



**University of Dundee**

## **SARS-CoV-2 positivity in asymptomatic-screened dental patients**

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# SARS-CoV-2 Positivity in Asymptomatic-Screened Dental Patients

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## Abstract

Enhanced community surveillance is a key pillar of the public health response to coronavirus disease 2019 (COVID-19). Asymptomatic carriage of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a potentially significant source of transmission, yet remains relatively poorly understood. Disruption of dental services continues with significantly reduced capacity. Ongoing precautions include preappointment and/or at appointment COVID-19 symptom screening and use of enhanced personal protective equipment (PPE). This study aimed to investigate SARS-CoV-2 infection in dental patients to inform community surveillance and improve understanding of risks in the dental setting. Thirty-one dental care centers across Scotland invited asymptomatic-screened patients aged over 5 y to participate. Following verbal consent and completion of sociodemographic and symptom history questionnaire, trained dental teams took a combined oropharyngeal and nasal swab sample using standardized Viral Transport Medium—containing test kits. Samples were processed by the Lighthouse Lab and patients informed of their results by SMS/email with appropriate self-isolation guidance in the event of a positive test. All positive cases were successfully followed up by the national contact tracing program. Over a 13-wk period (from August 3, 2020, to October 31, 2020), 4,032 patients, largely representative of the population, were tested. Of these, 22 (0.5%; 95% CI, 0.5%–0.8%) tested positive for SARS-CoV-2. The positivity rate increased over the period, commensurate with uptick in community prevalence identified across all national testing monitoring data streams. To our knowledge, this is the first report of a COVID-19 testing survey in asymptomatic-screened patients presenting in a dental setting. The positivity rate in this patient group reflects the underlying prevalence in community at the time. These data are a salient reminder, particularly when community infection levels are rising, of the importance of appropriate ongoing infection prevention control and PPE vigilance, which is relevant as health care team fatigue increases as the pandemic continues. Dental settings are a valuable location for public health surveillance.

**Keywords:** COVID-19, SARS virus, dentistry, outpatients, epidemiology, public health

## Introduction

The coronavirus disease (COVID-19) pandemic has wreaked a devastating global impact, causing harm to people’s health, society, and the economy (World Health Organization [WHO] 2020).

Trusted and reliable surveillance and epidemiology programs providing robust data are central to the efforts to monitor, understand, and respond to the pandemic—globally, these include the WHO (2020) and the Johns Hopkins Coronavirus Resource Center (2020), and in Scotland, there are Public Health Scotland (PHS; 2020a) resources.

The WHO declared COVID-19 a pandemic on March 11, 2020 (WHO 2020). At the time of this study (November 16, 2020), globally, there were over 54 million cases and over 1.3 million deaths estimated (rising to over 100 million cases and over 2 million deaths by end of January 2021) (Johns Hopkins Coronavirus Resource Center 2020).

Asymptomatic carriage of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a potentially significant source of transmission yet is poorly understood, with levels of

asymptomatic SARS-CoV-2 infection reported varying by setting, country, and over time (Oran et al. 2020). Asymptomatic positivity has ranged from as low as 5% in a small hospital

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A supplemental appendix to this article is available online.

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study from China in the early stages of the pandemic (Tian et al. 2020), to 17.9% on the cruise ship in Japan in February 2020 (Mizumoto et al. 2020), to 42.5% from the village-wide study in Italy during the first-wave lockdown (Lavezzo et al. 2020), and to 76.5% of those tested positive in the preliminary Office for National Statistics (ONS) population-level data from England (Petersen and Phillips 2020). In July 2020, a systematic review of 41 studies ( $n = 50,155$  participants) found a pooled percentage of 15.6% (95% confidence interval [CI], 10.1%–23.0%) but with significant heterogeneity and a very wide range (Kronbichler et al. 2020). In September 2020, 94 studies ( $n = 25,538$  participants) estimated 20% (95% CI, 17%–25%) asymptomatic carriage and suggested that inflated results from studies earlier on the pandemic were due to a limited range of symptoms being included (Buitrago-Garcia et al. 2020). More recently, in October 2020, a focused meta-analysis of 13 low risk of bias studies ( $n = 21,708$  participants) found 17% (95% CI, 14%–20%), with a range of 4% to 41% (Byambasuren et al. 2020). These reviews identified biases due to selection of study participants, calling for future population representative studies to determine the true proportion of asymptomatic SARS-CoV-2 infections as well as the prevalence in the population. Incomplete symptom assessment has also been considered important in overestimating the asymptomatic fraction (Meyerowitz et al. 2020).

