PHASE 4 OF THE STUDY ON THE SEROPREVALENCE OF ANTIBODIES TO SARS-COV-2 IN QUEBEC

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Introduction

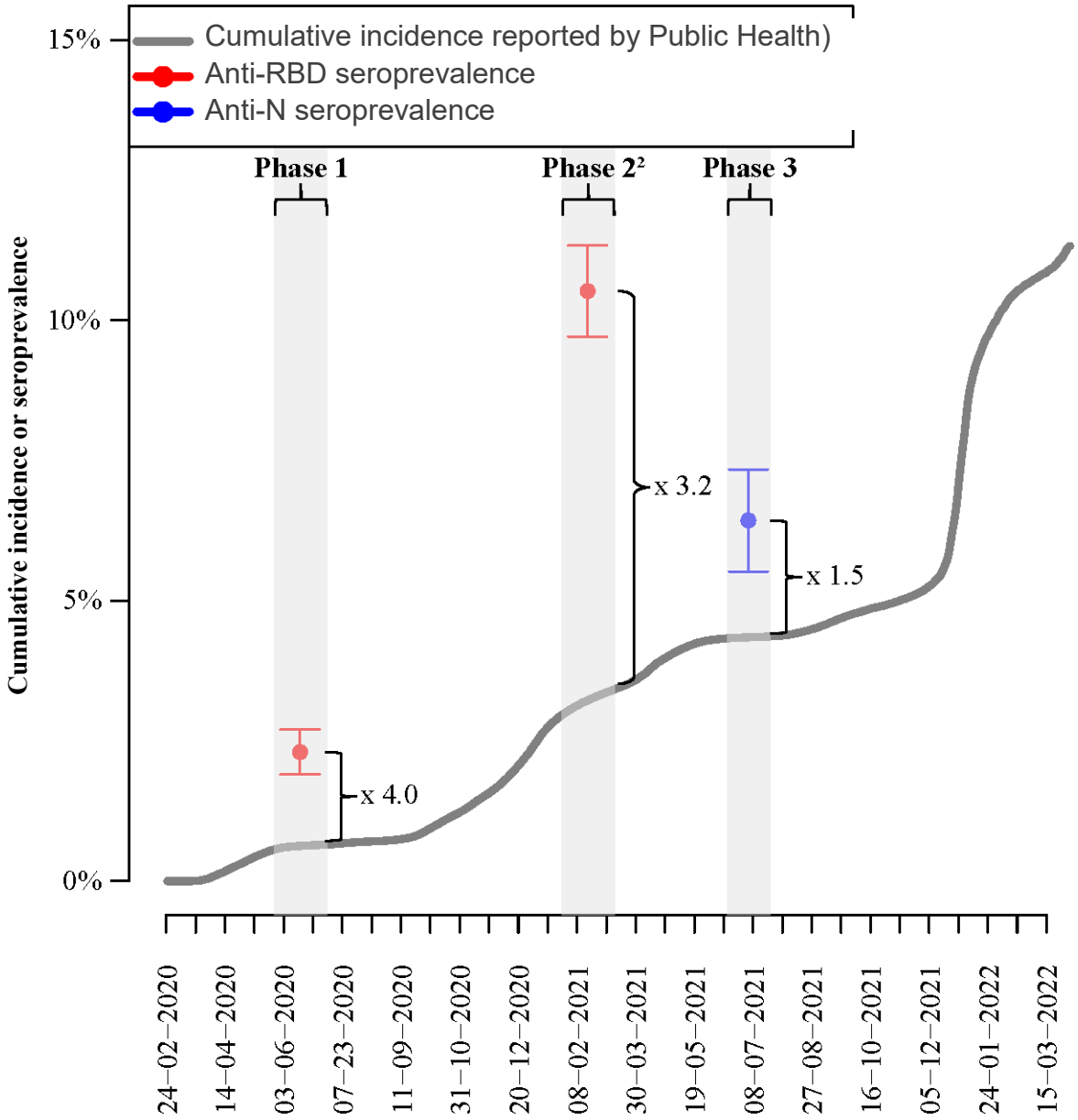
Phase 4 of the study on the seroprevalence of SARS-CoV-2 aims to document the seroprevalence of antibodies to SARS-CoV-2 in the Quebec population during the 5th wave caused by the B.1.1.529 variant (Omicron). This variant was first detected on November 25, 2021 in South Africa. The World Health Organization classified it as a variant of concern on November 26, 2021 due to its faster spread and immune evasion. In Quebec, the dominant strain was Omicron BA.1, which spread from December 2021 to March 2022 and was followed by the BA.2 sublineage.[1]

In phase 1 conducted between May and July 2020 (i.e., after the 1st wave of the COVID-19 pandemic), the weighted seroprevalence of antibodies targeting the receptor binding domain (RBD) of the spike protein (S) was estimated at 2.23% (confidence interval [CI] 95%=1.90%–2.56%); **Figure 1**).[2] In phase 2 conducted between January and March 2021 (i.e., after the peak of the 2nd wave), the weighted anti-RBD seroprevalence was estimated at 10.52% (CI 95%=9.71%–11.33%; **Figure 1**) in the unvaccinated population and 14.72% (CI 95%=13.81%–15.63%) in the general population studied.[3] In phase 3 conducted between June and July 2021 (i.e., after the 3rd wave, when a large part of the adult population had received at least one dose of the vaccine), the weighted anti-RBD seroprevalence was estimated at 89.61% (IC 95%=88.48%–90.75%) and the weighted anti-nucleocapsid (N) seroprevalence at 6.43% (CI 95%=5.52%–7.34%); **Figure 1**).[4]

The immunogen used in current vaccines is the S protein. By assessing both anti-N and anti-RBD (S) seroprevalence, phase 3 aimed to assess both vaccine-induced and infection induced immunity. The >80% weighted anti-RBD seroprevalence observed in phase 3 was consistent with the progress of the vaccination campaign. However, the anti-N seroprevalence seemed greatly underestimated and was not consistent with the anti-RBD seroprevalence observed in phase 2 (**Figure 1**). Seroreversion—a drop in anti-N levels and a loss of seropositivity over time—or excessively low induction of the primary anti-N response (below the seropositive threshold) in vaccinated individuals could explain these discrepancies.

