Assessment of SARS-CoV-2 Seropositivity During the First and Second Viral Waves in 2020 and 2021 Among Canadian Adults

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Abstract

**IMPORTANCE** The incidence of infection during SARS-CoV-2 viral waves, the factors associated with infection, and the durability of antibody responses to infection among Canadian adults remain undocumented.

**OBJECTIVE** To assess the cumulative incidence of SARS-CoV-2 infection during the first 2 viral waves in Canada by measuring seropositivity among adults.

**DESIGN, SETTING, AND PARTICIPANTS** The Action to Beat Coronavirus study conducted 2 rounds of an online survey about COVID-19 experience and analyzed immunoglobulin G levels based on participant-collected dried blood spots (DBS) to assess the cumulative incidence of SARS-CoV-2 infection during the first and second viral waves in Canada. A sample of 19 994 Canadian adults (aged ≥18 years) was recruited from established members of the Angus Reid Forum, a public polling organization. The study comprised 2 phases (phase 1 from May 1 to September 30, 2020, and phase 2 from December 1, 2020, to March 31, 2021) that generally corresponded to the first (April 1 to July 31, 2020) and second (October 1, 2020, to March 1, 2021) viral waves.

**MAIN OUTCOMES AND MEASURES** SARS-CoV-2 immunoglobulin G seropositivity (using a chemiluminescence assay) by major geographic and demographic variables and correlation with COVID-19 symptom reporting.

**RESULTS** Among 19 994 adults who completed the online questionnaire in phase 1, the mean (SD) age was 50.9 (15.4) years, and 10 522 participants (51.9%) were female; 2948 participants (14.5%) had self-identified racial and ethnic minority group status, and 1578 participants (8.2%) were self-identified Indigenous Canadians. Among participants in phase 1, 8967 had DBS testing. In phase 2, 14 621 adults completed online questionnaires, and 7102 of those had DBS testing. Of 19 994 adults who completed the online survey in phase 1, fewer had an educational level of some college or less (4747 individuals [33.1%]) compared with the general population in Canada (45.0%). Survey respondents were otherwise representative of the general population, including in prevalence of known risk factors associated with SARS-CoV-2 infection. The cumulative incidence of SARS-CoV-2 infection among unvaccinated adults increased from 1.9% in phase 1 to 6.5% in phase 2. The seropositivity pattern was demographically and geographically heterogeneous during phase 1 but more homogeneous by phase 2 (with a cumulative incidence ranging from 6.4% to 7.0% in most regions). The exception was the Atlantic region, in which cumulative incidence reached only 3.3% (odds ratio [OR] vs Ontario, 0.46; 95% CI, 0.21-1.02). A total of 47 of 188 adults (25.3%) reporting

(continued)
COVID-19 symptoms during phase 2 were seropositive, and the OR of seropositivity for COVID-19 symptoms was 6.15 (95% CI, 2.02-18.69). In phase 2, 94 of 444 seropositive adults (22.2%) reported having no symptoms. Of 134 seropositive adults in phase 1 who were retested in phase 2, 111 individuals (81.8%) remained seropositive. Participants who had a history of diabetes (OR, 0.58; 95% CI, 0.38-0.90) had lower odds of having detectable antibodies in phase 2.

CONCLUSIONS AND RELEVANCE The Action to Beat Coronavirus study found that the incidence of SARS-CoV-2 infection in Canada was modest until March 2021, and this incidence was lower than the levels of population immunity required to substantially reduce transmission of the virus. Ongoing vaccination efforts remain central to reducing viral transmission and mortality. Assessment of future infection-induced and vaccine-induced immunity is practicable through the use of serial online surveys and participant-collected DBS.

Introduction

As of December 15, 2021, Canada had reported more than 1.8 million cases of SARS-CoV-2 infection and approximately 30 000 deaths associated with COVID-19. Slightly fewer than 60% of cases and more than 80% of deaths occurred during the combined first (April to July 2020) and second (October 2020 to March 2021) viral waves, when the original SARS-CoV-2 was the predominant strain in circulation.1 Before the onset of the viral wave caused by the Omicron variant, which began on December 15, 2021, reported case rates in Canada (Figure 1) had been approximately one-third of those in the US and one-half of those in the United Kingdom.2 At the population level, the incidence of infection during each wave, the factors associated with infection, the levels and factors associated with asymptomatic infection, and the durability of antibody responses against infection remain uncertain.

