Title: National population prevalence of antibodies to SARS-CoV-2 in Scotland during the first and second waves of the COVID-19 pandemic

Abstract

Objectives: Studies that measure the prevalence of antibodies to SARS-CoV-2 ("seroprevalence") are essential to understand population exposure to SARS-CoV-2 among symptomatic and asymptomatic individuals. We aimed to measure seroprevalence in the Scottish population over the course of the COVID-19 pandemic – from prior to the first recorded case in Scotland, through to the second pandemic wave.

Study design: Serial cross-sectional.

Methods: We tested 41,477 residual samples retrieved from primary and antenatal care settings across Scotland for SARS-CoV-2 antibodies over a 12-month period from December 2019-December 2020 (pre-rollout of COVID-19 vaccination). Five-weekly rolling seroprevalence estimates were adjusted for the sensitivity and specificity of the assays and weighted to reference populations. Temporal trends in seroprevalence estimates and weekly SARS-CoV-2 PCR positive notifications were compared.

Results: Five-weekly rolling seroprevalence rates were 0% until the end of March, when they increased contemporaneously with the first pandemic wave. Seroprevalence rates remained stable through the summer (range 3%-5%) during a period of social restrictions, following which they increased concurrently with the second wave, reaching 9.6% (95% CI 8.4%-10.8%) in week beginning 28th December 2020. Seroprevalence rates were lower in rural vs. urban areas (adjusted odds ratio[AOR] 0.70, 95% CI 0.61-0.79) and among individuals aged 20-39 and 60+ (AORs 0.74, 95% CI 0.64-0.86, and 0.80, 95% CI 0.69-0.91, respectively) relative to those aged 0-19 years.

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Conclusions: After two waves of the COVID-19 pandemic, less than one in ten individuals in the Scottish population had antibodies to SARS-CoV-2. Seroprevalence may underestimate the true population exposure as a result of waning antibodies among individuals who were infected early in the first wave.

Keywords: SARS-CoV-2, COVID-19, seroprevalence, antibodies, cross-sectional

Text word count: 1,475

The first severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in Scotland was reported on 1 March 2020. The country has since experienced two waves of COVID-19 (the disease associated with SARS-CoV-2), which has resulted in one of the highest excess mortality rates worldwide. Estimates of the proportion of individuals in the population who have been exposed to SARS-CoV-2 – both symptomatic and asymptomatic – are necessary to understand the progression of the pandemic to date. Since PCR testing in the general population until recently has mainly been limited to those with symptoms, seroprevalence studies, which measure antibodies to SARS-CoV-2, provide a means of measuring population exposure. Furthermore, it is essential to have estimates of the extent of exposure from wild-type infection prior to rollout of vaccination. Most population-based seroprevalence surveys to date have focused on short time frames or sub-national populations; here, we examine serial cross-sectional estimates of seroprevalence over a 12-month period in the Scottish general population, using residual blood samples from different sources.

Residual blood samples, originally collected for other purposes, were identified from primary care (i.e. general practice) and antenatal care settings. Antenatal samples were retrospectively identified at regional laboratories across Scotland that store specimens taken for infectious disease screening at week 12 of pregnancy; these specimens are submitted from all antenatal settings in the regional health authority areas ("National Health Service (NHS) Boards") that are covered by these laboratories. Samples were available from ISO-week 1 (week beginning (w/b) 30th December 2019), to ISO-week 26 (w/b 29th June 2020); all available samples with sufficient volume were included (totaling 16,157). Primary care samples were retrieved from regional laboratories, which receive samples from general practices across the NHS Boards that they cover. Approximately 700 weekly samples were obtained between ISO week 17 (w/b 20th April 2020) and ISO-week 53 (w/b 28th December 2020), totaling 25,320

samples. Primary care samples were selected according to an age/sex/NHS Board sampling frame corresponding to the Scottish general population structure. Eleven and seven out of the 14 NHS Boards in Scotland participated in the primary care and antenatal surveillance, respectively, covering 91% and 73% of the Scottish population. This analysis was restricted to samples up until the end of December 2020 in order to measure exposure to wild-type infection and not vaccine response: vaccination rollout for target groups began in Scotland on 8th December 2020; however, given the lag time between exposure to vaccination and seroconversion,[1,2] antibodies resulting from vaccine response would likely not be detectable in seroprevalence estimates until early January 2021.

