

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open

Prospective investigation of SARS-CoV-2 seroprevalence in relation to natural infection and vaccination between October 2020 and September 2021 in the Czech Republic

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-068258
Article Type:	Original research
Date Submitted by the Author:	12-Sep-2022
Complete List of Authors:	Thon, Vojtěch; Masaryk University, RECETOX Piler, Pavel; Masaryk University, RECETOX Pavlik, Tomas; Masaryk University Andrýsková, Lenka; Masaryk University Doležel, Kamil; QualityLab Association Kostka, David; Health Insurance Company of the Ministry of the Interior of the Czech Republic Pikhart, Hynek; University College London, Department of Epidemiology and Public Health Bobak, Martin; University College London, Department of Epidemiology and Public Health Klánová, Jana; Masaryk University
Keywords:	EPIDEMIOLOGY, COVID-19, Public health < INFECTIOUS DISEASES

SCHOLARONE™
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

1
2
3 **Prospective investigation of SARS-CoV-2 seroprevalence in relation to natural infection and**
4 **vaccination between October 2020 and September 2021 in the Czech Republic**
5
6
7
8
9

10 Vojtěch Thon^{1#}, Pavel Piler^{1#}, Tomáš Pavlík², Lenka Andryšková¹, Kamil Doležel³, David Kostka⁴, Hynek
11 Pikhart^{1,5}, Martin Bobák^{1,5}, Jana Klánová¹
12
13
14
15

16 **Institutional affiliations of the authors:**

17
18
19 ¹ RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic.

20
21 ² Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Brno, Czech Republic.

22
23 ³ QualityLab Association, Prague, Czech Republic.

24
25 ⁴ Health Insurance Company of the Ministry of the Interior of the Czech Republic, Prague, Czech
26 Republic.
27

28
29 ⁵ Department of Epidemiology & Public Health, University College London, London WC1E 6BT, UK.
30
31
32
33

34 **Corresponding author:**

35
36 *Prof. Vojtěch Thon, M.D., Ph.D., vojtech.thon@recetox.muni.cz, RECETOX, Faculty of Science,
37 Masaryk University, Brno, Czech Republic
38
39
40
41
42

43 #These authors contributed equally.
44
45
46
47

48 **Keywords:**

49 SARS-CoV-2, seroprevalence, vaccination, epidemic growth, antibodies durability
50
51
52
53
54
55
56
57
58
59
60

Abstract

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

Design: Prospective national population 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 1, 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. 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 vaccination.

Results: Before vaccination (Phase 1), seroprevalence increased from 15% in October 2020 to 56% in March 2021. By the end of Phase 2, 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 1 but increased with age and body mass index. Only 9% of unvaccinated subjects who were seropositive in phase 1 became seronegative by phase 2.

Conclusions: The rapid increase in seropositivity during the 2nd wave of the COVID-19 epidemic (covered by phase 1 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

- Only a few nationwide prospective population-based studies have been published from the Central and Eastern European region.
- The PROSECO study covers the major epidemic wave as well as vaccination campaign and then it allows us to follow the dynamics of seroconversion of anti-SARS-CoV-2 IgG antibodies in the Czech population.
- Major strengths of our study are its size, coverage, start before vaccination period and on-going longitudinal follow-up.
- Detection of IgG antibodies against SARS-CoV-2 were performed by CE-marked serological tests in accredited clinical laboratories.
- The study response rate was 74% in the phase 2 (N=22,130 participants).

Introduction

During the COVID-19 pandemics, 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 pre- 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.

1
2
3 We have previously reported findings from a national cross-sectional survey of 30,000 persons in the
4
5 Czech Republic who were examined between October 2020 and March 2021, a period covering the
6
7 second wave of the epidemic, which was also the period before the start of national vaccination
8
9 campaign. We found that by March 2021, 53% of participants had measurable antibodies against SARS-
10
11 CoV-2[8]. This was consistent with governmental data using cumulative PCR testing data. These rates
12
13 were considerably higher than those reported in Western Europe[2, 9-11], due to a strong 2nd wave of
14
15 natural infection in the Czech Republic in autumn 2020[8].
16
17

18
19
20
21 In this report, we report longitudinal data on repeated assessment of the same population sample in
22
23 the period April 2021-Sept 2021, a period coinciding with the rollout of the national vaccination
24
25 programme. The objectives of this analysis were to 1) examine the trends in seropositivity before and
26
27 during the national vaccination campaign, 2) assess the contributions of natural infections and
28
29 vaccination to the seropositivity, 3) to assess seroconversion rates in previously seronegative persons,
30
31 4) to assess duration of immunity after natural infection, and 5) to estimate the rate ratio of
32
33 seroconversion and vaccination associated with sociodemographic indicators.
34
35
36
37
38

39 **Methods**

40 41 42 43 *Study design and participants*

44
45 Data for these analyses were derived from the first and second wave of the PROSECO study. The
46
47 PROSECO study design and population recruitment has been described elsewhere[8]. Briefly, phase 1
48
49 of the study recruited 30,054 unvaccinated adult volunteers from persons registered with the second
50
51 largest health insurance company in the Czech Republic. Participants provided blood sample between
52
53 October 2020 and March 2021, during the 2nd epidemic wave in the Czech Republic. Of those, 22,130
54
55 participants were re-examined during the national vaccination programme between April 2021 and
56
57 September 2021. Participants were invited for phase 2 in the same order as they participated in phase
58
59
60

1
2
3 1, so most subjects were re-examined 5-7 months after the first visit. Comparison of the persons
4 participating in both phases with those who only attended phase 1 is shown in **Supplementary Table**
5
6
7 **S1**. Those who participated in both assessments were older, more likely to be female, seropositive at
8
9 phase 1, more obese, and more likely to have history of chronic non-communicable diseases.
10
11
12

13
14 In phase 2, participants provided a second blood sample for detection of IgG antibodies against SARS-
15
16 CoV-2 and completed a questionnaire on personal information, self-reported results of RT-PCR tests
17
18 (if performed) and records of COVID vaccination. The second visit was organised at least 14 days after
19
20 any vaccination (if completed). Informed consent forms were obtained from all study participants
21
22 during each wave of the data collection. The study, including all aspects of data collection and data
23
24 analysis, was approved by the ELSPAC ethics committee under reference number
25
26 (C)ELSPAC/EK/5/2021.
27
28

29 *Laboratory analyses*

30
31 CE-marked serological tests were performed in accredited clinical laboratories. Antigen-specific
32
33 humoral immune response was analysed by detection of IgG antibodies against the spike protein using
34
35 commercial immunoassays LIAISON SARS-CoV-2 S1/S2 IgG (DiaSorin, Saluggia, Italy) and SARS-CoV-2
36
37 IgG II Quant (Abbott, Sligo, Ireland). Testing was conducted on the LIAISON XL (DiaSorin, Saluggia, Italy)
38
39 and on the Alinity (Abbott, Lake Forest, IL, USA) respectively. Samples were tested individually and
40
41 reported according to the manufactures' criteria.
42
43
44

45 *Statistical analysis*

46
47 The primary aim of this study was to estimate trends in seropositivity rates of the adult Czech
48
49 population. We estimated seroprevalence rates and 95% confidence intervals, we also standardized
50
51 the seroprevalence rates by age and sex, using the Czech population as a standard. We used a
52
53 multivariate Poisson regression model with a robust error variance to estimate the ratio of
54
55 seroconversion and vaccination associated with sociodemographic indicators. Differences in
56
57
58
59
60

1
2
3 prevalence were expressed as prevalence rate ratios (PRRs). We used standard descriptive statistics to
4
5 characterize the study data set.
6
7
8
9

10 Population data on COVID-19 were obtained from the Czech Central Information System of Infectious
11 Diseases (ISID), which includes records of all consecutive patients with COVID-19 in the Czech Republic
12 identified and confirmed by laboratory testing. ISID data are routinely collected in compliance with Act
13 No. 258/2000 Coll. on the Protection of Public Health and are publicly available in aggregated and
14 anonymized form of open or authenticated data sets. All analyses were conducted using Stata version
15
16
17
18
19
20
21 15.1 (StataCorp, College Station, Texas 77845 USA).
22
23
24

25 Results

26
27
28
29 This report is based on data from 22,130 subjects who participated in both phases of the study and
30 therefore had repeated antibody measurements. Characteristics of the analytical sample are shown in
31
32
33 **Table 1.** Just under 20% were under 40 years of age and 23% were older than 60 years, 62% were
34 females and 43% of participants had tertiary educational level, and 65% (14,483) subjects reported
35 vaccination by one of the four vaccines Comirnaty (BioNTech Manufacturing GmbH, Mainz, Germany),
36 Spikevax (previously COVID-19 Vaccine Moderna; Moderna Biotech Spain, S.L., Madrid, Spain),
37 Vaxzevria (previously COVID-19 Vaccine AstraZeneca; AstraZeneca AB, Södertälje, Sweeden), Jcovden
38 (previously COVID-19 Vaccine Janssen; Janssen-Cilag International NV, Beerse, Belgium) available in
39 the Czech Republic. The proportion of vaccinated persons increased with increasing age and increasing
40 body mass index while it was lower in previously seropositive subjects. On the other hand, there was
41 little variation in seroprevalence by sex and among ages groups. The proportion of self-reported
42 vaccination was similar to official figures for the general population in the Czech Republic for
43 September 2021 (see **Figure 1**).
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 **Figure 1** shows the temporal trends in outcomes related to COVID-19 over both phases of the study.
4
5 From March 2021 (end of phase 1), the seroprevalence increased from 56% to 91% in September 2021.
6
7 While the rapid increase in seropositivity rates during phase 1 was due to natural infection, a
8
9 substantial part of the increase during phase 2 was due to vaccination.
10
11

12
13
14 At phase one, 10,778 (49%) of participants were SARS-CoV-2 seropositive. Of the 11,352 seronegative
15
16 subjects at phase 1, 1,009 reported positive PCR test between first and second blood sample (**Table**
17
18 **2**). **Table 3** shows seroprevalence rates at phase 2 by SARS-CoV-2 infection status at phase 1 and
19
20 vaccination status. After standardisation to the Czech national population, the seroprevalence of anti-
21
22 SARS-CoV-2 IgG antibodies was 24% among those who were seronegative at phase 1 and unvaccinated
23
24 in phase 2; 90% among those who were seropositive at phase 1 or reported SARS-CoV-2 infection
25
26 before phase 2; 97% among infection free before but vaccinated at phase 2, and almost 100% among
27
28 those who both had SARS-CoV-2 infection before and were vaccinated at phase 2. In addition, only 9%
29
30 of 4,367 unvaccinated subjects who were seropositive in phase 1 became seronegative over the 5-7
31
32 months until phase 2. From 7,495 SARS-CoV-2 immune naïve persons, only 210 (2.8 %) did not produce
33
34 detectable IgG antibodies with 4-6 weeks after vaccination.
35
36
37
38
39
40

41 **Discussion**

42
43
44
45 In this prospective population-based study, we examined the changes in seroprevalence in a
46
47 population-based sample with IgG antibodies measured twice, the second measurement being 5-7
48
49 month after the first on average. We found that after the rapid increase in seroprevalence during first
50
51 phase (conducted in the 2nd wave of the COVID-19 epidemic in the Czech Republic), there was further
52
53 substantial increase in seroprevalence during the national vaccination campaign. By the end of phase
54
55 2 of the study, 91% of examined individuals had IgG antibodies against SARS-CoV-2; among vaccinated
56
57 persons this proportion was over 97%.
58
59
60

Strengths and limitations

The main methodological limitation of this study is the selection bias related to response rates. In phase 1, 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 30,000 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 1 also participated in phase 2; as described in the methods, the phase 2 sample included slightly more women (62%) than the phase 1 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 1 (October 2020 to March 2021) were 46.1% vs. 47.2% due to natural infection, in phase 2 (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 United Kingdom REACT-2 study[3], Blood donors study[6] and UK SARS-CoV-2 Immunity SIREN study[12]. In the week ending 28th March 2021, which corresponds with the end of Phase 1 and the beginning of Phase 2 of the nationwide Czech PROSECO study, 55% of the adult population in England was tested positive for antibodies against the coronavirus 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

1
2
3 occurred earlier, before an increase in natural infection, resulting in less lost lives. By the end of Phase
4
5 2 in September 2021 seroprevalence increased to 91% in the Czech cohort.
6
7

8
9
10 Studies in other European countries have documented the built-up of seroprevalence in 2021, e.g., an
11
12 82% among German blood donors by September 2021 (Robert Koch Institut, SeBluCo-Studie). An
13
14 Austrian cohort study of blood donors aged 18–70 years found that 10% of participants suffered with
15
16 prior SARS-CoV-2 infection, and the seroprevalence of anti-SARS-CoV-2 IgG antibodies increased from
17
18 30% in March 2021 to 85% in September 2021 (n = 19,792), with the bulk of seropositivity due to
19
20 vaccination. Anti-spike IgG seroprevalence was 99.6% among fully vaccinated individuals, 90% among
21
22 unvaccinated individuals with prior infection and 12% among unvaccinated individuals without known
23
24 prior infection[4, 13]. Comparable results on blood donors were reported in the US, such as 20% for
25
26 infection-induced antibodies and 83% for combined infection- and vaccine-induced antibodies in May
27
28 2021, and the estimated SARS-CoV-2 seroprevalence increased over time and varied by age, race and
29
30 ethnicity, and geographic region[14].
31
32
33
34
35

36
37 Again, this is consistent with our findings. The highest seroprevalence in our study was seen among
38
39 vaccinated persons with and without previous SARS-CoV-2 infection (99% and 97%, respectively), while
40
41 the lowest seroprevalence was found among unvaccinated persons with no signs of disease. Moreover,
42
43 only 2.8% of immune naïve persons did not produce detectable IgG antibodies with 4-6 weeks after
44
45 vaccination. Furthermore, our prospective study also addressed the decline in antibody positivity after
46
47 vaccination or after SARS-CoV-2 infection and we found that only among 9% of subjects who were
48
49 seropositive in phase 1 became seronegative over the 5-7 months until phase 2.
50
51
52

53
54 In conclusion, the rapid increase in seropositivity during the 2nd wave of the COVID-19 epidemic
55
56 (covered by phase 1 of the PROSECO study) was followed by a similarly steep rise in seroprevalence
57
58 during the national vaccination campaign, reaching seropositivity rates of over 97% among vaccinated
59
60

1
2
3 persons in the Czech Republic in the period of April 2021 to September 2021. The combination of
4
5 vaccination with the induction of a systemic immune response and natural infection with SARS-CoV-2
6
7 with the development of mucosal immunity is beneficial. It makes a significant contribution to good
8
9 effect for diagnostic purposes and prophylaxis and leads to the development of protective
10
11 immunity[15]. Seroconversion, as a marker of the ongoing immune response, is therefore an important
12
13 measure of population immunity level to guide policy response and should play an important role in
14
15 the WHO endorsed protocol for rapid adaptation and implementation of COVID-19 investigation[16].
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

Data availability statement

All data generated during the first and second phase of the PROSECO study is presented in this article. 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.