The Action to Beat Coronavirus (Ab-C) seroprevalence study included a representative sample of adult Canadians covering the first and second viral waves. We also estimated the incidence of seropositivity between the first and second waves, the association of age-specific mortality with SARS-CoV-2 seropositivity, and the incidence of asymptomatic infections by sex, age, self-identified...
Methods

The Ab-C study comprised 2 rounds of an online survey about COVID-19 experience and analysis of immunoglobulin G (IgG) antibody levels based on participant-collected dried blood spots (DBS) to assess the cumulative incidence of SARS-CoV-2 infection during the first and second viral waves in Canada. The study comprised 2 phases (phase 1 from May 1 to September 30, 2020, and phase 2 from December 1, 2020, to March 31, 2021) that generally corresponded to the first (April 1 to July 31, 2020) and second (October 1, 2020, to March 1, 2021) viral waves (Figure 1). Written or digital consent was obtained by all participants. The Ab-C study received ethical approval from Unity Health Toronto. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cohort studies.

Participants

In phase 1 (May to September 2020), we invited 44 270 members (of approximately 78 000 total members) of the Angus Reid Forum, an established nationwide polling panel of Canadian adults 18 years and older, to complete an online survey about SARS-CoV-2 symptoms and testing histories. The sampled population was stratified by age group (18-39 years, 40-59 years, 60-69 years, or ≥70 years), sex (male or female), educational level (high school or lower, some college or college or technical degree, some university, or university degree), and region by metropolitan census area to match the national demographic distribution.

In August 2021, we invited approximately 3100 additional Angus Reid Forum panel members from 17 regions (of 93 total regions nationwide) with high burden of infection based on a regression analysis of SARS-CoV-2 case counts (eFigure 1 in the Supplement). At the end of the online survey, respondents indicated their willingness to self-collect a blood sample from a finger prick, and those consented were sent a DBS collection kit. From December 2020 to January 2021, we invited all 19 994 participants from phase 1 to join phase 2, retaining the same sampling framework. The study investigators did not provide participants with financial compensation for participating in the study, but participants did earn a modest number of redeemable points from the Angus Reid Forum.

Study recruitment, flow, and exclusions (approximately 1% of individuals, mostly excluded because of incomplete test results) are shown in eFigure 2 in the Supplement. The timeline for phases 1 and 2 (in relation to Canada’s national weekly averages of confirmed COVID-19 cases) and phase 3 (in relation to weekly averages of vaccination) are provided in Figure 1. Phase 2 began in December 2020, just as SARS-CoV-2 vaccines were introduced. We excluded the 147 participants reporting they had received a vaccine from the phase 2 analysis.

Symptoms, Testing, and Vaccination History

The phase 1 online questionnaire solicited self-reported COVID-19 symptoms among household members by month of onset and experience with COVID-19 testing. The phase 2 questionnaire requested additional details about testing and vaccination history. We defined COVID-19 symptom positivity as a combination of fever plus any of the following: breathing difficulty, dry cough, loss of smell or taste, or discolored and/or swollen toe (commonly referred to as COVID toe). We asked participants if they had previously received a polymerase chain reaction (PCR) test or were awaiting receipt of a PCR test for SARS-CoV-2 infection, and we requested information about height and weight (to determine body mass index [calculated as weight in kilograms divided by height in meters squared]), the presence of hypertension and/or diabetes, current or past smoking, and other exposures.
**IgG Serologic Testing**

Participants collected 5 small circles of blood on special bar-coded filter paper, dried the sample for at least 2 hours, placed it in a 2-layer protective pouch, and returned it to St. Michael's Hospital in Toronto, Ontario (with postage prepaid). Mailing time across Canada ranged from 3 to 6 days. At arrival, samples were scanned, cataloged, and stored at 4 °C in larger boxes with additional desiccant, then monitored for humidity levels (kept at <20%). In phase 1, the Network Biology Collaborative Centre at Sinai Health, Toronto, conducted a high-throughput, highly sensitive chemiluminescence-based enzyme-linked immunosorbent assay targeting the spike protein as a trimer. In phase 2, antigens targeting the receptor binding domain (RBD) of the spike and nucleocapsid proteins were added. For each antigen, raw values were normalized to a blank-subtracted point in the linear range of a calibration standard curve to create a relative ratio. Based on a receiver operating characteristic curve analysis using 187 DBS samples from individuals outside the study population (supplied by the National Microbiology Laboratory of Canada), the sensitivity of the assays at a 1% false-positive rate was 98% for spike and RBD proteins and 92% for the nucleocapsid protein. The spike protein IgG antibodies used in this assay can persist for at least 115 days in symptomatic individuals who receive testing in clinical settings. Details of laboratory protocols, including reproducibility procedures, are available in eFigure 3 to eFigure 8 in the Supplement.