An opportunity for dentistry to contribute to the COVID-19 testing and (asymptomatic) surveillance effort in Scotland was proposed on the basis that patients were already making an essential visit outside lockdowns and that dental teams could accurately screen patients to ensure they had no COVID-19 symptoms and could readily be trained to undertake COVID-19 swab testing. Dental teams would also already be wearing appropriate personal protective equipment (PPE), which would both maximize PPE use and save the need for additional PPE given shortages at the time. Moreover, National Health Service (NHS) dental settings in Scotland had previously been shown to be a feasible source of participants representative of the population for human papillomavirus (HPV) testing (Conway et al. 2016).

This study aimed to demonstrate the public health role of dental teams in population screening for SARS-CoV-2 infection in asymptomatic-screened dental patients and to inform community surveillance and understanding of asymptomatic SARS-CoV-2 infection.

## Materials and Methods

This article follows Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines (von Elm et al. 2008). The West of Scotland NHS Research Ethics Service waived the requirement for research ethics approval. Information Governance approval was obtained via PHS (DPIA; DP20210155). A protocol for testing asymptomatic patients attending dental settings was developed (PHS 2020b). Setup meetings were held with each health board in which training, including a standard training video (PHS

2020c), was cascaded to clinical sites, using combinations of online and in-person training.

Dental patients were thoroughly screened to be asymptomatic of COVID-19 symptoms by a clinician on arrival at selected dental centers across Scotland following national guidance (Scottish Dental Clinical Effectiveness Programme [SDCEP] 2020). Any patients recording a positive response to any symptom/sign questions were excluded. Other inclusion criteria were patients able and willing to give verbal consent and patients older than 5 y. If the clinician deemed participation would compromise patient care, the patient was not invited to participate. A patient information letter (available in several languages) was provided to patients prior to or on their arrival at the clinic (PHS 2020b). The patients were further informed of the process, and verbal consent was obtained and recorded in the clinical notes and on the test kit registration portal (UK Government 2020a). Patients attending multiple clinic visits did not have repeat swab tests as part of the dental surveillance program.

A member of the dental team used a questionnaire to obtain information from the patient. This documented sociodemographic information (forename, surname, date of birth, gender, postcode, Community Health Index [CHI number—the unique NHS identifier in Scotland], and ethnicity), medical history (via a checklist of “comorbid conditions”: cancer, diabetes, chronic liver disease, chronic kidney disease, rheumatological disease, chronic respiratory disease, heart disease or hypertension, immunodeficiency/organ transplant, neurological conditions [including stroke]), previous/current history of potential COVID-19 symptoms, self-reported “shielding” (the policy in Scotland in which people with preexisting medical conditions were asked by their general practitioner [GP] to stay at home and minimize all contact with others for 12 wk during the first lockdown from March 2020 [Scottish Government 2020a]), and public health behaviors (mask wearing, physical distancing, home disinfection, extra handwashing, and use of hand sanitizer). The patient information from the questionnaire was used to populate the UK Government web-based COVID-19 test swab registration form (UK Government 2020a), which in addition collected test kit barcode, date and time of swab, patient email address, and mobile phone number.

In the dental surgery either prior to or after treatment, a combined oropharyngeal and nasal swab, using standardized Viral Transport Medium-containing (“non-Randox”) test kits, was taken by an appropriately trained member of the dental team wearing PPE and following infection protection control (IPC) guidance (NHS National Services Scotland 2020; NHS Scotland 2020). The swabs were transported to the Lighthouse Lab in Glasgow (LliG) (UK Government 2020b).

