Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Original article

Low prevalence of active COVID-19 in Slovenia: a nationwide population study of a probability-based sample

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ABSTRACT

Objectives: Accurate population-level assessment of the coronavirus disease 2019 (COVID-19) burden is fundamental for navigating the path forward during the ongoing pandemic, but current knowledge is scant. We conducted the first nationwide population study using a probability-based sample to assess active severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, combined with a longitudinal follow-up of the entire cohort over the next 6 months. Baseline SARS-CoV-2 RNA testing results and the first 3-week follow-up results are presented.

Methods: A probability-based sample of the Slovenian population comprising data from 2.1 million people was selected from the Central Population Register ($n = 3000$). SARS-CoV-2 RNA was detected in nasopharyngeal samples using the cobas 6800 SARS-CoV-2 assay. Each participant filled in a detailed baseline questionnaire with basic sociodemographic data and detailed medical history compatible with COVID-19. After 3 weeks, participants were interviewed for the presence of COVID-19–compatible clinical symptoms and signs, including in household members, and offered immediate testing for SARS-CoV-2 RNA if indicated.

Results: A total of 1368 individuals (46%) consented to participate and completed the questionnaire. Two of 1366 participants tested positive for SARS-CoV-2 RNA (prevalence 0.15%; posterior mean 0.18%, 95% Bayesian confidence interval 0.03–0.47; 95% highest density region (HDR) 0.01–0.41). No newly diagnosed infections occurred in the cohort during the first 3-week follow-up round.

Conclusions: The low prevalence of active COVID-19 infections found in this study accurately predicted the dynamics of the epidemic in Slovenia over the subsequent month. Properly designed and timely executed studies using probability-based samples combined with routine target-testing figures provide reliable data that can be used to make informed decisions on relaxing or strengthening disease mitigation strategies. **P. Maver Vodičar, Clin Microbiol Infect 2020;26:1514**

Introduction

The World Health Organization (WHO) announced a coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on 11 March 2020 after rapid global spread of the disease. Starting as a local emergence in late 2019 [1–3], almost 12 million cases and 550 000 SARS-CoV-2–related deaths had been confirmed worldwide as of 7 July 2020. Because of the wide variety of clinical presentation, which range from asymptomatic infection to severe respiratory failure requiring intensive care treatment and mechanical ventilation, the burden of disease varies across the world, and the true prevalence of both active and resolved COVID-19 is still largely unknown.

Countries' success in curbing the first wave of the SARS-CoV-2 epidemic was greatly influenced by the speed and extent of

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healthcare authorities' containment efforts and mitigation strategies to reduced the spread among the population, keeping the influx of new COVID-19 patients into hospitals manageable and sustainable for the healthcare system's capacities [4–7].

Cultural differences and community discipline were additional important factors influencing the level of compliance with decisions by healthcare authorities and politicians. Currently, the main uncertainty is how to determine the point on the epidemic curve when reopening society and cautiously relaxing measures is safe and sustainable. Accurate assessment of the COVID-19 burden at the community level is fundamental, and a survey using a probability-based sample is an essential component needed to make informed decisions on measures to help navigate the path forwards during the evolving epidemic [8].

We present the study design and baseline results of SARS-CoV-2 RNA detection in nasopharyngeal samples, as well as the first 3-week follow-up results of an ongoing nationwide population study on the SARS-CoV-2 burden in Slovenia using a probability-based population sample with a planned 6-month longitudinal follow-up. To our knowledge, this study is the first to use a probability-based sample representative of the whole country and across all age categories, combined with a longitudinal follow-up of the cohort over the next 6 months. The low prevalence of active COVID-19 infection assessed in this study provided important complementary data to daily epidemiologic surveillance and routine target-testing data. Combining both approaches accurately predicted the epidemic dynamic in Slovenia over the subsequent month, and provided a basis for informed decisions on the gradual and controlled relaxation of mitigation strategies, which ultimately led to Slovenia's being the first country in Europe to officially declare the end of the first wave of the epidemic, as of 31 May 2020.