Ethics statements

Informed consent forms were obtained from all study participants during each wave of the data collection. An ethics committee approval of all aspects of data collection, as well as of the secondary data analysis, was obtained from the ELSPAC ethics committee under reference number (C)ELSPAC/EK/5/2021.

Acknowledgements

We thank all collaborating nurses, laboratories from the QualityLab association, and administrative personnel and especially the 22,130 participants who invested their time and provided samples and information for this study.

A funding statements

The PROSECO study was sponsored by the Prevention Programme of the Health Insurance Company of the Ministry of the Interior of the Czech Republic. The RECETOX Research Infrastructure was supported by the Ministry of Education, Youth and Sports of the Czech Republic (LM2018121), and VT and PP from the CETOCOEN PLUS project of ESIF (CZ.02.1.01/0.0/0.0/15_003/0000469). This work was supported from the European Union's Horizon 2020 research and innovation programme under grant agreement No 857560 and the Ministry of Education, Youth and Sport of the Czech Republic/ESIF (CZ.02.1.01/0.0/0.0/17_043/0009632). This publication reflects only the author's view and the European Commission is not responsible for any use that may be made of the information it contains.

Competing interests

The authors declare no competing interests.

Author contributions

VT, PP, LA and JK were responsible for the design of the study. KD, DK and LA were responsible for the study operation, coordination of data acquisition and quality management of participating laboratories. VT, PP and TP developed the operationalized research question and the statistical analyses plan. TP performed the statistical analyses. The first draft was written by VT and PP. MB contributed to the writing and finalizing of the manuscript. MB and HP provided expertise in epidemiology. All authors contributed to data interpretation, critically reviewed the first draft, approved the final version and agreed to be accountable for the work.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Code availability

Statistical analyses were performed using STATA version 15.1 (StataCorp LLC, USA).

For peer review only

References:

1. Arora RK, Joseph A, Van Wyk J, et al. SeroTracker: a global SARS-CoV-2 seroprevalence dashboard. *Lancet Infect Dis* 2021;**21**(4):e75-e76 doi: 10.1016/s1473-3099(20)30631-9 [published Online First: 2020/08/09].
2. Vaughan A, Duffell EF, Friedl GS, et al. Seroprevalence of SARS-CoV-2 antibodies prior to the widespread introduction of vaccine programmes in the WHO European Region, January - December 2020: a systematic review. *medRxiv* 2021:2021.12.02.21266897 doi: 10.1101/2021.12.02.21266897.
3. Ward H, Whitaker M, Flower B, et al. Population antibody responses following COVID-19 vaccination in 212,102 individuals. *Nature Communications* 2022;**13**(1):907 doi: 10.1038/s41467-022-28527-x.
4. Siller A, Seekircher L, Wachter GA, et al. Seroprevalence, Waning and Correlates of Anti-SARS-CoV-2 IgG Antibodies in Tyrol, Austria: Large-Scale Study of 35,193 Blood Donors Conducted between June 2020 and September 2021. *Viruses* 2022;**14**(3) doi: 10.3390/v14030568 [published Online First: 20220309].
5. Stringhini S, Zaballa ME, Pullen N, et al. Seroprevalence of anti-SARS-CoV-2 antibodies 6 months into the vaccination campaign in Geneva, Switzerland, 1 June to 7 July 2021. *Euro Surveill* 2021;**26**(43) doi: 10.2807/1560-7917.es.2021.26.43.2100830.
6. Whitaker HJ, Elgohari S, Rowe C, et al. Impact of COVID-19 vaccination program on seroprevalence in blood donors in England, 2021. *The Journal of infection* 2021;**83**(2):237-79 doi: 10.1016/j.jinf.2021.04.037 [published Online First: 2021/05/11].
7. Soeorg H, Jögi P, Naaber P, Ottas A, Toompere K, Lutsar I. Seroprevalence and levels of IgG antibodies after COVID-19 infection or vaccination. *Infect Dis (Lond)* 2022;**54**(1):63-71 doi: 10.1080/23744235.2021.1974540 [published Online First: 20210914].
8. Piler P, Thon V, Andrišková L, et al. Nationwide increases in anti-SARS-CoV-2 IgG antibodies between October 2020 and March 2021 in the unvaccinated Czech population. *Communications Medicine* 2022;**2**(1):19 doi: 10.1038/s43856-022-00080-0.
9. Rostami A, Sepidarkish M, Leeftang MMG, et al. SARS-CoV-2 seroprevalence worldwide: a systematic review and meta-analysis. *Clin Microbiol Infect* 2021;**27**(3):331-40 doi: 10.1016/j.cmi.2020.10.020 [published Online First: 2020/11/25].
10. Bobrovitz N, Arora RK, Cao C, et al. Global seroprevalence of SARS-CoV-2 antibodies: A systematic review and meta-analysis. *PLOS ONE* 2021;**16**(6):e0252617 doi: 10.1371/journal.pone.0252617.
11. Grant R, Dub T, Andrianou X, et al. SARS-CoV-2 population-based seroprevalence studies in Europe: a scoping review. *BMJ Open* 2021;**11**(4):e045425 doi: 10.1136/bmjopen-2020-045425.
12. Hall V, Foulkes S, Insalata F, et al. Protection against SARS-CoV-2 after Covid-19 Vaccination and Previous Infection. *The New England journal of medicine* 2022;**386**(13):1207-20 doi: 10.1056/NEJMoa2118691 [published Online First: 2022/02/16].
13. Siller A, Wachter GA, Neururer S, et al. Prevalence of SARS-CoV-2 antibodies in healthy blood donors from the state of Tyrol, Austria, in summer 2020. *Wien Klin Wochenschr* 2021;**133**(23-24):1272-80 doi: 10.1007/s00508-021-01963-3 [published Online First: 20211026].
14. Jones JM, Stone M, Sulaeman H, et al. Estimated US Infection- and Vaccine-Induced SARS-CoV-2 Seroprevalence Based on Blood Donations, July 2020-May 2021. *JAMA* 2021;**326**(14):1400-09 doi: 10.1001/jama.2021.15161.
15. Russell MW, Moldoveanu Z, Ogra PL, Mestecky J. Mucosal Immunity in COVID-19: A Neglected but Critical Aspect of SARS-CoV-2 Infection. *Front Immunol* 2020;**11**:611337 doi: 10.3389/fimmu.2020.611337 [published Online First: 2020/12/18].
16. Bergeri I, Lewis HC, Subissi L, et al. Early epidemiological investigations: World Health Organization UNITY protocols provide a standardized and timely international investigation

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

framework during the COVID-19 pandemic. *Influenza Other Respir Viruses* 2022;**16**(1):7-13
doi: 10.1111/irv.12915 [published Online First: 20211005].

For peer review only

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

	No. of participants	No. of seropositive	No. of vaccinated participants	Model of antibodies of any origin (N = 22,130)			Model of propensity to vaccination (N = 22,130)			Model of antibodies in unvaccinated participants (N = 7,647)			
				% of seropositive	PRR (CI)	p value	% of vaccinated	PRR (CI)	p value	N of participants	% of seropositive	PRR (CI)	p value
Sex:													
Female	13,824	12,067	8,844	87.29%	1.00	-	63.98%	1.00		4,980	67.25%	1.00	-
Male	8,306	7,282	5,639	87.67%	0.99	0.012	67.89%	1.05	<0.001	2,667	65.47%	0.95	<0.001
Age groups:													
18-29	1,491	1,202	770	80.62%	1.00	-	51.64%	1.00		721	61.72%	1.00	-
30-39	2,774	2,275	1,534	82.01%	1.02	0.215	55.30%	1.03	0.420	1,240	61.05%	0.97	0.338
40-49	6,700	5,725	4,177	85.45%	1.01	0.194	62.34%	1.17	<0.001	2,523	64.05%	0.97	0.226
50-59	6,049	5,405	4,061	89.35%	1.03	0.003	67.14%	1.23	<0.001	1,988	70.32%	1.04	0.170
60+	5,116	4,742	3,941	92.69%	1.05	<0.001	77.03%	1.37	<0.001	1,175	74.81%	1.09	0.001
Education													
basic	1,952	1,744	1,295	89.34%	1.00	-	66.34%	1.00		657	70.02%	1.00	-
medium	8,024	7,119	5,348	88.72%	1.00	0.972	66.65%	1.02	0.275	2,676	69.21%	1.02	0.337
high	7,544	6,689	5,223	88.67%	1.00	0.890	69.23%	1.08	<0.001	2,321	65.75%	1.02	0.394
missing	4,610	3,797	2,617	82.36%	0.97	0.003	56.77%	0.87	<0.001	1,993	63.07%	1.00	0.923
COVID in history													
Seronegative	11,352	8,935	7,882	78.71%	1.00	-	69.43%	1.00		3,470	36.54%	1.00	-
Seropositive - no symptoms	5,597	5,374	3,458	96.02%	1.28	<0.001	61.78%	0.75	<0.001	2,139	90.04%	3.45	<0.001
Seropositive - with symptoms	5,181	5,040	3,143	97.28%	1.32	<0.001	60.66%	0.78	<0.001	2,038	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	8,192	6,964	5,038	85.01%	1.04	0.127	61.50%	1.09	0.141	3,154	63.44%	1.17	0.009
25-29.9	8,080	7,167	5,488	88.70%	1.05	0.077	67.92%	1.15	0.020	2,592	68.36%	1.18	0.006
30+	4,802	4,369	3,312	90.98%	1.06	0.046	68.97%	1.16	0.013	1,490	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	8,688	86.10%	1.00	-	62.56%	1.00		5,200	65.23%	1.00	-
Yes	7,152	6,500	5,161	90.88%	1.00	0.818	72.16%	1.06	<0.001	1,991	71.97%	1.00	0.813
missing	1,090	891	634	81.74%	1.02	0.266	58.17%	0.91	0.002	456	59.21%	1.12	0.005
Vaccination													
Vaccination No	7,647	5,095	0	66.63%	1.00	-	0.00%						
Vaccination Yes	14,483	14,254	14,483	98.42%	1.52	<0.001	100.00%						
Total	22,130	19,349	14,483							7,647			

<http://bmjopen.bmj.com/> on April 20, 2024 by guest. Protected by copyright.

Table 2 Number of subjects with history of positive PCR test by seropositivity at Phase 1.