The testing system used at the LliG consists of the ThermoFisher Scientific KingFisher Flex System Nucleic acid extraction, the TaqPath COVID19 CEIVD RTPCR Kit, a multiplex assay that targets 3 SARS-CoV-2 genomic regions (ORF1ab, S protein, and N protein) and bacteriophage MS2 (internal control), and it is run on the Applied Biosystems 7500 Fast RealTime PCR Instrument (used with 7500 Software

v2.3). Data were analyzed using UgenTec Fast Finder 3.300.5 (TaqMan 2019-nCoV Assay Kit V2 UK NHS ABI 7500 v2.1; Ugentec). The assay plugin contains an assay-specific algorithm and decision mechanism that allows conversion of the qualitative amplification assay polymerase chain reaction (PCR) raw data from the ABI 7500 Fast into test results with minimal manual intervention. Samples are called positive in the presence of at least single N gene and/or ORF1ab but may be accompanied with the S gene (1, 2, or 3 gene positives). The S gene was not considered a reliable single gene positive.

Patients were informed directly of their results (positive, negative, or void) by SMS/email, which included guidance to self-isolate for those with a positive test. Positive test results entered the Case Management System (CMS) used by health protection teams in local boards for contact tracing follow-up (known as the NHS Scotland's "Test and Protect" system; NHS Inform 2020). Those patients with void (test "could not be read") results were followed up by the National Steering Group, as the standard SMS message (intended primarily for symptomatic testing) recommended a retest, which was not required for an unclear asymptomatic surveillance result. Local health protection teams followed up all positive tests with clinical teams in the dental settings to confirm IPC guidance was followed (PHS 2020b).

Positivity percentages (rates) with 95% CIs were calculated using Wilson's method (and 3-wk rolling averages) on a weekly basis and incorporated into the PHS enhanced surveillance of COVID-19 in Scotland weekly report for the Scottish Government. Rates and 95% CIs were similarly calculated for the 13 wk overall (and also for the first 6 wk and second 7 wk). For data visualization comparison, routine daily testing data for the same time period were extracted from the PHS daily positive test monitoring dashboard (PHS 2020a). Growth rates were estimated using Poisson regression models with a log link, and an interaction test was used to compare the growth rate between our dental and general (medical) practice (GP) primary care surveillance data (PHS 2020d).

Participant characteristic proportions and confidence intervals were calculated using Wilson's method. These were compared against population demographics by linking our data into the Early Pandemic Evaluation and Enhanced Surveillance of COVID-19 (EAVE-II) linked data set in PHS, which has demographic and GP clinical information from the whole population of Scotland (Simpson et al. 2020). Population weightings were calculated via comparing the participants to the EAVE-II data set on age group, socioeconomic deprivation, and comorbid risk groups. Gender was not included in the calculation of the weights as the proportions of men and women in our dental surveillance sample matched the population proportions.

Analysis was undertaken to investigate the impact of using an imprecise test by assessing the impact on positivity by various scenarios of specificity and sensitivity. Very high levels of specificity were used in line with data from the Office for National Statistics (ONS; 2020a) (99.92%) and PHS (unpublished; 99.68%), alongside lower levels of sensitivity, as in ONS (2020a), of 60% (with 95% probability between 45% and 75%) or medium sensitivity of 90% (with 95% probability

between 85% and 95%). This was carried out by a parametric bootstrap analysis with specificities and sensitivities coming from beta distributions and test positivity from a binomial. All analyses were undertaken in BusinessObjects (SAP), SPSS (SPSS, Inc.), and R version 3.6.1 (Free Software foundation's GNU General Public License).