Methods

Study design

This Slovenian national study using a probability-based sample was planned in two phases (Fig. 1). The first cross-sectional phase, which took place between 20 April and 1 May 2020, was performed to determine the burden of active SARS-CoV-2 infections in the general population that may have gone undetected within the past and current testing approaches and epidemiologic contact tracing. Selected persons were sent an invitation letter and a two-page questionnaire by postal mail about their household structure, their recent contacts and travel history, and their own and household members' potential COVID-19–compatible symptoms experienced in the past 2 months. According to General Data Protection Regulation rules, we were only allowed to contact these persons using regular post and publicly available landline and mobile telephone numbers. Respondents expressed their willingness via telephone or e-mail; however, in line with the study's protocol, refusals were asked no further questions and were not reapproached. Those who did not respond were sent a reminder by post 1 week after the first invitation. The study design is shown in Fig. 1.

The second phase included longitudinal tracking of the cohort, allowing close and active monitoring of the epidemic dynamics at the population level in the following 6 months. Participants were interviewed by medically qualified personnel every 3 weeks for the presence of COVID-19–compatible clinical symptoms and signs, including in household members. If infection was suspected, they were offered immediate testing. In addition, the participants were provided with a dedicated mobile number and e-mail address so they could actively report their own and household members' health status at any time during the study. The first follow-up calls took place between 18 May and 24 May 2020.

After 6 months of follow-up, exit anti–SARS-CoV-2 serologic testing of the entire cohort is planned for October 2020 to determine the cumulative incidence of SARS-CoV-2 infections in the general population.

Participants

The sample was created following a well-established national practice for probability-based official, academic and public health surveys. The sample was selected on 31 March 2020 from the Central Population Register (CPR), which is maintained by the Ministry of the Interior and which includes all permanent and temporary residents of Slovenia (Fig. 1). The chosen gross sample size ($n = 3000$) matched the available resources and time limitations (1–2 weeks of fieldwork) and was also sufficient for the study aims. The sampling design minimized fieldwork costs, which involved ten to 12 trained medical teams with ambulances per day, by selecting 300 census enumeration areas as primary sampling units. The selection of these units followed the implicit stratification according to region and settlement type. Within each unit, ten persons were randomly selected; data from the CPR on age, sex, region and size and type of settlement were also attached.

All study participants provided written informed consent; for participants under 18, consent was provided by parents or guardians. To ensure confidentiality, all samples and questionnaires were coded and analysed anonymously. The study was approved by the National Medical Ethics Committee of the Republic of Slovenia (consent 0120-199/2020/19) and registered with ClinicalTrials.gov (NCT04376996).

SARS-CoV-2 RNA testing

Detection of SARS-CoV-2 RNA in nasopharyngeal samples was performed using the clinically validated, fully automated sample-to-result two-target PCR-based assay cobas 6800 SARS-CoV-2 (Roche, Branchburg, NJ, USA) according to the manufacturer's instructions, as previously described in detail [9]. Briefly, the sample was considered positive if either both the *ORF1* (target 1) and *E* (target 2) genes, or only the *ORF1* gene tested positive. Internal validation showed the assay's excellent 100% sensitivity and 100% specificity [9]. The assay received US Food and Drug Administration emergency use authorization on 12 March 2020.

Statistical analysis

We estimated the prevalence θ with a binomial beta conjugate model with noninformative (Jeffrey's) prior on prevalence:

$$y \sim Binomial(n,\ \theta);\ \theta \sim B\left(\frac{1}{2},\ \frac{1}{2}\right),$$

with n being the sample size and y the number of positive cases. The analysis was performed in R software [10]. We used 1000 warmup and 1 million sampling iterations, which is sufficient for the sampling-based approximation error to be lower than the number of decimal places reported. Confidence intervals (CI) are based on the 2.5% and 97.5% percentiles of the posterior distribution.

Results

The response rate, adjusted for noneligible persons, was 47% American Association for Public Opinion Research (AAPOR) (Fig. 1). The study included 1368 participants, 663 men (48.5%) and 705

women (51.5%). The mean age was 46.0 years (range, 3 months to 99 years). Of these, 1366 participants were tested for SARS-CoV-2 RNA between 20 April and 1 May 2020. The sample matched the population structure well; the differences in sex, region and settlement type were not statistically significant (χ^2, $p > 0.01$). The age structure was mismatched only for the age groups 0 to 10 years (7.3% instead of 11.0%) and 51 to 60 years (18.3% instead of 14.0%). However, as a result of small differences, the weighting procedures had little effect, and when optimizing the mean squared error, the corresponding reduction in the bias component was smaller than the related increase in the variance component due to weighting.