**COVID-19 Mortality**

We collected age, sex, and location of COVID-19 deaths, as defined by the World Health Organization (International Classification of Diseases, Tenth Revision, diagnostic codes U07.1 and U07.2), from Statistics Canada and provincial data sources. Based on these data and the national population and death totals for each age group (20-39 years, 40-59 years, 60-69 years, and ≥70 years), we estimated infection fatality rates (IFRs) as the number of deaths divided by the cumulative incidence of infection for each viral wave. To ensure comparability, we excluded the larger number of deaths occurring in long-term care facilities and nursing homes because those populations were not included in our sampling framework.

**Primary Outcome and Seropositivity Thresholds**

The primary outcome was IgG antibody seropositivity (detailed definitions available in eTable 1 and eTable 2 in the Supplement). We categorized phase 1 results as seropositive if they were 3 SD or greater from the mean of negative control samples, yielding a spike protein relative ratio greater than 0.39. We also calculated a more lenient threshold of 3 SD or greater from the mean of the presumed negative distribution (0.27). For phase 2, in an effort to reduce false positivity, we applied higher cutoff criteria based on 3 SD or greater from the mean of the log density distribution of samples from phase 1, yielding a spike protein relative ratio greater than 0.34 and a stricter cutoff of greater than 0.48 based on receiver operating characteristic curve analyses. We also included the 2 new antigens (relative ratio, ≥0.32 for RBD proteins and ≥0.64 for the nucleocapsid protein based on receiver operating characteristic curve analyses) in phase 2, with strict and lenient cutoff criteria being the same for these 2 antigens (eFigure 8 in the Supplement). Our main definition of seropositivity in phase 2 was the presence of antigens for any of the following: spike protein (lenient cutoff), RBD proteins, or nucleocapsid protein. We also reported results using the strict cutoff for the spike protein.

**Statistical Analysis**

We standardized cumulative incidence for age and educational level to the 2016 census population. We used Spearman correlation analysis to explore the persistence of IgG antibodies over time and logistic regression analysis to examine the individual factors associated with IgG antibody status and asymptomatic infection, with the regression analysis considering province or region (Atlantic, British Columbia, Ontario, Prairies, or Quebec), household size (live alone, 2 people, 3 people, 4 people, or ≥5 people), age group (18-39 years, 40-59 years, 60-69 years, or ≥70 years), sex (male
or female), educational level (some college or less, college graduate, or university graduate), self-identified racial or ethnic minority status or self-identified Indigenous Canadian status, weight (body mass index <25 [underweight or normal weight], 25-29 [overweight], or ≥30 [obese]), smoking status (current, former, or never), presence of diabetes (yes or no) or hypertension (yes or no), and COVID-19 symptom history (yes or no).

All significance tests were 2-sided. The a priori significance level was \( P = .05 \). All analyses were performed using Stata software, version 16 (StataCorp LLC). 13

Results

Of 44 270 invited Angus Reid Forum panel members, 19 994 completed the online survey in phase 1 (May to September 2020; response rate, 45.2%) and 14 621 completed the survey in phase 2 (December 2020 to March 2021; with surveys completed by January 2021; response rate, 73.1% of participants in phase 1). We analyzed 8967 DBS samples in phase 1 and 7102 DBS samples in phase 2; an additional 64 samples had an inadequate amount of dried blood available for analysis. In phase 1, the mean (SD) age of the cohort was 50.9 (15.4 years), and 10 522 participants (51.9%) were female. The older age distribution of the study participants vs the Canadian census population occurred because of intentional oversampling of individuals 60 years and older. The demographic and health characteristics of those who completed surveys and provided DBS compared with the Canadian census population are shown in eTable 3 in the Supplement. The overall distribution of the 19 994 participants in phase 1 was similar to that of the census population, 3,14,15 with the exception of fewer adults with an educational level of some college or less in the Ab-C study (4747 individuals [33.1%]) compared with the census population (45.0%). Hence, we adjusted for educational level in the regression analyses and when calculating all subsequent estimates of cumulative incidence. In phase 1, the study sample had fewer racial or ethnic minority adults (2948 individuals [14.5%]) but more Indigenous Canadian adults (1578 individuals [8.2%]) than the census population (22.0% and 5.0%, respectively). Compared with the census population, study participants had a similar prevalence of obesity (27.1% vs 27.0%), current or former smoking (50.7% vs 54.0%), diabetes (9.7% vs 9.0%), and hypertension (26.5% vs 23.0%). 16-19 The phase 1 and 2 population distributions of those who completed surveys and those who provided DBS remained similar.

