Title: Canadian COVID-19 Population Serological Survey Utilizing Antenatal Serum Samples

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Title

Canadian COVID-19 Population Serological Survey Utilizing Antenatal Serum Samples

Abstract

Background: Insufficient data on the rate and distribution of SARS-CoV-2 infection in Canada continues to present a significant challenge to the public health response to the COVID-19 pandemic. Our objective was to assess SARS-CoV-2 seroprevalence in a representative population of reproductive age females throughout Canada, across three waves of infection in 2020.

Methods: This is a Canadian retrospective serological surveillance study utilising existing serological prenatal samples across ten provinces over three time periods: February 3-21 2020, August 24-September 11 2020 and November 16-December 4 2020. Age and postal code administrative data were included to allow comparison with concurrent PCR-positivity rates and regional distribution of SARS-CoV-2 spread.

Results: The seropositivity rates were 1.5 to 10 fold higher than the documented concurrent PCR positive rates in these jurisdictions. Presence of seropositivity as early as February in all jurisdictions reflects the extent of SARS-CoV-2 transmission in the early phases, prior to pandemic declaration.

Interpretation: This study presents one of the largest national Canadian SARS-CoV-2 seroprevalence datasets to date. Its results are more generalizable than many reports owing to the minimally biased nature of sample acquisition (routine prenatal testing). Overall seropositivity was below 6%, indicating widespread vulnerability to SARS-CoV-2 prior to the advent of vaccination in Canada. During the time periods sampled, public health tracking systems in all provinces were underreporting infections by 4 fold on average.

Introduction

The COVID-19 pandemic has presented great challenges to healthcare systems and policymakers worldwide since its declaration in March 2020 (1). It has been critical that public health networks collaborate to ensure the rapid dissemination of information and data to inform policies, yet obtaining accurate assessments of community spread of SARS-CoV-2 has been difficult from the outset. Initially in Canada, case detection relied on polymerase chain reaction (PCR) assays on specimens obtained via symptomatic testing and contact tracing. Tracking using PCR alone underestimates cases due to missed asymptomatic or mild cases, individuals electing not to be tested, and variable testing capacity (2-6). In other global settings using similar testing criteria, it is estimated that 33-66% of all cases were undetected due to being asymptomatic (2,3). Although various SARS-CoV-2 serosurveillance studies have been performed within Canada, including those involving postal recruitment or utilising residual sera from blood donors and clinical samples, each study has challenges that limit generalisability to the broader Canadian population, particularly in regard to geographic location (7-12).

Antenatal sera are collected as part of standard prenatal care across Canada to screen for infections and immunity to viruses such as HIV, rubella, and hepatitis B, or for aneuploidy. In some jurisdictions, antenatal specimens are stored for later use or reference. With a very high uptake among all pregnant persons ranging from 93-96% in Canada (13-15), these sera are highly representative of the greater population of reproductive age and encompass the diversity of Canadian residents by geographic location, socioeconomic status, and race/ethnicity. These samples provide an opportunity to test and assess population seroprevalence among a minimally biased sample throughout the pandemic.

Our objective was to assess SARS-CoV-2 seroprevalence in adults by testing available antenatal serum samples across Canada. To capture progression of the pandemic, three sampling periods were selected for assessment of seroprevalence with comparison to concurrent PCR-positivity rates. This serial, cross-sectional study represents one of the largest antenatal seroprevalence studies of the pandemic to date, and is the first Canadian study to present data from three different time points spanning the first year of the pandemic (16,17).

Methods

Sample Selection

As part of a national serological surveillance project, we present data from ten provinces: British Columbia (BC), Alberta (AB), Saskatchewan (SK), Manitoba (MB), Ontario (ON), Quebec (QC), Newfoundland and Labrador (NL), Nova Scotia (NS), New Brunswick (NB), and Prince Edward Island (PEI). Where possible, stored antenatal samples were retrieved from three sampling periods between February 3, 2020 and December 4, 2020; period A: February 3-21, 2020 prior to the first wave and nationwide restrictions, B: August 24 – September 11, 2020 which corresponds to seroprevalence after the first wave, and C: November 16 – December 4, 2020 corresponding to the second wave of SARS-CoV-2 in Canada. BC, AB, and SK participated in time period A with the addition of data from NL and NS for time period B. All ten provinces participated in time period C. Of note, all three time periods pre-dated SARS-CoV-2 vaccine roll out so results uniformly represent response to infection. Given the relatively continuous flow of samples from pregnant persons, selection of all eligible samples (e.g. adequate volume and integrity) from a set time period was conducted to provide proportionate sampling based on population size in each jurisdiction. Antenatal sera are routinely stored for all pregnant persons in Canada. To reduce bias, participating laboratory sites in all provinces included all samples with adequate residual sera within the selected time period.