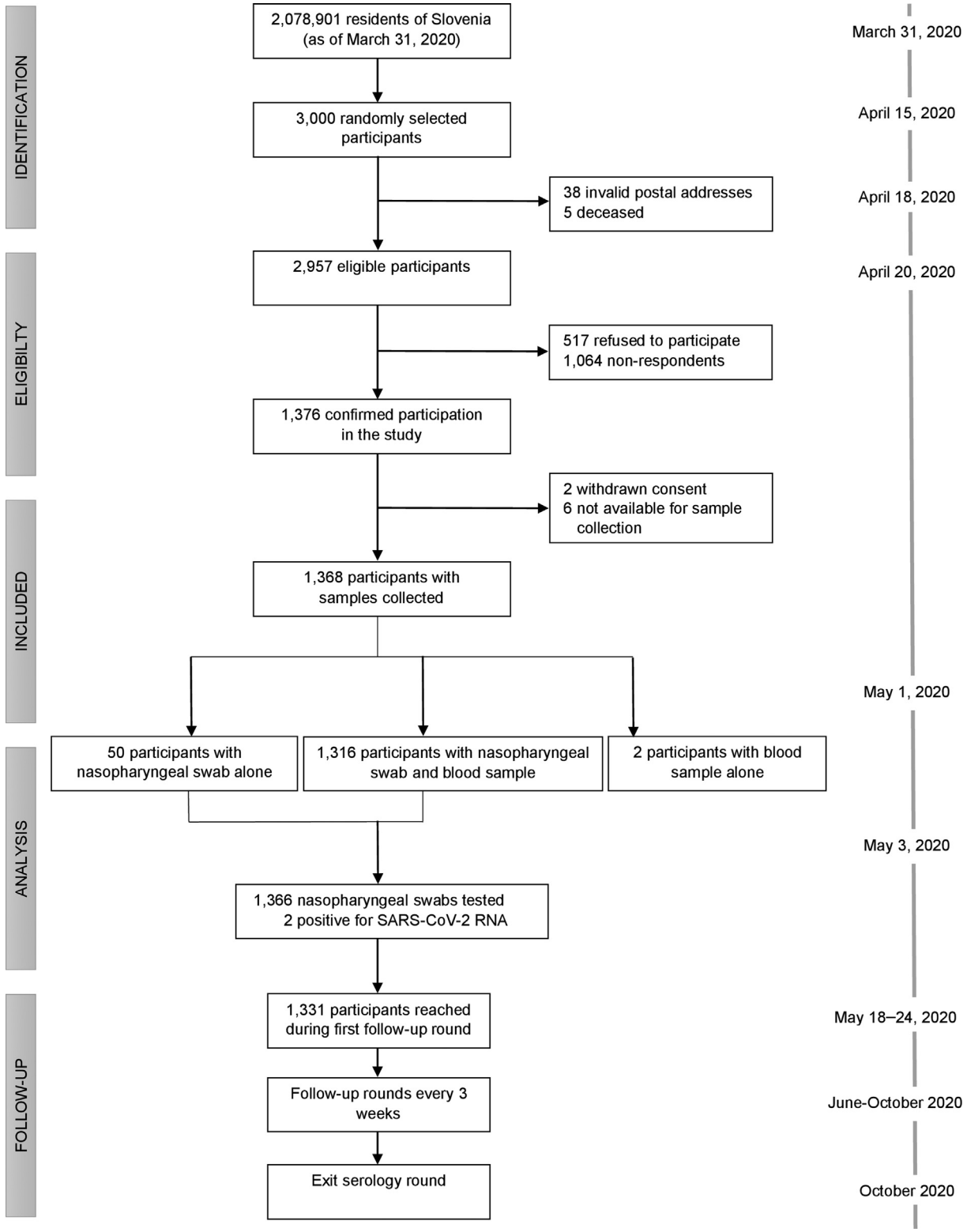

Fig. 1. Study design.

Therefore, the study results, as reported here, are based on the unweighted data.

