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SARS-CoV-2 seroprevalence survey estimates are affected by anti-nucleocapsid antibody decline

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Abstract

We analyzed 21,676 residual specimens from Ontario, Canada collected between March-August, 2020 to investigate the effect of antibody decline on SARS-CoV-2 seroprevalence estimates. Testing specimens orthogonally using the Abbott (anti-nucleocapsid) and then the Ortho (anti-spike) assays, seroprevalence estimates ranged from 0.4%-1.4%, despite ongoing disease activity. The geometric mean concentration (GMC) of antibody-positive specimens decreased over time (p=0.015), and the GMC of antibody-negative specimens increased over time (p=0.0018). The association between the two tests decreased each month (p<0.001), suggesting anti-N antibody decline. Lowering the Abbott index cut-off from 1.4 to 0.7 resulted in a 16% increase in positive specimens.

Key-words: COVID-19, SARS-CoV-2, serology, seroprevalence

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Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), emerged as a novel pathogen in late 2019. By mid-September, 2020 it has resulted in over 28 million cases and nearly one million deaths [1]. In Ontario, Canada, the first pandemic wave peaked in mid-April [2]. This was followed by lower but sustained disease activity between June and August, with elevated case counts in various hot-spots in the province. By September 22, 2020 there were approximately 48,000 cases reported provincially for a population of 14.8 million residents [2].

As part of Ontario's pandemic response, Public Health Ontario (PHO) initiated a SARS-CoV-2 serosurveillance program to estimate the proportion of the population that has been infected, identify demographic risk factors for infection, and enhance the pandemic response [3]. The program utilizes residual specimens submitted to the PHO Laboratory from March, 2020 onwards. We tested specimens using an orthogonal approach in order to minimize false positives, particularly when population prevalence is low, in line with a recommendation by the Centers for Disease Control and Prevention [4]. Despite ongoing disease activity [2], we were surprised to discover that there were no substantial changes in Ontario seroprevalence estimates over the first five serosurveys conducted from March to August, 2020, with very low seroprevalence estimates throughout this period [3 and unpublished]. Recent studies by others have reported declining antibody levels in confirmed SARS-CoV-2 cases. However, these findings have generally been based on symptomatic, confirmed individuals or small groups of asymptomatic cases, with limited reporting of antibody decline at the population level [5–7]. We therefore investigated population-level antibody decline using Ontario serosurveillance data.

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Methods

Specimen source

We conducted five cross-sectional SARS-CoV-2 serosurveys (March 27 – April 30; May 24 – 31; June 5 – 30; July 4 – 31; August 1-21, 2020), using residual sera, plasma and blood specimens remaining after routine clinical testing at the PHO Laboratory. Specimens were selected to represent both sexes, all age groups and all geographic regions in Ontario.

Laboratory testing

Using an orthogonal testing approach, we first tested specimens with the Abbott Architect SARS-CoV-2 IgG test (Abbott Laboratories, USA), which detects anti-nucleocapsid (N) antibodies, then tested positive samples with the Ortho-Clinical Diagnostics VITROS Anti-SARS-CoV-2 IgG test (Ortho-Clinical Diagnostics, Inc., USA), which detects anti-spike (S) antibodies [8]. We used the manufacturers' recommended index value cut-off of 1.4 and 1.0 for the Abbott and Ortho assays, respectively. Specimens that were positive using both tests were considered positive for SARS-CoV-2 antibodies. To explore the effect of lowering the Abbott index value on population seroprevalence, we analyzed a subset of specimens with an index value of 0.7, otherwise maintaining the same orthogonal approach as above.

Statistical analysis

To investigate changes in the average antibody concentration over time, we calculated the geometric mean concentration (GMC) of serology specimens for each collection period. Since GMC cannot be calculated with zero values, we first replaced zero index values with a value of $0.01/\sqrt{2}$ [9], with 0.01 being the lowest non-zero value reported by the Abbott instrument. We used linear regression to assess the trend in GMC across months and truncated linear regression to examine the association between the Abbott and Ortho index values through time [10]. Truncation was used to account for potential bias

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introduced by using orthogonal testing since this analysis only includes samples where the Abbott result was ≥1.4. We fit one model for the overall association as well as one model for each of the five monthly serosurveys. To investigate the relationship between the Abbott and Ortho results over time, we fit a regression model with the statistical interaction between date and the slope to measure the change in slope through time.

Ethics

Ethics approval for the serosurveys was granted by the PHO Ethics Review Board.