Laboratory Assays

Samples were assayed for SARS-CoV-2 antibody by the most sensitive and specific assay platform available in each reference laboratory in each province with assay sensitivities ranging from 96%-63% (Supplementary Table 1) (18). These included: the OrthoDiagnostics VITROS Total assay (used by BC, SK, ON, NB, and NS), the DiaSorin LIAISON IgG assay (MB, and QC), the EUROIMMUN assay (used by NL), Abbott assays (used by AB), and the Roche S Total assay (used by PEI).

Statistical Analysis

Sample results from each province were shared with the coordinating centre in Vancouver. Raw seroprevalence

was estimated as the number of positive samples over the total, and stratified by age within each province with exact 95% confidence intervals, except for AB and PEI, where age-linkage was not obtainable. We used the direct method to age-standardize the seroprevalence within each province using Statistics Canada (StatCan) data on the proportion of females in each province in each age range. Confidence intervals for the age-standardized estimates were calculated using the gamma method (19) as implemented in the package dsrTest (20). All analyses were carried out in R version 4.1.1 (2021-08-10) (21).

PCR Confirmed Cases Comparison

The cumulative number of PCR-positive pregnant cases per province was obtained from the Canadian Surveillance of COVID-19 in Pregnancy: Epidemiology, Maternal and Infant Outcomes (CANCOVID-Preg) project (22), while the number of cumulative PCR-positive cases for SARS-CoV-2 infection for females aged 20-49 by region was obtained from StatCan (23). Province-specific data for females aged 20-49 were not available for all provinces therefore several are combined to represent regions: 1) BC ; 2) Prairies including AB, SK, MB, and the Northwest Territories (NT); 3) ON and Nunavut (NU); 4) QC; 5) Atlantic including NB, NS, PEI, and NL. To estimate the PCR-positive rate in each province for 1) the pregnant population, and 2) the population of females aged 20-49 we used data from StatCan. The number of births in 2020 was used as a proxy for the number of pregnant people in each province adjusted for the relevant time period to use as a denominator for PCR-positive rates in pregnancy (24) and the number of females aged 20-49 in each region was used as the denominator for calculating regional PCR-confirmed-positive result rates among this age-group more generally (23). Both seroprevalence and PCR rate are presumed to be cumulative for the purposes of comparison for the ten-month period spanned by this analysis. Rate ratios and confidence intervals of seroprevalence to PCR rates were calculated using a Chi-square approximation.

Ethics Approval

Ethics approval was granted for each site individually to allow for laboratory testing and data transfer to the central coordination centre of the study. Coordination and data management were established through the Women's Health Research Institute (WHRI) at BC Women's Hospital and Health Centre (H20-02252).

Results

Period A February 3-21 2020

Sampling period A was selected to determine if there was any notable SARS-CoV-2 circulating within the general population prior to declaration of the COVID-19 pandemic. A total of 7329 antenatal serum samples from BC, AB, and SK were available for this three-week period. The median age was similar in BC and SK with values of 32 years (range: 15-60) and 30 years (range: 14-41), respectively. The raw seroprevalence in BC was 0.07% (95% CI: 0.01-0.24), in AB was 0.35% (95%CI: 0.19-0.59), and in SK was 0.18% (95% CI: 0.01-1.02), while the age-adjusted seroprevalence in BC was 0.03% (95% CI: 0.00-0.10), and in SK was 0.19% (95% CI: 0.00-0.71). No formal reporting of PCR-positive cases occurred during this sampling period.

Period B August 24- September 11 2020

Testing of samples from sampling period B was performed to determine the seropositivity rate after the first wave and to indicate susceptibility for the second wave following natural infection. For sampling period B, BC and SK retrieved and centrally tested 2427 antenatal samples. Raw seroprevalence was 0.34% (95% CI: 0.12-0.73) in BC, 1.56% (95% CI: 1.15-2.06) in AB, 0.66% (95% CI: 0.18-1.68) in SK, 0.41% (95% CI: 0.05-1.47) in NS, and age-adjusted seroprevalence in both BC and SK was 0.29% with confidence intervals 0.07-0.65 and 0.08-0.63, respectively. NL tested 33 serological samples for this period and detected no confirmed seropositive samples.

Period C November 16 – December 4 2020

This period reflects the weeks just prior to access to COVID-19 vaccines in Canada. Ten provinces (BC, AB, SK, MB, ON, QC, NL, NS, NB, and PEI) retrieved and tested 14 372 antenatal samples for sampling period C. Age

adjusted data were available for eight provinces (BC, SK, MB, ON, QC, NS, NB, and NL) and raw rates only for AB, and PEI.