A total of 168 seropositive adults were identified in phase 1 using the strict cutoff criteria. Samples were considered to be seropositive if a positive result was found for at least 1 of 3 antigens using both the lenient and strict cutoffs (eTable 1 in the Supplement). A total of 455 seropositive adults were identified in phase 2 (377 of whom had a positive response using the strict cutoff). Among 136 seropositive adults identified using the lenient cutoff criteria in phase 1, 123 individuals (90.4%) became seronegative in phase 2. Of the 455 seropositive adults in phase 2, 334 individuals had positive results for antibodies against the spike protein, 184 had positive results for antibodies against RBD proteins, and 187 had positive results for antibodies against the nucleocapsid protein, with high correlations among the antigens (nucleocapsid protein vs receptor binding domain: \( F = 6493.26 \); nucleocapsid protein vs full-length spike protein: \( F = 3634.98 \); receptor binding domain vs full-length spike protein: \( F = 3128.04 \); \( P < .001 \) for all comparisons) (eTable 2 in the Supplement). Among the 6955 participants who received testing in phase 2, 177 had positive results for at least 2 antigens (unadjusted cumulative incidence, 2.5%), and 73 had positive results for all 3 antigens (unadjusted cumulative incidence, 1.0%).

A total of 168 seropositive adults of 8967 who had DBS testing in phase 1 represented an education-weighted cumulative incidence of 1.9% (95% CI, 0.7%-4.7%), and 455 seropositive adults of 6955 who had DBS testing in phase 2 represented an education-weighted cumulative incidence of 6.5% (95% CI, 4.6%-9.1%), 377 of whom met the strict cutoff criteria (education-weighted cumulative incidence, 5.4%; 95% CI, 4.1%-7.1%) (Table 1). Based only on spike protein results for the purpose of comparison with phase 1 results, 334 adults were seropositive in phase 2, for an education-weighted cumulative incidence of 4.7% (95% CI, 2.8%-7.8%).
Table 1. SARS-CoV-2 Seropositivity in Canada in the First and Second Viral Waves and Factors Associated With Infection in the Second Wave

| Variable                        | Phase 1 (n = 8967)
|---------------------------------|------------------------
|                                 | Seronegative, No. | Seropositive, No. | Incidence, %  |
| Total participants              | 8799               | 168               | 1.9          |
| High-risk regions               | 3267               | 82                | 2.5          |

| Province                        | Phase 2 (n = 6955)
|---------------------------------|------------------------
|                                 | Seronegative, No. | Seropositive, No. | Incidence, %  |
| Total participants              | 6500               | 455               | 6.5          |
| High-risk regions               | 2408               | 185               | 7.1          |

| Province                        | Phase 2, OR (95% CI)
|---------------------------------|------------------------
| Atlantic                        | 0.46 (0.21-1.02)       |
| British Columbia (some Yukon Territory) | 1.03 (0.74-1.44)       |
| Ontario                         | 1 (Reference)          |
| Prairies (some Northwest Territories) | 1.08 (0.48-2.45)       |
| Quebec                          | 0.99 (0.63-1.56)       |

| Sex                             | Phase 2, OR (95% CI)
|---------------------------------|------------------------
| Female                          | 0.99 (0.80-1.21)       |
| Male                            | 1 (Reference)          |

| Age group, y                    | Phase 2, OR (95% CI)
|---------------------------------|------------------------
| 18-39                           | 1 (Reference)          |
| 40-59                           | 1.01 (0.55-1.84)       |
| 60-69                           | 1.23 (0.62-2.42)       |
| ≥70                             | 1.10 (0.72-1.70)       |

| Educational level               | Phase 2, OR (95% CI)
|---------------------------------|------------------------
| Some college or less            | 1 (Reference)          |
| College graduate                | 1.12 (0.51-2.46)       |
| University graduate             | 0.94 (0.39-2.27)       |

| Racial and/or ethnic minority   | Phase 2, OR (95% CI)
|---------------------------------|------------------------
| No                              | 1 (Reference)          |
| Yes                             | 1.49 (0.50-4.44)       |

| Indigenous Canadian             | Phase 2, OR (95% CI)
|---------------------------------|------------------------
| No                              | 1 (Reference)          |
| Yes                             | 0.84 (0.23-3.07)       |

| COVID-19 symptomatic            | Phase 2, OR (95% CI)
|---------------------------------|------------------------
| No                              | 1 (Reference)          |
| Yes                             | 6.15 (2.02-18.69)      |