Results

We tested a total of 21,676 specimens over the five time periods. Of these, 3/827 (0.4%, 95% confidence interval (CI) 0.1, 1.1) were positive in March-April, 15/1,061 (1.4%, 95% CI 0.7, 2.1) in May, 79/7,023 (1.1%, 95% CI 0.9, 1.4) in June, 70/7,001 (1.0%, 95% CI 0.8, 1.2) in July, and 64/5,764 (1.1%, 95% CI 0.8, 1.4) in August (Table 1). Overall, 318 specimens were positive for SARS-CoV-2 antibodies using the Abbott assay, of which 231 (72.6%) were also positive using the Ortho assay.

To explore whether SARS-CoV-2 antibody decline could account for the lack of increase in seroprevalence estimates over time, we first compared the GMCs for each sampling period. GMCs of specimens that were positive by the Abbott assay (n=318), which was the only assay with which all specimens were tested, were relatively stable for our first three sampling periods. However, a decline was observed from 3.7 (95% CI 3.4, 4.1) in June to 3.3 (95% CI 3.0, 3.7) in July, and then to 3.1 (95% CI 2.8, 3.4) in August (linear regression p for all sampling periods =0.015) (Table 1). Conversely, the GMC of specimens that tested negative by the Abbott assay (n=21,358) increased incrementally from May to August (linear regression p for all sampling periods =0.0018). The statistically significant decrease and increase in GMCs over time for both Abbott positive and negative specimens, respectively, suggests that population-level SARS-CoV-2 antibodies has declined over time. The GMC of positive and negative

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specimens using the Ortho assay fluctuated over time (Table 1), but changes were not statistically significant (linear regression p=0.29 and 0.35, respectively).

To explore how antibody decline might impact our seroprevalence estimates, we examined the relationship between the Abbott and Ortho assay results over time for the 318 specimens that were tested using both assays. Overall, there was a strong association between the assays, such that each Ortho index value increase of 10 was associated with an Abbott index value increase of 1.4 (95% CI 1.2, 1.7). Additionally, we found that the magnitude of the association decreased over time (Figure 1). Specifically, we found that in April, each Ortho index value increase of 10 was associated with an Abbott index value increase of 2.5 (95% CI 0.005, 5.1), while in June the association was 1.5 (95% CI 1.2, 1.8), and in August it was 1.3 (95% CI 0.4, 2.3). Overall, there was an index value decrease of 0.3 (95% CI 0.1, 0.5) in the slope of the association for each additional month since April (p<0.001).

Our observed trend of anti-N antibody decline using the Abbott assay has potential implications on seroprevalence estimates, because using our orthogonal approach specimens that test negative using the Abbott assay would not be subsequently tested using the Ortho assay, and would be deemed negative. To further explore this, we tested a subset of specimens collected in August, 2020 (N = 4,166) with the same orthogonal approach, but lowered the Abbott index value to 0.7 to account for anti-N antibody decline. While using a cut-off of 1.4 resulted in 43/4,166 specimens (1.0%) that were positive by both assays, lowering the index resulted in seven additional positive specimens, for a total of 50/4,166 (1.2%).

Discussion

Our investigation of population-level antibodies to SARS-CoV-2 revealed a decline in population level anti-N antibodies detected by the Abbott Architect SARS-CoV-2 IgG assay, over a five month period. This finding has implications for serosurveys that use the Abbott Architect assay or other anti-N assays, alone

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or as part of an orthogonal approach. Our results suggest that the anti-N antibody decline is substantial enough to affect the results of population seroprevalence surveys, especially in high prevalence settings. Due to overall low seroprevalence in Ontario, our observed antibody decline represented only a small change in estimates, from 1.0% to 1.2%. However, lowering the Abbott index cut-off resulted in a 16% increase in positive specimens, which would make a substantial difference in jurisdictions where population seroprevalence was higher. Since seroprevalence data are often used to estimate the proportion of individuals infected, including those who are not captured as cases through RT-PCR testing, antibody decline may also result in an underestimate of SARS-CoV-2 infections at the population level.

Others investigating SARS-CoV-2 antibody decline have reported conflicting results. Assessing individual immune responses, Long et al. [6] found decline in both anti-N and anti-S IgG antibody levels by eight weeks after hospital discharge. Conversely, Wu and colleagues reported stable anti-S and anti-N IgG activity six months post-infection [11]. Similarly, an analysis of >1,000 specimens from Icelandic patients using various assays directed against both anti-N and anti-S antibodies found durable IgG responses four months after diagnosis [5]. Exploration of antibody decline at the population level is less common. However, Public Health England recently reported an increase in equivocal serosurveillance results using an anti-S IgG assay [12]. It is likely that contrasting study results are related to differences between the populations sampled, time of sampling in relation to onset of infection, as well as the variety of assays used [5].