Raw and Age-Adjusted Seropositivity by Province for Time Period C

With the data available in time period C for all ten provinces, age-adjusted rates and PCR comparisons were possible by province for all but AB and PEI. Figure 1 demonstrates the range of raw and age-adjusted seropositivity for this period with the highest rates evident in QC at 5.95% (95% CI 2.66-10.44) and the lowest rates occurring in NL at 0.24% (95% CI 0.01-0.87).

Age-stratified seroprevalences are shown in Figure 2. The figure omits the youngest (<20), and oldest (≥45) age groups due to low numbers.

Comparison over time was possible with BC, AB, and SK data for all three time periods, and with NL and NS for two time periods, and is reflected in Figure 3, showing increases in seropositivity for time period C.

PCR versus Seroprevalence Comparison

The rate of PCR-positive confirmed SARS-CoV-2 infections in pregnant people and the rate of PCR-positive confirmed SARS-CoV-2 infections in females aged 20-49 by region was compared to the seroprevalence for time period C. Seroprevalence was higher than PCR-confirmed-positive case rate for all regions and both populations. In addition, the individual provinces showed higher seroprevalence compared to the regional PCR-positive rate for all provinces except SK. The seroprevalence was on average 4.3 times higher than the PCR positivite rate for the ten provinces.

Geographic Seropositivity Mapping

Figure 4 demonstrates the positive and negative sera results geographically. Positive serum sampling is demonstrated in remote parts of BC, SK, and MB as well as on the Eastern coast in regions which may have underestimated the amount of community spread during this time period.

Interpretation

Data from this national serosurveillance study demonstrate several key findings regarding SARS-CoV-2 spread in Canada. First, SARS-CoV-2 community spread prior to declaration of the pandemic in March 2020 was more expansive than previously thought (based on PCR-based case detection). During sampling period A, it was believed that there was no community spread in BC, AB and no infections in SK. Despite this, the age-adjusted seroprevalence of 0.03% in BC, 0.19% in SK and raw rate of 0.35% in AB, demonstrate early spread through the general population within these provinces. Following the first wave of infection in Canada, seroprevalence was slightly elevated but remained fairly low in BC and SK at 0.29%, but had increased to 1.56% in AB. During the second wave of infection (sampling period C), seroprevalence varied widely across provinces, from 0.24% in NL to 5.95% in QC. The evolution of the pandemic between provinces and within each province has shown distinctive differences likely due to varying public health policy and population density. Nonetheless, data from all provinces demonstrated significant vulnerability to subsequent waves of the pandemic.

Prior to this study, Canadian seroprevalence estimates from testing of residual serum by Canadian Blood Services (CBS) (7,10) showed similar results to ours for sampling period A and B but with some important differences within sampling period C. CBS samples from November 2020 demonstrated a lower seroprevalence in ON (3.86% vs 0.77%), NS (1.41% vs 0.19%), and PEI (1.11% vs 0%) but higher seroprevalence in SK (1.81% vs 4.17%) and MB (4.28% vs 8.56%). These discrepancies may reflect the selection bias of healthy blood donors in the CBS sampling methodology, which may be different to the potential biases inherent in antenatal serum screening. Although antenatal sera results may reflect variability in fertility rates in certain sub-populations that can impact generalizability of results, compared to other sampling they cover broad geographies, cultures, and socioeconomic groups due to high uptake rates of prenatal infection screening (13-15).

Our findings also demonstrate important differences when compared to positive SARS-CoV-2 PCR test rates for the same time periods, with seropositivity rates found to be consistently higher. This is reflected in the results seen in BC and MB for time period C. We detected an age-adjusted seropositivity rate of 1.87% (95% CI 1.12-

2.80) in BC during sampling period C, while the PCR comparison rate among women aged 20-49 years in BC to December 4, 2020 was 1.13 (95%CI 1.12-1.16). The PCR-positive rate among pregnant women in BC for the same time period from the CANCOVID-Preg project was 0.62% (95% CI 0.55-0.70). In comparison to the pregnant PCR-positive test rate, age-adjusted seroprevalence was almost 3 times higher (RR 2.99, 95% CI 2.16-3.95). In MB, the age-adjusted seroprevalence in our study was 5.62% (95% CI 1.42-12.42), while the rate of PCR-positive tests in women aged 20-49 in the prairie provinces to December 4, 2020 was 2.11 (95%CI 2.08 – 2.13) giving an estimated rate ratio 2.79 (1.27-4.55). Despite varying provincial PCR testing protocols and provisions, these findings of higher seroprevalence when compared to PCR rates in nearly all of the provinces demonstrate an important level of undetected SARS-CoV-2 spread. They also suggest a useful mechanism for public health monitoring of the pandemic that overcomes the significant cost and variable availability of PCR testing.