This document presents the results of phase 4 of the study, which assessed seroprevalence in January 2022, February 2022 and March 2022. The study was conducted by Héma-Québec with financial support from Quebec's Ministère de la Santé et des Services sociaux (MSSS) and the Public Health Agency of Canada (through the Vaccine Surveillance Reference Group and the COVID-19 Immunity Task Force) toward collecting the samples used in the study. The opinions expressed in this document do not necessarily reflect those of the Public Health Agency of Canada or of the MSSS.

Figure 1. Seroprevalences weighted in phases 1-3, and cumulative incidence of PCR-confirmed infections reported by Public Health[1]



Note:

1. The error bars represent confidence intervals of 95%; the shaded areas represent the periods during which the samples were collected
2. Seroprevalence in the unvaccinated population

Phase 4 method

Data and donor sources

Samples were taken from plasma donors residing in 12 of the 18 health regions (HR) in Quebec. All the plasma donors whose samples were analyzed for anti-SARS-CoV-2 consented to take part in the study. They are all part of the PLASCOV Biobank project.[5] As in phases 1, 2 and 3 of the study, minors (<18 years) and donors who had COVID-19 symptoms in the 14 days preceding their donation were excluded, as they were not eligible for regular plasma donations. Héma-Québec obtained participants' vaccination status (including vaccination date(s) and type of vaccine) through the Système d'information-protection en maladies infectieuses (SI-PMI) and the Trajectoire de santé publique (TSP) system from Quebec's MSSS.

Study design

Samples from regular plasma donations collected between January 17 and 18, 2022, February 14 and 15, 2022, and March 16 and 18, 2022, were used in this study. Seroprevalence was measured separately for each of these sampling periods.

Anti-N ELISA— conventional approach

In phase 4, the samples were tested using the anti-nucleocapsid (N) ELISA (also used in phase 3 of the study) since these antibodies are only present in individuals who were infected with SARS-CoV-2. This test is similar to the anti-RBD ELISA used in earlier phases, except that: (1) a recombinant form of the N protein (and not the RBD) was adsorbed to the plates; and (2) the seropositive threshold of the study was set to an absorbance (optical density) of 0.350 using logistic regression and a receiver operating characteristic curve. With this threshold, the test has a sensitivity of 98.1% and a specificity of 98.5%, as assessed in a group of negative individuals (n=66) and a group of unvaccinated individuals who previously contracted SARS-CoV-2 (n=52) (i.e., infection confirmed by PCR). However, we realized in phase 3 of the seroprevalence study that this sensitivity and specificity might not be transposable to our population-based seroprevalence study to estimate asymptomatic infections. This is mainly due to the mitigating effect of the anti-N humoral immune response in individuals who had been vaccinated and then got infected. Anti-N may also be less sensitive for establishing cumulative seroprevalence since the start of the pandemic because seroreversion occurs quicker for anti-N responses than for anti-RBD responses.

Anti-N ELISA — ratio approach

A new calculation method, the "ratio approach", was developed to compensate for the lack of sensitivity of the conventional approach. This method allows us to assess infection rates during a given period rather than cumulatively from the start of the pandemic and requires at least two longitudinal samples per individual. For the Omicron wave, we use a sample collected before Omicron (i.e., before but as close as possible to December 15, 2021), and a sample collected on the dates of the identified prevalence points. A ratio is then calculated between the absorbance (result of anti-N test) after and before Omicron. Note that absorbances lower than 0.100 for samples corresponding to the identified prevalence points (i.e, January, February or March) are considered background noise and a ratio is not calculated in that event.

We tested this approach on 178 frequent plasma donors (including two unvaccinated donors) who contracted SARS-CoV-2 during the Omicron wave, namely after December 15, 2021, and whose infections were confirmed

by PCR according to the information in the provincial register (TSP). Using the 0.350 seropositive threshold (i.e., as in the conventional approach), only 64.6% of these donors were seropositive. Using an anti-N ratio of >1.5 as our seropositive threshold, we found that 94.9% of donors were seropositive. This new method provides a more accurate measure of the proportion of individuals with (recent) infections in a given observation period. These results also show that in some 30% of vaccinated individuals, a recent infection will not raise anti-N sufficiently to move from seronegative to seropositive status, highlighting the limitations of using anti-N serology to evaluate the prevalence of SARS-CoV-2 infections since the start of the pandemic.

Statistical analyses

Means and standard deviations were reported for continuous variables, and proportions were reported for discrete variables. Anti-N seroprevalence was also weighted based on the age and sex of the Quebec population, and the demographic weight of health regions (HRs) in Quebec.

Results

Donor characteristics

In total, 568 samples were collected between January 17 and 18, 2022, 496 between February 14 and 15, 2022, and 554 between March 16 and 18, 2022. Two hundred thirty-seven donors gave samples both in the period of January 17 to 18, 2022 and February 14 to 15, 2022. We then made sure all samples collected in March came from donors who had not given samples in January and February. In all three sampling periods, the proportion of men was higher than women, and donors in the greater Montréal area and its belt were underrepresented (**Table 1**). Nearly all the plasma donors were Caucasian; few were in the 5th quintile (Q5, the most deprived one) of the material and social deprivation indexes.

Table 1. Donor characteristics stratified by prevalence point

	January 17-18, 2022 N=568	February 14-15, 2022 N=496	March 16 to 18, 2022 N=554
Age, average ± SD	53 ± 17	56 ± 16	54 ± 15
Women, n (%)	211 (37.5)	166 (33.47)	153 (27.47)
Montréal and belt, n (%)	59 (10.39)	48 (9.68)	77 (13.90)
Caucasian, n (%)	549 (96.65)	484 (97.58)	541 (97.13)
Material deprivation (Q5)[1,2], n (%)	41 (7.51)	44 (9.32)	50 (9.14)
Social deprivation (Q5)[1,2], n (%)	90 (16.48)	79 (16.74)	107 (19.56)
PCR-confirmed infection since the start of the pandemic, n (%)	32 (5.6)	34 (6.85)	49 (8.91)

Abbreviations: PCR = polymerase chain reaction; Q = quintile; SD = standard deviation