There are several modifications that can be made to SARS-CoV-2 serosurveillance testing protocols to address the issue of antibody decline, while still maintaining an orthogonal approach. We have shown here that lowering the antibody index value of the Abbott anti-N assay to increase sensitivity to account for potential antibody decline would result in a higher proportion of positive specimens. However, we recognize that this would change the test characteristics markedly. Using sera from SARS-CoV-2 RT-PCR

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positive patients, a study by Bryan and colleagues determined that lowering the Abbott assay index cut-off from 1.4 to 0.7 resulted in test sensitivity (≥7 days from symptom onset) rising from 84.2% to 88.0%, and test specificity decreasing from 100.0% to 99.6% [13]. However, retaining an orthogonal approach may mitigate the reduced specificity. Alternatively, switching to a different anti-N assay may alleviate the issue of antibody decline. Although anti-N antibodies have been shown to decline faster than anti-S antibodies, the magnitude of decline seems to be somewhat assay-specific, and therefore switching anti-N assays may result in more durable serology measures [14]. Consideration should be given to the portion of the N antigen that is used in serology assays, as this may impact the ability to detect a durable antibody response.

Our analysis included >21,000 specimens over five months, representing SARS-CoV-2 unexposed individuals as well as infected individuals of all ages, who likely experienced a spectrum of SARS-CoV-2 disease symptoms and severity. This is a strength of our study, since many analyses of SARS-CoV-2 antibody decline to date have focused on individual humoral responses using specimens from symptomatic individuals who presented to medical care, or small groups of asymptomatic individuals [5–7]. While these studies are essential for characterizing individual immune responses to SARS-CoV-2, they have a limited ability to shed light on population-level antibody decline, which may have different kinetics than those observed in small groups of previously infected individuals [5]. However, our study was limited by the cross-sectional nature of our serosurveys. Since our specimens were de-identified, we could not follow individual antibody concentrations longitudinally to explore the determinants of antibody decline, such as disease severity, age or sex. This also limited our ability to explore the reasons behind the trend of increasing GMC in Abbott-negative specimens. While we suspect that the negative specimens included samples from infected individuals that declined into the non-reactive range of the test, our cross-sectional approach does not allow us to investigate this further. Lastly, due to our testing algorithm, only a subset of our specimens were tested using the Ortho assay. Changes in the Ortho GMC

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were not statistically significant, and we suspect this may be due to selection bias towards specimens with higher antibody concentrations in this group. Although we took this selection bias into account by using a truncated regression model to compare our two assays, it still limited our ability to characterize anti-S antibody levels over time.

As the COVID-19 pandemic progresses, it is becoming clear that although serology tests may not always correlate to virus neutralization [15], they are integral to understanding population exposure, since not every infected individual is tested and reported as a case. New solutions to mitigate the issue of antibody decline are a priority area of research.

Author contributions

All authors have contributed to this work and have approved the final version of the manuscript. SB conceived the idea for the study. SB, KB, SO, EJ and VT analyzed the data, and all authors provided subject-matter expertise with interpreting the data.

Conflicts of interest

The authors have no conflicts of interest to declare

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Table 1: Serology test results and geometric mean concentration of Ontario serosurveillance specimens, March – August, 2020

Test results	Specimen collection period				
	March - April	May	June	July	August
Abbott					
Positive n/N	5/827	20/1,061	106/7,023	97/7,001	90/5,764
Positive AI GMC (95% CI)*	3.4 (1.9, 6.0)	3.7 (3.0, 4.5)	3.7 (3.4, 4.1)	3.3 (3.0, 3.7)	3.1 (2.8, 3.4)
Negative n/N	822/827	1,041/1,061	6,917/7,023	6,904/7,001	5,674/5,764
Negative AI GMC (95% CI)*	0.034 (0.032, 0.037)	0.032 (0.031, 0.034)	0.033 (0.032, 0.033)	0.034 (0.033, 0.035)	0.035 (0.034, 0.035)
Ortho[a]					
Positive n/N	3/5	15/20	79/106	70/97	64/90
Positive AI GMC (95% CI)	22 (13, 38)	15 (11, 22)	18 (15, 21)	16 (13, 19)	15 (13, 18)
Negative n/N	2/5	5/20	27/106	27/97	26/90
Negative AI GMC (95% CI)	0.046 (0.0055, 0.38)	0.047 (0.012, 0.18)	0.029 (0.020, 0.042)	0.021 (0.015, 0.030)	0.028 (0.019, 0.042)
Orthogonally positive					
n/N	3/827	15/1,061	79/7,023	70/7,001	64/5,764
% (95% CI)	0.4 (0.1, 1.1)	1.4 (0.7, 2.1)	1.1 (0.9, 1.4)	1.0 (0.8, 1.2)	1.1 (0.8, 1.4)