Samples were anonymised prior to testing: only age (and sex for primary care samples) and NHS Board were attached to the result. Primary care samples were sent to the Scottish Microbiology Reference laboratory, where they were tested for the presence of anti-SARS-CoV-2 IgG antibodies using the LIAISON®SARS-CoV-2 S1/S2 IgG assay (DiaSorin, Italy). Antenatal samples were tested at the regional laboratories where they were stored using either the Abbott (Abbott SARS-CoV-2 N IgG), Roche (Roche Elecsys Anti-SARS-CoV-2 N IgM/ IgG) or Siemens (Siemens Atellica IM 1300 S1 total antibody) assay, depending on the laboratory where testing was undertaken. We identified positive results in the antenatal samples in the weeks prior to the first confirmed case of COVID-19 in Scotland (n=9). While there is evidence to suggest that COVID-19 was circulating before it was first identified in numerous countries,[3,4] we considered the possibility that these were false positives and therefore confirmatory testing was undertaken by re-testing on a different assay (Roche/Siemens) to the original (Abbott). Samples were re-tested (one re-test per sample) in ascending chronological order until 10 consecutive negative samples were obtained. The confirmatory result is presented here.

Local unpublished evaluations determined sensitivities and specificities of the assays (Table 1). Seroprevalence rates were adjusted for sensitivity and specificity,[5] and weighted to a reference

population (the Scottish general population for primary care; maternities in Scotland for antenatal), using bootstrap methods. Five-weekly rolling seroprevalence estimates were calculated to smooth out week-to-week variation; these were plotted against the last week in each five-week grouping (e.g. weeks 17-21 were plotted at week 21). Seroprevalence estimates were compared to SARS-CoV-2 PCR positive notifications obtained from Public Health Scotland. Weekly totals for the latter were plotted against a 3-week lag, to account for the delay between exposure to the virus and seroconversion (estimated to be between 2-4 weeks).[6] Chi-square tests for trend (χ^2_{trend}) were conducted on the weekly unadjusted seroprevalence data to determine statistical significance. Mantel-Haenszel odds ratios, adjusted for ISO-week, were calculated to examine differences in seroprevalence by sex, age group, and region (categorized into urban/rural) among the primary care samples.

Rolling 5-weekly seroprevalence rates (Figure 1a) in the antenatal samples were 0% from ISO weeks 5 (w/b Monday 27th January 2020) through to 13 (w/b Monday 23rd March), then subsequently increased from 0.1% (95% CI 0.0%-0.3%) in ISO-week 14 to 2.8% (95% CI 2.2%-3.5%) in ISO-week 20 (χ^2_{trend}=14.72, p=0.0001). The increase in seroprevalence occurred contemporaneously with the increase in SARS-CoV-2 PCR positive cases associated with first wave of the COVID-19 pandemic in Scotland (Figure 1b). Seroprevalence estimates were comparable between the two sources across the weeks where data were available from both (ISO-weeks 21-26). The primary care seroprevalence estimates were stable across the period until approximately ISO-week 43 (w/b 19th October 2020) (χ^2_{trend}=0.41, p=0.5241), at which point there was a sharp increase, reaching 9.6% (95% CI 8.4%-10.8%) in ISO-week 53 (w/b 28th December 2020) (χ^2_{trend}=23.28, p<0.001). This second increase in seroprevalence occurred concurrently with the increase in SARS-CoV-2 cases associated with the second wave of the pandemic (Figure 1b).

Seroprevalence rates by sex, age, and region are presented in the supplementary files. Seroprevalence between males and females (Figure S1) was similar across the time series (p=0.8318; Table S1).

Seroprevalence was lower in the 20-39 and 60+ age groups compared to those aged 0-19 years ($p<0.001$ and $p=0.0013$, respectively; Table S1, Figure S2). Regional comparisons showed higher seroprevalence in NHS Boards with large urban centres compared to those with primarily rural populations ($p<0.001$; Table 1, Figures S3 and S4).

Our findings provide an indication of the general population exposure to SARS-CoV-2 in Scotland, prior to the mass rollout of vaccination. To our knowledge, this is the first study to present national seroprevalence rates over two waves of infection. Our results are consistent with no population exposure in the early months of 2020, and a sharp increase in seroprevalence associated with onset of the first pandemic wave in March 2020. The stable seroprevalence rates thereafter coincide with a period of varying intensity of social restrictions imposed from the first lockdown, on 23rd March, onward. A second sharp increase in seroprevalence was observed from October, coinciding with the second pandemic wave. Our seroprevalence findings are consistent with the PCR data during the period of restrictions from March to October; it is likely that the infection rates across this period, which otherwise would have caused an increase in seroprevalence, were counterbalanced by waning antibody among individuals who had been exposed early on in the pandemic.[7] We also found that seroprevalence rates were higher in urban areas and in those aged 0-19 years.