This study presents one of the largest serosurveillance studies performed to date on a representative sample of Canadian reproductive age adults. However, the national nature of this study resulted in the use of varied assays at regional laboratories across participating provinces. Due to the retrospective nature of sampling, samples were not available from many provinces for the sampling periods A and B, resulting in a smaller sample size for these time periods as compared to sampling period C. Additionally, given that waning of SARS-CoV-2 antibodies remains an area of investigation, a lack of detectable antibody does not necessarily indicate a lack of prior infection. Seroprevalence was presumed to be cumulative for the purposes of comparison with PCR-confirmed cases and it is therefore possible, with waning antibody levels, that the percentage of individuals with prior SARS-CoV-2 infection is higher than our seroprevalence estimates. However, as the only accepted correlate of immunity is neutralization of viral strain cultures among convalescent patients, the seroprevalence estimates presented here likely represent the upper limit of individuals who were potentially immune to SARS-CoV-2 prior to vaccination (25). Of importance, the delay in approvals for doing the assays and sharing the data greatly limited the public health value of this information to inform real time decisions. This is a concern that has been echoed by the Pan Canadian Health Data Strategy expert advisory group (26) and highlights how administrative

delays in the context of a pandemic need to be improved upon for effective future public health responses in Canada.

Conclusion

This national serosurveillance project using minimally-biased antenatal serum samples as a window into the general population demonstrates more expansive spread of SARS-CoV-2 prior to pandemic declaration than previously thought. We also document variability in how each province experienced waves of SARS-CoV-2 infection and show that seroprevalence rates were 1.5-10 times greater than reported PCR-positive case rates. Despite this undetected spread, seroprevalence overall remained low in Canada until the end of 2020 and at a level suggesting widespread susceptibility to SARS-CoV-2 infection prior to the introduction of vaccination in 2021. Antenatal serosamples represent a highly valuable window into the population health burden of this pandemic and other infectious diseases of public health signficance. In the future, they can be deployed in a timely and effective manner to inform and guide public health responses.

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References:

1. World Health Organization (WHO). WHO Director- General's opening remarks at the media briefing on COVID-19 – 11 March 2020. 2020. Available https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020 (accessed 8 August 2021).

2. Oran DP, Topol EJ. The proportion of SARS-CoV-2 Infections that are asymptomatic: A systematic review. Annals of Int Med 2021; 174(5):655-662

3. Kalish H, Klump-Thomas C, Hunsberger S, et al. Undiagnosed SARS-CoV-2 seropositivty during the first 6 months of the COVID-19 pandemic in the United States. Sci Transl Med 2021;13

4. Vos ERA, den Hartog G, Schepp RM, et al. Nationwide seroprevalence of SARS-CoV-2 and identification of risk factors in the general population of the Netherlands during the first epidemic wave. J Epidemiol Community Health 2021;75:489-95

5. Burgess S, Ponsford MJ, Gill D. Are we underestimating seroprevalence of SARS-CoV-2? Brit Med J 2020; 370:m3364

6. Ng DL, Goldof Gm, Shy BR, et al. SARS-CoV-2 seroprevalence and neutralizing activity in donor and patient blood. Nat Community 2020; 11:4698

7. Saeed S, Drews SJ, Pambrun C, et al. SARS-CoV-2 seroprevalence among blood donors after the first COVID-19 wave in Canada. Transfusion 2021; 61:862-72

8. LeBlanc JJ, Gubbay JB, Li Y, et al. Real-Time PCR-based SARS-CoV-2 detection in Canadian Laboratories. J Clinical Virology 2020; 128

9. Charlton CL, Nguyen LT, Bailey A, et al. Pre-vaccine positivity of SARS-CoV-2 antibodies in Alberta, Canada during the first two waves of the COVID-19 pandemic. Microbiol Spectr 2021; 9:e00291-21

10. Skowronski DM, Sekirov I, Sabaiduc S, et al. Low SARS-CoV-2 sero-prevalence based on anonymized residual sero-survey before and after first wave measures in British Columbia, Canada, March-May 2020. medRxiv 2020.07.13.20153148.