Notes:

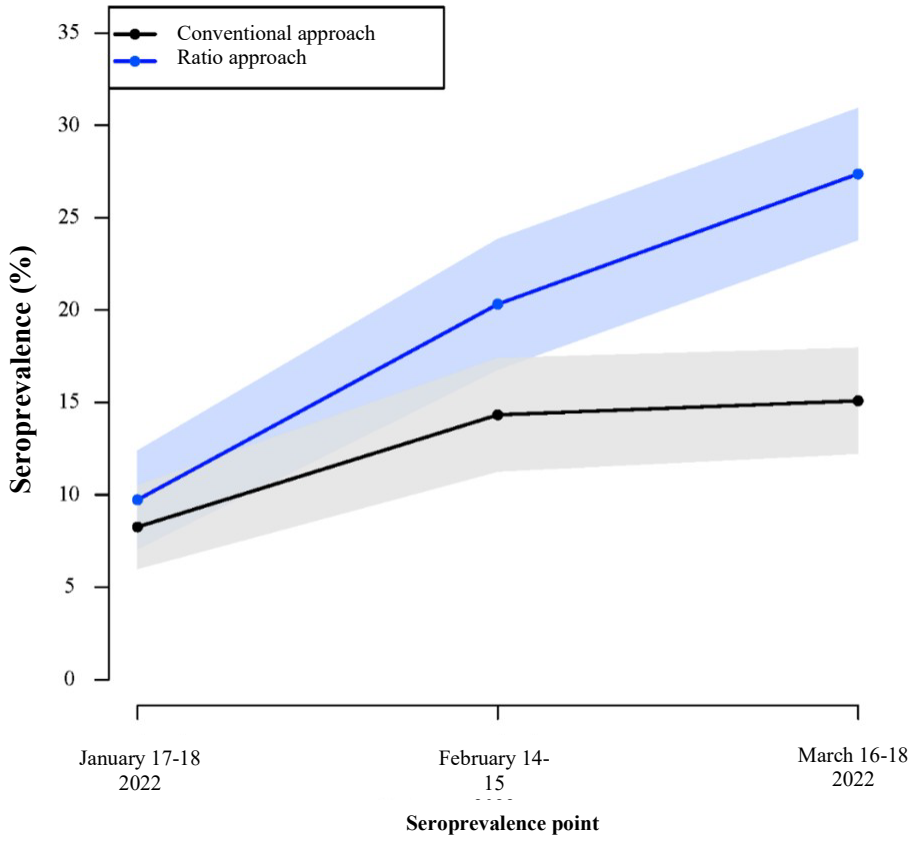
1. Data missing for 31 donors in January, 33 donors in February and 16 donors in March
2. Index of material deprivation in Quebec based on 6-character postal code. The index was divided into quintiles (Q: groups of 20%), allowing to differentiate the Quebec population based on its degree of deprivation, from the least deprived group (Q1) to the most deprived group (Q5). The material deprivation and social deprivation quintiles can be considered together or separately. These quintiles reflect considerable socio-economic variations. Across Quebec, each year the index is produced, material deprivation is linked to lower income, lower levels of education, lower employment rates and a greater proportion of widowed, separated or divorced individuals and single-parent families

Observed seroprevalences

With the conventional approach (anti-N ELISA with a seropositive threshold of 0.350), 43 (7.57%) of donors were considered seropositive on January 17-18, 2022, 69 (13.91%) on February 14-15, 2022, and 84 (15.16%) on March 16-18, 2022 (Table 2). Note that this doesn't just reflect recent infections (i.e., during the Omicron wave) but can also reflect older infections since certain donors who had been infected several months prior were still anti-N seropositive. The age, sex and health region (HR)-weighted seroprevalence was 8.26% (CI 95%=6.00%–10.52%) on January 17-18, 2022, 14.33% (CI 95%=11.27%–17.40%) on February 14-15, 2022, and 15.09% (CI 95%=12.23%–17.95%) on March 16-18, 2022 (Table 2 and Figure 2).

With the ratio approach (seropositive with anti-N ratio of >1.5), 39 (7.72%) of donors were considered seropositive on January 17-18, 2022, 90 (18.15%) on February 14-15, 2022, and 137 (24.60%) on March 16-18, 2022 (Table 3). The age, sex and HR-weighted seroprevalence was 9.73% (CI 95%=7.08%–12.38%) from January 17-18, 2022, 20.32% (CI 95%=16.80%–23.84%) from February 14-15, 2022, and 27.37% (CI 95%=23.80%–30.93%) from March 16 to 18, 2022 (Table 3 and Figure 2).

Figure 2. Seroprevalences weighted in the three sampling periods assessed[1]



Note:

1. The shaded areas represent confidence intervals of 95%

Health region-stratified seroprevalence

With the conventional approach, Montréal and its belt had a weighted seroprevalence of 8.83% (CI 95%= 3.60% – 14.05%) on January 17-18, 2022, 13.19% (CI 95%= 6.23% – 20.14%) on February 14-15, 2022 and 17.22% (CI 95%= 11.16% – 23.29%) on March 16-18, 2022 (**Table 2**). The other regions showed a similar trend, except in March where the seroprevalence was 14.39% (CI 95%= 11.15% – 17.62%) for other regions compared to 17.22% (CI 95%= 11.16% – 23.29%) for Montréal and its belt.

With the ratio approach, Montréal and its belt had a weighted seroprevalence of 13.75% (CI 95%=6.52% – 20.97%) on January 17-18, 2022, 21.98% (CI 95%=13.47% – 30.49%) on February 14-15, 2022 and 36.36% (CI 95%=25.62% – 47.11%) on March 16-18, 2022 (**Table 3**). The other regions showed a similar trend, with lower rates in March especially, at 24.24% (CI 95%= 20.29–28.19) compared to 36.36% (CI 95%= 25.62 – 47.11) for Montréal and its belt.