Despite two waves of infection, our estimate of seroprevalence of 9.6% at the end of December 2020 suggests that the majority of the Scottish population has still not been exposed to SARS-CoV-2. This finding is corroborated by seroprevalence data from other western countries in similar phases of the pandemic: a study of blood donors/pregnant women in Stockholm, Sweden, showed approx. 15% seroprevalence by mid-Dec 2020 (this is comparable to our estimates from urban areas such as Greater Glasgow, which approached 13% by end December).[8] A household survey from Geneva found a slightly higher seroprevalence of 21%.[9]

Seroprevalence may, however, underestimate true population exposure for several reasons: lack of antibody persistence[7] and the role of other immune responses in neutralizing infection (there is evidence that some individuals who are exposed to SARS-CoV-2 do not develop measurable antibodies, suggesting the role of cellular immunity).[10] The ability of binding antibodies to confer immunity to SARS-CoV-2 infection is also not known and antibody neutralisation activity may be less among asymptomatic individuals.[11]

Limitations of our study include the uncertainty in the sensitivity and specificity of the assays, and uncertainty with regard to the representativeness of our samples with regard to the general Scottish population. To address this, we weighted the data to standard reference populations to account for any oversampling according to age/sex/geography; furthermore, primary care results are very consistent with data from a general household survey undertaken across Scotland.[12] The seroprevalence rates from antenatal samples were slightly lower than those from primary care: we hypothesise that pregnant women were taking extra precautions to avoid infection in the context of the COVID-19 pandemic.

Author statements

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Competing interests: PM owns shares of Astra Zeneca. The remaining authors have no competing interests to declare.

Ethical approval: Approval for the COVID-19 serological surveillance work was given by the Head of Information Governance and Statistical Governance at Public Health Scotland.

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180 **Table 1.** Sensitivities and specificities of the assays used to test samples for antibodies to SARS-CoV-2

Assay	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**Samples tested**
Abbott	95.5% (95% CI 90.4%-98.3%)	99.8% (98.8%-100.0%)	Antenatal
DiaSorin	87.5% (95% CI 78.2%-93.8%)	98.6% (95%CI 97.0%-99.5%)	Primary care
Roche	92.3% (95% CI 85.4%-96.6%)	100% (95% CI 98.7%-100.0%)	Antenatal
Siemens	98.5% (95% CI 95.7%-99.7%)	100% (95% CI 98.4%-100.0%)	Antenatal