| Smoking status                  | Phase 2, OR (95% CI)
|---------------------------------|------------------------
| Current                         | 0.71 (0.46-1.11)       |
| Former                          | 1.15 (1.05-1.27)       |
| Never                           | 1 (Reference)          |

| BMI                             | Phase 2, OR (95% CI)
|---------------------------------|------------------------
| Underweight or normal weight (<25) | 1 (Reference)          |
| Overweight (25-29)              | 0.99 (0.40-2.46)       |
| Obesity (≥30)                   | 0.94 (0.34-2.63)       |

| Diabetes                        | Phase 2, OR (95% CI)
|---------------------------------|------------------------
| No                              | 1 (Reference)          |
| Yes                             | 0.58 (0.38-0.90)       |

| Hypertension                    | Phase 2, OR (95% CI)
|---------------------------------|------------------------
| No                              | 1 (Reference)          |
| Yes                             | 0.96 (0.85-1.10)       |

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); NA, not applicable; OR, odds ratio.

a Variables were adjusted for all others in the table.
b Strict cutoff criteria were used for the main seropositivity results in phase 1.
c Lenient and strict cutoff criteria were used for the main seropositivity results in phase 2.
d COVID-19 symptoms were defined as a combination of fever (or fever with hallucinations) with difficulty breathing, dry cough, loss of smell or taste, or discoloration and/or swelling in toe (COVID toe).
e Incidence was weighted by educational level.
In phase 2, the overall cumulative incidence and factors associated with infection using the lenient or strict cutoffs for the spike protein were similar; therefore, the main analysis of seropositivity used the lenient cutoff criteria (unless otherwise stated). We performed testing for a total of 6338 adults in both phases 1 and 2, 6204 of whom were seronegative in phase 1. Of those, 296 individuals became seropositive in phase 2, representing an education-weighted incidence of 4.8% (95% CI, 4.0%-5.7%) (eTable 4 in the Supplement).

In phase 1, cumulative incidence peaked at ages 18 to 39 years (2.4%) and decreased at older ages (1.8% at 40-59 years, 1.9% at 60-69 years, and 0.7% at ≥70 years) but was similar across age groups in phase 2 (6.7% at 18-39 years, 6.1% at 40-59 years, 6.9% at 60-69 years, and 6.1% at ≥70 years). Ontario had the highest incidence (2.6%) in phase 1, which was approximately double that of several other provinces or regions (eg, 1.3% in British Columbia and the Atlantic provinces). By phase 2, almost all provinces and regions had a cumulative incidence ranging from 6.4% (British Columbia) to 7.0% (Prairies). The exceptions were the Atlantic provinces (New Brunswick, Newfoundland, Nova Scotia, and Prince Edward Island), where cumulative incidence was 3.3% (odds ratio [OR] vs Ontario, 0.46; 95% CI, 0.21-1.02), with similar results for incident seropositivity. Seropositivity increased among racial or ethnic minority adults, from 1.7% in phase 1 to 8.6% in phase 2, but this increase was not statistically significant.

In multivariable analyses of cumulative infection, after adjustment for demographic characteristics, risk factors, and COVID-19 symptom history, racial or ethnic minority group status was not associated with cumulative or between-phase seropositivity (Table 2 and eTable 4 in the Supplement). Participants with a history of diabetes (OR, 0.58; 95% CI, 0.38-0.90) had lower odds of having detectable antibodies in phase 2.

In both phases combined, 16 348 of 34 615 respondents (47.2%) experienced at least 1 of the symptoms included in the survey, and 1672 respondents (4.8%) met the study definition of COVID-19 symptom positivity (1191 adults [5.8%] during phase 1, peaking in March 2020, and 481 adults [3.2%] in the interval after completion of the first survey, peaking in December 2020). In phase 1, 54 of 715 adults (7.5%) reporting COVID-19 symptoms were seropositive. In phase 2, 47 of 188 adults (25.3%) reporting COVID-19 symptoms were seropositive, and the OR of seropositivity for COVID-19 symptoms was 6.15 (95% CI, 2.02-18.69). In phase 2, 94 of 444 seropositive adults (22.2% weighted for education) reported having no symptoms. There was variability in the associations of sex, education, and ethnicity with asymptomatic infection.