Seropositivity at Phase 1	SARS-CoV-2 infection reported (PCR)			
	Prior 1 st BS	Between 1 st and 2 nd BS	Never	Total
No	1,080	1,009	9,263	11,352
Yes	6,397	95	4,286	10,778
Total	7,477	1,104	13,549	22,130

BS = blood sample collection

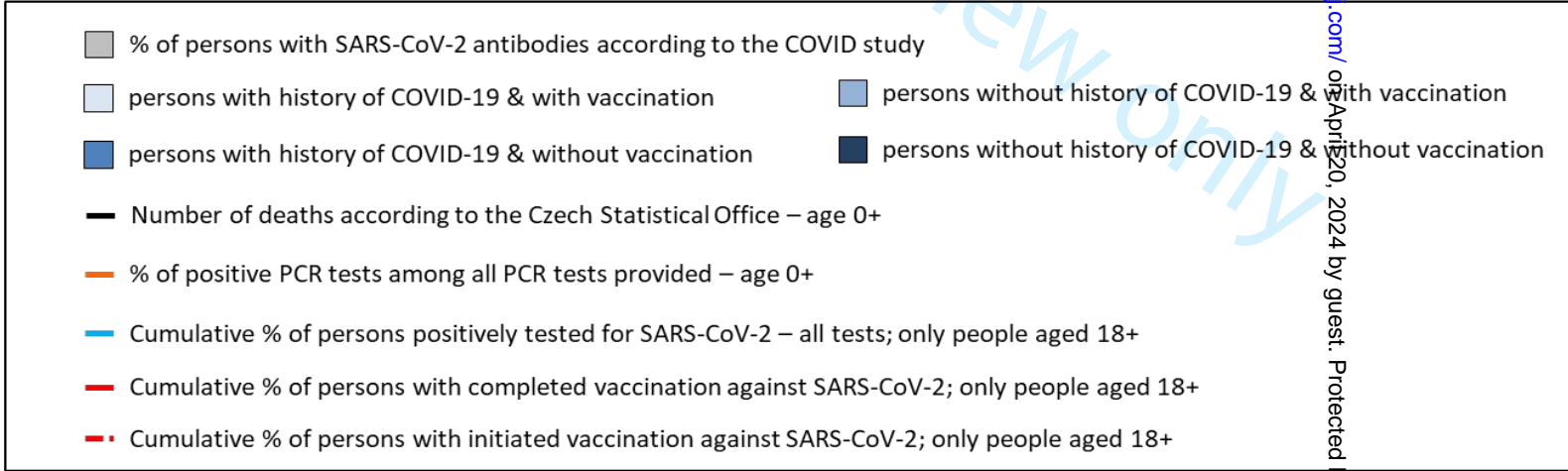
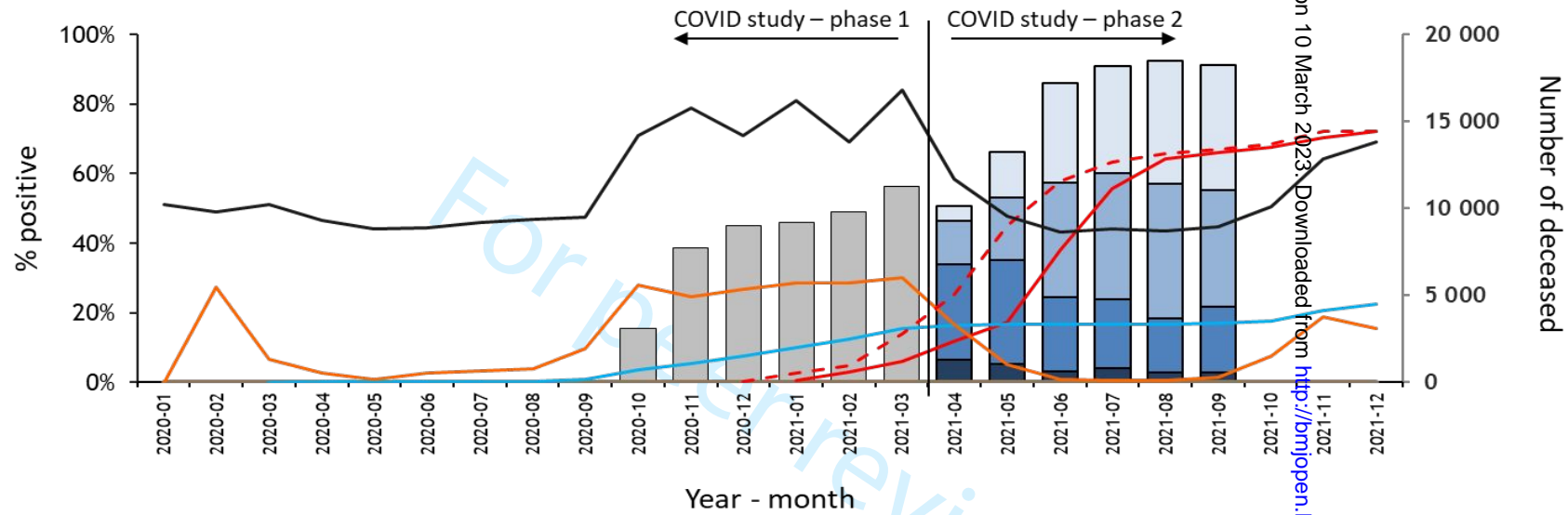
Table 3 Seroprevalence at phase 2 by SARS-CoV-2 infection and vaccination status.

	Positive		Negative		Total	Estimated seroprevalence in general population		p value
	N	%	N	%		%	95% CI	
SARS-CoV-2- & no vaccination	728	25.56%	2,120	74.44%	2,848	23.97%	22.18 – 25.85%	p<0.001
SARS-CoV-2+ & no vaccination	4,367	91.00%	432	9.00%	4,799	89.57%	88.33 – 90.70%	
SARS-CoV-2- & With vaccination	7,285	97.20%	210	2.80%	7,495	97.36%	96.72 – 97.88%	
SARS-CoV-2+ & With vaccination	6,969	99.73%	19	0.27%	6,988	99.81%	99.68 – 99.89%	
Total	19,349	87.43%	2,781	12.57%	22,130	84.37%	83.64 – 85.07%	

SARS-CoV-2- = seronegative at phase 1 AND self-report of negative or not done PCR test between phase 1 and 2

SARS-CoV-2+ = seropositive at phase 1 OR self-report of positive PCR test between phase 1 and 2

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



Supplementary table S1: Comparison of the persons participating in both phases with those who only attended phase 1

	Persons participating in both P1 and P2	%	Persons not participating in P2	%
Total	22,130	100%	7,924	100%
Sex				
Female	13,824	62.5%	4,438	56.0%
Male	8,306	37.5%	3,486	44.0%
Age groups				
18-29	1,491	6.7%	1,069	13.5%
30-39	2,774	12.5%	1,485	18.7%
40-49	6,700	30.3%	2,431	30.7%
50-59	6,049	27.3%	1,658	20.9%
60+	5,116	23.1%	1,281	16.2%
COVID in history				
Seronegative	11,352	51.3%	4,641	58.6%
Seropositive – no symptoms	5,597	25.3%	1,757	22.2%
Seropositive – with symptoms	5,181	23.4%	1,526	19.3%
BMI				
under 18.5	256	1.2%	85	1.1%
18.5-24.9	8,192	37.0%	2,791	35.2%
25-29.9	8,080	36.5%	2,360	29.8%
30 and more	4,802	21.7%	1,189	15.0%
missing	800	3.6%	1,499	18.9%
NCDs in history				
No	13,888	62.8%	6,563	82.8%
Yes	7,152	32.3%	539	6.8%
missing	1,090	4.9%	822	10.4%

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-068258.R1
Article Type:	Original research
Date Submitted by the Author:	05-Jan-2023
Complete List of Authors:	Thon, Vojtěch; Masaryk University, RECETOX Piler, Pavel; Masaryk University, RECETOX Pavlik, Tomas; Masaryk University Andrýsková, Lenka; Masaryk University Doležel, Kamil; QualityLab Association Kostka, David; Health Insurance Company of the Ministry of the Interior of the Czech Republic Pikhart, Hynek; University College London, Department of Epidemiology and Public Health Bobak, Martin; University College London, Department of Epidemiology and Public Health Klánová, Jana; Masaryk University
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Immunology (including allergy), Infectious diseases
Keywords:	EPIDEMIOLOGY, COVID-19, Public health < INFECTIOUS DISEASES

SCHOLARONE™
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

1
2
3 1 **Investigation of SARS-CoV-2 seroprevalence in relation to natural infection and vaccination between**
4
5 2 **October 2020 and September 2021 in the Czech Republic: a prospective national cohort study**
6
7

8 3
9
10 4 Vojtěch Thon^{1#}, Pavel Piler^{1#}, Tomáš Pavlík², Lenka Andryšková¹, Kamil Doležel³, David Kostka⁴, Hynek
11
12 5 Pikhart^{1,5}, Martin Bobák^{1,5}, Jana Klánová¹
13
14 6

15
16 7 **Institutional affiliations of the authors:**

17
18 8 ¹ RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic.

19
20 9 ² Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Brno, Czech Republic.

21
22 10 ³ QualityLab Association, Prague, Czech Republic.

23
24 11 ⁴ Health Insurance Company of the Ministry of the Interior of the Czech Republic, Prague, Czech
25
26 12 Republic.
27

28
29 13 ⁵ Department of Epidemiology & Public Health, University College London, London WC1E 6BT, UK.
30
31
32 14

33
34 15 **Corresponding author:**

35
36 16 *Prof. Vojtěch Thon, M.D., Ph.D., vojtech.thon@recetox.muni.cz, RECETOX, Faculty of Science,
37
38 17 Masaryk University, Brno, Czech Republic
39
40
41 18

42
43 19 #These authors contributed equally.
44
45
46 20

47 21 **Keywords:**

48
49 22 SARS-CoV-2, seroprevalence, vaccination, epidemic growth, antibodies durability
50
51 23
52 24
53
54
55 25
56
57
58
59
60

1
2
3 1 **Abstract**
4
5 2
6
7

8 3 **Objective:** Examine changes in SARS-CoV-2 seropositivity before and during the national vaccination
9
10 4 campaign in the Czech Republic.

11
12 5 **Design:** Prospective national population-based cohort study.

13
14 6 **Setting:** Masaryk University, RECETOX, Brno.

15
16 7 **Participants:** 22,130 persons provided blood samples at two time points approximately 5-7 months
17
18 8 apart, between October 2020 and March 2021 (Phase 1, before vaccination), and between April and
19
20 9 September 2021 (during vaccination campaign).

21
22
23 10 **Outcome measures:** Antigen-specific humoral immune response was analysed by detection of IgG
24
25 11 antibodies against the SARS-CoV-2 spike protein by commercial chemiluminescent immunoassays.
26
27 12 Participants completed a questionnaire that included personal information, anthropometric data, self-
28
29 13 reported results of previous RT-PCR tests (if performed), history of symptoms compatible with COVID-
30
31 14 19, and records of COVID vaccination. Seroprevalence was compared between calendar periods,
32
33 15 previous RT-PCR results, vaccination, and other individual characteristics.

34
35
36 16 **Results:** Before vaccination (Phase 1), seroprevalence increased from 15% in October 2020 to 56% in
37
38 17 March 2021. By the end of Phase 2, in September 2021, prevalence increased to 91%; the highest
39
40 18 seroprevalence was seen among vaccinated persons with and without previous SARS-CoV-2 infection
41
42 19 (99.7% and 97.2%, respectively), while the lowest seroprevalence was found among unvaccinated
43
44 20 persons with no signs of disease (26%). Vaccination rates were lower in persons who were seropositive
45
46 21 in phase 1 but increased with age and body mass index. Only 9% of unvaccinated subjects who were
47
48 22 seropositive in phase 1 became seronegative by phase 2.

49
50
51
52 23 **Conclusions:** The rapid increase in seropositivity during the 2nd wave of the COVID-19 epidemic
53
54 24 (covered by phase 1 of this study) was followed by a similarly steep rise in seroprevalence during the
55
56 25 national vaccination campaign, reaching seropositivity rates of over 97% among vaccinated persons.
57
58
59 26

1 Strengths and limitations of this study

- 3 • The PROSECO study provide nationwide data from the Central European region heavily
4 affected by COVID-19.
- 5 • The levels of anti-SARS-CoV-2 antibodies and the dynamics of seroconversion were assessed
6 using a harmonized network of accredited clinical laboratories.
- 7 • Major strengths of the study are its size, coverage, start before vaccination period, evaluation
8 of natural SARS-CoV-2 infection & on-going longitudinal follow-up inclusive of vaccination.
- 9 • The duration of anti-SARS-CoV-2 antibodies after infection in unvaccinated subjects is
10 assessed.
- 11 • The main limitation relates to the fact that study subjects were volunteers.

1 Introduction

2
3
4
5
6
7
8 3 During the COVID-19 pandemic, monitoring of the seroprevalence of antibodies in the population is an
9
10 4 important tool to design and adjust preventive strategies. As a part of this process, it is essential to
11
12 5 assess the contribution of natural infections and vaccination to the immune response to SARS-CoV-2.
13
14 6 The Serotracker platform has recorded hundreds of SARS-CoV-2 serological studies worldwide
15
16 7 (serotracker.com)[1]. Most national seroprevalence studies were performed before the start of
17
18 8 massive vaccination programme in Europe[2] but there are only few published European
19
20 9 seroprevalence studies covering both pre- and after vaccination campaign periods. Overall, these
21
22 10 studies, mainly based in Western Europe, reported rising seroprevalence after the national vaccination
23
24 11 programmes[3-7]. However, very few published studies have been conducted in Central and Eastern
25
26 12 Europe, where the dynamics of both the epidemics and vaccine uptake differed from the Western
27
28 13 European countries.
29

30
31
32 14
33
34 15 We have previously reported findings from a national cross-sectional survey of 30,000 persons in the
35
36 16 Czech Republic who were examined between October 2020 and March 2021, a period covering the
37
38 17 second wave of the epidemic, which was also the period before the start of national vaccination
39
40 18 campaign. We found that by March 2021, 53% of participants had measurable antibodies against SARS-
41
42 19 CoV-2[8]. This was consistent with governmental data using cumulative PCR testing data. These rates
43
44 20 were considerably higher than those reported in Western Europe[2 9-11], due to a strong 2nd wave of
45
46 21 natural infection in the Czech Republic in autumn 2020[8].
47
48
49

50 22
51
52 23 In this report, we report longitudinal data on repeated assessment of the same population sample in
53
54 24 the period April 2021-Sept 2021, a period coinciding with the rollout of the national vaccination
55
56 25 programme. The objectives of this analysis were to 1) examine the trends in seropositivity before and
57
58 26 during the national vaccination campaign, 2) assess the contributions of natural infections and
59
60

1
2
3 1 vaccination to the seropositivity, 3) to assess seroconversion rates in previously seronegative persons,
4
5 2 4) to assess duration of seropositivity after natural infection, and 5) to estimate the rate ratio of
6
7 seroconversion and vaccination associated with sociodemographic indicators.
8
9
10 4