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Table 2. Region-stratified anti-N seroprevalence (conventional approach)

	January 17-18, 2022		February 14-15, 2022		March 16 to 18, 2022	
	n/N	Seroprevalence (%),[1] (CI 95%)	n/N	Seroprevalence (%),[1] (CI 95%)	n/N	Seroprevalence (%),[1] (CI 95%)
Health regions						
01-Bas-Saint-Laurent	0/2	-	0/1	-	0/1	-
02-Saguenay–Lac-Saint-Jean	7/109	6.71 (1.03–12.39)	11/94	9.36 (2.23–16.49)	14/93	17.26 (8.02–26.50)
03-Capitale-Nationale	7/92	9.25 (4.03–14.46)	16/86	17.86 (10.53–25.20)	8/67	10.23 (4.06–16.40)
04-Mauricie-et-Centre-du-Québec	5/77	5.70 (0.00–11.60)	12/70	19.28 (8.13–30.42)	17/92	22.05 (11.48–32.63)
05-Estrie	3/119	2.31 (0.00–5.70)	9/83	13.70 (5.31–22.09)	13/83	15.04 (5.89–24.19)
06-Montréal	1/30	3.30 (0.00–7.88)	3/13	23.08 (6.88–39.27)	5/37	10.51 (5.72–21.30)
07-Outaouais	11/78	10.21 (3.14–17.29)	10/77	12.52 (4.65–20.40)	13/90	14.37 (6.38–22.35)
08-Abitibi-Témiscamingue	-	-	-	-	-	-
09-Côte-Nord	-	-	-	-	-	-
10-Nord-du-Québec	-	-	-	-	-	-
11-Gaspésie–Îles-de-la-Madeleine	-	-	-	-	0/1	-
12-Chaudière-Appalaches	5/23	23.62 (9.88–37.35)	4/25	18.44 (5.73–31.16)	2/23	9.58 (0.65–18.51)
13-Laval	1/8	12.67 (0.00–29.08)	1/8	12.67 (0.00–29.08)	2/15	15.35 (1.51–29.18)
14-Lanaudière	-	-	0/5	-	2/9	21.61 (2.08–41.14)
15-Laurentides	0/7	-	1/6	22.22 (0.00–49.38)	1/7	14.29 (0.00–42.81)
16-Montérégie	3/22	14.12 (3.65–24.59)	2/28	7.42 (0.42–14.41)	7/39	18.30 (9.64–26.97)
17-Nunavik	0/1	-	-	-	-	-
18-Terres-Cries-de-la-Baie-James	-	-	-	-	-	-
Greater area						
Montréal and belt[2]	5/59	8.83 (3.60 – 14.05)	6/48	13.19 (6.23 – 20.14)	13/77	17.22 (11.16 – 23.29)
Other regions[3]	38/509	8.12 (5.61–10.63)	63/448	14.59 (11.17–18.00)	71/480	14.39 (11.15–17.62)
Total	**43/568**	**8.26 (6.00–10.52)**	**69/496**	**14.33 (11.27 – 17.40)**	**84/554**	**15.09 (12.23–17.95)**

Abbreviations: CI = confidence interval

Notes:

1. Weighted based on health region (HR), and distribution by sex and age in each HR (2011 census)
2. Montréal, Laval and Montréal's belt, which includes the Lanaudière region, the local health and social services networks of Deux-Montagnes – Mirabel Sud, Rivière-du-Nord – Mirabel-Nord and Thérèse-De Blainville in the Laurentides region, and the local health and social services networks of Champlain and Pierre-Boucher in Montérégie

3. The other regions include the other Laurentides and Montérégie local health and social services networks, as well as the health regions of Bas-Saint-Laurent, Saguenay-Lac-Saint- Jean, Capitale-Nationale, Mauricie-et-Centre-du-Québec, Estrie, Outaouais, Abitibi-Témiscamingue, Côte-Nord, Nord-du-Québec, Gaspésie-Îles-de-la- Madeleine, Chaudière-Appalaches, Nunavik, Terres-Cries-de-la-Baie James

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Table 3. Region-stratified Anti-N seroprevalence (ratio approach)

	January 17-18, 2022		February 14-15, 2022		March 16 to 18, 2022	
	n/N	Seroprevalence (%),[1,2] (CI 95%)	n/N	Seroprevalence (%),[1,2] (CI 95%)	n/N	Seroprevalence (%),[1,2] (CI 95%)
Health regions						
01-Bas-Saint-Laurent	0/2	-	0/1	-	0/1	-
02-Saguenay–Lac-Saint-Jean	2/101	3.73 (0.00–8.26)	16/94	17.94 (8.55–27.34)	23/93	29.54 (18.38–40.69)
03-Capitale-Nationale	6/77	8.95 (3.28–14.63)	14/86	18.37 (10.96–25.79)	9/67	13.16 (6.28–20.04)
04-Mauricie-et-Centre-du-Québec	5/72	9.06 (1.24–16.87)	16/70	25.28 (13.00–37.56)	22/92	27.49 (16.10–38.87)
05-Estrie	8/107	6.63 (0.70–12.56)	15/83	20.31 (10.49–30.12)	20/83	24.11 (13.16–35.06)
06-Montréal	0/21	-	4/13	30.77 (13.03–48.51)	14/37	37.84 (26.79–48.89)
07-Outaouais	7/73	7.07 (0.74–13.40)	13/77	20.55 (10.94–30.16)	19/90	24.27 (14.51–34.02)
08-Abitibi-Témiscamingue	-	-	-	-	-	-
09-Côte-Nord	-	-	-	-	-	-
10-Nord-du-Québec	-	-	-	-	-	-
11-Gaspésie–Îles-de-la-Madeleine	-	-	-	-	0/1	-
12-Chaudière-Appalaches	5/20	32.52 (15.96–49.08)	4/25	22.38 (8.72–36.04)	7/23	28.94 (15.19–42.70)
13-Laval	2/7	29.02 (5.06–52.98)	2/8	25.34 (3.88–46.80)	3/15	20.18 (4.78–35.59)
14-Lanaudière	-	-	1/5	20.00 (0.00–44.79)	5/9	56.79 (33.28–80.29)
15-Laurentides	1/6	22.22 (0.00–49.38)	2/6	44.44 (11.98–76.91)	4/7	57.14 (31.22–83.07)
16-Montérégie	3/18	17.39 (4.74–30.04)	3/28	11.12 (2.73–19.51)	11/39	28.76 (18.62–38.90)
17-Nunavik	0/1	-	-	-	-	-
18-Terres-Cries-de-la-Baie-James	-	-	-	-	-	-
Greater area						
Montréal and belt[3]	6/46	13.75 (6.52 – 20.97)	10/48	21.98 (13.47 – 30.49)	28/77	36.36 (25.62 – 47.11)
Other regions[4]	33/459	8.84 (6.04–11.64)	80/448	19.95 (16.09–23.81)	109/480	24.24 (20.29–28.19)
Total	**39/466**	**9.73 (7.08–12.38)**	**90/496**	**20.32 (16.80–23.84)**	**137/557**	**27.37 (23.80–30.93)**