## Results

By week 13 of the program, 31 dental centers from 13 of the 14 health boards across Scotland were recruiting patients into the program. Tests were carried out by 287 dental team members. There were 4,032 tests processed during this period. Compared to the Scottish COVID-19 surveillance population distribution, the sample had a similar sex distribution, slightly fewer participants from the least deprived communities, and fewer children (Table 1). The sample had similar numbers of recorded comorbid conditions but also had a high proportion (9.9%) of participants who self-reported that they had been shielding compared with the population numbers reported by PHS ( $n = 179,728$ ; 3.3% of the population; Scottish Government 2020a).

There were 22 positive tests in total during the 13 wk (Table 2; cycle thresholds [Ct] reported in Appendix Table 1), with an overall test positivity rate of 0.5% (95% CI, 0.4%–0.8%). None of the positive tests had the S-gene dropout (Appendix Table 1) suggestive of the "new UK variant" referred to as SARS-CoV-2 VUI 202012/01. Analysis with population weighting had a minimal impact on the overall population percentage positive (0.6%; 95% CI, 0.4%–0.9%). There was an increase from an average of 0.0% (95% CI, 0.0%–0.4%) during the first 6 wk when no patients tested positive to 0.7% (95% CI, 0.5%–1.1%) in the latter 7 wk (Table 2 and Fig. 1). This trend followed the second-wave uptick observed in the daily positive test data (Fig. 2).

Over the period from week 30 to week 44, the estimated growth in the epidemic measured via the GP Primary Care Surveillance Program of symptomatic patients was 0.31 (95% CI, 0.28%–0.35%) per week (data not shown; PHS 2020d; Simpson et al. 2020) and for the dental surveillance of asymptomatic individuals was 0.29 (95% CI, 0.10%–0.48%) per week. There was no difference in growth rates ( $P = 0.92$ ), although the dental growth rate was estimated with lower precision due to the small numbers of positive cases.

We estimated the adjusted positivity for both a very high or high specificity each combined with moderate or low sensitivity. With a very high specificity (99.92%) and moderate sensitivity of 90%, the adjusted overall positivity was 0.5% (0.3%–0.8%). With a very high specificity (99.92%) and a low sensitivity of 60%, the adjusted overall positivity increased to 0.8% (0.4%–1.4%). With a slightly lower specificity (99.68%) and moderate sensitivity (90%), the adjusted positivity rate was 0.3% (0.0%–0.6%). And at the slightly lower specificity (99.68%) with low (60%) sensitivity, the adjusted positivity was 0.4% (0.0%–1.0%).

Of the tests that were classified "void" ( $n = 170$ , 4.2%), the majority ( $n = 108$ ) occurred in a 3-wk (weeks 5–7) cluster

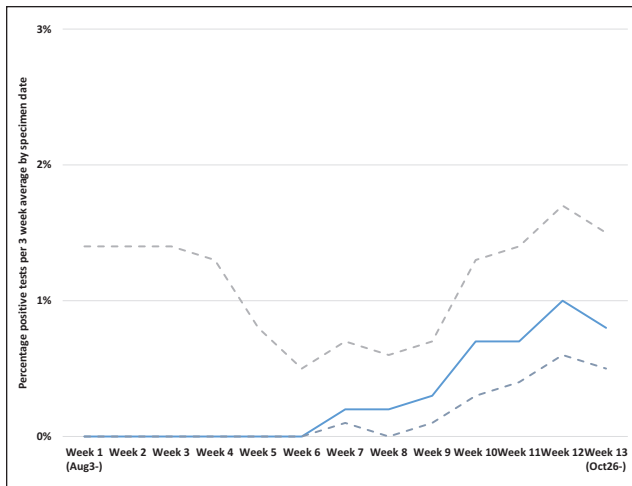
**Table 1.** Characteristics of Participant Dental Patients with Swab Test Results (N = 4,032).