11 5 **Methods**

12 6 13 14 15 6 16 7 *Study design and participants*

17
18 Data for these analyses were derived from the first and second wave of the PROSECO study. The
19 8 PROSECO study design and population recruitment has been described elsewhere[8]. Briefly, phase 1
20 9 PROSECO study design and population recruitment has been described elsewhere[8]. Briefly, phase 1
21 9 PROSECO study design and population recruitment has been described elsewhere[8]. Briefly, phase 1
22 10 of the study recruited 30,054 unvaccinated adult volunteers from persons registered with the second
23 10 of the study recruited 30,054 unvaccinated adult volunteers from persons registered with the second
24 10 of the study recruited 30,054 unvaccinated adult volunteers from persons registered with the second
25 11 largest health insurance company in the Czech Republic. Participants provided blood sample between
26 11 largest health insurance company in the Czech Republic. Participants provided blood sample between
27 11 largest health insurance company in the Czech Republic. Participants provided blood sample between
28 12 October 2020 and March 2021, during the 2nd epidemic wave in the Czech Republic. Of those, 22,130
29 12 October 2020 and March 2021, during the 2nd epidemic wave in the Czech Republic. Of those, 22,130
30 13 participants were re-examined during the national vaccination programme between April 2021 and
31 13 participants were re-examined during the national vaccination programme between April 2021 and
32 14 September 2021. Participants were invited for phase 2 in the same order as they participated in phase
33 14 September 2021. Participants were invited for phase 2 in the same order as they participated in phase
34 15 1, so most subjects were re-examined 5-7 months after the first visit. Comparison of the persons
35 15 1, so most subjects were re-examined 5-7 months after the first visit. Comparison of the persons
36 16 participating in both phases with those who only attended phase 1 is shown in **Supplementary Table**
37 16 participating in both phases with those who only attended phase 1 is shown in **Supplementary Table**
38 17 **S1**. Those who participated in both assessments were older, more likely to be female, seropositive at
39 17 **S1**. Those who participated in both assessments were older, more likely to be female, seropositive at
40 18 phase 1, more obese, and more likely to have history of chronic non-communicable diseases.
41 18 phase 1, more obese, and more likely to have history of chronic non-communicable diseases.
42 19
43 19

44 19
45 20 In phase 2, participants provided a second blood sample for detection of IgG antibodies against SARS-
46 20 In phase 2, participants provided a second blood sample for detection of IgG antibodies against SARS-
47 21 CoV-2 and completed a questionnaire on personal information, including educational level, weight and
48 21 CoV-2 and completed a questionnaire on personal information, including educational level, weight and
49 22 height (to calculate BMI) and smoking status. Self-reported data about common non-communicable
50 22 height (to calculate BMI) and smoking status. Self-reported data about common non-communicable
51 23 disorders (diabetes, hypertension, asthma and chronic obstructive pulmonary disease (COPD)) were
52 23 disorders (diabetes, hypertension, asthma and chronic obstructive pulmonary disease (COPD)) were
53 24 also collected together with self-reported results of RT-PCR tests (if performed) and records of COVID
54 24 also collected together with self-reported results of RT-PCR tests (if performed) and records of COVID
55 25 vaccination. The second visit was organised at least 14 days after any vaccination (if completed).
56 25 vaccination. The second visit was organised at least 14 days after any vaccination (if completed).
57 26 Informed consent forms were obtained from all study participants during each wave of the data
58 26 Informed consent forms were obtained from all study participants during each wave of the data
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 collection. The study, including all aspects of data collection and data analysis, was approved by the
2 ELSPAC ethics committee under reference number (C)ELSPAC/EK/5/2021.

4 *Laboratory analyses*

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

12 *Statistical analysis*

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

21 We adjusted the estimated values of seroprevalence for the sensitivity and specificity of serological
22 tests used in this study, employing a standard correction formula based on Bayesian approach:
23 $\text{seroprevalence} = (\text{proportion positive} + \text{specificity} - 1) / (\text{sensitivity} + \text{specificity} - 1)$ [12]. As serological
24 tests were performed using chemiluminescent immunoassay methods, the range of standardized
25 seroprevalence values given by the 95% confidence interval was adjusted based on the range of
26 sensitivity and specificity values given by their 95% confidence intervals declared by the

1
2
3 1 manufacturers: DiaSorin LIAISON 95%CI for sensitivity 86.8-99.5%; 95%CI for specificity 97.5-99.2%,
4
5 2 Abbott Alinity 95%CI for sensitivity 96.5-100%; 95%CI for specificity 99.2-99.8%. Combination of the
6
7 3 most likely values of standardized seroprevalence, sensitivity and specificity yielded a range of values
8
9 4 where the test-adjusted seroprevalence is likely to occur (**Supplementary Table S2**).

10
11
12 5
13
14 6 Population data on COVID-19 were obtained from the Czech Central Information System of Infectious
15
16 7 Diseases (ISID), which includes records of all consecutive patients with COVID-19 in the Czech Republic
17
18 8 identified and confirmed by laboratory testing. ISID data are routinely collected in compliance with Act
19
20 9 No. 258/2000 Coll. on the Protection of Public Health and are publicly available in aggregated and
21
22 10 anonymized form of open or authenticated data sets. All analyses were conducted using Stata version
23
24 11 15.1 (StataCorp, College Station, Texas 77845 USA).

30 13 **Results**

31
32 14
33
34 15 This report is based on data from 22,130 subjects who participated in both phases of the study and
35
36 16 therefore had repeated antibody measurements. Characteristics of the analytical sample are shown in
37
38 17 **Table 1**. Just under 20% were under 40 years of age and 23% were older than 60 years, 62% were
39
40 18 females and 43% of participants had tertiary educational level, and 65% (14,483) subjects reported
41
42 19 vaccination by one of the four vaccines Comirnaty (BioNTech Manufacturing GmbH, Mainz, Germany),
43
44 20 Spikevax (previously COVID-19 Vaccine Moderna; Moderna Biotech Spain, S.L., Madrid, Spain),
45
46 21 Vaxzevria (previously COVID-19 Vaccine AstraZeneca; AstraZeneca AB, Södertälje, Sweden), Jcovden
47
48 22 (previously COVID-19 Vaccine Janssen; Janssen-Cilag International NV, Beerse, Belgium) available in
49
50 23 the Czech Republic. The proportion of vaccinated persons increased with increasing age and increasing
51
52 24 body mass index while it was lower in previously seropositive subjects. On the other hand, there was
53
54 25 little variation in seroprevalence by sex and among ages groups. Individuals with history of chronic
55
56 26 diseases were more likely to be vaccinated. A higher age of 60+ years was associated with a higher
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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 1 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 (see **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 1), the seroprevalence increased from 56% to 91% in September 2021. While the rapid increase in seropositivity rates during phase 1 was due to natural infection, a substantial part of the increase during phase 2 was due to vaccination.

At phase one, 10,778 (49%) of participants were SARS-CoV-2 seropositive. Of the 11,352 seronegative subjects at phase 1, 1,009 reported positive PCR test between first and second blood sample (**Table 2**). **Table 3** shows seroprevalence rates at phase 2 by SARS-CoV-2 infection status at phase 1 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 1 and unvaccinated in phase 2; 90% among those who were seropositive at phase 1 or reported SARS-CoV-2 infection before phase 2; 97% among infection free before but vaccinated at phase 2, and almost 100% among those who both had SARS-CoV-2 infection before and were vaccinated at phase 2. In addition, only 9% of 4,367 unvaccinated subjects who were seropositive in phase 1 became seronegative over the 5-7 months until phase 2. From 7,495 SARS-CoV-2 immune naïve persons, only 210 (2.8 %) did not produce detectable IgG antibodies with 4-6 weeks after vaccination.

1 Discussion

2
3
4
5
6
7
8 In this prospective population-based study, we examined the changes in seroprevalence in a
9
10 population-based sample with IgG antibodies measured twice, the second measurement being 5-7
11
12 month after the first on average. We found that after the rapid increase in seroprevalence during first
13
14 phase (conducted in the 2nd wave of the COVID-19 epidemic in the Czech Republic), there was further
15
16 substantial increase in seroprevalence during the national vaccination campaign. By the end of phase
17
18 2 of the study, 91% of examined individuals had IgG antibodies against SARS-CoV-2; among vaccinated
19
20 persons this proportion was over 97%.
21
22
23
24

25 *Strengths and limitations*

26
27 The main methodological limitation of this study is the selection bias related to response rates. In
28
29 phase 1, the response rates could not be established, since the number of persons who were invited
30
31 by their insurance companies to participate in the study was known, as only the first 30,000 of those
32
33 who attended were accepted in the study. These respondents were volunteers who were not entirely
34
35 representative for the national population [8]. In addition, only about 74% of those who participated
36
37 in phase 1 also participated in phase 2; as described in the methods, the phase 2 sample included
38
39 slightly more women (62%) than the phase 1 had (61%).
40
41
42
43
44

45 Notwithstanding this limitation, the availability of repeated antibody measurements on a large number
46
47 of individuals with high-quality chemiluminescent immunoassay is a major strength, since the
48
49 prospective design allows assessment of antibody response in different groups of people. Both sex
50
51 groups showed comparable seropositivity in both phases of the PROSECO study; the male and female
52
53 rates in phase 1 (October 2020 to March 2021) were 46.1% vs. 47.2% due to natural infection, in phase
54
55 2 (April 2021 to September 2021) the rates increase to 87.7% vs. 87.3%, respectively, mostly due to
56
57 vaccination.
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
601
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Our results are in line with other national studies of antibody prevalence, such as the United Kingdom REACT-2 study[3], Blood donors study[6] and UK SARS-CoV-2 Immunity SIREN study[13]. In the week ending 28th March 2021, which corresponds with the end of Phase 1 and the beginning of Phase 2 of the nationwide Czech PROSECO study, 55% of the adult population in England was tested positive for antibodies against the coronavirus 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 2 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, e.g., 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 = 19,792), with the bulk of seropositivity due to vaccination. Anti-spike 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 US, such as 20% for infection-induced antibodies and 83% for combined infection- 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].

1
2
3 1 Again, this is consistent with our findings. The highest seroprevalence in our study was seen among
4
5 2 vaccinated persons with and without previous SARS-CoV-2 infection (99% and 97%, respectively), while
6
7 3 the lowest seroprevalence was found among unvaccinated persons with no signs of disease. Moreover,
8
9 4 only 2.8% of immune naïve persons did not produce detectable IgG antibodies with 4-6 weeks after
10
11 5 vaccination. Furthermore, our prospective study also addressed the decline in antibody positivity after
12
13 6 vaccination or after SARS-CoV-2 infection and we found that only among 9% of subjects who were
14
15 7 seropositive in phase 1 became seronegative over the 5-7 months until phase 2.
16
17
18
19 8

20
21 9 In conclusion, the rapid increase in seropositivity during the 2nd wave of the COVID-19 epidemic
22
23 10 (covered by phase 1 of the PROSECO study) was followed by a similarly steep rise in seroprevalence
24
25 11 during the national vaccination campaign, reaching seropositivity rates of over 87% among general
26
27 12 population and 97% among vaccinated persons in the Czech Republic in the period of April 2021 to
28
29 13 September 2021. Vaccination rates were lower in persons who were seropositive in phase 1 but
30
31 14 increased with age and body mass index. Only 9% of unvaccinated subjects who were seropositive in
32
33 15 phase 1 became seronegative by phase 2. The combination of vaccination with the induction of a
34
35 16 systemic immune response and natural infection with SARS-CoV-2 with the development of mucosal
36
37 17 immunity is beneficial. It makes a significant contribution to good effect for diagnostic purposes and
38
39 18 prophylaxis and leads to the development of protective immunity[16]. Seroconversion, as a marker of
40
41 19 the ongoing immune response, is therefore an important measure of population immunity level to
42
43 20 guide policy response[17].
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 2 3 1 **Data availability statement**

4
5 2 All data generated during the first and second phase of the PROSECO study is presented in this article.
6 3 Anonymised data can be made available from the corresponding author upon request once all study
7 4 phases are completed and data validated. Release of data is a subject of approval of the Ethical and
8 5 Scientific boards of the PROSECO study.
9

10 11 6 12 7 **Ethics statements**

13
14 8 Informed consent forms were obtained from all study participants during each wave of the data
15 9 collection. An ethics committee approval of all aspects of data collection, as well as of the secondary
16 10 data analysis, was obtained from the ELSPAC ethics committee under reference number
17 11 (C)ELSPAC/EK/5/2021.
18
19

20 12 21 13 **Patient and public involvement**

22 14 No patient was involved in the design or implementation of this study. Study participants were
23 15 individually informed about their results, and they have access to the final publication of the study
24 16 results.
25
26
27

28 17 29 18 **Acknowledgements**

30 19 We thank all collaborating nurses, laboratories from the QualityLab association, and administrative
31 20 personnel and especially the 22,130 participants who invested their time and provided samples and
32 21 information for this study.
33
34
35

36 22 37 23 **A funding statements**

38 24 The PROSECO study was sponsored by the Prevention Programme of the Health Insurance Company
39 25 of the Ministry of the Interior of the Czech Republic. The RECETOX Research Infrastructure was
40 26 supported by the Ministry of Education, Youth and Sports of the Czech Republic (LM2018121), and VT
41 27 and PP from the CETOCOEN PLUS project of ESIF (CZ.02.1.01/0.0/0.0/15_003/0000469). This work was
42 28 supported from the European Union's Horizon 2020 research and innovation programme under grant
43 29 agreement No 857560 and the Ministry of Education, Youth and Sport of the Czech Republic/ESIF
44 30 (CZ.02.1.01/0.0/0.0/17_043/0009632). This publication reflects only the author's view and the
45 31 European Commission is not responsible for any use that may be made of the information it contains.
46
47
48
49

50 32 51 33 **Competing interests**

52 34 The authors declare no competing interests.
53
54
55

56 35 57 36 **Author contributions**

1 VT, PP, LA and JK were responsible for the design of the study. KD, DK and LA were responsible for the
2 study operation, coordination of data acquisition and quality management of participating
3 laboratories. VT, PP and TP developed the operationalized research question and the statistical
4 analyses plan. TP performed the statistical analyses. The first draft was written by VT and PP. MB
5 contributed to the writing and finalizing of the manuscript. MB and HP provided expertise in
6 epidemiology. All authors contributed to data interpretation, critically reviewed the first draft,
7 approved the final version and agreed to be accountable for the work.