Among 134 adults with seropositivity for the spike protein in phase 1 who had DBS testing in phase 2, 111 individuals (81.8% weighted for education) retained antibodies between the testing dates (for at least 7 months). The proportion of those with persistent antibodies was stable over the 3- to 7-month period between the 2 phases (eg, strict cutoff: 72% [95% CI, 66%-78%] at 3 months vs 66% [95% CI, 52%-78%] at 7 months; lenient cutoff: 94% [95% CI, 66%-99%] at 3 months vs 84% [95% CI, 71%-92%] at 7 months). The Spearman correlation analysis revealed a nonsignificant decrease in seropositivity (strict cutoff: \( p = -0.04; P = .66 \); lenient cutoff: \( p = -0.04; P = .62 \)) (Figure 2). We examined the factors associated with retention of antibodies, focusing on the strict cutoff criteria because they enabled inclusion of a larger sample of adults who lost seropositivity (\( n = 46 \)), whom we compared with the 88 adults who retained seropositivity. An increase in persistence of seropositivity was associated with belonging to a racial or ethnic minority group (OR, 1.69; 95% CI, 1.27-2.08), or being a current or former smoker (OR, 2.09; 95% CI, 1.00-4.38). Diabetes was associated with lower odds of seropositivity (OR, 0.38; 95% CI 0.21-0.68) (eTable 5 in the Supplement). However, the numbers were too small to identify any conclusive patterns.

Because the phase 1 sample was broadly representative of the Canadian adult population, we were able to derive plausible estimates of the total number of Canadians who were seropositive for SARS-CoV-2 and compare seropositivity with non–nursing home deaths associated with COVID-19 to derive the IFR for each viral wave (Table 3). In wave 1, we estimated that 0.57 million adult Canadians had SARS-CoV-2 antibodies between April 1 and September 30, 2020, and 0.25 million (43.8%) of those were young adults aged 20 to 39 years. By the end of wave 2 (October 1, 2020, to March 1,
2021), we estimated that 1.9 million adults had seropositivity (excluding the approximately 0.2 million Canadians residing in nursing homes or long-term care facilities). In wave 2, approximately 32% of all Canadians with SARS-CoV-2 infection were 60 years or older compared with 21% of all Canadians with SARS-CoV-2 infection in wave 1. A total of 4908 of 7040 COVID-19-associated deaths (69.7%) nationwide between April 2020 and March 20211 (excluding those in nursing homes and long-term care facilities) occurred during wave 2. However, the IFR per 1000 infections was lower overall in wave 2 (2.58; 95% CI, 1.81-3.59) than in wave 1 (3.73; 95% CI, 1.53-10.24). Because of reasonably wide estimates of seropositivity, the IFRs between the phases did not substantially differ. There was a suggestion of a substantially greater reduction in IFR among those 70 years and older (from 48.01 [95% CI, 14.61-168.02] in wave 1 to 12.07 [95% CI, 6.57-22.31] in wave 2), but the 95% CIs were wide.

**Discussion**

The Ab-C study found that the cumulative incidence of SARS-CoV-2 infection among Canadian adults in the community remained relatively low throughout the first 2 viral waves until March 2021, which

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**Table 2. Factors Associated With Seropositive Asymptomatic and Symptomatic SARS-CoV-2 Infections in Phase 2**

| Variable* | No. | OR (95% CI) |
|-----------|-----|-------------|
|           | Asymptomatic | Symptomatic | |
| Sex       |               |             | |
| Female    | 42            | 221         | 0.46 (0.17-1.28) |
| Male      | 52            | 128         | 1 [Reference] |
| Age group, y |         |             | |
| 18-39     | 16            | 107         | 1 [Reference] |
| 40-59     | 22            | 120         | 1.40 (0.23-8.63) |
| 60-69     | 35            | 91          | 2.43 (0.39-15.01) |
| ≥70       | 21            | 32          | 3.78 (1.01-14.12) |
| Educational level | |             | |
| Some college or less | 27 | 58 | 1 [Reference] |
| College graduate | 30 | 123 | 0.54 (0.15-2.02) |
| University graduate | 37 | 169 | 0.53 (0.39-0.73) |
| Racial and/or ethnic minority | |         | |
| No        | 83            | 293         | 1 [Reference] |
| Yes       | 11            | 57          | 0.79 (0.05-12.10) |
| Indigenous Canadian | |         | |
| No        | 91            | 319         | 1 [Reference] |
| Yes       | 3             | 31          | 0.25 (0.04-1.78) |
| Smoking status | |         | |
| Current   | 5             | 24          | 0.92 (0.14-5.95) |
| Former    | 43            | 134         | 1.01 (0.21-4.78) |
| Never     | 46            | 189         | 1 [Reference] |
| BMI       |               |             | |
| Underweight or normal weight (<25) | 37 | 109 | 1 [Reference] |
| Overweight (25-30) | 26 | 130 | 0.43 (0.09-2.01) |
| Obese (≥30) | 26 | 85  | 0.64 (0.30-1.37) |
| Diabetes  |               |             | |
| No        | 84            | 327         | 1 [Reference] |
| Yes       | 9             | 20          | 1.25 (0.21-7.47) |
| Hypertension |          |             | |
| No        | 62            | 265         | 1 [Reference] |
| Yes       | 31            | 83          | 1.04 (0.24-4.48) |

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); OR, odds ratio.