11. Lewin A, Therrien R, De Serres G, Grégoire Y, Perreault J, Drouin M, Fournier MJ, Tremblay T, Beaudoin J, Beaudoin-Bussières G, Prévost J, Gendron-Lepage G, Finzi A, Bernier F, Bazin R, Germain M, Delage G. SARS-CoV-2 seroprevalence among blood donors in Québec, and analysis of symptoms associated with seropositivity: a nested case-control study. Can J Public Health. 2021 Aug;112(4):576-586. doi: 10.17269/s41997-021-00531-6. Epub 2021 May 17. PMID: 33999398; PMCID: PMC8127462.

12. Tang X, Sharma A, Pasic M, Brown P, Colwill K, Gelband H, Birnboim HC, Nagelkerke N, Bogoch II, Bansal A, Newcombe L, Slater J, Rodriguez PS, Huang G, Fu SH, Meh C, Wu CN, Kaul R, Langlois MA, Morawski E, Hollander A, Eliopoulos D, Aloi B, Lam T, Abe KT, Rathod B, Fazel-Zarandi M, Wang J, Iskilova M, Pasculescu A, Caldwell L, Barrios-Rodiles M, Mohammed-Ali Z, Vas N, Santhanam DR, Cho ER, Qu K, Jha S, Jha V, Suraweera W, Malhotra V, Mastali K, Wen R, Sinha S, Reid A, Gingras AC, Chakraborty P, Slutsky AS, Jha P. SARS-CoV-2 Seroprevalence During the First and Second Pandemic Waves in Canada. SSRN. 12 Aug 2021. Doi: http://dx.doi.org/10.2139/ssrn.3903944

13. Biondi MJ, Marchand-Austin A, Cronin K, Nanwa N, Ravirajan V, Mandel E, Goneau LW, Mazzulli T, Shah H, Capraru C, Janssen HLA, Sander B, Feld JJ. Prenatal hepatitis B screening, and hepatitis B burden among children, in Ontario: a descriptive study. CMAJ. 2020 Oct 26;192(43):E1299-E1305. doi: 10.1503/cmaj.200290.

14. Remis RS, Merid MF, Palmer RW, Whittingham E, King SM, Danson NS, Vernich L, Swantee C, Major C. High uptake of HIV testing in pregnant women in Ontario, Canada. PLoS One. 2012;7(11):e48077. doi: 10.1371/journal.pone.0048077. Epub 2012 Nov 9.

15. Plitt SS, Smith TR, Berry W, Osman M, Senthilselvan A, Charlton CL. Examination of a Canadian provincial prenatal HIV screening program: 2010 to 2014. Can J Public Health. 2020 Aug;111(4):555-561. doi: 10.17269/s41997-019-00281-6. Epub 2020 Feb 3.

16. 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. Published 2021 Jun 23. doi:10.1371/journal.pone.0252617

17. McAuley A, Gousias P, Hasan T, Rashid L, Richardson C, Reid G, Templeton K, McGuire J, Wise H, McVicar L, Jenks S, Gunn R, Dickson E, Stock SJ, Stockton A, Waugh C, Wood R, McMenamin J, Robertson C, Goldberg DJ, Palmateer NE. National population prevalence of antibodies to SARS-CoV-2 among pregnant women in Scotland during the second wave of the COVID-19 pandemic: a prospective national serosurvey. Public Health. 2021 Oct;199:17-19. doi: 10.1016/j.puhe.2021.08.005.

18. Stone M, Grebe E, Sulaeman H, Di Germanio C, Dave H, Kelly K, Biggerstaff B, O.Crews B, Tran N, Jerome KR, Denny TN, Hogema B, Destree M, Jones JM, Thornburg N, Simmons G, Krajden M, Kleinman S, Dumont LJ, Busch MP. Evaluation of commercially available high-throughput SARS-CoV-2 serological assays for surveillance and related applications. medRxiv 15 Sept 2021. Doi: 10.1101/2021.09.04.21262414

19. Tiwari RC, Clegg LX, & Zou Z (2006). 'Efficient interval estimation for age-adjusted cancer rates.' Statistical Methods in Medical Research 15: 547–569. doi: 10.1177/0962280206070621

20. Michael Nelson (2017). dsrTest: Tests and Confidence Intervals on Directly Standardized Rates for Several Methods. R package version 0.2.1.https://CRAN.R-project.org/package=dsrTest.