8 **Code availability**

9 Statistical analyses were performed using STATA version 15.1 (StataCorp LLC, USA).

10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60**References:**

1. Arora RK, Joseph A, Van Wyk J, et al. SeroTracker: a global SARS-CoV-2 seroprevalence dashboard. *Lancet Infect Dis* 2021;**21**(4):e75-e76 doi: 10.1016/s1473-3099(20)30631-9 [published Online First: 2020/08/09].
2. Vaughan A, Duffell EF, Friedl GS, et al. Seroprevalence of SARS-CoV-2 antibodies prior to the widespread introduction of vaccine programmes in the WHO European Region, January - December 2020: a systematic review. *medRxiv* 2021:2021.12.02.21266897 doi: 10.1101/2021.12.02.21266897.
3. Ward H, Whitaker M, Flower B, et al. Population antibody responses following COVID-19 vaccination in 212,102 individuals. *Nature Communications* 2022;**13**(1):907 doi: 10.1038/s41467-022-28527-x.
4. Siller A, Seekircher L, Wachter GA, et al. Seroprevalence, Waning and Correlates of Anti-SARS-CoV-2 IgG Antibodies in Tyrol, Austria: Large-Scale Study of 35,193 Blood Donors Conducted between June 2020 and September 2021. *Viruses* 2022;**14**(3) doi: 10.3390/v14030568 [published Online First: 20220309].
5. Stringhini S, Zaballa ME, Pullen N, et al. Seroprevalence of anti-SARS-CoV-2 antibodies 6 months into the vaccination campaign in Geneva, Switzerland, 1 June to 7 July 2021. *Euro Surveill* 2021;**26**(43) doi: 10.2807/1560-7917.es.2021.26.43.2100830.
6. Whitaker HJ, Elgohari S, Rowe C, et al. Impact of COVID-19 vaccination program on seroprevalence in blood donors in England, 2021. *The Journal of infection* 2021;**83**(2):237-79 doi: 10.1016/j.jinf.2021.04.037 [published Online First: 2021/05/11].
7. Soeorg H, Jögi P, Naaber P, Ottas A, Toompere K, Lutsar I. Seroprevalence and levels of IgG antibodies after COVID-19 infection or vaccination. *Infect Dis (Lond)* 2022;**54**(1):63-71 doi: 10.1080/23744235.2021.1974540 [published Online First: 20210914].
8. Piler P, Thon V, Andrišková L, et al. Nationwide increases in anti-SARS-CoV-2 IgG antibodies between October 2020 and March 2021 in the unvaccinated Czech population. *Communications Medicine* 2022;**2**(1):19 doi: 10.1038/s43856-022-00080-0.
9. Rostami A, Sepidarkish M, Leeftang MMG, et al. SARS-CoV-2 seroprevalence worldwide: a systematic review and meta-analysis. *Clin Microbiol Infect* 2021;**27**(3):331-40 doi: 10.1016/j.cmi.2020.10.020 [published Online First: 2020/11/25].
10. Bobrovitz N, Arora RK, Cao C, et al. Global seroprevalence of SARS-CoV-2 antibodies: A systematic review and meta-analysis. *PLOS ONE* 2021;**16**(6):e0252617 doi: 10.1371/journal.pone.0252617.
11. Grant R, Dub T, Andrianou X, et al. SARS-CoV-2 population-based seroprevalence studies in Europe: a scoping review. *BMJ Open* 2021;**11**(4):e045425 doi: 10.1136/bmjopen-2020-045425.
12. Rothman KJ, Greenland S, Lash TL. *Modern Epidemiology*. Philadelphia PA: Lippincott Williams & Wilkins, 2008.
13. Hall V, Foulkes S, Insalata F, et al. Protection against SARS-CoV-2 after Covid-19 Vaccination and Previous Infection. *The New England journal of medicine* 2022;**386**(13):1207-20 doi: 10.1056/NEJMoa2118691 [published Online First: 2022/02/16].
14. Siller A, Wachter GA, Neururer S, et al. Prevalence of SARS-CoV-2 antibodies in healthy blood donors from the state of Tyrol, Austria, in summer 2020. *Wien Klin Wochenschr* 2021;**133**(23-24):1272-80 doi: 10.1007/s00508-021-01963-3 [published Online First: 20211026].
15. Jones JM, Stone M, Sulaeman H, et al. Estimated US Infection- and Vaccine-Induced SARS-CoV-2 Seroprevalence Based on Blood Donations, July 2020-May 2021. *JAMA* 2021;**326**(14):1400-09 doi: 10.1001/jama.2021.15161.
16. Russell MW, Moldoveanu Z, Ogra PL, Mestecky J. Mucosal Immunity in COVID-19: A Neglected but Critical Aspect of SARS-CoV-2 Infection. *Front Immunol* 2020;**11**:611337 doi: 10.3389/fimmu.2020.611337 [published Online First: 2020/12/18].

- 1
2
3 1 17. Bergeri I, Lewis HC, Subissi L, et al. Early epidemiological investigations: World Health
4 2 Organization UNITY protocols provide a standardized and timely international investigation
5 3 framework during the COVID-19 pandemic. *Influenza Other Respir Viruses* 2022;**16**(1):7-13
6 4 doi: 10.1111/irv.12915 [published Online First: 20211005].
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

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

	No. of participants	No. of seropositive	No. of vaccinated participants	Model of antibodies of any origin (N = 22,130)			Model of propensity to vaccination (N = 22,130)			Model of antibodies in unvaccinated participants (N = 7,647)			
				% of seropositive	PRR (CI)	p value	% of vaccinated	PRR (CI)	p value	N of participants	% of seropositive	PRR (CI)	p value
Sex:													
Female	13,824	12,067	8,844	87.29%	1.00	-	63.98%	1.00		4,980	67.25%	1.00	-
Male	8,306	7,282	5,639	87.67%	0.99	0.012	67.89%	1.05	<0.001	2,667	65.47%	0.95	<0.001
Age groups:													
18-29 years	1,491	1,202	770	80.62%	1.00	-	51.64%	1.00		721	61.72%	1.00	-
30-39 years	2,774	2,275	1,534	82.01%	1.02	0.215	55.30%	1.03	0.420	1,240	61.05%	0.97	0.338
40-49 years	6,700	5,725	4,177	85.45%	1.01	0.194	62.34%	1.17	<0.001	2,523	64.05%	0.97	0.226
50-59 years	6,049	5,405	4,061	89.35%	1.03	0.003	67.14%	1.23	<0.001	1,988	70.32%	1.04	0.170
60+ years	5,116	4,742	3,941	92.69%	1.05	<0.001	77.03%	1.37	<0.001	1,175	74.81%	1.09	0.001
Education													
Basic	1,952	1,744	1,295	89.34%	1.00	-	66.34%	1.00		657	70.02%	1.00	-
Medium	8,024	7,119	5,348	88.72%	1.00	0.972	66.65%	1.02	0.275	2,676	69.21%	1.02	0.337
High	7,544	6,689	5,223	88.67%	1.00	0.890	69.23%	1.08	<0.001	2,321	65.75%	1.02	0.394
Missing	4,610	3,797	2,617	82.36%	0.97	0.003	56.77%	0.87	<0.001	1,993	63.07%	1.00	0.923
COVID in history													
Seronegative	11,352	8,935	7,882	78.71%	1.00	-	69.43%	1.00		3,470	36.54%	1.00	-
Seropositive - no symptoms	5,597	5,374	3,458	96.02%	1.28	<0.001	61.78%	0.75	<0.001	2,139	90.04%	3.45	<0.001
Seropositive - with symptoms	5,181	5,040	3,143	97.28%	1.32	<0.001	60.66%	0.78	<0.001	2,038	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	8,192	6,964	5,038	85.01%	1.04	0.127	61.50%	1.09	0.141	3,154	63.44%	1.17	0.009
25-29.9	8,080	7,167	5,488	88.70%	1.05	0.077	67.92%	1.15	0.020	2,592	68.36%	1.18	0.006
30+	4,802	4,369	3,312	90.98%	1.06	0.046	68.97%	1.16	0.013	1,490	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	8,688	86.10%	1.00	-	62.56%	1.00		5,200	65.23%	1.00	-
Yes	7,152	6,500	5,161	90.88%	1.00	0.818	72.16%	1.06	<0.001	1,991	71.97%	1.00	0.813
missing	1,090	891	634	81.74%	1.02	0.266	58.17%	0.91	0.002	456	59.21%	1.12	0.005
Vaccination													
Vaccination No	7,647	5,095	0	66.63%	1.00	-	0.00%						
Vaccination Yes	14,483	14,254	14,483	98.42%	1.52	<0.001	100.00%						
Total	22,130	19,349	14,483							7,647			

BMI = body mass index; 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 above 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 above 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 (COPD)) 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

Table 2 Number of subjects with history of positive PCR test by seropositivity at Phase 1.

Seropositivity at Phase 1	SARS-CoV-2 infection reported (PCR)			Total
	Prior 1 st BS	Between 1 st and 2 nd BS	Never	
No	1,080	1,009	9,263	11,352
Yes	6,397	95	4,286	10,778
Total	7,477	1,104	13,549	22,130

BS = blood sample collection

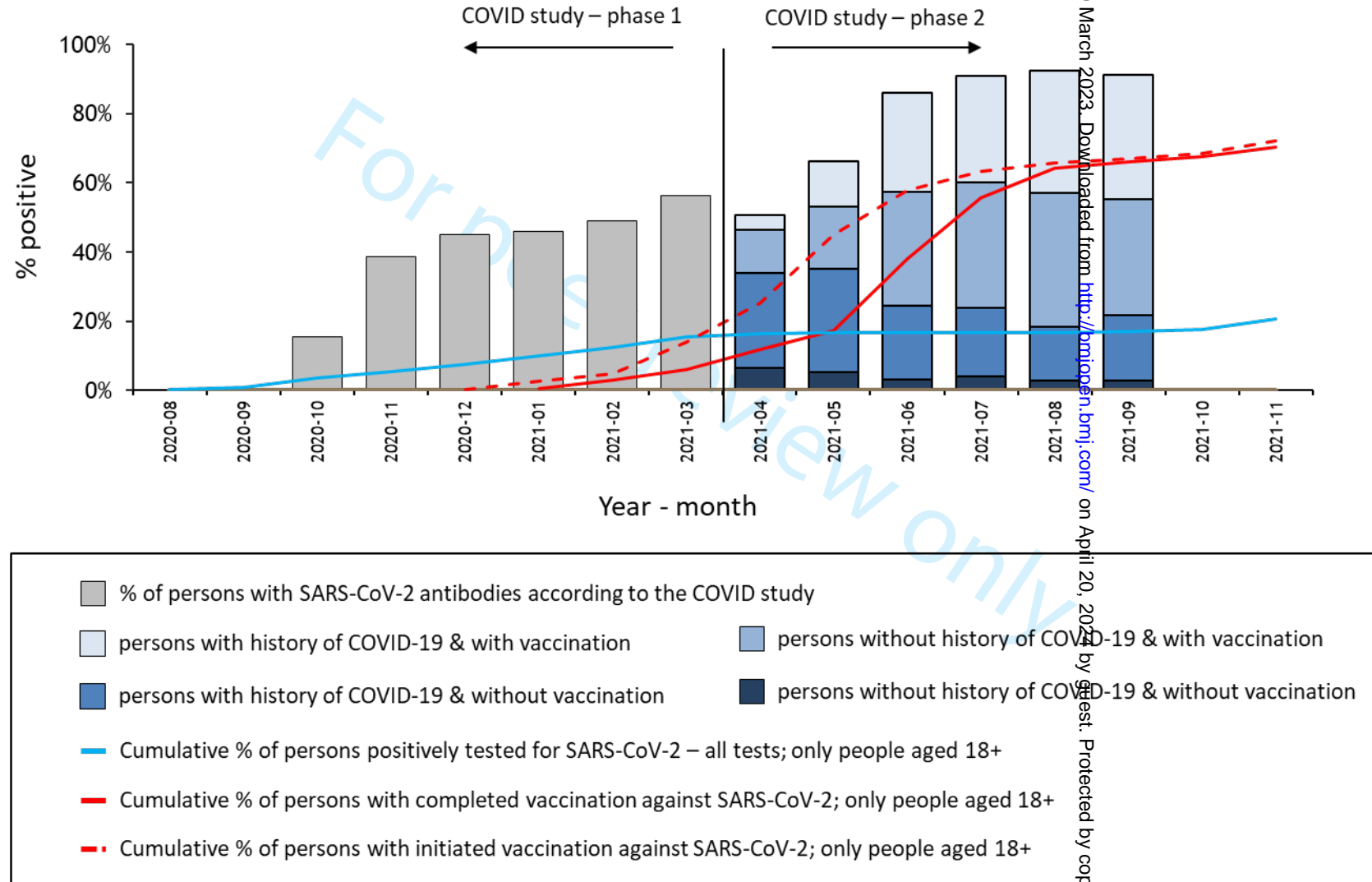
Table 3 Seroprevalence at phase 2 by SARS-CoV-2 infection and vaccination status.