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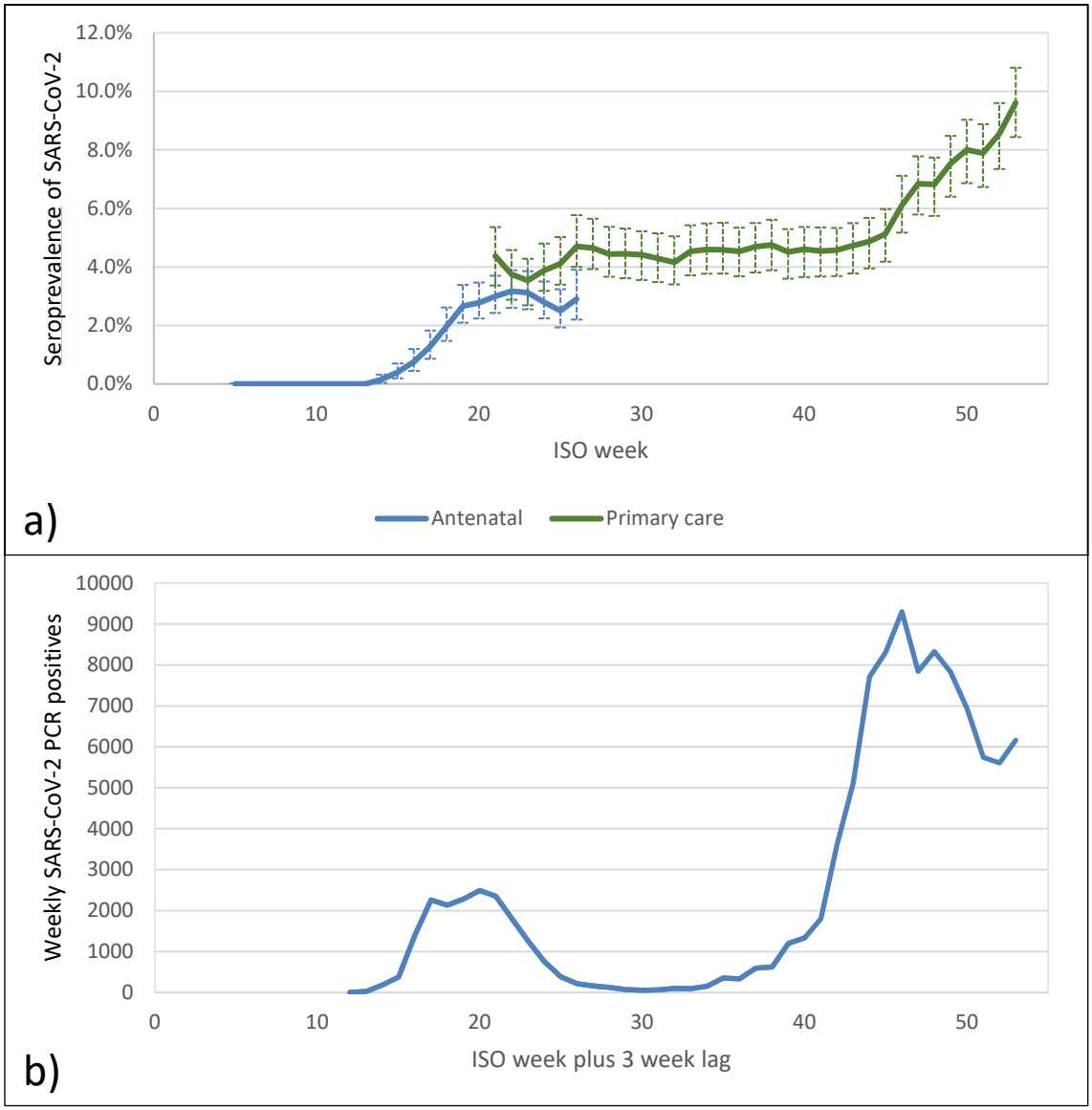

Figure 1. Comparison of (a) 5-weekly rolling SARS-CoV-2 seroprevalence by source of residual samples with (b) confirmed weekly SARS-CoV-2 PCR positives reported to Public Health Scotland. Dashed lines indicate 95% confidence intervals. SARS-CoV-2 PCR positives have been plotted against ISO week +3 on the x-axis to account for the delay between exposure to the virus (infection) and formation of antibodies (seroconversion).

189 **SUPPLEMENTARY FILES**

190 **Table S1.** Adjusted Mantel-Haenszel odds ratios for the association between covariates and SARS-CoV-2

191 seroprevalence: data from residual samples obtained from primary care across Scotland[a]

Model	Covariate	AOR_{MH} (95% CI)	p-value
1 (Sex)	Male	1 (Ref)	
	Female	1.01 (0.91-1.12)	0.8318
2 (Age group)	0-19 years	1 (Ref)	
	20-39 years	0.74 (0.64-0.86)	<0.001
	40-59 years	0.89 (0.77-1.03)	0.1104
	60+ years	0.80 (0.69-0.91)	0.0013
3 (Regional urban/rural classification)	Urban	1 (Ref)	
	Rural	0.70 (0.61-0.79)	<0.001