21. R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

22. Money D. 2020. Maternal and Infant Outcomes (March 1, 2020 to March 31, 2021) from Five Canadian Provinces Report #4: Released June 3, 2021. Canadian Surveillance of COVID-19 in Pregnancy: Epidemiology, Maternal and Infant Outcomes. Available from: https://ridprogram.med.ubc.ca/cancovid-preg/

23. Statistics Canada. Preliminary dataset on confirmed cases of COVID-19, Public Health Agency of Canada, 2020-2021**.** Issue Number: 2020001. Release date November 12, 2021. Available from: https://www150.statcan.gc.ca/n1/en/catalogue/132600032020001

24. Statistics Canada. Table 13-10-0415-01 Live births, by month

DOI: https://doi.org/10.25318/1310041501-eng

25. Khoury, D.S., Cromer, D., Reynaldi, A. et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med **27,** 1205–1211 (2021). https://doi.org/10.1038/s41591-021-01377-8

26. Public Health Agency of Canada. Pan Canadian Health Data Strategy. Expert Advisory Group-Report 2. Building Canada's Health Data Foundation. November 2021. Available from URL: https://www.canada.ca/en/public-health/corporate/mandate/about-agency/external-advisory-bodies/list/pan-canadian-health-data-strategy-reports-summaries/expert-advisory-group-report-02-building-canada-health-data-foundation.html

Figure 1: Raw and age-adjusted seroprevalence by province for Period C. Error bars indicate 95% CI and sample sizes are given below province names.

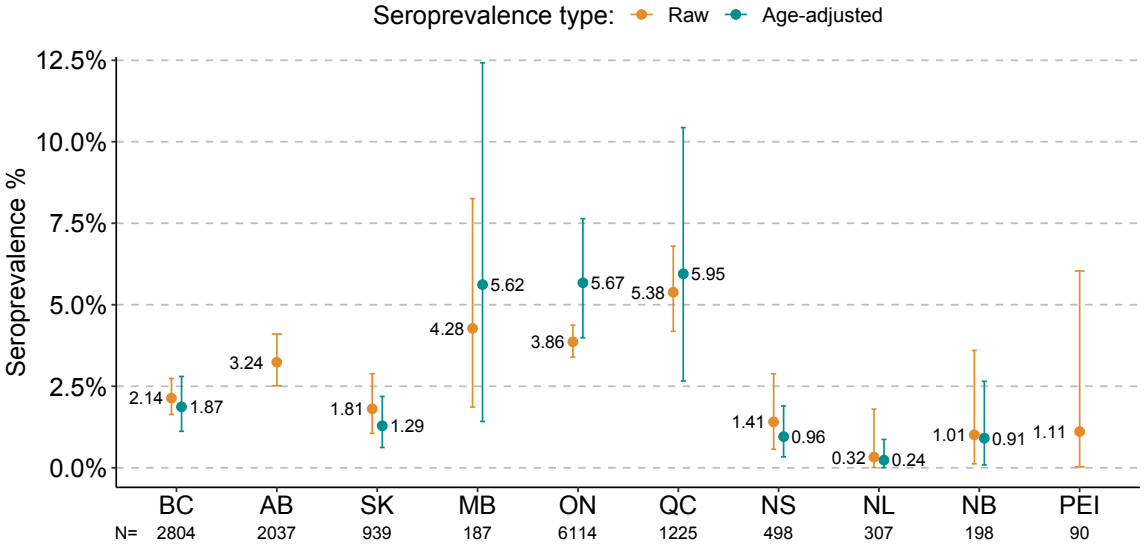

Figure 2: Age-stratified seroprevalence for time period C for each province. Error bars indicate 95% CI, and those that extend past 20% have been cut off for ease of visual comparison among provinces.

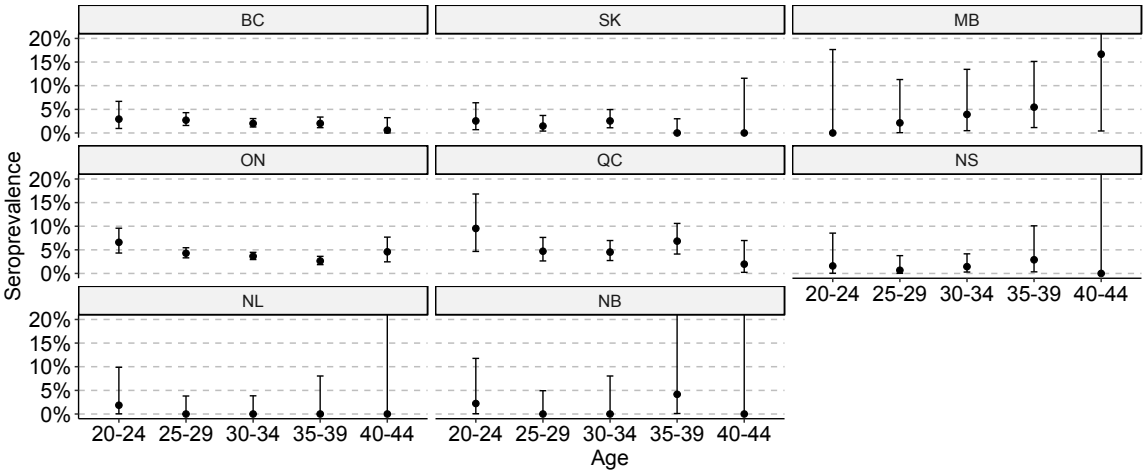

Figure 3: Age-adjusted seroprevalence over all three time periods in BC, AB and SK, and two time periods for NS and NL. The upper confidence interval for NL in August 2020 extends beyond the boundaries of the graph and was truncated for easier visualization of the other provinces.