	Positive		Negative		Total	Estimated seroprevalence in general population		
	N	%	N	%		%	95% CI	p value
SARS-CoV-2- & no vaccination	728	25.56%	2,120	74.44%	2,848	23.97%	22.18 – 25.85%	p<0.001
SARS-CoV-2+ & no vaccination	4,367	91.00%	432	9.00%	4,799	89.57%	88.33 – 90.70%	
SARS-CoV-2- & With vaccination	7,285	97.20%	210	2.80%	7,495	97.36%	96.72 – 97.88%	
SARS-CoV-2+ & With vaccination	6,969	99.73%	19	0.27%	6,988	99.81%	99.68 – 99.89%	
Total	19,349	87.43%	2,781	12.57%	22,130	84.37%	83.64 – 85.07%	

SARS-CoV-2- = seronegative at phase 1 AND self-report of negative or not done PCR test between phase 1 and 2

SARS-CoV-2+ = seropositive at phase 1 OR self-report of positive PCR test between phase 1 and 2

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



Supplementary table S1: Comparison of the persons participating in both phases with those who only attended phase 1

	Persons participating in both P1 and P2	%	Persons not participating in P2	%
Total	22,130	100%	7,924	100%
Sex				
Female	13,824	62.5%	4,438	56.0%
Male	8,306	37.5%	3,486	44.0%
Age groups				
18-29	1,491	6.7%	1,069	13.5%
30-39	2,774	12.5%	1,485	18.7%
40-49	6,700	30.3%	2,431	30.7%
50-59	6,049	27.3%	1,658	20.9%
60+	5,116	23.1%	1,281	16.2%
COVID in history				
Seronegative	11,352	51.3%	4,641	58.6%
Seropositive – no symptoms	5,597	25.3%	1,757	22.2%
Seropositive – with symptoms	5,181	23.4%	1,526	19.3%
BMI				
under 18.5	256	1.2%	85	1.1%
18.5-24.9	8,192	37.0%	2,791	35.2%
25-29.9	8,080	36.5%	2,360	29.8%
30 and more	4,802	21.7%	1,189	15.0%
missing	800	3.6%	1,499	18.9%
NCDs in history				
No	13,888	62.8%	6,563	82.8%
Yes	7,152	32.3%	539	6.8%
missing	1,090	4.9%	822	10.4%

Supplementary table S2: Overview of seroconversion by general population over time (04/2021-09/2021) - PHASE 1 - with adjustment for sensitivity and specificity of serological tests

N = 22,130	Total	Positive		Estimated seroprevalence in general population		Seroprevalence adjusted for sensitivity and specificity of serological tests	
		N	%	%	95% CI	Lower bound	Upper bound
SARS-CoV-2- & No vaccination	2,848	728	25.56%	23.97%	22.18-25.85%	20.19%	29.62%
SARS-CoV-2+ & No vaccination	4,799	4,367	91.00%	89.57%	88.33-90.70%	88.06%	100.00%
SARS-CoV-2- & With vaccination	7,495	7,285	97.20%	97.36%	96.72-97.88%	96.67%	100.00%
SARS-CoV-2+ & With vaccination	6,988	6,969	99.73%	99.81%	99.68-99.89%	99.70%	100.00%
Total	22,130	19,349	87.43%	84.37%	83.64-85.07%	83.25%	98.00%

SARS-CoV-2- = seronegative at phase 1 AND self-report of negative or not done PCR test between phase 1 and 2

SARS-CoV-2+ = seropositive at phase 1 OR self-report of positive PCR test between phase 1 and 2

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-068258.R2
Article Type:	Original research
Date Submitted by the Author:	23-Jan-2023
Complete List of Authors:	Thon, Vojtěch; Masaryk University, RECETOX Piler, Pavel; Masaryk University, RECETOX Pavlik, Tomas; Masaryk University Andrýsková, Lenka; Masaryk University Doležel, Kamil; QualityLab Association Kostka, David; Health Insurance Company of the Ministry of the Interior of the Czech Republic Pikhart, Hynek; University College London, Department of Epidemiology and Public Health Bobak, Martin; University College London, Department of Epidemiology and Public Health Klánová, Jana; Masaryk University
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Immunology (including allergy), Infectious diseases
Keywords:	EPIDEMIOLOGY, COVID-19, Public health < INFECTIOUS DISEASES

SCHOLARONE™
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

1
2
3 1 **Investigation of SARS-CoV-2 seroprevalence in relation to natural infection and vaccination between**
4
5 2 **October 2020 and September 2021 in the Czech Republic: a prospective national cohort study**
6
7

8 3
9
10 4 Vojtěch Thon^{1#}, Pavel Piler^{1#}, Tomáš Pavlík², Lenka Andryšková¹, Kamil Doležel³, David Kostka⁴, Hynek
11
12 5 Pikhart^{1,5}, Martin Bobák^{1,5}, Jana Klánová¹
13
14 6

15
16 7 **Institutional affiliations of the authors:**

17
18 8 ¹ RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic.

19
20 9 ² Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Brno, Czech Republic.

21
22 10 ³ QualityLab Association, Prague, Czech Republic.

23
24 11 ⁴ Health Insurance Company of the Ministry of the Interior of the Czech Republic, Prague, Czech
25
26 12 Republic.

27
28 13 ⁵ Department of Epidemiology & Public Health, University College London, London WC1E 6BT, UK.
29
30 14

31
32 15 **Corresponding author:**

33
34 16 *Prof. Vojtěch Thon, M.D., Ph.D., vojtech.thon@recetox.muni.cz, RECETOX, Faculty of Science,
35
36 17 Masaryk University, Brno, Czech Republic
37
38 18

39
40 19 #These authors contributed equally.
41
42 20

43
44 21 **Keywords:**

45
46 22 SARS-CoV-2, seroprevalence, vaccination, epidemic growth, antibodies durability
47
48 23
49
50 24
51
52 25
53
54
55
56
57
58
59
60

1
2
3 1 **Abstract**
4
5 2
6
7

8 3 **Objective:** Examine changes in SARS-CoV-2 seropositivity before and during the national vaccination
9
10 4 campaign in the Czech Republic.

11
12 5 **Design:** Prospective national population-based cohort study.

13
14 6 **Setting:** Masaryk University, RECETOX, Brno.

15
16 7 **Participants:** 22,130 persons provided blood samples at two time points approximately 5-7 months
17
18 8 apart, between October 2020 and March 2021 (Phase 1, before vaccination), and between April and
19
20 9 September 2021 (during vaccination campaign).

21
22
23 10 **Outcome measures:** Antigen-specific humoral immune response was analysed by detection of IgG
24
25 11 antibodies against the SARS-CoV-2 spike protein by commercial chemiluminescent immunoassays.
26
27 12 Participants completed a questionnaire that included personal information, anthropometric data, self-
28
29 13 reported results of previous RT-PCR tests (if performed), history of symptoms compatible with COVID-
30
31 14 19, and records of COVID vaccination. Seroprevalence was compared between calendar periods,
32
33 15 previous RT-PCR results, vaccination, and other individual characteristics.

34
35
36 16 **Results:** Before vaccination (Phase 1), seroprevalence increased from 15% in October 2020 to 56% in
37
38 17 March 2021. By the end of Phase 2, in September 2021, prevalence increased to 91%; the highest
39
40 18 seroprevalence was seen among vaccinated persons with and without previous SARS-CoV-2 infection
41
42 19 (99.7% and 97.2%, respectively), while the lowest seroprevalence was found among unvaccinated
43
44 20 persons with no signs of disease (26%). Vaccination rates were lower in persons who were seropositive
45
46 21 in phase 1 but increased with age and body mass index. Only 9% of unvaccinated subjects who were
47
48 22 seropositive in phase 1 became seronegative by phase 2.

49
50
51
52 23 **Conclusions:** The rapid increase in seropositivity during the 2nd wave of the COVID-19 epidemic
53
54 24 (covered by phase 1 of this study) was followed by a similarly steep rise in seroprevalence during the
55
56 25 national vaccination campaign, reaching seropositivity rates of over 97% among vaccinated persons.
57
58
59 26
60

1 Strengths and limitations of this study

- 2
- 3
- 4
- 5
- 6
- 7
- 8 • The PROSECO study provide nationwide data from the Central European region heavily
- 9 affected by COVID-19.
- 10
- 11
- 12 • The levels of anti-SARS-CoV-2 antibodies and the dynamics of seroconversion were assessed
- 13 using a harmonized network of accredited clinical laboratories.
- 14
- 15
- 16
- 17 • Major strengths of the study are its size, coverage, start before vaccination period, evaluation
- 18 of natural SARS-CoV-2 infection & on-going longitudinal follow-up inclusive of vaccination.
- 19
- 20
- 21 • The duration of anti-SARS-CoV-2 antibodies after infection in unvaccinated subjects is
- 22 assessed.
- 23
- 24
- 25
- 26 • The main limitation relates to the fact that study subjects were volunteers at the baseline, and
- 27 this may affect the representativeness of the cohort.
- 28
- 29
- 30
- 31
- 32
- 33
- 34
- 35
- 36
- 37
- 38
- 39
- 40
- 41
- 42
- 43
- 44
- 45
- 46
- 47
- 48
- 49
- 50
- 51
- 52
- 53
- 54
- 55
- 56
- 57
- 58
- 59
- 60

1 Introduction

2
3
4
5
6
7
8 During the COVID-19 pandemic, monitoring of the seroprevalence of antibodies in the population is an
9
10 important tool to design and adjust preventive strategies. As a part of this process, it is essential to
11
12 assess the contribution of natural infections and vaccination to the immune response to SARS-CoV-2.
13
14 The Serotracker platform has recorded hundreds of SARS-CoV-2 serological studies worldwide
15
16 (serotracker.com)[1]. Most national seroprevalence studies were performed before the start of
17
18 massive vaccination programme in Europe[2] but there are only few published European
19
20 seroprevalence studies covering both pre- and after vaccination campaign periods. Overall, these
21
22 studies, mainly based in Western Europe, reported rising seroprevalence after the national vaccination
23
24 programmes[3-7]. However, very few published studies have been conducted in Central and Eastern
25
26 Europe, where the dynamics of both the epidemics and vaccine uptake differed from the Western
27
28 European countries.
29
30
31
32
33
34

35 We have previously reported findings from a national cross-sectional survey of 30,000 persons in the
36
37 Czech Republic who were examined between October 2020 and March 2021, a period covering the
38
39 second wave of the epidemic, which was also the period before the start of national vaccination
40
41 campaign. We found that by March 2021, 53% of participants had measurable antibodies against SARS-
42
43 CoV-2[8]. This was consistent with governmental data using cumulative PCR testing data. These rates
44
45 were considerably higher than those reported in Western Europe[2 9-11], due to a strong 2nd wave of
46
47 natural infection in the Czech Republic in autumn 2020[8].
48
49
50
51

52 In this report, we report longitudinal data on repeated assessment of the same population sample in
53
54 the period April 2021-Sept 2021, a period coinciding with the rollout of the national vaccination
55
56 programme. The objectives of this analysis were to 1) examine the trends in seropositivity before and
57
58 during the national vaccination campaign, 2) assess the contributions of natural infections and
59
60

1
2
3 1 vaccination to the seropositivity, 3) to assess seroconversion rates in previously seronegative persons,
4
5 2 4) to assess duration of seropositivity after natural infection, and 5) to estimate the rate ratio of
6
7 seroconversion and vaccination associated with sociodemographic indicators.
8
9
10 4

11 5 **Methods**

12 6 13 14 15 6 16 7 *Study design and participants*

17 7
18
19 8 Data for these analyses were derived from the first and second wave of the PROSECO study. The
20
21 9 PROSECO study design and population recruitment has been described elsewhere[8]. Briefly, phase 1
22
23 10 of the study recruited 30,054 unvaccinated adult volunteers from persons registered with the second
24
25 11 largest health insurance company in the Czech Republic. Participants provided blood sample between
26
27 12 October 2020 and March 2021, during the 2nd epidemic wave in the Czech Republic. Of those, 22,130
28
29 13 participants were re-examined during the national vaccination programme between April 2021 and
30
31 14 September 2021. Participants were invited for phase 2 in the same order as they participated in phase
32
33 15 1, so most subjects were re-examined 5-7 months after the first visit. Comparison of the persons
34
35 16 participating in both phases with those who only attended phase 1 is shown in **Supplementary Table**
36
37 17 **S1**. Those who participated in both assessments were older, more likely to be female, seropositive at
38
39 18 phase 1, more obese, and more likely to have history of chronic non-communicable diseases.
40
41
42
43 19

44
45 20 In phase 2, participants provided a second blood sample for detection of IgG antibodies against SARS-
46
47 21 CoV-2 and completed a questionnaire on personal information, including educational level, weight and
48
49 22 height (to calculate BMI) and smoking status. Self-reported data about common non-communicable
50
51 23 disorders (diabetes, hypertension, asthma and chronic obstructive pulmonary disease (COPD)) were
52
53 24 also collected together with self-reported results of RT-PCR tests (if performed) and records of COVID
54
55 25 vaccination. The second visit was organised at least 14 days after any vaccination (if completed).
56
57 26 Informed consent forms were obtained from all study participants during each wave of the data
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 collection. The study, including all aspects of data collection and data analysis, was approved by the
2 ELSPAC ethics committee under reference number (C)ELSPAC/EK/5/2021.