Category	No. (%)	95% CI	Pop. %
<b>Age, y</b>			
5 to 18	218 (5.4)	4.8–6.1	15.6
19 to 44	1,710 (42.4)	40.9–43.9	35.4
45 to 64	1,332 (33.0)	31.6–34.5	28.9
65+	703 (17.4)	16.3–18.6	20
Missing	69 (1.7)	1.4–2.2	
<b>Sex</b>			
Female	2,137 (53.0)	51.5–54.5	51.3
Male	1,877 (46.6)	45–48.1	48.7
Unknown/missing	18 (0.4)	0.3–0.7	
<b>SIMD 2020</b>			
1 Most deprived	864 (21.4)	20.2–22.7	20.1
2	868 (21.5)	20.3–22.8	19.6
3	765 (19.0)	17.8–20.2	19.5
4	761 (18.9)	17.7–20.1	19.8
5 Least deprived	514 (12.7)	11.8–13.8	20.0
Missing	260 (6.4)	5.7–7.2	1.0
<b>Ethnicity</b>			
Black and minority ethnic	161 (4.0)	3.4–4.6	—
White	3,779 (93.7)	92.9–94.4	—
Missing	92 (2.3)	1.9–2.8	—
<b>Comorbid conditions</b>			
0	1,666 (41.3)	39.8–42.8	59.3
1	692 (17.2)	16.0–18.4	16.0
2	549 (13.6)	12.6–14.7	14.2
3 to 4	353 (8.8)	7.9–9.7	8.6
5	61 (1.5)	1.2–1.9	2.0
Missing	711 (17.6)	16.5–18.8	
<b>Shielding group</b>			
Yes	401 (9.9)	9.1–10.9	—
No	3,624 (89.9)	88.9–90.8	—
Missing	7 (0.2)	0.1–0.4	—

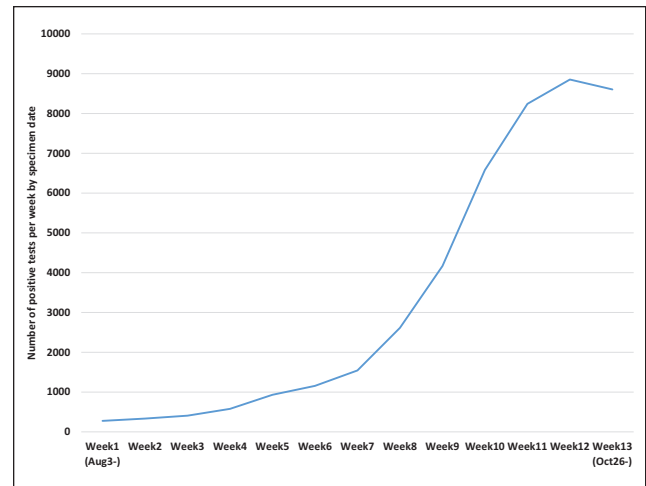
Pop., population distribution of known variables; SIMD, Scottish Index of Multiple Deprivation; —, not available in population data set.

**Table 2.** Numbers of Samples, Test Results, and Percentage Positive for Severe Acute Respiratory Syndrome Coronavirus 2 per Week (by Specimen Date; Dental Settings; Scotland; August 3, 2020, to October 31, 2020).

Week (Date)	No. of Samples	No. of Void Tests	No. of Positive Tests	% Swab Positive	95% CI
Week 1 (August 3–)	72	1	0	0	0–5.1
Week 2	109	1	0	0	0–3.4
Week 3	87	3	0	0	0–4.2
Week 4	85	0	0	0	0–4.3
Week 5	317	43	0	0	0–1.2
Week 6	334	55	0	0	0–1.1
Week 7	390	16	2	0.5	0.1–1.9
Week 8	397	7	0	0	0–1.0
Week 9	398	2	1	0.3	0–1.4
Week 10	419	13	7	1.7	0.8–3.4
Week 11	432	5	1	0.2	0.0–1.3
Week 12	510	9	6	1.2	0.5–2.5
Week 13 (October 26–)	482	15	5	1.0	0.4–2.4
Total (weeks 1–6)	1,004	103	0	0	0–0.4
Total (weeks 7–13)	3,028	67	22	0.7	0.5–1.1
Total (weeks 1–13)	4,032	170	22	0.5	0.4–0.8



**Figure 1.** Percentage positive tests (solid line) with upper and lower 95% confidence intervals (dashed lines) per 3-wk average by specimen date; dental settings; Scotland; August 3, 2020, to October 31, 2020.



