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SARS-CoV-2 incidence, seroprevalence, and antibody dynamics in a rural, population-based cohort: March 2020–July 2022

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Abstract

Studies of SARS-CoV-2 incidence are important for response to continued transmission and future pandemics. We followed a rural community cohort with broad age representation with active surveillance for SARS-CoV-2 identification from November 2020 through July 2022. Participants provided serum specimens at regular intervals and after SARS-CoV-2 infection or vaccination. We estimated the incidence of SARS-CoV-2 infection identified by study reverse transcriptase–polymerase chain reaction, electronic health record documentation or self-report of a positive test, or serology. We also estimated the seroprevalence of SARS-CoV-2 spike and nucleocapsid antibodies measured by ELISA. Overall, 65% of the cohort had ≥ 1 SARS-CoV-2 infection by July 2022, and 19% of those with primary infection were reinfected. Infection and vaccination contributed to high seroprevalence: 98% (95% CI, 95–99) of participants were spike or nucleocapsid seropositive at the end of follow-up. Among those seropositive, 82% were vaccinated. Participants were more likely to be seropositive to spike than nucleocapsid after infection. Infection among seropositive individuals could be identified by increases in nucleocapsid, but not spike, ELISA optical density values. Nucleocapsid antibodies waned more quickly after infection than spike antibodies. High levels of SARS-CoV-2 population immunity, as found in this study, are leading to changing epidemiology necessitating ongoing surveillance and policy evaluation.

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Conflict of interest

All authors declare no conflicts of interest.

Disclaimer

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Keywords

COVID-19; SARS-CoV-2; incidence; ELISA; rural; cohort; seroprevalence; vaccine

Introduction

Since the SARS-CoV-2 virus emerged in late 2019, multiple pandemic waves have resulted in widespread social and economic disruption and > 6.5 million confirmed deaths worldwide.¹ A large excess-mortality study estimated that the true worldwide death toll attributable to COVID-19 is 2.9 to 3.3 times higher than the number of confirmed deaths.² Although death is the most impactful outcome of the pandemic, estimating the total number of SARS-CoV-2 infections also has public health utility. Throughout the pandemic, studies of the incidence of SARS-CoV-2 infection have informed public health mitigation measures, vaccination policy and distribution goals, and forecasts of future pandemic states.^{3–7} Studies of the incidence of SARS-CoV-2 and its variants have ongoing importance for estimating levels and patterns of hybrid immunity in the population, informing the response to future pandemics, and providing a historical record of the impact of the COVID-19 pandemic.

Study designs for determining SARS-CoV-2 incidence vary, but all must address limitations of the diagnostic methods used to identify infections. Cross-sectional serosurveys have been used to identify the proportion of the population who have SARS-CoV-2 antibodies indicative of past infection.^{8–12} Before vaccines were available, infection could be inferred by the presence of antibodies targeting the spike or nucleocapsid proteins; however, vaccine- and infection-induced spike antibodies cannot be distinguished. As a result, seroprevalence studies carried out in vaccinated populations have typically used nucleocapsid antibody to identify infection. These studies may fail to identify prior infections, because of antibody waning, and their utility for detecting reinfection is unclear.^{13,14} Furthermore, serosurveys do not provide information on the timing of infection and can be subject to issues of selection bias and generalizability, depending on the sampling scheme.

Other incidence studies have focused on SARS-CoV-2 cases identified during acute infection by reverse-transcriptase–polymerase chain reaction (RT-PCR) or rapid antigen tests performed by health care providers or public health professionals, which are required to be reported to health departments in the United States. Because the availability and likelihood of testing have changed throughout the pandemic, these studies must account for time-varying under-ascertainment, often by incorporating seroprevalence data.^{15–17} However, these studies typically do not follow individuals longitudinally, which limits their ability to estimate the incidence of re-infection that increased substantially after the emergence of the Omicron variant.^{18,19}

In this study, we followed a cohort with broad age representation in rural, central Wisconsin with the aims of estimating the incidence and seroprevalence of SARS-CoV-2 infection. We also describe the serologic response to infection and vaccination, and the persistence of antibodies after SARS-CoV-2 infection.