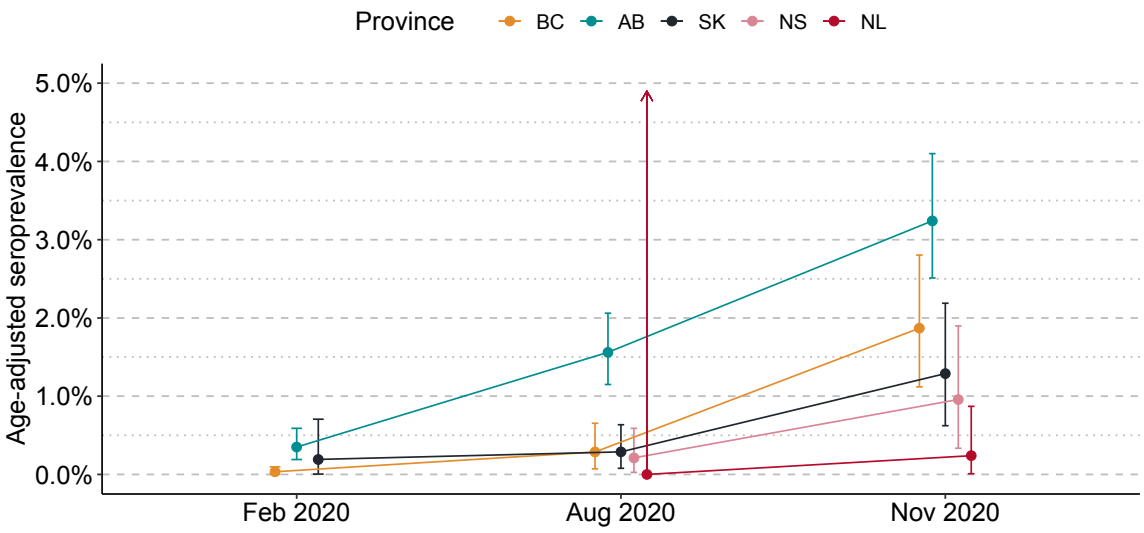

Figure 4: Location of positive antenatal serum results for SARS-CoV-2. Blue cross indicates seropositive samples and red cross indicates seronegative samples. Regional mapping of samples was not possible for samples from AB, NL and NB.