**Figure 2.** Number of positive tests per week by specimen date; all settings Scotland; August 3, 2020, to October 31, 2020—PHS (2020a) COVID-19 monitoring dashboard data.

(Table 2). At this time, testing demand frequently exceeded laboratory processing capacity, causing delays (Scottish Government 2020b). Indeed, analysis of the time between date of sample taken and date of processing in the lab was significantly ( $P < 0.01$ ) longer in the void samples (median 5.0 d, mean 4.2 d) compared with the processed (median 3.0 d, mean 3.1 d) test groups (data not shown).

## Discussion

To the best of our knowledge, this is the first report of SARS-CoV-2 positivity in asymptomatic dental patients and documentation that asymptomatic carriage in this population shows a pattern of epidemic growth consistent with the surveillance of symptomatic individuals (PHS 2020a, 2020d; Simpson et al. 2020).

Our results followed the trends in the national recorded positive tests by specimen date (Fig. 2), which had under 1,000 tests per week reported across all testing in Scotland in the comparable first 5 wk, rising to nearly 9,000 cases per week by week 12 (PHS 2020a). However, true comparisons with these data are not possible because the denominator of numbers of tests taken is not reported, and these data will include both symptomatic and asymptomatic. Our results are also similar to those from the ONS Coronavirus (COVID-19) Infection Survey, which commenced in Scotland in October 2020—a large household population-based study assessing the incidence of infection in the UK general population. Data are produced fortnightly and have shown a rise from 0.6% (95% CI, 0.4%–0.9%) at the beginning of October 2020 to 0.9% (95% CI, 0.6%–1.2%) by the end of the month (Scottish Government 2020c). The ONS survey has no symptom-related exclusion criteria, and analyses from the pilot study in England found three-quarters ( $n = 88/115$ ) of the participants who tested positive for SARS-CoV-2 were asymptomatic among the total

36,061 tested between April 26 and June 27, 2020 (Petersen and Phillips 2020). A more recently published systematic review (Yanes-Lane et al. 2020) identified only 2 general population studies, which found the proportion of asymptomatic COVID-19 at the time of testing to be 20% in Luxembourg ( $n = 1$  out of the 5 positives from 1,842 tested) (Snoeck et al. 2020) and 75% in Italy ( $n = 6$  out of the 8 positives from 2,322 tested) (Lavezzo et al. 2020). In the same systematic review, they identified 5 small cohorts of obstetric patients from the United States and Japan—the proportion of asymptomatic patients was between 84% and 100% in the smallest studies (none larger than  $n = 155$ ) and 45% in the biggest (20 out of 757 patients with positive tests).

There have been relatively few studies investigating SARS-CoV-2 infection in COVID-19 asymptomatic-screened patients in clinical outpatient health care (including dental) settings. One survey conducted in outpatient gastrointestinal clinics prior to endoscopy found 3 from 2,611 (0.1%; 95% CI, 0.0%–0.3%) asymptomatic patients tested positive for SARS-CoV-2 (Hayee et al. 2020). Other clinical-based testing studies have been undertaken in patients admitted to the hospital, for example, prior to surgery (Gruskay et al. 2020).