Methods

Study population

Prospective Assessment of COVID-19 in a Community (PACC) is a longitudinal cohort whose participants were recruited from the Marshfield Epidemiologic Study Area (MESA)–Central (hereafter, we use MESA, rather than MESA–Central, to refer to this study area). MESA is a well-characterized population cohort of 53 079 individuals living in 14 zip code areas comprising approximately 1300 square miles surrounding the city of Marshfield in central Wisconsin in the United States. The vast majority of MESA residents receive health care from the Marshfield Clinic Health System, and their electronic health record (EHR) data are available for institutional review board–approved research.²⁰

From November 2020 through March 2021, MESA residents of any age were randomly selected by 10-year age group for recruitment and enrollment into the PACC cohort with oversampling of young children and older adults (Table S1; Figure S1). Individuals living in institutional or group settings, those not planning to reside in the MESA study area for the following year, and those participating in a clinical trial evaluating investigational COVID-19 vaccines or drugs were excluded from participation. Informed consent was obtained from participants or parents of minor participants prior to participation with a waiver of documentation. Children ages 7 to 17 years also provided assent for participation. The study was reviewed and approved by the Marshfield Clinic Research Institute Institutional Review Board. The Centers for Disease Control and Prevention (CDC) determined these activities were conducted consistent with applicable federal law and CDC policy (see 45 C.F.R. part 46²¹; 21 C.F.R. part 56²²).

Participants were interviewed at enrollment and surveyed 26 and 52 weeks after enrollment to collect information on demographic characteristics, including age, sex, race/ethnicity, and chronic health conditions, including asthma, cancer, chronic kidney disease, chronic obstructive pulmonary disease or other chronic lung disease, hypertension, immunocompromised state, cardiac disease (eg, heart failure, coronary artery disease, cardiomyopathy), diabetes, chronic liver disease, or mental health conditions. COVID-19 vaccination was defined by documentation in the EHR or Wisconsin Immunization Registry.

Identification of SARS-CoV-2 infection

Beginning at enrollment, participants were instructed to report new acute respiratory illnesses (ARIs) as soon as possible after symptom onset. Study activities and specimen collection timelines are presented in Figure S2. Participants were also asked to complete weekly surveys on the presence or absence of ARI symptoms over the prior week. Participants reporting an ARI with new onset in the past 7 days with symptoms of cough, fever or chills, sore throat, muscle or body aches, loss of smell or taste, shortness of breath, or diarrhea were asked to self-collect a nasal swab specimen. Based on age and timing of enrollment, a subset of participants (48%) was assigned to enhanced surveillance with weekly specimen collection, regardless of symptoms, to identify asymptomatic infection for their first 6 months of follow-up. All participants collected weekly specimens from February through July 2022. Nasal swab specimens collected as part of routine and enhanced study

surveillance were tested for SARS-CoV-2 identification by RT-PCR in the Marshfield Clinic Research Institute Integrated Research and Development Laboratory. Additional RT-PCR positive results of tests performed in clinical settings were identified via Marshfield Clinic Health System EHR.

Participants were also asked to self-report positive SARS-CoV-2 tests performed outside of the study during surveys administered at enrollment, at 26 and 52 weeks after enrollment, and weekly after February 2022. Those reporting a positive result from a test performed outside of the study were asked to provide the month and year of the positive result; exact dates were imputed as the 15th day of the reported month. Hereafter, those reporting a positive SARS-CoV-2 test are referred to as having evidence of infection by self-report.

Participants initially consented to participate for a 12 month period, but beginning in February 2022, they were invited to re-consent to continued participation through the end of July 2022. We were able to obtain EHR documentation retrospectively and self-report of positive SARS-CoV-2 tests for participants who re-consented but had a period of lapsed consent (Figure S3). Active study surveillance with specimen collection to identify SARS-CoV-2 infections was not performed during periods of lapsed consent.

Blood was collected at enrollment (November 2020–March 2021) and at scheduled visits approximately 12 weeks (January–June 2021) and 24 weeks (April–September 2021) after enrollment (Figures S2 and S3). A fourth round of serum collection began in February 2022 (February–May), with a fifth specimen collected approximately 12 weeks later (May–July 2022). Individuals with SARS-CoV-2 infection identified by RT-PCR had serum specimens collected at 4 and 16 weeks after infection. Similarly, individuals who received COVID-19 vaccination had serum specimens collected 4–6 weeks after each dose. Postinfection and postvaccination blood collections counted toward scheduled post-enrollment visits if collected within 30 days of the scheduled date to reduce the total number of blood sample collections required for participants.