Of 1366 nasopharyngeal swabs, two tested positive for SARS-CoV-2 RNA using the cobas 6800 SARS-CoV-2 assay, corresponding to a prevalence of 0.15% (posterior mean = 0.18%, 95% Bayesian CI 0.03–0.47; 95% highest density region (HDR) 0.01–0.41). Both cobas SARS-CoV-2 RNA–positive samples were additionally confirmed to be positive by two-target reverse transcriptase PCRs (SARS-CoV-2 specific and pan-*Sarbecovirus*) using commercially available primers and FAM-labeled hydrolysis probes [11]. No correction of the estimate of prevalence for sensitivity or specificity was performed. One participant was newly diagnosed with COVID-19 and one had previous PCR-confirmed SARS-CoV-2 infection; both participants experienced COVID-19–defining symptoms 2 and 5 weeks before study sampling, respectively.

Between 18 May and 24 May 2020, all enrolled participants were contacted again. Of 1331 participants (97.3%) reached by 24 May 2020, a total of 29 reported acute respiratory symptoms and/or fever during 3 weeks after initial sampling and were offered SARS-CoV-2 RNA testing. During detailed telephone medical consultation, for 22 participants it was jointly agreed not to test for SARS-CoV-2 RNA because of the high probability that the symptoms recalled were linked to other medical conditions. Finally, seven participants were tested for SARS-CoV-2 RNA; all had negative results. In addition, five participants informed us that they sought testing for SARS-CoV-2 RNA during the 3 weeks after the initial sampling at their own discretion and for nonmedical reasons; all were SARS-CoV-2 RNA negative and reported no COVID-19–compatible symptoms.

Discussion

Despite almost 12 million recorded cases, knowledge about the population COVID-19 burden is scant. To address this knowledge gap, the WHO recently recommended nationwide population-based, age-stratified epidemiologic surveys and designed an investigation study protocol to facilitate the collection and sharing of COVID-19 epidemiologic data in a standardized format [12]. Each country that performs such a survey may tailor different aspects of the study protocol (including the diagnostic approach) according to its public health, laboratory and clinical capacities, availability of resources and cultural acceptance [12]. However, as of early June 2020, very few population studies have been performed using a probability-based sample assessing the COVID-19 burden on a national or broader regional level, and even fewer have been published in the peer-reviewed literature [13,14].

To our knowledge, so far, the only peer-reviewed study surveying the active COVID-19 burden using a national probability-based sample was performed in April 2020 in Iceland [13]. In the probability-based sample arm, 2283 participants (20–70 years old) were tested, and 0.6% (95% CI 1.3–0.9) samples were positive for SARS-CoV-2 RNA. A similar prevalence (0.8%; 95% CI 0.6–1.0) was recorded in an open-invitation arm (10 797 participants), but it was significantly higher in the targeted-testing arm (13.3%). Although not directly comparable because of different testing approaches, different age populations tested (0–99 years vs. 20–70 years) and the different epidemic dynamic of both countries, the diagnostic yield of targeted testing in Slovenia was also expectedly higher (2.6%; 4.1% in the four diagnostically most intensive weeks) than that assessed in our study's probability-based sample (0.15%) (Fig. 2).

In addition to the Icelandic and Slovenian studies, our intensive language-nonrestricted literature search identified a non–peer-reviewed notice of two rounds of an Austrian cross-sectional study on a probability-based sample that estimated active COVID-19 prevalence at 0.33% (95% CI 0.12–0.76) in the first-round survey, which further decreased in the second-round survey [15–17]. A non–peer-reviewed CON-VINCE Luxembourgian study estimated an active COVID-19 prevalence of 0.3% (95% CI 0.03–0.56) in people 18 to 79 years old [18]. Although these studies surveyed different age populations using different diagnostic approaches and are highly sensitive to differences in timing within the epidemic curve, all yielded results comparable to our study. As a result of the non–peer-reviewed nature of the identified reports, direct comparison of results using a critical scientific approach is impossible [19,20]; however, this is temporary because many studies are currently underway or have reporting backlogs.

Although baseline blood samples were already collected, because of the current uncertainty regarding the accuracy of anti–SARS-CoV-2 assays (especially specificity and consequently low positive predictive value when testing low-prevalent and random populations) [21–24], we have decided to publish seroepidemiologic part of our study after collecting both baseline and exit blood samples for each study participant. We hope that in the meantime a consensus regarding reference-standard serologic assays will have been reached or some form of confirmatory algorithm for screen-positive results developed. A similar approach was also recently taken by the US Centers for Disease Control and Prevention [25].