* Variables were adjusted for all others in the table.
was consistent with modest COVID-19–associated mortality outside of nursing homes and long-term care facilities, reflecting some success in curbing community spread of infection. Adult seropositivity more than tripled nationwide, from 1.9% to 6.5%, between the first and second viral waves, but increased less in Atlantic provinces, where travel restrictions limited introduction of the virus, and swift responses moderated outbreaks.46 By March 1, 2021, approximately 0.9 million cases were PCR-confirmed, suggesting an unadjusted ratio of approximately 2 infections to each confirmed PCR-positive case, which was lower than the ratio of infections to confirmed PCR-positive cases reported in England,47,48 Spain,23 and the US.24 Canadian adult seropositivity in the second viral wave was lower than that reported among adults included in national seroprevalence studies in England and Spain21-23 and from convenience samples in the US24 but higher than that reported in a national study in Iceland.25

We found that, at the population level, most infections produced persistent antibodies against the spike protein for a minimum of 7 months based on the timing of reported symptoms. Similar persistence of antibodies has been reported in selected cities in Italy and among health care workers,26,27 but few national studies have been conducted.

To our knowledge, the Ab-C study is among the few to examine demographic and risk factors associated with seropositivity, persistence of antibody status over time, and asymptomatic infections. Racial and ethnic minority participants had nonsignificantly higher cumulative or between-phase seropositivity, which is consistent with reports of a higher number of cases and deaths among these groups in Canada.28 Participants who currently smoked had nonsignificantly lower odds of cumulative or between-phase seropositivity, which has been reported inconsistently with other studies.29

Table 3. Age-Specific Distribution of SARS-CoV-2 Infections, Deaths, and Infection Fatality Rates in Canada in First and Second Viral Waves

| Age group, y | Population, millions | Cumulative incidence of infections, % (95% CI) | No. of infections | Fatality rate per 1000 infections (95% CI) |
|--------------|----------------------|-----------------------------------------------|-----------------|---------------------------------------|
|              |                      | Wave 1 | Wave 2 | Wave 1 | Wave 2 | Wave 1 | Wave 2 |
| 20-39        | 10.4                 | 2.4 (0.9-6.3) | 6.7 (3.6-12.0) | 250 | 698 | 0.10 (0.04-0.26) | 0.12 (0.07-0.23) |
| 40-59        | 9.9                  | 1.8 (0.5-5.7) | 6.1 (4.6-8.0) | 179 | 605 | 0.78 (0.25-2.82) | 0.77 (0.58-1.02) |
| 60-69        | 4.7                  | 1.9 (0.9-4.1) | 6.9 (5.1-9.3) | 90 | 326 | 5.20 (2.41-10.98) | 3.27 (2.43-4.43) |
| ≥70b         | 4.5                  | 0.7 (0.2-2.3) | 6.1 (3.3-11.2) | 31 | 272 | 48.01 (14.61-168.02) | 12.07 (6.57-22.31) |
| All age groups | 29.7               | 1.9 (0.7-4.7) | 6.5 (4.6-9.1) | 571 | 1902 | 3.73 (1.53-10.24) | 2.58 (1.81-3.59) |

a Wave 1 occurred from April 1 to September 30, 2020, and wave 2 from October 1, 2020, to March 1, 2021.
b A total of 7040 confirmed deaths associated with COVID-19 were reported in Canada from April 1, 2020, to March 1, 2021.1 Of those, 2132 deaths occurred in wave 1, and 4908 deaths occurred in wave 2. This total excludes deaths that occurred in long-term care facilities and nursing homes.
in other epidemiologic studies. The association between a history of diabetes and reductions in the risk of cumulative or between-phase seropositivity was unexpected because both diabetes and smoking are established risk factors associated with COVID-19 hospitalization and mortality. Possible subtle biases in self-reported health status and the relatively small sample included in the present study suggest further epidemiologic studies are needed to understand the largely unknown host factors or intermediate factors associated with disease risk (unrelated to SARS-CoV-2 infection itself) that might make infection more severe or have implications for the durability of the immune response. The archived DBS in the Ab-C study may aid such exploration.