Abbreviations: CI = confidence interval

Notes:

1. Weighted based on HR, and distribution by sex and age in each HR (2011 census)
2. An anti-N ratio of ≥1.5 is considered a recent infection. Samples with anti-N ODs of < 0.10 are directly identified as negative samples
3. Montréal, Laval and Montréal's belt, which includes the Lanaudière region, the local health and social services networks of Deux-Montagnes – Mirabel Sud, Rivière-du-Nord – Mirabel-Nord and Thérèse-De Blainville in the Laurentides region, and the local health and social services networks of Champlain and Pierre-Boucher in Montérégie

4. The other regions include the other Laurentides and Montérégie local health and social services networks, as well as the health regions of Bas-Saint-Laurent, Saguenay-Lac-Saint- Jean, Capitale-Nationale, Mauricie-et-Centre-du-Québec, Estrie, Outaouais, Abitibi-Témiscamingue, Côte-Nord, Nord-du-Québec, Gaspésie-Îles-de-la- Madeleine, Chaudière-Appalaches, Nunavik, Terres-Cries-de-la-Baie James

Age-stratified seroprevalence

With the conventional approach, in March, the oldest donors (40-59 and 60+) had a higher weighted seroprevalence than the youngest donors (18-24 and 25-39), 16.10 (CI 95%= 11.28% – 20.91%) and 16.34% (CI 95%=11.46% – 21.21%) versus 6.10% (CI 95%=0.00% – 12.99%) and 14.33% (CI 95%=7.78% – 20.87%) respectively (Table 4). When we look at each age group in January, February and March, we can see that seroprevalence was very low in 18-24 year-olds in January, then went up in February and dropped in March. This age group's small sample size likely explains these variations. In individuals aged 25-39, seroprevalence remained constant over the three months. It increased significantly in February in individuals aged 40-59, then stabilized. In individuals aged 60+, we observe a gradual rise in seroprevalence over these three months.

With the ratio approach, in March, the weighted seroprevalence declined as donor age increased, going from 40.25% (CI 95%=26.11% – 54.38%) for 18-24 year-olds to 18.73% (CI 95%=13.59% – 23.88%) for individuals aged 60+ (Table 5). The trend is similar in January and February. The biggest rise in seroprevalence between January and March was observed in the 60+ group (2.82 vs 18.73, 6.6 times higher).

Table 4. Region and age-stratified anti-N seroprevalence (conventional approach)

JANUARY	18-24 years		25-39 years		40-59 years		60+ years	
	(n/N)	Seroprevalence (%),[1] (CI 95%)	(n/N)	Seroprevalence (%),[1] (CI 95%)	(n/N)	Seroprevalence (%),[1] (CI 95%)	(n/N)	Seroprevalence (%),[1] (CI 95%)
Greater area								
Montréal-belt[2]	1/6	16.67 (0.00 – 37.75)	2/11	18.18 (2.06 – 34.30)	1/19	5.26 (0.00 – 12.36)	1/23	4.84 (0.00 – 11.39)
Others	0/47	-	9/80	14.55 (7.46 – 21.64)	9/136	7.49 (3.32 – 11.65)	20/246	7.88 (3.53 – 12.24)
Total	**1/53**	**2.81 (0.00 -6.65)**	**11/91**	**15.87 (8.72 – 21.74)**	**10/155**	**7.05 (3.42 – 10.67)**	**21/269**	**7.22 (3.52 – 10.91)**

FEBRU	18-24		25-39		40-59		60+	
	(n/N)	Seroprevalence (%),[1] (CI 95%)	(n/N)	Seroprevalence (%),[1] (CI 95%)	(n/N)	Seroprevalence (%),[1] (CI 95%)	(n/N)	Seroprevalence (%),[1] (CI 95%)
Greater area								
Montréal-belt[2]	1/6	16.67 (0.00 – 37.75)	1/4	25.00 (0.00 – 55.01)	2/14	14.29 (1.32 – 27.25)	2/24	9.30 (4.43 – 17.98)
Others	6/26	18.50 (6.41 – 30.60)	8/58	13.68 (6.58 – 20.78)	18/106	18.40 (11.52 – 25.29)	31/258	11.22 (6.32 – 16.11)
Total	**7/32**	**18.08 (7.58 – 28.57)**	**9/62**	**14.61 (7.61 – 21.60)**	**20/120**	**17.63 (11.53 – 23.74)**	**33/282**	**10.81 (6.53 – 15.08)**

MARCH	18-24 years		25-39 years		40-59 years		60+ years	
	(n/N)	Seroprevalence (%),[1] (CI 95%)	(n/N)	Seroprevalence (%),[1] (CI 95%)	(n/N)	Seroprevalence (%),[1] (CI 95%)	(n/N)	Seroprevalence (%),[1] (CI 95%)
Greater area								
Montréal-belt[2]	1/7	14.29 (0.00 – 32.62)	2/13	15.38 (1.52 – 29.25)	4/28	14.32 (5.14 – 23.51)	6/29	21.94 (10.82 – 33.05)
Others	1/25	2.54 (0.00 – 7.98)	10/69	14.00 (6.58 – 21.42)	27/159	16.68 (11.05 – 22.32)	33/227	14.56 (9.22 – 19.89)
Total	**2/32**	**6.10 (0.00 – 12.99)**	**12/82**	**14.33 (7.78 – 20.87)**	**31/187**	**16.10 (11.28 – 20.91)**	**39/256**	**16.34 (11.46 – 21.21)**

Notes:

1. Weighted based on HR, and distribution by sex and age in each HR (2011 census)
2. Montréal, Laval and Montréal's belt, which includes the Lanaudière region, the local health and social services networks of Deux-Montagnes – Mirabel Sud, Rivière-du-Nord – Mirabel-Nord and Thérèse-De Blainville in the Laurentides region, and the local health and social services networks of Champlain and Pierre-Boucher in Montérégie

Table 5. Region and age-stratified anti-N seroprevalence (ratio approach)

JANUARY	18-24 (n/N)	18-24 Seroprevalence (%),[1] (CI 95%)	25-39 (n/N)	25-39 Seroprevalence (%),[1] (CI 95%)	40-59 (n/N)	40-59 Seroprevalence (%),[1] (CI 95%)	60+ (n/N)	60+ Seroprevalence (%),[1] (CI 95%)
Greater area								
Montréal-belt[2]	1/6	16.67 (0.00 – 37.75)	2/6	33.33 (6.66 – 60.01)	3/17	17.65 (4.83 – 30.46)	0/17	-
Others	5/38	14.07 (4.13 – 24.00)	9/73	13.77 (6.47 – 21.07)	10/117	9.26 (4.24 – 14.29)	9/231	3.44 (0.35 – 6.53)
Total	**6/44**	**14.59 (5.59 – 23.60)**	**11/79**	**16.17 (8.87 – 23.48)**	**13/134**	**11.02 (6.20 – 15.85)**	**9/248**	**2.82 (0.28 – 5.36)**

FEBRUARY	18-24 years (n/N)	18-24 years Seroprevalence (%),[1] (CI 95%)	25-39 years (n/N)	25-39 years Seroprevalence (%),[1] (CI 95%)	40-59 years (n/N)	40-59 years Seroprevalence (%),[1] (CI 95%)	60+ years (n/N)	60+ years Seroprevalence (%),[1] (CI 95%)
Greater area								
Montréal-belt[2]	1/6	16.67 (0.00 – 37.75)	2/4	50.00 (15.35 – 84.65)	5/14	35.71 (17.97 – 53.46)	2/24	9.30 (0.62 – 17.98)
Others	9/26	31.06 (16.65 – 45.47)	16/58	24.64 (15.74 – 33.55)	26/106	23.80 (16.24 – 31.36)	29/258	11.62 (6.65 – 16.59)
Total	**10/32**	**27.71 (15.50 – 39.92)**	**18/62**	**16.71 (17.95 – 35.48)**	**31/120**	**26.03 (19.00 – 33.06)**	**31/251**	**11.13 (6.80 – 15.46)**

MARCH	18-24 years (n/N)	18-24 years Seroprevalence (%),[1] (CI 95%)	25-39 years (n/N)	25-39 years Seroprevalence (%),[1] (CI 95%)	40-59 years (n/N)	40-59 years Seroprevalence (%),[1] (CI 95%)	60+ years (n/N)	60+ years Seroprevalence (%),[1] (CI 95%)
Greater area								
Montréal-belt[2]	5/7	71.43 (47.76 – 95.09)	9/13	69.23 (51.49 – 86.97)	7/28	25.07 (13.70 – 36.43)	7/29	24.30 (12.79 – 35.82)
Others	6/25	26.71 (11.44 – 41.98)	21/69	28.50 (18.85 – 38.15)	50/159	28.90 (22.05 – 35.75)	31/227	16.97 (11.29 – 22.64)
Total	**11/32**	**40.25 (26.11 – 54.38)**	**30/82**	**38.12 (29.05 – 47.20)**	**57/187**	**27.94 (22.07 – 33.82)**	**39/256**	**18.73 (13.59 – 23.88)**

Notes:

1. Weighted based on HR, and distribution by sex and age in each HR (2011 census)
2. Montréal, Laval and Montréal's belt, which includes the Lanaudière region, the local health and social services networks of Deux-Montagnes – Mirabel Sud, Rivière-du-Nord – Mirabel-Nord and Thérèse-De Blainville in the Laurentides region, and the local health and social services networks of Champlain and Pierre-Boucher in Montérégie

Material deprivation index-stratified seroprevalence

With the conventional approach, the weighted seroprevalence tended to increase in keeping with the material deprivation index, going from 13.11% (CI 95%=8.17%–18.06%) for the first quintile (Q1) of the index to 24.50% (CI 95%=11.96%–37.04%) for Q5 (**Table 6** and **Figure 3**). However, it remained similar between quintiles in the social deprivation index (Q1: 16.26% [CI 95%=10.15%–22.38%]; Q5: 16.14% [9.39%–22.89%]).

With the ratio approach, the trend described above for the material deprivation index was much less noticeable (or even nonexistent): The weighted seroprevalence went from 28.15% (CI 95%=21.56%–34.73%) for Q1 to 33.29% (CI 95%=19.55%–47.04%) for Q5 (**Table 6** and **Figure 3**). It was also similar between social deprivation index quintiles (Q1: 32.00% [CI 95%=24.28%–39.73%]; Q5: 24.35% [CI 95%=16.47%–32.23%]).

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Table 6. March 16-17, 2022 seroprevalences using the conventional and ratio approaches stratified by material deprivation index and social deprivation index[1]