[a] = only Abbott-positive specimens were tested; GMC = geometric mean concentration; AI = antibody index; CI= confidence interval;* = statistically significant trend across sampling periods

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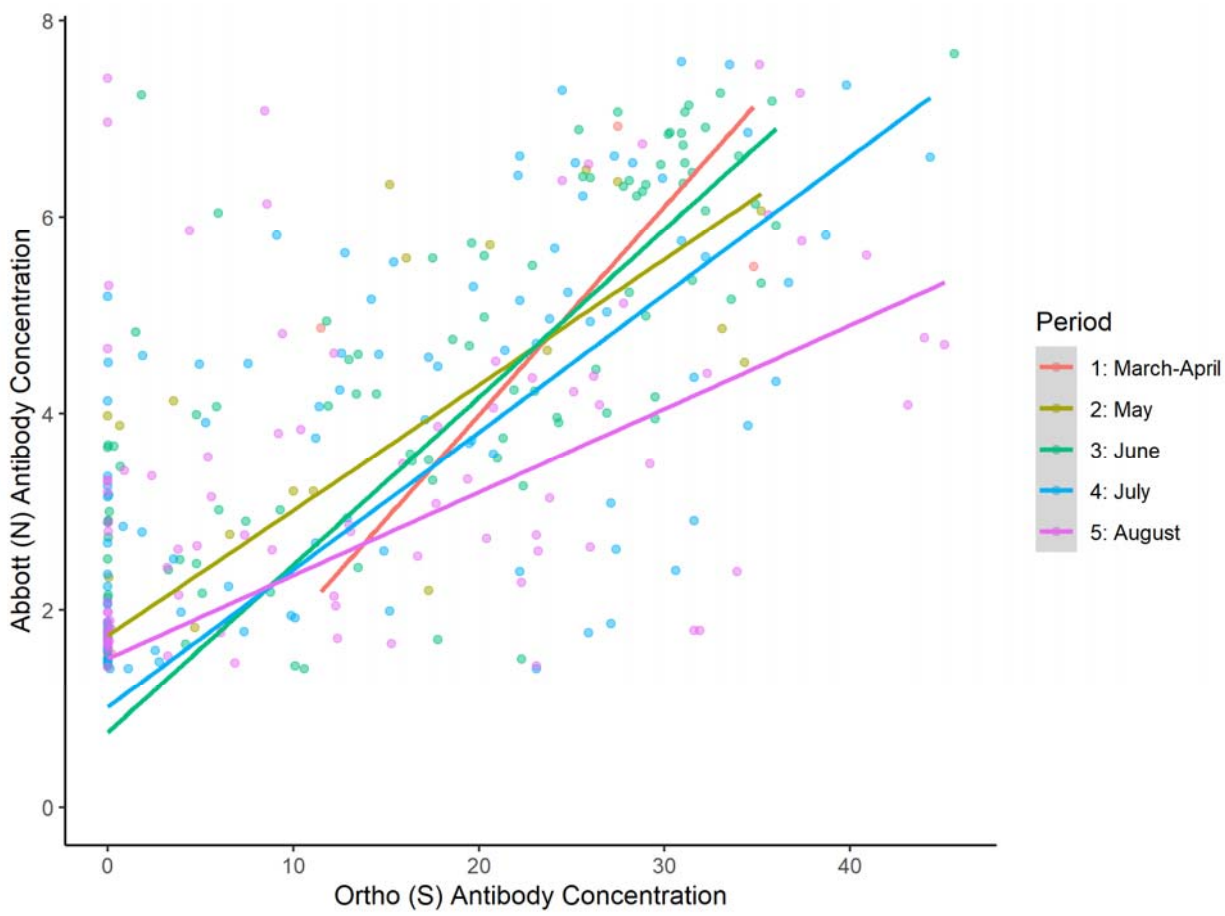


Figure 1. The association between Ortho and Abbott antibody index values by month, March to August, 2020.

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4-5
		(*b*) Describe any methods used to examine subgroups and interactions	4-5
		(*c*) Explain how missing data were addressed	4-5
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	4-5
		(*e*) Describe any sensitivity analyses	4-6
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5-6
		(b) Indicate number of participants with missing data for each variable of interest	N/A
Outcome data	15*	Report numbers of outcome events or summary measures	5-6
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5-6

		(*b*) Report category boundaries when continuous variables were categorized	5-6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	5-6
Discussion			
Key results	18	Summarise key results with reference to study objectives	7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8-9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7-9
Generalisability	21	Discuss the generalisability (external validity) of the study results	8
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	9

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.