As summarized in Fig. 2, as of 10 June 2020, a total of 86 994 SARS-CoV-2 tests (41 426 tests per million inhabitants) have been performed in Slovenia, which has detected 1488 laboratory-confirmed COVID-19 cases and reported 109 deaths (https://covid-19.sledilnik.org/en/stats). This study further confirmed the effectiveness of the draconian containment measures in Slovenia during the first wave of the epidemic, which were introduced simultaneously with the official declaration of the epidemic on 12 March 2020. On 16 March, public transport was shut down, and all educational institutions (preschools, schools and universities) and public institutions such as museums, libraries, theatres and sport facilities were closed. Nonessential medical procedures were cancelled, all nonessential shops and services were closed, and public gatherings were prohibited. In addition, international travel was restricted, and national borders were completely closed. On 29 March 2020, population mobility was further restricted to home municipalities, with strict police control. Containment and mitigation efforts, the early availability of reliable and clinically validated PCR tests, and prompt and central reporting of the results and immediate epidemiologic contact tracing were fundamental in limiting the epidemic's spread in Slovenia in its early phases. However, the national testing recommendations changed several times during the course of the epidemic, starting with a very conservative approach and initially testing only those with severe clinical presentation from 14 March to 7 April 2020 (mainly due to the limited supply of reagents and consumables), then later expanding recommendations to include patients with milder disease if they were over 60 or had any risk factors for a more severe disease course. In addition, the contact tracing recommendations also changed several times; unfortunately, limited personnel capacity meant that no direct epidemiologic contact tracing was in place and no quarantine officially introduced from 30 March to 20 April 2020.

There are some important limitations of our study that must be considered. Although SARS-CoV-2 RNA testing is indispensable for estimating the burden of active COVID-19 infections in epidemiologic surveys using a probability-based sample, the prevalence of disease assessed at a single time point can be predictive only for a limited time frame. Furthermore, such an approach has the limited potential to detect smaller focal outbreaks and is probably most appropriate in environment with low virus circulation. An additional limitation is that negative SARS-CoV-2 RNA testing result