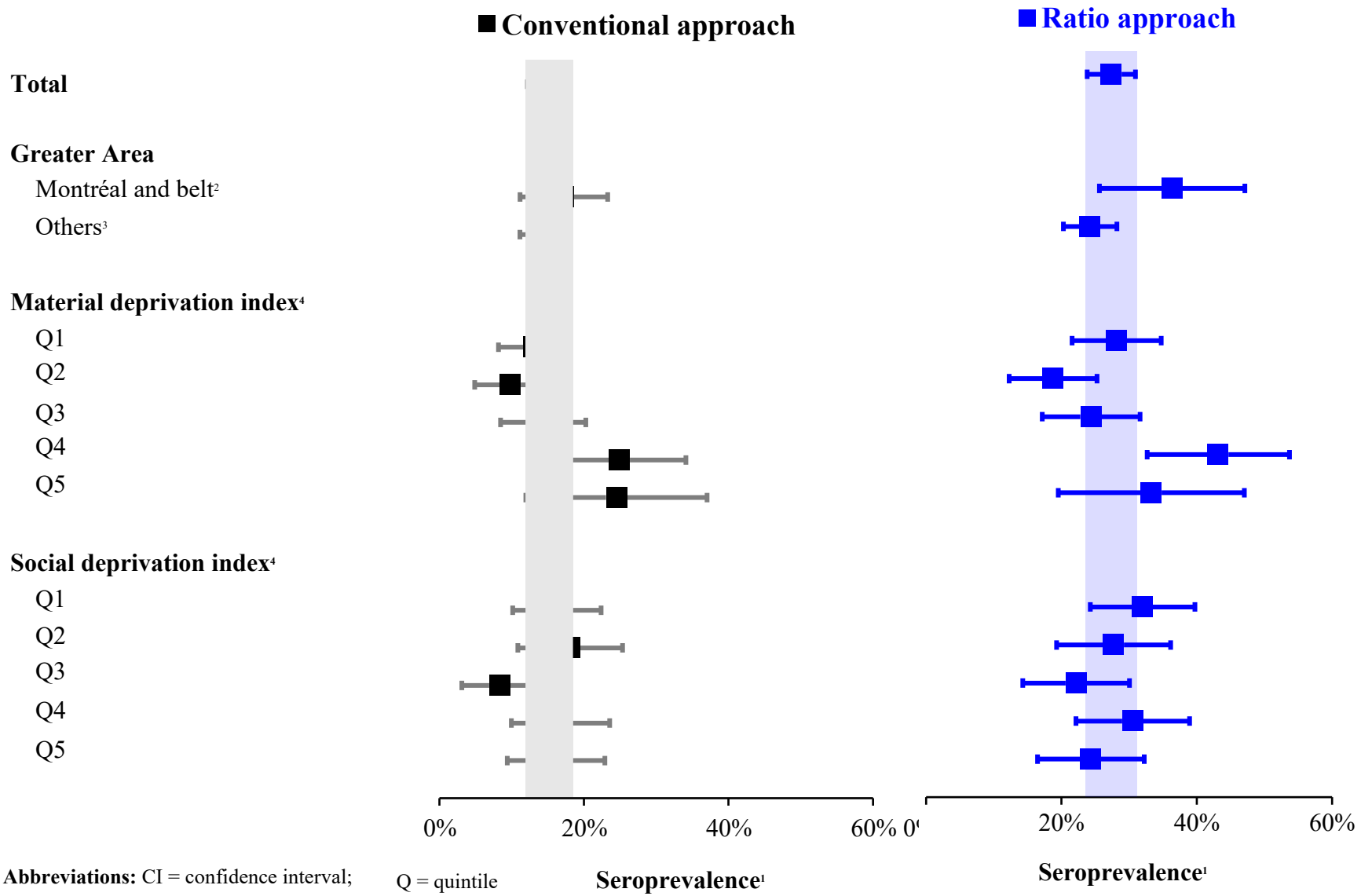
	Conventional approach				Ratio approach			
	Material deprivation		Social deprivation		Material deprivation		Social deprivation	
Quintile	n/N	Seroprevalence (%),[2] (CI 95%)	n/N	Seroprevalence (%),[2] (CI 95%)	n/N	Seroprevalence (%),[2] (CI 95%)	n/N	Seroprevalence (%),[2] (CI 95%)
Q1	21/148	13.11 (8.17–18.06)	18/116	16.26 (10.15–22.38)	41/148	28.15 (21.56–34.73)	31/116	32.00 (24.28–39.73)
Q2	13/137	9.85 (4.89–14.80)	19/109	18.09 (10.85–25.34)	21/137	18.77 (12.28–25.26)	30/109	27.72 (19.30–36.15)
Q3	18/130	14.36 (8.47–20.26)	9/107	8.35 (3.10–13.60)	30/130	24.39 (17.17–31.61)	20/107	22.17 (14.29–30.05)
Q4	18/76	24.93 (15.75–34.11)	18/102	16.76 (9.95–23.56)	27/76	43.18 (32.67–53.69)	29/102	30.55 (22.16–38.95)
Q5	13/50	24.50 (11.96–37.04)	19/107	16.14 (9.39–22.89)	15/50	33.29 (19.55–47.04)	24/107	24.35 (16.47–32.23)

Abbreviations: CI = confidence interval; Q = quintile

Notes:

1. Index of material deprivation in Quebec based on 6-character postal code. The index was divided into quintiles (Q: groups of 20%), allowing to differentiate the Quebec population based on its degree of deprivation, from the least deprived group (Q1) to the most deprived group (Q5). The material deprivation and social deprivation quintiles can be considered together or separately. These quintiles reflect considerable socio-economic variations. Across Quebec, each year the index is produced, material deprivation is linked to lower income, lower levels of education, lower employment rates and a greater proportion of widowed, separated or divorced individuals and single-parent families
2. 16 participants missing
3. Weighted based on HR, and distribution by sex and age in each HR (2011 census)

Figure 3. Seroprevalence observed between March 16 and 18, 2022, stratified by region, material deprivation index and social deprivation index[1]



Abbreviations: CI = confidence interval; Q = quintile

Notes:

1. The intervals represent CIs of 95%
2. Montréal, Laval and Montréal's belt, which includes the Lanaudière region, the local health and social services networks of Deux-Montagnes – Mirabel Sud, Rivière-du-Nord – Mirabel-Nord and Thérèse-De Blainville in the Laurentides region, and the local health and social services networks of Champlain and Pierre-Boucher in Montérégie
3. The other regions include the other Laurentides and Montérégie local health and social services networks, as well as the health regions of Bas-Saint-Laurent, Saguenay-Lac-Saint- Jean, Capitale-Nationale, Mauricie-et-Centre-du-Québec, Estrie, Outaouais, Abitibi-Témiscamingue, Côte-Nord, Nord-du-Québec, Gaspésie-Îles-de-la- Madeleine, Chaudière-Appalaches, Nunavik, Terres-Cries-de-la-Baie James
4. Index of material deprivation in Quebec based on 6-character postal code. The index was divided into quintiles (Q: groups of 20%), allowing to differentiate the Quebec population based on its degree of deprivation, from the least deprived group (Q1) to the most deprived group (Q5). The material deprivation and social deprivation quintiles can be considered together or separately. These quintiles reflect considerable socio-economic variations. Across Quebec, each year the index is produced, material deprivation is linked to lower income, lower levels of education, lower employment rates and a greater proportion of widowed, separated or divorced individuals and single-parent families
5. Weighted based on HR, and distribution by sex and age in each HR (2011 census)