192 AOR_{MH} = adjusted Mantel-Haenszel odds ratio; CI = confidence interval

193 [a]All models have been adjusted for ISO-week

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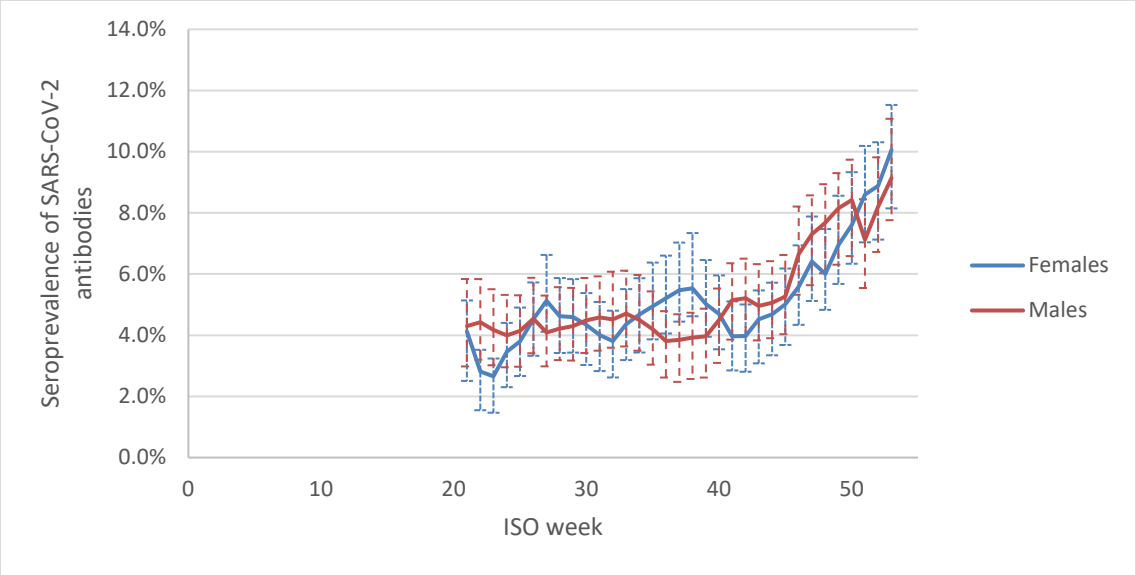


Figure S1. 5-weekly rolling SARS-CoV-2 seroprevalence by sex: data from residual samples obtained from primary care across Scotland. Dashed lines indicate 95% confidence intervals.

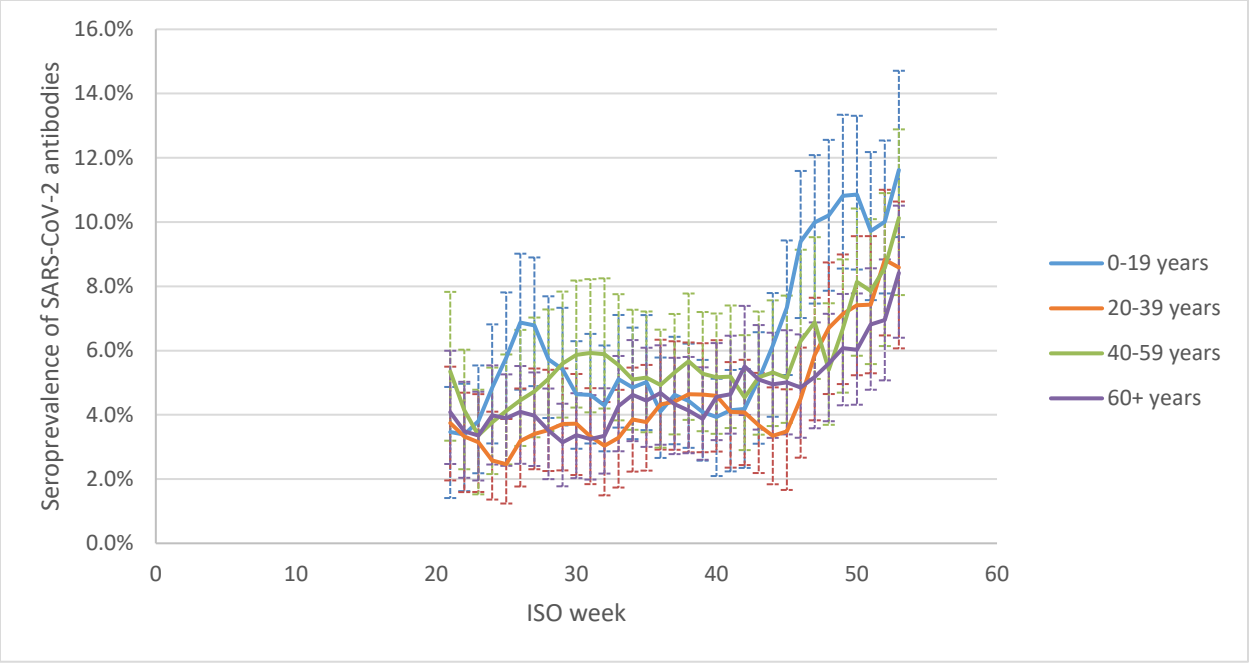
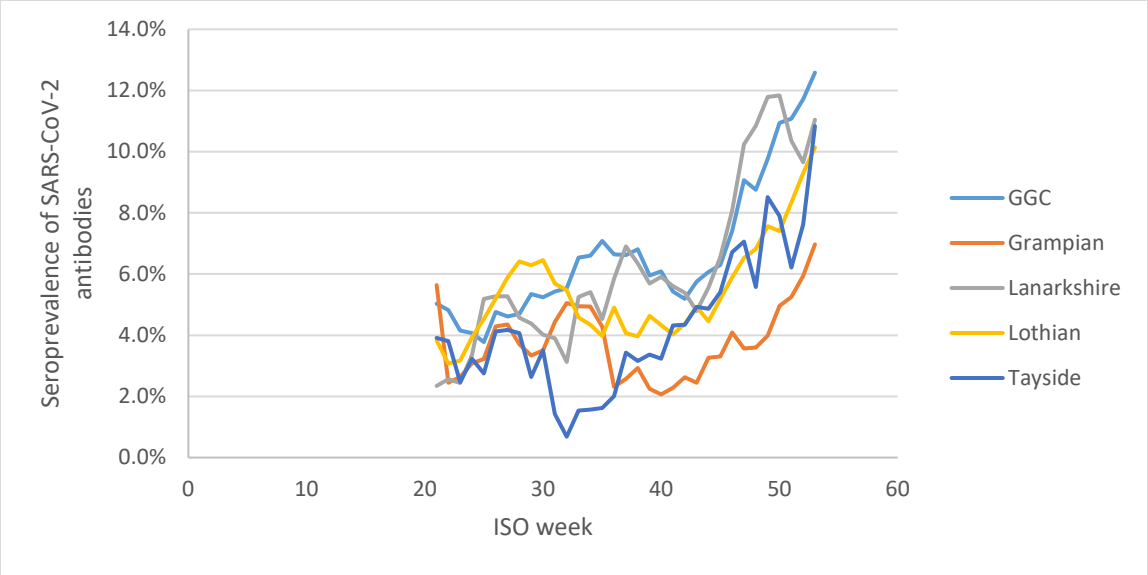