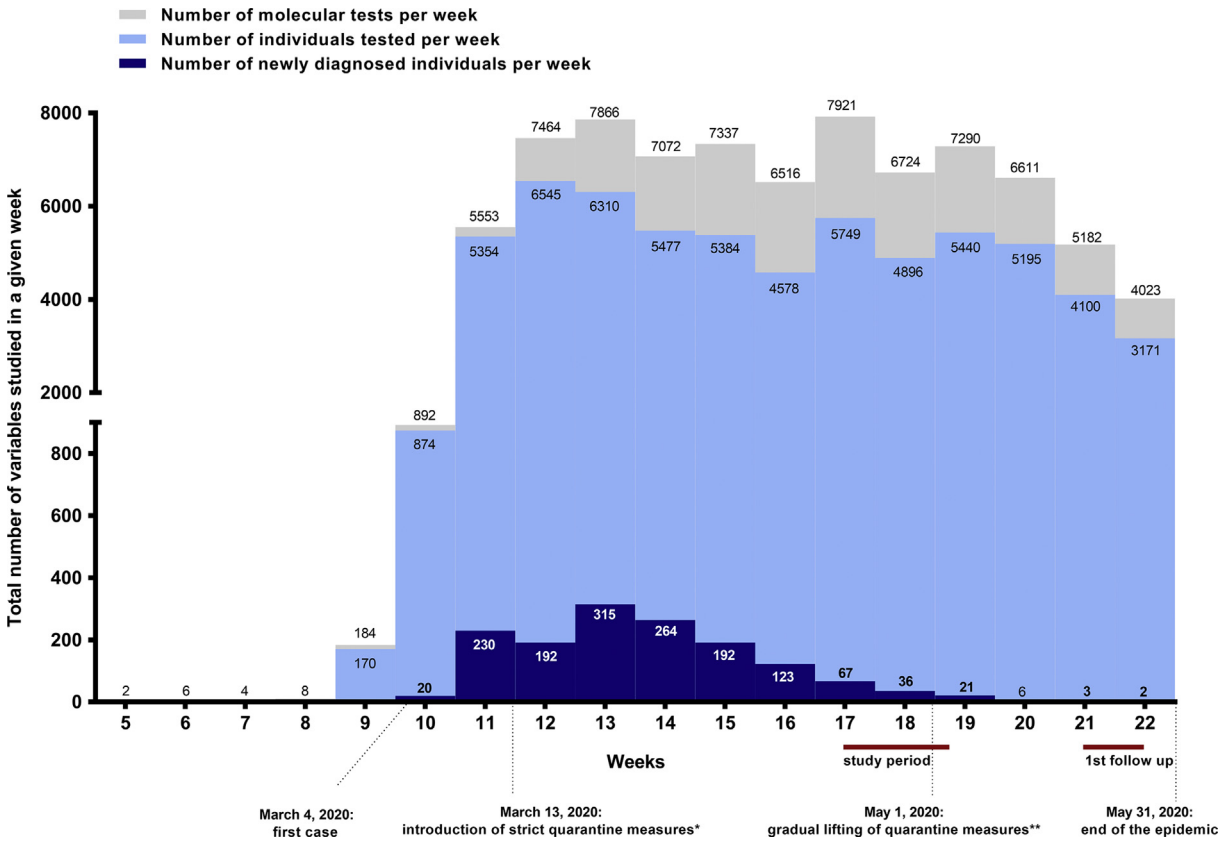

Fig. 2. Total number of molecular tests performed, individuals tested and newly diagnosed individuals with SARS-CoV-2 infection in a given week in the COVID-19 epidemic in Slovenia. On 4 March 2020, the first case of COVID-19 infection was confirmed in Slovenia. *As of 13 March 2020, strict quarantine measures were introduced, including closing the borders with neighbouring countries (Italy, Croatia, Austria and Hungary); closing preschools, primary schools, high schools and universities; shutdown of public transport; closure of all nonessential shops and services, including cancellation of nonessential medical procedures; and, later, restriction on movement outside one's municipality of residence. **The gradual lifting of quarantine measures started on 1 May 2020, with removal of restrictions on travel outside the municipality of residence, reopening all health and dental services, restarting public transport and reopening preschools and schools for selected age groups.

does not necessarily rule out COVID-19 if the sample is taken during a diagnostic window or in case of suboptimal quality of sampling. Lastly, nonrespondents could present potential limitation of our study. However, the study sample matched the population structure well, and weighting did not change the prevalence estimates. Additionally, for nonresponse bias, a content-specific missing data mechanism must exist linking participation with prevalence, and there is little evidence to support the assumption that persons who are more likely to be infected might also be more willing to participate. Thus, we believe that nonresponse bias in the present study is relatively small, and the study reliably estimated the true prevalence of active COVID-19 infection in Slovenia.

Properly designed and executed studies using probability-based sample combined with target-testing figures are extremely important for accurate and timely disease burden estimates and close monitoring of epidemic dynamics. They cannot be replaced by modeling studies and extensive testing campaigns using an open-invitation (nonprobability) sample. We believe that our study provides timely insight into the COVID-19 burden in the general population; our strategies may be considered a suitable alternative to more expensive large-scale testing campaigns using an open-invitation sample. The study results also confirmed the overall effectiveness of the timely, strict implementation of rigorous lockdown measures in Slovenia. The study contributed to the accurate prediction of disease dynamics and subsequent near disappearance of active cases in the following weeks. Only 37 new cases were identified in the entire country in the month after the study, despite extensive testing (25 093 tests; average 810 tests per day; average 385 tests per day per million inhabitants) (Fig. 2).

On the basis of the favourable epidemiologic situation in the country, supported by the study results, mobility restrictions within the country were lifted on 1 May 2020, followed by gradual reopening of healthcare and dental services (9 May), reopening of public transport (11 May) and partial opening of preschools and schools (16 May). All of this ultimately led to Slovenia's being the first country in Europe to officially declare the end of the first wave of the epidemic as of 31 May 2020. With close follow-up of our cohort, coupled with ongoing, extensive routine and commercial testing in the following weeks (500 to 900 tests per day per million inhabitants), we hope to be able to closely monitor the epidemic dynamics in the coming months and predict possible COVID-19 recurrence in Slovenia, allowing us to remain alert and prepared to take the necessary preventive measures in a timely manner.

Transparency declaration

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article or decision to submit the work for publication. All authors report no conflicts of interest relevant to this article.

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