The estimates provided in our surveillance study are percentage of dental patients testing positive for the SARS-CoV-2 (the positivity rate). We were unable to report the population prevalence rate because without a true gold-standard diagnostic test, we do not know the accurate sensitivity (true-positive rate) and specificity (true-negative rate). Given the low number of positive tests in the study, even if all the positives were false, the specificity would be very high, confirmed in the ONS population pilot in England (Pouwels et al. 2021). The test sensitivity has previously been estimated to be between 85% and 98% (Pouwels et al. 2021). As underlying prevalence rises, high test specificity would not lead to changes in false positives; however, with low test sensitivity, there would be an increase in the numbers of false negatives, and the proportion

of all positives that are false would also decrease (ONS 2020b). We explored the potential impact of varying degrees of specificity and sensitivity. As our program uses the same Lighthouse Lab testing as the ONS survey, the most likely scenario takes their reported higher specificity level (Pouwels et al. 2021) alongside the moderate level of sensitivity (which is the most likely scenario as the underlying population prevalence has been rising over the survey period) (ONS 2020b). In this scenario, we find our overall positivity rate unchanged.

The Ct values show a significant number of positive tests, which were reported as “weak/close to the limit of detection”; while they are likely true analytical positives, the health protection teams would ordinarily risk assess and propose a repeat test. In practice, in the height of the pandemic, these positives (irrespective of symptoms) were taken at face value, and all patients were advised to self-isolate and followed up for contact tracing. Similarly, the sheer volume of testing from multiple locations and surveillance streams during the pandemic caused lab capacity and delay issues in processing, which led to some test results being void.

This surveillance program had several advantages, including using trained dental teams for the collection of high-quality and complete data and samples, although supervised self-collected specimens were recently found to perform similarly to clinician-collected samples (Kojima et al. 2020). Moreover, there was no need for the clinical teams to use additional PPE as they were already wearing it to provide dental care, and the patients could attend for their treatment despite periods of lockdown restriction. There was a thorough process of clinicians screening to ensure patients were asymptomatic. However, participants were not followed up; therefore, we cannot determine if patients were truly asymptomatic or actually presymptomatic and developed symptoms subsequently. Current evidence from other longitudinal studies suggests that three-quarters of those with a PCR positive test in the absence of symptoms remain asymptomatic (Oran and Topol 2021). All dental settings where positive tests were detected were followed up by local health protection teams, and in all cases, assurances were given that IPC guidance had been followed.

Convenience sampling comprised dental patients who volunteered to participate; it was not possible to collect information on nonparticipants, but it delivered a sample largely representative of the Scottish population. Although large numbers of dental patients were recruited, the number of participants with positive test outcomes was too small to test for associations between positivity and demographic characteristics. However, there were sufficient numbers to monitor the trends of SARS-CoV-2 in the asymptomatic population over time. This dental surveillance program continued through the winter.

## Conclusions

To our knowledge, this is the first COVID-19 surveillance survey in dental settings, and we have demonstrated the feasibility of developing and implementing a surveillance testing protocol at a rapid pace in response to the pandemic. Participating patients were largely representative of the Scottish population.

The positivity rate in this patient group reflects the underlying prevalence in the community at the time.

Our data have contributed to Public Health Scotland’s enhanced surveillance work, which is monitoring how COVID-19 is spreading through the population of Scotland via collation of a wide variety of data about COVID-19 from a range of sources. As the pandemic has evolved, this surveillance work has supported the Scottish Government with the national response to the pandemic.

These data are a salient reminder, particularly when community infection levels are rising, of the importance of appropriate ongoing infection prevention control and PPE vigilance, which is relevant as health care team fatigue increases as the pandemic continues.

Our data suggest that dental settings are a valuable location for public health surveillance.

## Author Contributions

D.I. Conway, contributed to conception, design, data analysis, and interpretation, drafted the manuscript; S. Culshaw, contributed to conception, design, data acquisition, and interpretation, drafted the manuscript; M. Edwards, contributed to conception, design, and data interpretation, drafted the manuscript; C. Clark, C. Watling, contributed to data analysis, critically revised the manuscript; C. Robertson, contributed to data analysis and interpretation, critically revised the manuscript; R. Braid, E. O’Keefe, N. McGoldrick, J. Burns, S. Provan, contributed to data acquisition, critically revised the manuscript; H. VanSteenhouse, J. Hay, R. Gunson, contributed to data acquisition and analysis, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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