Figure S2. 5-weekly rolling SARS-CoV-2 seroprevalence by age group: data from residual samples obtained from primary care across Scotland. Dashed lines indicate 95% confidence intervals.



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206 **Figure S3.** 5-weekly rolling SARS-CoV-2 seroprevalence by NHS Board (restricted to NHS Boards with a

207 large urban population): data from residual samples obtained from primary care across Scotland.

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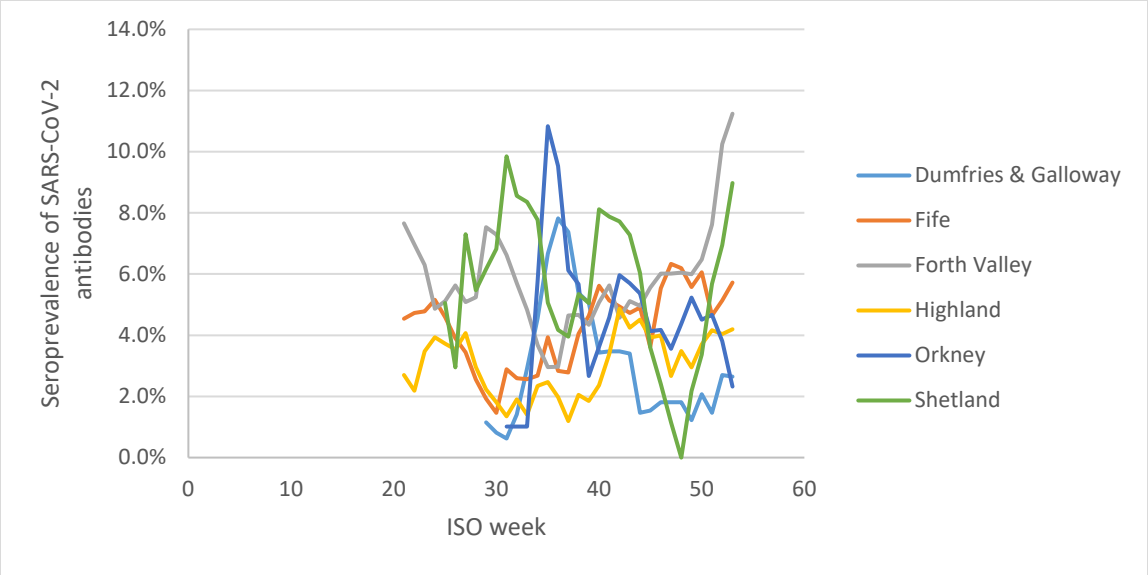

Figure S4. 5-weekly rolling SARS-CoV-2 seroprevalence by NHS Board (NHS Boards with primarily rural populations): data from residual samples obtained from primary care across Scotland.