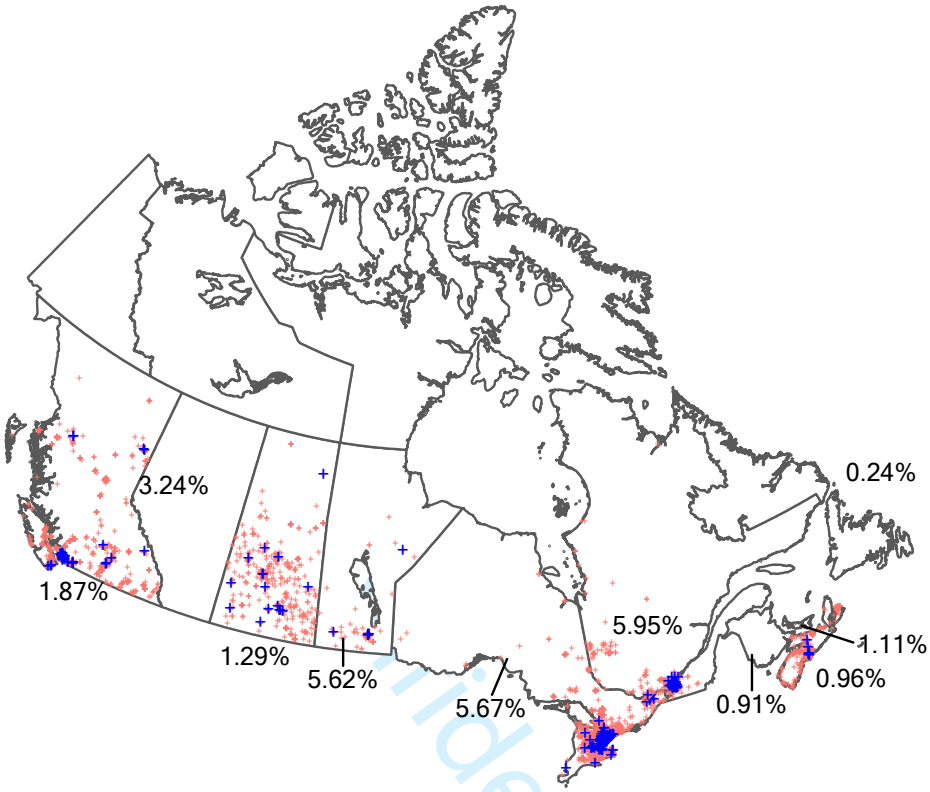

Table 1: Comparison of PCR-confirmed-positive rates among pregnant people from CANCOVID-Preg, and among females aged 20-49 from StatCan, by region for time period C and results of SARS-CoV-2 sero-screening adjusted for age (November/December 2020). Rate ratios shown for comparison with age-adjusted seroprevalence estimate. Rates for AB and PEI are not age-adjusted. Shown as rates per 100. PCR-positive rates not available for pregnant people in SK.

Region	Province	Age-adjusted Seroprevalence (95% CI)	PCR positive rate in pregnant people (95% CI)	Rate ratio (95%CI)	PCR positive rate in Canadian females aged 20-49 (95% CI)	Rate ratio (95%CI)
1	**BC**	1.87 (1.12-2.80)	0.62 (0.55-0.70)	2.99 (2.16-3.95)***	1.13 (1.12 – 1.16)	1.84 (1.37-2.36)***
2	**AB**	3.24 (2.51-4.10)	0.86 (0.78-0.95)	3.77 (2.86-4.79)***	2.11 (2.08 – 2.13)	1.54 (1.19-1.92)**
2	**SK**	1.29 (0.62-2.19)	-	-	2.11 (2.08 – 2.13)	0.61 (0.30-0.96)
2	**MB**	5.62 (1.42-12.42)	1.24 (1.08-1.44)	4.73 (2.13-7.73)***	2.11 (2.08 – 2.13)	2.79 (1.27-4.55)*
3	**ON**	5.67 (3.93-7.50)	0.47 (0.43-0.51)	12.07 (10.61-13.69)***	1.28 (1.27 - 1.29)	4.42 (3.97-4.87)***
4	**QC**	5.95 (2.66-10.44)	1.01 (0.94-1.08)	5.90 (4.60-7.33)***	2.45 (2.42 - 2.47)	2.43 (1.90-2.99)***
5	**NS**	0.96 (0.33-1.90)	0.17 (0.09-0.30)	5.91 (1.28-15.35)**	0.11 (0.10 - 0.12)	8.90 (1.80-17.28)**
5	**NL**	0.24 (0.01-0.87)	0.00 (0.00-0.13)	-[1]	0.11 (0.10 - 0.12)	2.88 (0.00-9.02)
5	**NB**	0.91 (0.09-2.65)	0.05 (0.01-0.15)	15.69 (2.64-93.37)*	0.11 (0.10 - 0.12)	8.93 (0.00-22.79)

5	**PEI**	1.11 (0.03-6.04)	0.00 (0.00-0.36)	-[1]	0.11 (0.10 - 0.12)	9.83 (0.00-30.97)

[1]Rate ratio not possible to calculate

*** indicates p-value <0.01, ** indicates p-value <0.001, *** indicates p-value <0.0001**

Supplementary Table 1: Assays and their Specifications by Province

Province	Assay	Description of assay (IgG, IgA, IgM)	Specifications
BC & SK	OrthoDiagnostics VITROS Anti-SARS-CoV-2 Total	IgG, IgA, IgM	Anti-S
AB	Abbott Architect SARS-CoV-2 IgG	IgG	Anti-N
MB	DiaSorin LIAISON SARS-CoV-2 S1/S2 IgG	IgG	Anti-S
ON	OrthoDiagnostics VITROS Anti-SARS-CoV-2 Total	IgG, IgA, IgM	Anti-S
QC	DiaSorin LIAISON SARS-CoV-2 TrimericS IgG	IgG	Anti-S
NL	EUROIMMUN	IgG	Anti-S
NS	OrthoDiagnostics VITROS Anti-SARS-CoV-2 Total	IgG, IgA, IgM	Anti-S
PEI	Roche Diagnostics Elecsys Anti-SARS-CoV-2 Total Antibody	IgG, IgA, IgM	Anti-N
NB	OrthoDiagnostics VITROS Anti-SARS-CoV-2 Total	IgG, IgA, IgM	Anti-S