Limitations

Though the ratio approach helps detect the vast majority of recent infections, the maximum period during which the anti-N ratio remains above 1.5 is not known. The longest observation period in this study was 4 months (March 2022 vs < December 15, 2021). We do know that 29 donors who were tested during this period had a positive PCR test result between December 2021 and February 2022 (14 in December, 11 in January and 4 in February); all of these donors had an anti-N ratio of $\geq$ 1.5. More specifically, donors who were infected in December 2021 and tested in March 2022 had an anti-N ratio ranging from 2.0 to 13.5 (median = 4.8). This data suggests that the anti-N ratio could be applied to observation periods of at least 4 months. On the other hand, the proportion of false positives obtained with the anti-N ratio approach remains unknown, even though we can reasonably assume that this proportion is minimal given the specificity of the anti-N test. Lastly, donors with symptoms of infection are asked not to donate for 14 days.
Accordingly, the results are likely representative of the seroprevalence 14 days before each sampling period.

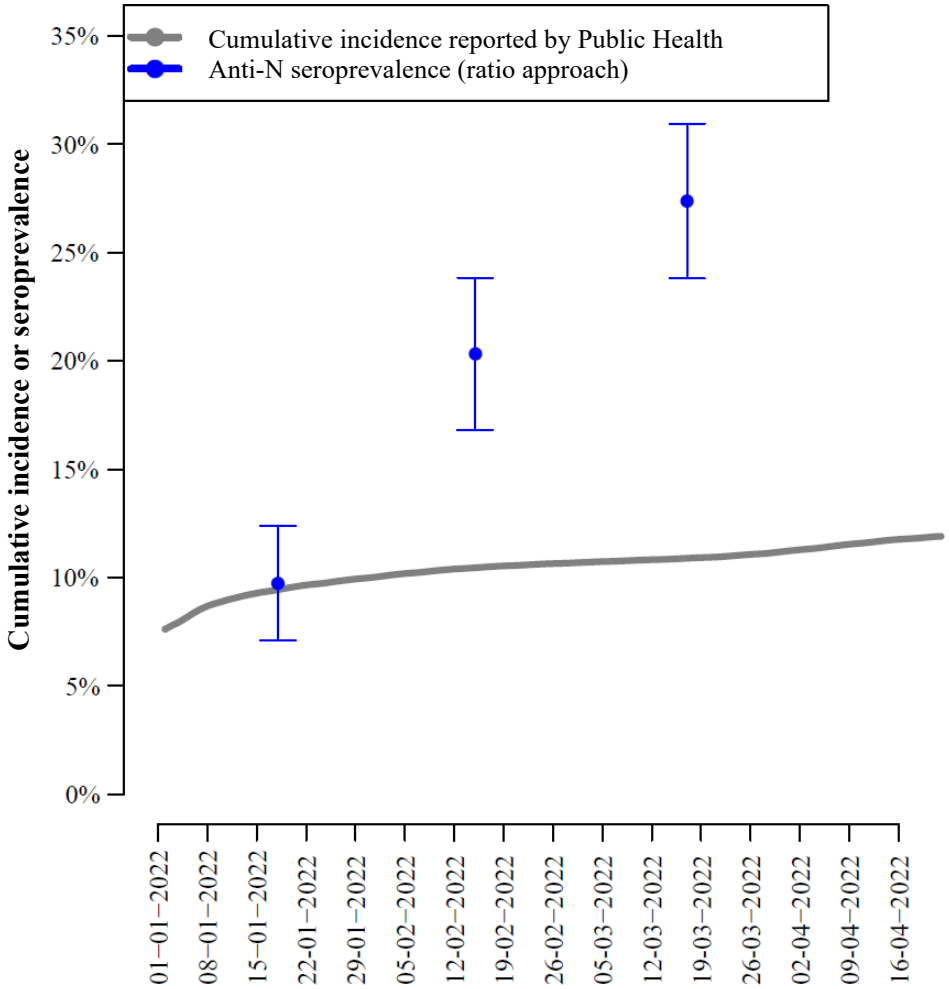
Conclusion

Phase 4 of the seroprevalence study shows that the anti-N positive threshold previously set to identify recent infections at the start of the pandemic is no longer sensitive enough now that most of the population has been vaccinated. Moreover, anti-N antibody levels decrease with time and can become negative after a few months. They are thus good markers of recent infection but cannot be used to estimate the cumulative incidence of infection since the start of the pandemic. Evaluating the progress of the anti-N signal in serial samples (ratio approach) does, however, allow us to identify the vast majority of recent PCR-confirmed infections. This approach allows us to paint a more accurate picture of how infection evolves in the general population over a given period. From March 16 to 18, 2022, the weighted anti-N seroprevalence was 15.09% with the conventional approach and 27.37% with the ratio approach. At the Quebec scale, this seroprevalence is equivalent to approximately 1.9 million infected adults (or 2.4 million infected individuals in the general population if we include children) (**Figure 4**).

As far as we know, our ratio approach is the first to track the incidence of recent infections in a vaccinated population without relying entirely on statistical modelling. Shioda et al. (city of New York and Connecticut)[20] and Chen et al. (United Kingdom)[21] used Bayesian approaches to estimate the cumulative incidence of SARS-CoV-2 based on anti-S seroprevalence data while accounting for seroreversion. However, the uncertainty inherent to this type of modelling, especially as regards the applicability of the inferences, significantly limits this approach.

This approach makes it possible to continue estimating the incidence of Omicron (BA.1) and its BA.2 sublineage using new samples collected in the coming weeks in order to measure the magnitude of Omicron and its variants.

Figure 4. Changes in seroprevalence throughout the three sampling periods of phase 4 and cumulative incidence of PCR-confirmed infections reported by Public Health.



Note:

1. The intervals represent CIs of 95%

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