Serum specimens were tested by ELISA assay based on the Krammer group protocol in a single 1:40 dilution to determine seropositivity against full-length spike and nucleocapsid SARS-CoV-2 proteins.^{23,24} Seropositivity thresholds were derived from logistic regression on ELISA optical density (OD) measurements against prepandemic and convalescent SARS-CoV-2–positive samples, with OD readings of 0.506 and 0.664 corresponding to a 50% probability of infection for spike and nucleocapsid, respectively.²⁴ In a validation study, the spike threshold corresponded to a sensitivity of 91% and specificity of 99%; the sensitivity of the nucleocapsid threshold was 87% and the specificity was 94%.²⁴ This study treats samples with OD measurements above these thresholds as seropositive. Spike seropositivity could result from infection or vaccination; nucleocapsid seropositivity is only expected after infection.

Based on testing at enrollment, individuals were defined as having infection prior to enrollment if they were (1) spike seropositive and had not yet received any COVID-19 vaccine doses, or (2) nucleocapsid seropositive regardless of COVID-19 vaccination status. Serologic evidence of new infection during the study period was defined for nucleocapsid

as a seronegative preinfection specimen and a seropositive postinfection specimen or an increase in OD value from preinfection to postinfection by more than 0.256 units if both specimens were seropositive. For spike, evidence of new infection was similarly defined among individuals with no evidence of vaccination between pre- and postinfection specimens using an OD change threshold of 0.545. The OD change thresholds were determined by maximizing sensitivity while maintaining $\geq 95\%$ specificity comparing OD changes between paired pre- and postinfection specimens and paired specimens with no intervening infection or vaccination, as further described in Appendix S1 (Figures S4–S7).

Statistical analysis

Participants enrolled in the PACC cohort were described in terms of race/ethnicity, sex, chronic health conditions, and vaccination status by age group. Age groups for analysis were defined based on those used in COVID-19 vaccine authorizations and recommendations, as follows: < 5 years, 5–11 years, 12–17 years, 18–29 years, 30–59 years, 60–79 years, and ≥ 80 years.

The monthly proportion seropositive to spike, nucleocapsid, or either spike or nucleocapsid proteins was estimated using least-square mean predictions from age-adjusted generalized estimating equation logistic regression models clustering on individual with an exchangeable correlation structure using the *geepack* and *lsmeans* R packages. The monthly proportion spike or nucleocapsid seropositive was also plotted by age group and by combined vaccination and infection status. Only results of ELISA assays using serum collected at scheduled blood sample collections (ie, excluding additional postinfection and postvaccination specimens) were used to calculate monthly seropositivity proportions to avoid double counting infection and vaccination events.

Incidence of SARS-CoV-2 infection per 10 000 person days in PACC was calculated during the following periods based on circulating variant proportions in Wisconsin: pre-Delta period (March 1, 2020–June 30, 2021); Delta period (July 1, 2021–December 19, 2021); Omicron period (December 20, 2021–July 31, 2022); and full study period (March 1, 2020–July 1, 2022). Person-time was calculated as the time under study from the start of each analysis period to study withdrawal, infection, or the end of the analysis period. Incidence was calculated separately by age-group, type of evidence of infection (serologic, self-report of a positive test, study RT-PCR or EHR documentation of a positive clinical test, or any combination of these sources), and number of infections or reinfections (first, second, third). Primary infection was defined by serologic evidence of infection, self-report of a positive test, study RT-PCR, or EHR documentation of a positive test; reinfections were defined by self-report, study RT-PCR, or EHR documentation of a SARS-CoV-2 infection occurring > 90 days from a previous infection. For the period prior to study enrollment, infections were identified by serology, EHR documentation, or self-report of a positive test. Binomial 95% CIs were calculated by the Wilson method using the *Hmisc* package in R.