4 *Laboratory analyses*

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

12 *Statistical analysis*

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

21 We adjusted the estimated values of seroprevalence for the sensitivity and specificity of serological
22 tests used in this study, employing a standard correction formula based on Bayesian approach:
23 $\text{seroprevalence} = (\text{proportion positive} + \text{specificity} - 1) / (\text{sensitivity} + \text{specificity} - 1)$ [12]. As serological
24 tests were performed using chemiluminescent immunoassay methods, the range of standardized
25 seroprevalence values given by the 95% confidence interval was adjusted based on the range of
26 sensitivity and specificity values given by their 95% confidence intervals declared by the

1
2
3 1 manufacturers: DiaSorin LIAISON 95%CI for sensitivity 86.8-99.5%; 95%CI for specificity 97.5-99.2%,
4
5 2 Abbott Alinity 95%CI for sensitivity 96.5-100%; 95%CI for specificity 99.2-99.8%. Combination of the
6
7 3 most likely values of standardized seroprevalence, sensitivity and specificity yielded a range of values
8
9 4 where the test-adjusted seroprevalence is likely to occur (**Supplementary Table S2**).

10
11
12 5
13
14 6 Population data on COVID-19 were obtained from the Czech Central Information System of Infectious
15
16 7 Diseases (ISID), which includes records of all consecutive patients with COVID-19 in the Czech Republic
17
18 8 identified and confirmed by laboratory testing. ISID data are routinely collected in compliance with Act
19
20 9 No. 258/2000 Coll. on the Protection of Public Health and are publicly available in aggregated and
21
22 10 anonymized form of open or authenticated data sets. All analyses were conducted using Stata version
23
24 11 15.1 (StataCorp, College Station, Texas 77845 USA).

30 13 **Results**

31
32 14
33
34 15 This report is based on data from 22,130 subjects who participated in both phases of the study and
35
36 16 therefore had repeated antibody measurements. Characteristics of the analytical sample are shown in
37
38 17 **Table 1**. Just under 20% were under 40 years of age and 23% were older than 60 years, 62% were
39
40 18 females and 43% of participants had tertiary educational level, and 65% (14,483) subjects reported
41
42 19 vaccination by one of the four vaccines Comirnaty (BioNTech Manufacturing GmbH, Mainz, Germany),
43
44 20 Spikevax (previously COVID-19 Vaccine Moderna; Moderna Biotech Spain, S.L., Madrid, Spain),
45
46 21 Vaxzevria (previously COVID-19 Vaccine AstraZeneca; AstraZeneca AB, Södertälje, Sweden), Jcovden
47
48 22 (previously COVID-19 Vaccine Janssen; Janssen-Cilag International NV, Beerse, Belgium) available in
49
50 23 the Czech Republic. The proportion of vaccinated persons increased with increasing age and increasing
51
52 24 body mass index while it was lower in previously seropositive subjects. On the other hand, there was
53
54 25 little variation in seroprevalence by sex and among ages groups. Individuals with history of chronic
55
56 26 diseases were more likely to be vaccinated. A higher age of 60+ years was associated with a higher
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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 1 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 (see **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 1), the seroprevalence increased from 56% to 91% in September 2021. While the rapid increase in seropositivity rates during phase 1 was due to natural infection, a substantial part of the increase during phase 2 was due to vaccination.

At phase one, 10,778 (49%) of participants were SARS-CoV-2 seropositive. Of the 11,352 seronegative subjects at phase 1, 1,009 reported positive PCR test between first and second blood sample (**Table 2**). **Table 3** shows seroprevalence rates at phase 2 by SARS-CoV-2 infection status at phase 1 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 1 and unvaccinated in phase 2; 90% among those who were seropositive at phase 1 or reported SARS-CoV-2 infection before phase 2; 97% among infection free before but vaccinated at phase 2, and almost 100% among those who both had SARS-CoV-2 infection before and were vaccinated at phase 2. In addition, only 9% of 4,367 unvaccinated subjects who were seropositive in phase 1 became seronegative over the 5-7 months until phase 2. From 7,495 SARS-CoV-2 immune naïve persons, only 210 (2.8 %) did not produce detectable IgG antibodies with 4-6 weeks after vaccination.

1 Discussion

2
3
4
5
6
7
8 In this prospective population-based study, we examined the changes in seroprevalence in a
9
10 4 population-based sample with IgG antibodies measured twice, the second measurement being 5-7
11
12 5 month after the first on average. We found that after the rapid increase in seroprevalence during first
13
14 6 phase (conducted in the 2nd wave of the COVID-19 epidemic in the Czech Republic), there was further
15
16 7 substantial increase in seroprevalence during the national vaccination campaign. By the end of phase
17
18 8 2 of the study, 91% of examined individuals had IgG antibodies against SARS-CoV-2; among vaccinated
19
20 9 persons this proportion was over 97%.

10 11 *Strengths and limitations*

12 The main methodological limitation of this study is the selection bias related to response rates. In
13
14 13 phase 1, the response rates could not be established, since the number of persons who were invited
15
16 14 by their insurance companies to participate in the study was known, as only the first 30,000 of those
17
18 15 who attended were accepted in the study. These respondents were volunteers who were not entirely
19
20 16 representative for the national population [8]. In addition, only about 74% of those who participated
21
22 17 in phase 1 also participated in phase 2; as described in the methods, the phase 2 sample included
23
24 18 slightly more women (62%) than the phase 1 had (61%).

25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

20 Notwithstanding this limitation, the availability of repeated antibody measurements on a large number
21
22 of individuals with high-quality chemiluminescent immunoassay is a major strength, since the
23
24 prospective design allows assessment of antibody response in different groups of people. Both sex
25
26 groups showed comparable seropositivity in both phases of the PROSECO study; the male and female
27
28 rates in phase 1 (October 2020 to March 2021) were 46.1% vs. 47.2% due to natural infection, in phase
29
30 2 (April 2021 to September 2021) the rates increase to 87.7% vs. 87.3%, respectively, mostly due to
31
32 vaccination.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1

2

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

Our results are in line with other national studies of antibody prevalence, such as the United Kingdom REACT-2 study[3], Blood donors study[6] and UK SARS-CoV-2 Immunity SIREN study[13]. In the week ending 28th March 2021, which corresponds with the end of Phase 1 and the beginning of Phase 2 of the nationwide Czech PROSECO study, 55% of the adult population in England was tested positive for antibodies against the coronavirus 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 2 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, e.g., 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 = 19,792), with the bulk of seropositivity due to vaccination. Anti-spike 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 US, such as 20% for infection-induced antibodies and 83% for combined infection- 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].

1
2
3 1 Again, this is consistent with our findings. The highest seroprevalence in our study was seen among
4
5 2 vaccinated persons with and without previous SARS-CoV-2 infection (99% and 97%, respectively), while
6
7 3 the lowest seroprevalence was found among unvaccinated persons with no signs of disease. Moreover,
8
9 4 only 2.8% of immune naïve persons did not produce detectable IgG antibodies with 4-6 weeks after
10
11 5 vaccination. Furthermore, our prospective study also addressed the decline in antibody positivity after
12
13 6 vaccination or after SARS-CoV-2 infection and we found that only among 9% of subjects who were
14
15 7 seropositive in phase 1 became seronegative over the 5-7 months until phase 2.
16
17
18
19
20

21 9 In conclusion, the rapid increase in seropositivity during the 2nd wave of the COVID-19 epidemic
22
23 10 (covered by phase 1 of the PROSECO study) was followed by a similarly steep rise in seroprevalence
24
25 11 during the national vaccination campaign, reaching seropositivity rates of over 87% among general
26
27 12 population and 97% among vaccinated persons in the Czech Republic in the period of April 2021 to
28
29 13 September 2021. Vaccination rates were lower in persons who were seropositive in phase 1 but
30
31 14 increased with age and body mass index. Only 9% of unvaccinated subjects who were seropositive in
32
33 15 phase 1 became seronegative by phase 2. The combination of vaccination with the induction of a
34
35 16 systemic immune response and natural infection with SARS-CoV-2 with the development of mucosal
36
37 17 immunity is beneficial. It makes a significant contribution to good effect for diagnostic purposes and
38
39 18 prophylaxis and leads to the development of protective immunity[16]. Seroconversion, as a marker of
40
41 19 the ongoing immune response, is therefore an important measure of population immunity level to
42
43 20 guide policy response[17].
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 2 3 1 **Data availability statement**

4
5 2 All data generated during the first and second phase of the PROSECO study is presented in this article.
6 3 Anonymised data can be made available from the corresponding author upon request once all study
7 4 phases are completed and data validated. Release of data is a subject of approval of the Ethical and
8 5 Scientific boards of the PROSECO study.
9

10 11 6 12 7 **Ethics statements**

13
14 8 Informed consent forms were obtained from all study participants during each wave of the data
15 9 collection. An ethics committee approval of all aspects of data collection, as well as of the secondary
16 10 data analysis, was obtained from the ELSPAC ethics committee under reference number
17 11 (C)ELSPAC/EK/5/2021.
18
19

20 12 21 22 13 **Patient and public involvement**

23 14 No patient was involved in the design or implementation of this study. Study participants were
24 15 individually informed about their results, and they have access to the final publication of the study
25 16 results.
26
27

28 17 29 30 18 **Acknowledgements**

31 19 We thank all collaborating nurses, laboratories from the QualityLab association, and administrative
32 20 personnel and especially the 22,130 participants who invested their time and provided samples and
33 21 information for this study.
34
35

36 22 37 38 23 **A funding statements**

39 24 The PROSECO study was sponsored by the Prevention Programme of the Health Insurance Company
40 25 of the Ministry of the Interior of the Czech Republic. The RECETOX Research Infrastructure was
41 26 supported by the Ministry of Education, Youth and Sports of the Czech Republic (LM2018121), and VT
42 27 and PP from the CETOCOEN PLUS project of ESIF (CZ.02.1.01/0.0/0.0/15_003/0000469). This work was
43 28 supported from the European Union's Horizon 2020 research and innovation programme under grant
44 29 agreement No 857560 and the Ministry of Education, Youth and Sport of the Czech Republic/ESIF
45 30 (CZ.02.1.01/0.0/0.0/17_043/0009632). This publication reflects only the author's view and the
46 31 European Commission is not responsible for any use that may be made of the information it contains.
47
48
49

50 32 51 52 33 **Competing interests**

53 34 The authors declare no competing interests.
54
55

56 35 57 36 **Author contributions**

1 VT, PP, LA and JK were responsible for the design of the study. KD, DK and LA were responsible for the
2 study operation, coordination of data acquisition and quality management of participating
3 laboratories. VT, PP and TP developed the operationalized research question and the statistical
4 analyses plan. TP performed the statistical analyses. The first draft was written by VT and PP. MB
5 contributed to the writing and finalizing of the manuscript. MB and HP provided expertise in
6 epidemiology. All authors contributed to data interpretation, critically reviewed the first draft,
7 approved the final version and agreed to be accountable for the work.

8 **Code availability**

9 Statistical analyses were performed using STATA version 15.1 (StataCorp LLC, USA).

10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60**References:**

1. Arora RK, Joseph A, Van Wyk J, et al. SeroTracker: a global SARS-CoV-2 seroprevalence dashboard. *Lancet Infect Dis* 2021;**21**(4):e75-e76 doi: 10.1016/s1473-3099(20)30631-9 [published Online First: 2020/08/09].
2. Vaughan A, Duffell EF, Friedl GS, et al. Seroprevalence of SARS-CoV-2 antibodies prior to the widespread introduction of vaccine programmes in the WHO European Region, January - December 2020: a systematic review. *medRxiv* 2021:2021.12.02.21266897 doi: 10.1101/2021.12.02.21266897.
3. Ward H, Whitaker M, Flower B, et al. Population antibody responses following COVID-19 vaccination in 212,102 individuals. *Nature Communications* 2022;**13**(1):907 doi: 10.1038/s41467-022-28527-x.
4. Siller A, Seekircher L, Wachter GA, et al. Seroprevalence, Waning and Correlates of Anti-SARS-CoV-2 IgG Antibodies in Tyrol, Austria: Large-Scale Study of 35,193 Blood Donors Conducted between June 2020 and September 2021. *Viruses* 2022;**14**(3) doi: 10.3390/v14030568 [published Online First: 20220309].
5. Stringhini S, Zaballa ME, Pullen N, et al. Seroprevalence of anti-SARS-CoV-2 antibodies 6 months into the vaccination campaign in Geneva, Switzerland, 1 June to 7 July 2021. *Euro Surveill* 2021;**26**(43) doi: 10.2807/1560-7917.es.2021.26.43.2100830.
6. Whitaker HJ, Elgohari S, Rowe C, et al. Impact of COVID-19 vaccination program on seroprevalence in blood donors in England, 2021. *The Journal of infection* 2021;**83**(2):237-79 doi: 10.1016/j.jinf.2021.04.037 [published Online First: 2021/05/11].
7. Soeorg H, Jögi P, Naaber P, Ottas A, Toompere K, Lutsar I. Seroprevalence and levels of IgG antibodies after COVID-19 infection or vaccination. *Infect Dis (Lond)* 2022;**54**(1):63-71 doi: 10.1080/23744235.2021.1974540 [published Online First: 20210914].
8. Piler P, Thon V, Andrišková L, et al. Nationwide increases in anti-SARS-CoV-2 IgG antibodies between October 2020 and March 2021 in the unvaccinated Czech population. *Communications Medicine* 2022;**2**(1):19 doi: 10.1038/s43856-022-00080-0.
9. Rostami A, Sepidarkish M, Leeftang MMG, et al. SARS-CoV-2 seroprevalence worldwide: a systematic review and meta-analysis. *Clin Microbiol Infect* 2021;**27**(3):331-40 doi: 10.1016/j.cmi.2020.10.020 [published Online First: 2020/11/25].
10. Bobrovitz N, Arora RK, Cao C, et al. Global seroprevalence of SARS-CoV-2 antibodies: A systematic review and meta-analysis. *PLOS ONE* 2021;**16**(6):e0252617 doi: 10.1371/journal.pone.0252617.
11. Grant R, Dub T, Andrianou X, et al. SARS-CoV-2 population-based seroprevalence studies in Europe: a scoping review. *BMJ Open* 2021;**11**(4):e045425 doi: 10.1136/bmjopen-2020-045425.
12. Rothman KJ, Greenland S, Lash TL. *Modern Epidemiology*. Philadelphia PA: Lippincott Williams & Wilkins, 2008.
13. Hall V, Foulkes S, Insalata F, et al. Protection against SARS-CoV-2 after Covid-19 Vaccination and Previous Infection. *The New England journal of medicine* 2022;**386**(13):1207-20 doi: 10.1056/NEJMoa2118691 [published Online First: 2022/02/16].
14. Siller A, Wachter GA, Neururer S, et al. Prevalence of SARS-CoV-2 antibodies in healthy blood donors from the state of Tyrol, Austria, in summer 2020. *Wien Klin Wochenschr* 2021;**133**(23-24):1272-80 doi: 10.1007/s00508-021-01963-3 [published Online First: 20211026].
15. Jones JM, Stone M, Sulaeman H, et al. Estimated US Infection- and Vaccine-Induced SARS-CoV-2 Seroprevalence Based on Blood Donations, July 2020-May 2021. *JAMA* 2021;**326**(14):1400-09 doi: 10.1001/jama.2021.15161.
16. Russell MW, Moldoveanu Z, Ogra PL, Mestecky J. Mucosal Immunity in COVID-19: A Neglected but Critical Aspect of SARS-CoV-2 Infection. *Front Immunol* 2020;**11**:611337 doi: 10.3389/fimmu.2020.611337 [published Online First: 2020/12/18].