The asymptomatic proportion of 22.2% among seropositive adults was similar to the proportion reported in England but higher than the proportions reported in 2 systematic reviews. Nonetheless, retrospective collection of symptom data may not reflect the true proportion of individuals with symptomatic SARS-CoV-2 infection. The IFR differed more than 100-fold between those 70 years or older and those aged 20 to 39 years, as documented in earlier reviews.

Nationally representative studies are needed in various settings. As observed in the Ab-C study, full representativeness is difficult to achieve, but ensuring that the serially assessed populations are comparable over time may reduce the role of biases at enrollment when explaining seropositivity patterns. The performance of such nationally representative studies is important to monitor differences in vaccine-induced immunity across age groups (particularly older adults) and across vulnerable groups. These studies can include more detailed examination of the persistence of antibodies among such groups and identify age-specific factors among those who maintain or lose antibodies. Such information can, in turn, inform the need for booster vaccination or periodic revaccination. The home-based DBS collection used in the Ab-C study is highly practicable and compatible with physical distancing requirements for COVID-19 control. We began a third assessment in June to July 2021 to capture the fourth viral wave, which was mostly associated with the delta variant, and to examine the early results of vaccination, and we plan to conduct fourth and fifth phases in 2022 to assess the levels and durability of vaccine-induced immunity as well as any possible future viral waves, including those associated with the Omicron variant or future variants.

**Strengths and Limitations**

This study has several strengths. The Ab-C study was representative of Canadian adults with regard to demographic patterns and risk factor prevalence. The study used high-quality assays, with strategies to minimize false-positive results.

The study also has limitations. Antibody assays and related laboratory procedures to optimize SARS-CoV-2 antibody detection have been developed only recently and continue to undergo refinement. Although we could not define seropositivity identically in the 2 phases because of improvements in laboratory methods, epidemiologic patterns and factors associated with infection were similar for only the spike protein using strict and lenient cutoff criteria among seropositive adults in phase 2. Moreover, the 3 antigens tested in phase 2 were highly correlated. Our assay results may be less comparable with those of seroprevalence studies using other assays. Given that almost all SARS-CoV-2 assays have been recently developed, cross-comparisons with multiple assays are challenging. To improve comparisons over time and across assays, national authorities might organize a testing scheme with defined and blinded sample panels that can be provided to relevant laboratories. A similar strategy was used to successfully improve HIV diagnostic testing. Serial assessments such as ours can help to better correct for possible false-positive results. Most of the adults with seropositivity based on strict cutoff criteria in phase 1 retained seropositivity at stable levels in phase 2, whereas adults with seropositivity based on lenient cutoff criteria in phase 1 did not, perhaps because these individuals did, in fact, have false-positive results or faster decreases in antibody levels. Follow-up in planned phase 3 (July to August 2021) and phase 4 (January 2022) will further enable understanding of the role of false positivity. In addition, the present study sample enrolled fewer adults with lower educational levels compared with the general Canadian population.
We adjusted for differences in educational level, but there might have been unrecorded factors associated with COVID-19 risk that had consequences for participation.

**Conclusions**

The Ab-C study documented a low cumulative incidence of IgG antibodies against the SARS-CoV-2 spike protein in Canada during the first viral wave (<2%) and modest levels of IgG antibodies against the spike protein, nucleocapsid protein, and RBD proteins by the second viral wave (6.5%). These findings were consistent with those reported in earlier studies involving convenience samples from blood donors and residual sera from public health laboratory specimens. Together, these studies suggest that infection-induced seropositivity did not increase population immunity to levels sufficient to substantially reduce transmission of the virus. Ongoing vaccination of the population remains central to reducing viral transmission, morbidity, and mortality.
Conflict of Interest Disclosures: Dr Bogoch reported serving as a consultant for BlueDot and the National Hockey League Players’ Association outside the submitted work. Dr Chakraborty reported receiving grants from PerkinElmer outside the submitted work. Dr Slutsky reported receiving consulting fees from Apeiron Biologics, Cellenkos, Diffusion Pharmaceuticals, and GlaxoSmithKline outside the submitted work. No other disclosures were reported.

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SUPPLEMENT.

eMethods. Angus Reid Forum Sampling, High-Burden Regions, and Laboratory Testing Procedures

eTable 1. Seropositivity Definitions in Phase 1 and Phase 2

eTable 2. Seropositivity by Antigen in Phase 2

eTable 3. Sample Characteristics and Representativeness of Phase 1 and Phase 2 Surveys and DBS Samples

eTable 4. New Seropositivity in Phase 2 Among Those Who Did Not Have Definite Seropositivity in Phase 1

eTable 5. Persistent and Nonpersistent Antibodies and Factors Associated With Those Antibodies in the Study

eReferences