We translated incidence among PACC participants to the MESA source population by computing and then applying analytic weights (MESA population size divided by PACC sample size). Weights were computed within cells defined by unique combinations of age group, day within the study period, and number of COVID-19 vaccine doses received to

date. Cells where the PACC sample size was 0 were not included in the analyses. The weighted estimates and corresponding 95% CIs were calculated using SAS (SAS Institute Inc.) and accounted for the PACC study's stratified sample design.

We evaluated spike- and nucleocapsid-specific responses to SARS-CoV-2 infection by calculating the numbers and proportions of individuals with serologic evidence of infection following a positive SARS-CoV-2 test. Analyses were stratified by pre-event seropositivity and analysis period (pre-Delta variant, Delta variant, Omicron variant, and full study period). This analysis included individuals with SARS-CoV-2 infection identified by study testing, self-report, or EHR documentation with spike and nucleocapsid ELISA results from paired specimens collected ≤ 180 days before and 14 to 180 days after the infection. Infected individuals were excluded if they were vaccinated or infected more than once between the pre- and postinfection collection dates (ie, serum pairs only bracketed a single immunizing event).

The durability of spike- and nucleocapsid-specific binding antibodies after infection was assessed by plotting the trajectories of ELISA OD values by time since SARS-CoV-2 infection identified by study testing, self-report, or EHR documentation as further described in Appendix S1. The proportion of individuals still seropositive by number of days from positive test was estimated by interval-censored Kaplan-Meier analysis using the *survival* package in R. This analysis included individuals with self-reported or RT-PCR-identified infection who had nucleocapsid ELISA results for ≥ 1 specimen collected after infection and whose first-collected serum specimen after infection was seropositive. Person-time began on the date of positive SARS-CoV-2 test and ended at the collection date of (1) the last serum specimen if all were seropositive, (2) the first seronegative serum specimen, (3) the last serum specimen prior to evidence of reinfection (self-report, RT-PCR, or serologic evidence of infection). Primary analyses were limited to ≤ 350 days of follow-up because relatively few individuals had follow-up longer than that in the spike analysis; full follow-up data were considered in secondary analyses.

Results

A total of 1520 people were enrolled in the cohort, which included broad representation across all age groups; 57% participants were female, 46% reported ≥ 1 chronic health condition, and 9% received ≥ 1 COVID-19 vaccine doses prior to enrollment (Table 1). By the end of follow-up, 25% of the cohort remained unvaccinated against COVID-19, whereas 49% had received ≥ 3 vaccine doses; 98% of all vaccine doses received in the cohort were mRNA vaccines. Older age, female sex, non-Hispanic White race, and presence of chronic health conditions were all significantly associated with increased vaccination (Table 1). Of the 1520 participants, 361 withdrew from the study before the end of the analysis period on July 31, 2022. The majority of loss to follow-up occurred at the end participants' initial 1 year enrollment (November 2021-March 2022; Figure S3). Those lost to follow-up were more likely to be 18–29 years of age and to be unvaccinated and less likely to be non-Hispanic White and have ≥ 1 chronic conditions (Table S2). However, on average, characteristics of participants who participated for the entire period remained comparable

to all enrolled participants with the exception that they were somewhat less likely to be unvaccinated (21% vs 25%; $P = .04$).

Overall, 992 of 1520 individuals (65%) had 1196 SARS-CoV-2 infections identified by study RT-PCR, EHR documentation, self-report, or serology (Table 2). Daily age- and vaccine dose-specific weighting to the MESA source population corresponded to an estimated 40 516 first infections among the 53 079 MESA residents (76%) and an incidence of approximately 12 first infections per 10 000 person-days from local start of the pandemic, March 1, 2020–July 31, 2022. Incidence of first infection was lowest among adults ≥ 60 years of age (7 per 10 000 person-days), and ranged from 10 to 13 per 10 000 person-days in younger age groups. Incidence also varied by period of variant circulation (Table 2). Incidence of first infection was 6.5 (95% CI, 5.9–7.1), 9.1 (95% CI, 7.8–10.7), and 34.2 (95% CI, 31.0–37.7) per 10 000 person-days in local periods of pre-Delta, Delta, and Omicron circulation, respectively. In all periods, incidence of re-infection was substantially lower than that of first infection. However, incidence of second infections during the Omicron period was higher than the incidence of first infection in any prior period, and third infections were only observed during the Omicron period.