- 1
2
3 1 17. Bergeri I, Lewis HC, Subissi L, et al. Early epidemiological investigations: World Health
4 2 Organization UNITY protocols provide a standardized and timely international investigation
5 3 framework during the COVID-19 pandemic. *Influenza Other Respir Viruses* 2022;**16**(1):7-13
6 4 doi: 10.1111/irv.12915 [published Online First: 20211005].
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

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

	No. of participants	No. of seropositive	No. of vaccinated participants	Model of antibodies of any origin (N = 22,130)			Model of propensity to vaccination (N = 22,130)			Model of antibodies in unvaccinated participants (N = 7,647)			
				% of seropositive	PRR (CI)	p value	% of vaccinated	PRR (CI)	p value	N of participants	% of seropositive	PRR (CI)	p value
Sex:													
Female	13,824	12,067	8,844	87.29%	1.00	-	63.98%	1.00		4,980	67.25%	1.00	-
Male	8,306	7,282	5,639	87.67%	0.99	0.012	67.89%	1.05	<0.001	2,667	65.47%	0.95	<0.001
Age groups:													
18-29 years	1,491	1,202	770	80.62%	1.00	-	51.64%	1.00		721	61.72%	1.00	-
30-39 years	2,774	2,275	1,534	82.01%	1.02	0.215	55.30%	1.03	0.420	1,240	61.05%	0.97	0.338
40-49 years	6,700	5,725	4,177	85.45%	1.01	0.194	62.34%	1.17	<0.001	2,523	64.05%	0.97	0.226
50-59 years	6,049	5,405	4,061	89.35%	1.03	0.003	67.14%	1.23	<0.001	1,988	70.32%	1.04	0.170
60+ years	5,116	4,742	3,941	92.69%	1.05	<0.001	77.03%	1.37	<0.001	1,175	74.81%	1.09	0.001
Education													
Basic	1,952	1,744	1,295	89.34%	1.00	-	66.34%	1.00		657	70.02%	1.00	-
Medium	8,024	7,119	5,348	88.72%	1.00	0.972	66.65%	1.02	0.275	2,676	69.21%	1.02	0.337
High	7,544	6,689	5,223	88.67%	1.00	0.890	69.23%	1.08	<0.001	2,321	65.75%	1.02	0.394
Missing	4,610	3,797	2,617	82.36%	0.97	0.003	56.77%	0.87	<0.001	1,993	63.07%	1.00	0.923
COVID in history													
Seronegative	11,352	8,935	7,882	78.71%	1.00	-	69.43%	1.00		3,470	36.54%	1.00	-
Seropositive - no symptoms	5,597	5,374	3,458	96.02%	1.28	<0.001	61.78%	0.75	<0.001	2,139	90.04%	3.45	<0.001
Seropositive - with symptoms	5,181	5,040	3,143	97.28%	1.32	<0.001	60.66%	0.78	<0.001	2,038	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	8,192	6,964	5,038	85.01%	1.04	0.127	61.50%	1.09	0.141	3,154	63.44%	1.17	0.009
25-29.9	8,080	7,167	5,488	88.70%	1.05	0.077	67.92%	1.15	0.020	2,592	68.36%	1.18	0.006
30+	4,802	4,369	3,312	90.98%	1.06	0.046	68.97%	1.16	0.013	1,490	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	8,688	86.10%	1.00	-	62.56%	1.00		5,200	65.23%	1.00	-
Yes	7,152	6,500	5,161	90.88%	1.00	0.818	72.16%	1.06	<0.001	1,991	71.97%	1.00	0.813
missing	1,090	891	634	81.74%	1.02	0.266	58.17%	0.91	0.002	456	59.21%	1.12	0.005
Vaccination													
Vaccination No	7,647	5,095	0	66.63%	1.00	-	0.00%						
Vaccination Yes	14,483	14,254	14,483	98.42%	1.52	<0.001	100.00%						
Total	22,130	19,349	14,483							7,647			

BMI = body mass index; 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 above 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 above 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 (COPD)) 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

Table 2 Number of subjects with history of positive PCR test by seropositivity at Phase 1.

Seropositivity at Phase 1	SARS-CoV-2 infection reported (PCR)			Total
	Prior 1 st BS	Between 1 st and 2 nd BS	Never	
No	1,080	1,009	9,263	11,352
Yes	6,397	95	4,286	10,778
Total	7,477	1,104	13,549	22,130

BS = blood sample collection

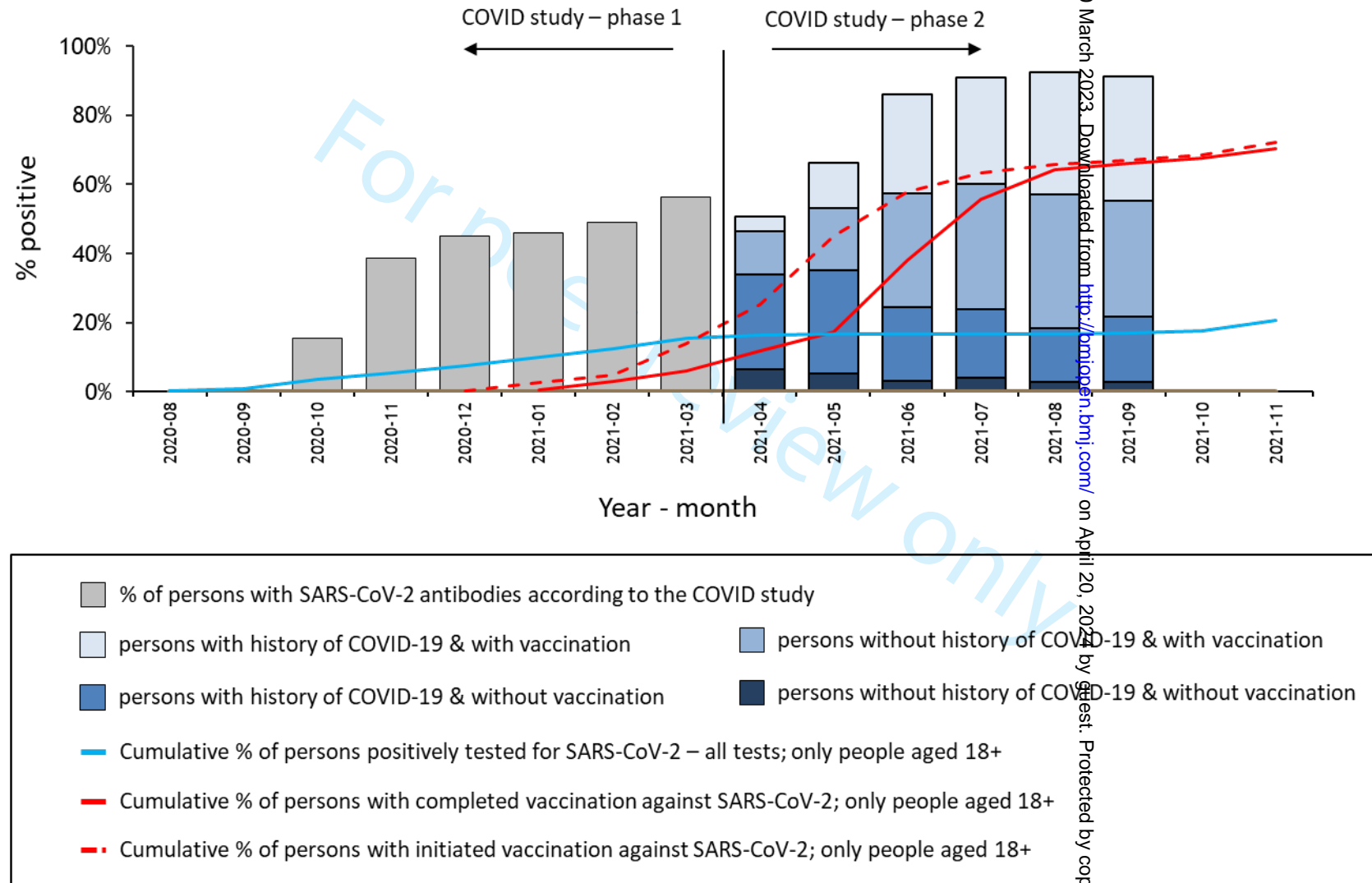
Table 3 Seroprevalence at phase 2 by SARS-CoV-2 infection and vaccination status.

	Positive		Negative		Total	Estimated seroprevalence in general population		
	N	%	N	%		%	95% CI	p value
SARS-CoV-2- & no vaccination	728	25.56%	2,120	74.44%	2,848	23.97%	22.18 – 25.85%	p<0.001
SARS-CoV-2+ & no vaccination	4,367	91.00%	432	9.00%	4,799	89.57%	88.33 – 90.70%	
SARS-CoV-2- & With vaccination	7,285	97.20%	210	2.80%	7,495	97.36%	96.72 – 97.88%	
SARS-CoV-2+ & With vaccination	6,969	99.73%	19	0.27%	6,988	99.81%	99.68 – 99.89%	
Total	19,349	87.43%	2,781	12.57%	22,130	84.37%	83.64 – 85.07%	

SARS-CoV-2- = seronegative at phase 1 AND self-report of negative or not done PCR test between phase 1 and 2

SARS-CoV-2+ = seropositive at phase 1 OR self-report of positive PCR test between phase 1 and 2

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



Supplementary table S1: Comparison of the persons participating in both phases with those who only attended phase 1

	Persons participating in both P1 and P2	%	Persons not participating in P2	%
Total	22,130	100%	7,924	100%
Sex				
Female	13,824	62.5%	4,438	56.0%
Male	8,306	37.5%	3,486	44.0%
Age groups				
18-29	1,491	6.7%	1,069	13.5%
30-39	2,774	12.5%	1,485	18.7%
40-49	6,700	30.3%	2,431	30.7%
50-59	6,049	27.3%	1,658	20.9%
60+	5,116	23.1%	1,281	16.2%
COVID in history				
Seronegative	11,352	51.3%	4,641	58.6%
Seropositive – no symptoms	5,597	25.3%	1,757	22.2%
Seropositive – with symptoms	5,181	23.4%	1,526	19.3%
BMI				
under 18.5	256	1.2%	85	1.1%
18.5-24.9	8,192	37.0%	2,791	35.2%
25-29.9	8,080	36.5%	2,360	29.8%
30 and more	4,802	21.7%	1,189	15.0%
missing	800	3.6%	1,499	18.9%
NCDs in history				
No	13,888	62.8%	6,563	82.8%
Yes	7,152	32.3%	539	6.8%
missing	1,090	4.9%	822	10.4%

Supplementary table S2: Overview of seroconversion by general population over time (04/2021-09/2021) - PHASE 1 - with adjustment for sensitivity and specificity of serological tests

N = 22,130	Total	Positive		Estimated seroprevalence in general population		Seroprevalence adjusted for sensitivity and specificity of serological tests	
		N	%	%	95% CI	Lower bound	Upper bound
SARS-CoV-2- & No vaccination	2,848	728	25.56%	23.97%	22.18-25.85%	20.19%	29.62%
SARS-CoV-2+ & No vaccination	4,799	4,367	91.00%	89.57%	88.33-90.70%	88.06%	100.00%
SARS-CoV-2- & With vaccination	7,495	7,285	97.20%	97.36%	96.72-97.88%	96.67%	100.00%
SARS-CoV-2+ & With vaccination	6,988	6,969	99.73%	99.81%	99.68-99.89%	99.70%	100.00%
Total	22,130	19,349	87.43%	84.37%	83.64-85.07%	83.25%	98.00%

SARS-CoV-2- = seronegative at phase 1 AND self-report of negative or not done PCR test between phase 1 and 2

SARS-CoV-2+ = seropositive at phase 1 OR self-report of positive PCR test between phase 1 and 2