural infection, in phase II (April 2021 to September 2021) the rates increase to 87.7% vs 87.3%, respectively, mostly due to vaccination.

Our results are in line with other national studies of antibody prevalence, such as the UK REal-time Assessment of Community Transmission study (REACT-2),³ Blood donors study⁶ and UK SARS-CoV-2 Immunity

Table 2 Number of subjects with history of positive PCR test by seropositivity at phase I

Seropositivity at phase I	SARS-CoV-2 infection reported (PCR)			
	Prior first BS	Between first and second BS	Never	Total
No	1080	1009	9263	11352
Yes	6397	95	4286	10778
Total	7477	1104	13549	22130

BS, blood sample.

**Table 3** Seroprevalence at phase II by SARS-CoV-2 infection and vaccination status

	Positive		Negative		Total	Estimated seroprevalence in general population		P value
	N	%	N	%		%	95% CI	
SARS-CoV-2- and no vaccination	728	25.56	2120	74.44	2848	23.97	22.18% to 25.85%	
SARS-CoV-2+ and no vaccination	4367	91.00	432	9.00	4799	89.57	88.33% to 90.70%	
SARS-CoV-2- and with vaccination	7285	97.20	210	2.80	7495	97.36	96.72% to 97.88%	<0.001
SARS-CoV-2+ and with vaccination	6969	99.73	19	0.27	6988	99.81	99.68% to 99.89%	
Total	19349	87.43	2781	12.57	22130	84.37	83.64% to 85.07%	

SARS-CoV-2- = seronegative at phase I AND self-report of *negative* or not done PCR test between phase I and II.

SARS-CoV-2+ = seropositive at phase I OR self-report of *positive* PCR test between phase I and II.

SIREN study.¹³ In the week ending 28 March 2021, which corresponds with the end of phase I and the beginning of phase II of the nationwide Czech PROSECO study, 55% of the adult population in England was tested positive for antibodies against the COVID-19 SARS-CoV-2, these proportions were 49% in Wales, 59% in Scotland and 64% in Northern Ireland. The temporal trends were also comparable. By end of September 2021, the prevalence in England it was estimated as 92% of the adult population (and 90%, 91% and 91% in Wales, Scotland and Northern Ireland, respectively (UK Office for National Statistics, www.ons.gov.uk)). It is important to highlight that, unlike the Czech Republic, in the UK vaccination occurred earlier, before an increase in natural infection, resulting in less lost lives. By the end of phase II in September 2021, seroprevalence increased to 91% in the Czech cohort.

Studies in other European countries have documented the built-up of seroprevalence in 2021, for example, an 82% among German blood donors by September 2021 (Robert Koch Institut, SeBluCo-Studie). An Austrian cohort study of blood donors aged 18–70 years found that 10% of participants suffered with prior SARS-CoV-2 infection, and the seroprevalence of anti-SARS-CoV-2 IgG antibodies increased from 30% in March 2021 to 85% in September 2021 (n=19792), with the bulk of seropositivity due to vaccination. Antispike IgG seroprevalence was 99.6% among fully vaccinated individuals, 90% among unvaccinated individuals with prior infection and 12% among unvaccinated individuals without known prior infection.^{4 14} Comparable results on blood donors were reported in the USA, such as 20% for infection-induced antibodies and 83% for combined infection-induced and vaccine-induced antibodies in May 2021, and the estimated SARS-CoV-2 seroprevalence increased over time and varied by age, race and ethnicity, and geographic region.¹⁵

Again, this is consistent with our findings. The highest seroprevalence in our study was seen among vaccinated

persons with and without previous SARS-CoV-2 infection (99% and 97%, respectively), while the lowest seroprevalence was found among unvaccinated persons with no signs of disease. Moreover, only 2.8% of immune-naïve persons did not produce detectable IgG antibodies with 4–6 weeks after vaccination. Furthermore, our prospective study also addressed the decline in antibody positivity after vaccination or after SARS-CoV-2 infection and we found that only among 9% of subjects who were seropositive in phase I became seronegative over the 5–7 months until phase II.

In conclusion, the rapid increase in seropositivity during the second wave of the COVID-19 epidemic (covered by phase I of the PROSECO study) was followed by a similarly steep rise in seroprevalence during the national vaccination campaign, reaching seropositivity rates of over 87% among general population and 97% among vaccinated persons in the Czech Republic in the period of April 2021 to September 2021. Vaccination rates were lower in persons who were seropositive in phase I but increased with age and BMI. Only 9% of unvaccinated subjects who were seropositive in phase I became seronegative by phase II. The combination of vaccination with the induction of a systemic immune response and natural infection with SARS-CoV-2 with the development of mucosal immunity is beneficial. It makes a significant contribution to good effect for diagnostic purposes and prophylaxis and leads to the development of protective immunity.¹⁶ Seroconversion, as a marker of the ongoing immune response, is therefore an important measure of population immunity level to guide policy response.¹⁷

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study, including all aspects of data collection and data analysis, was approved by the ELSPAC ethics committee under reference number (C)ELSPAC/EK/5/2021. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. All data relevant to the study are included in the article or uploaded as supplementary information. Anonymised data can be made available from the corresponding author upon request once all study phases are completed and data validated. Release of data is a subject of approval of the Ethical and Scientific boards of the PROSECO study.

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