A total of 6602 serum specimens were collected across 5 scheduled visits (average: 4.3 specimens per participant; SD 1.0). The timing of the collection of these specimens relative to infection and vaccination in the cohort are presented in Figure 1A. Seroprevalence of SARS-CoV-2 antibodies binding either the spike or nucleocapsid protein increased from 6% (95% CI, 3–9) at baseline in November 2020 to $> 94\%$ in all months from February 2022 onward. The time-series trajectory of seroprevalence was consistent with the cumulative incidence of infection and vaccination in the cohort. Throughout the follow-up period, more individuals were seropositive for spike-binding antibodies than were seropositive for nucleocapsid-binding antibody (Figure 1B). As expected, the trajectory of nucleocapsid antibody seroprevalence more closely followed SARS-CoV-2 cumulative incidence than spike seroprevalence. Overall seroprevalence of spike or nucleocapsid antibody was low, with little variation by age prior to the availability of COVID-19 vaccines (Figure 1C). Increases in overall seroprevalence over time were consistent with age-specific vaccine availability and uptake. At the end of follow-up in July 2022, all age groups had seroprevalence $> 95\%$ except for children < 5 years (60%) for whom vaccines had only recently been authorized. Among all seropositive participants in summer 2022, 6% were unvaccinated without reported or documented infection, 12% were unvaccinated with history of infection, 41% were vaccinated with history of infection, and 41% were vaccinated without history of infection (Figure 1D).

To assess the performance of our seropositivity and OD change thresholds for accurately identifying infections, we estimated the proportion of individuals with serologic evidence of infection after a study or EHR-documented or self-reported positive SARS-CoV-2 test (Table 3). In each variant period, individuals who were spike and nucleocapsid seronegative prior to infection became spike seropositive after infection more than 95% of the time. However, further spike ELISA OD increases were only observed among 5% of individuals infected despite spike seropositivity. Postinfection nucleocapsid seropositivity among individuals who were spike and nucleocapsid seronegative prior to infection ranged

from 66% in the pre-Delta period to 89% in the Delta period. In contrast to spike, further nucleocapsid ELISA OD increases were common among individuals infected despite nucleocapsid seropositivity (70% overall among those seropositive for nucleocapsid only or both nucleocapsid and spike).

We examined the persistence of SARS-CoV-2 antibodies to determine the extent to which antibody waning might have affected our seroprevalence estimates. We found that spike antibodies declined relatively slowly after infection (rate of ELISA OD change per 100 days: -0.04 ; 95% CI, -0.02 to -0.05), and no participants were observed to revert to seronegative status after being spike seropositive (Figure 2A). In contrast, nucleocapsid antibodies exhibited notable waning in the first year after infection (Figure 2B); only 58% of infected individuals remained seropositive after 300 days (Figure 2C). The decline in ELISA OD per 100 days was significantly slower (-0.10 ; 95% CI, -0.09 to -0.11) when using all follow-up data compared with the primary analysis restricting to ≤ 350 days of observation (-0.17 ; 95% CI, -0.15 to -0.19 ; Figure S8). Consistent with this, waning appeared to slow after about 1 year following infection; 52% of infected individuals remained seropositive at 600 days. The rate of spike antibody waning did not change when using full follow-up data vs restricting to ≤ 350 days of observation (Figure S8).

Discussion

This study provides unique insights into the epidemiology of SARS-CoV-2 in a rural population during a period of rapid virus evolution. We observed a high cumulative incidence over 2 years, with a markedly higher incidence during the period of Omicron variant circulation compared with the Delta and pre-Delta periods. COVID-19 vaccine uptake was also relatively high. As a result of this high incidence of infection and vaccination, 98% of the cohort was seropositive to either SARS-CoV-2 spike or nucleocapsid protein by July 2022. Although seropositivity was high, it is important to note that we measured antibodies that bind SARS-CoV-2 proteins in ELISA assays. Levels of binding antibodies may not correlate with protection from infection as well as neutralizing antibody, particularly for SARS-CoV-2 variants of concern.²⁵

Our estimates of nucleocapsid seroprevalence (62% in February 2022; Figure 1B) are similar to those from a national seroprevalence study estimating that 67% of Wisconsin residents had antibody to the SARS-CoV-2 nucleocapsid protein, indicating prior infection, by February 2022.^{11,26} However, our results also highlight limitations of studies estimating incidence based on seroprevalence alone. Reinfection was common in our study; 19% of individuals with ≥ 1 infection had a second infection, and 7% of reinfected individuals had a third infection. Our estimates of reinfection may even be underestimated because serology was only used to define primary infection in incidence analyses. Seroprevalence studies using a simple seropositivity endpoint will likely miss most reinfections.

The results of our analysis of serologic response to infection suggest that increases in the SARS-CoV-2 nucleocapsid, but not spike, protein ELISA OD value can be used to identify reinfection in seroprevalence studies with longitudinal sampling. We also found that about 20% of previously seronegative individuals with SARS-CoV-2 infection did not become

nucleocapsid seropositive after infection (or revert to seronegative status prior to collection of their postinfection specimen). Among those who became seropositive, approximately 40% reverted to seronegative status in less than 1 year. This is consistent with findings of previous studies demonstrating waning of antibodies to the nucleocapsid protein but relatively stable spike protein antibody levels over time.¹³ Methods to correct for assay sensitivity and antibody waning should be used to improve estimates of SARS-CoV-2 incidence.^{16,27}

The first SARS-CoV-2 infections in Wisconsin were identified in March 2020, but cohort enrollment did not begin until November 2020. As a result, we may have underestimated the incidence of infections that occurred early in the pandemic. However, our findings that nearly all individuals with SARS-CoV-2 infection became spike-protein seropositive, and that this antibody response was long-lasting, suggest that the magnitude of this underestimation likely is small. The cohort also experienced loss to follow-up, particularly at the end of the participants' initial 1 year enrollment, which generally coincided with the early period of Omicron circulation. Those lost to follow-up were more likely to be unvaccinated than those who were not. As a result we may have underestimated Omicron incidence and overestimated overall seroprevalence. We did not ascertain asymptomatic infection in all participants and did not distinguish between symptomatic asymptomatic infections in our analyses. If asymptomatic participants are less likely to have an antibody response after infection, our incidence and seroprevalence estimates may have been underestimated.

Greater than 70% of PACC participants completed primary series vaccination, whereas the counties that include MESA zip codes ranged in primary series completion from 35% to 58%.²⁸ Consistent with this, we estimated that 11% more MESA residents had ≥ 1 SARS-CoV-2 infection during the study period compared with PACC participants (76% vs 65%). The differences in incidence estimates between PACC and MESA were largest during periods of Delta and Omicron circulation after COVID-19 vaccines became available. This finding highlights the importance of accounting for differences in vaccine uptake when extrapolating estimates of SARS-CoV-2 incidence beyond the population directly under study. These results also highlight heterogeneity in vaccine uptake in rural settings. Additional research is needed to identify those populations least likely to be vaccinated and strategies for increasing vaccine uptake. For example, local data indicate that children living on farms are less likely to be vaccinated than other children living in the same communities.²⁹

Increases in reinfection observed in this and other studies after the emergence of the Omicron variant suggest that antigenic drift will play a prominent role in ongoing SARS-CoV-2 transmission.³⁰ However, infections among those previously exposed, through vaccination or prior infection, are less likely to result in severe outcomes.^{31,32} This changing epidemiology will necessitate ongoing epidemiologic and virologic surveillance and continued revision of vaccination policy and recommendations. Prospective longitudinal studies, such as PACC, are essential to informing these activities. As SARS-CoV-2 transitions to endemicity, immunity is still uneven, and some populations may continue to have increased burden of infection and disease for the foreseeable future. In the United

States, vaccine uptake has remained low among young children, and there is considerable variation in vaccination coverage among adults, due to vaccine hesitancy and issues of access.^{33,34} Globally, issues of vaccine equity have contributed to a substantial number of preventable deaths, and more than one-third of the global population is SARS-CoV-2 seronegative.^{35,36} Solving these issues in the context of a transition to endemicity will require sustained public health and research efforts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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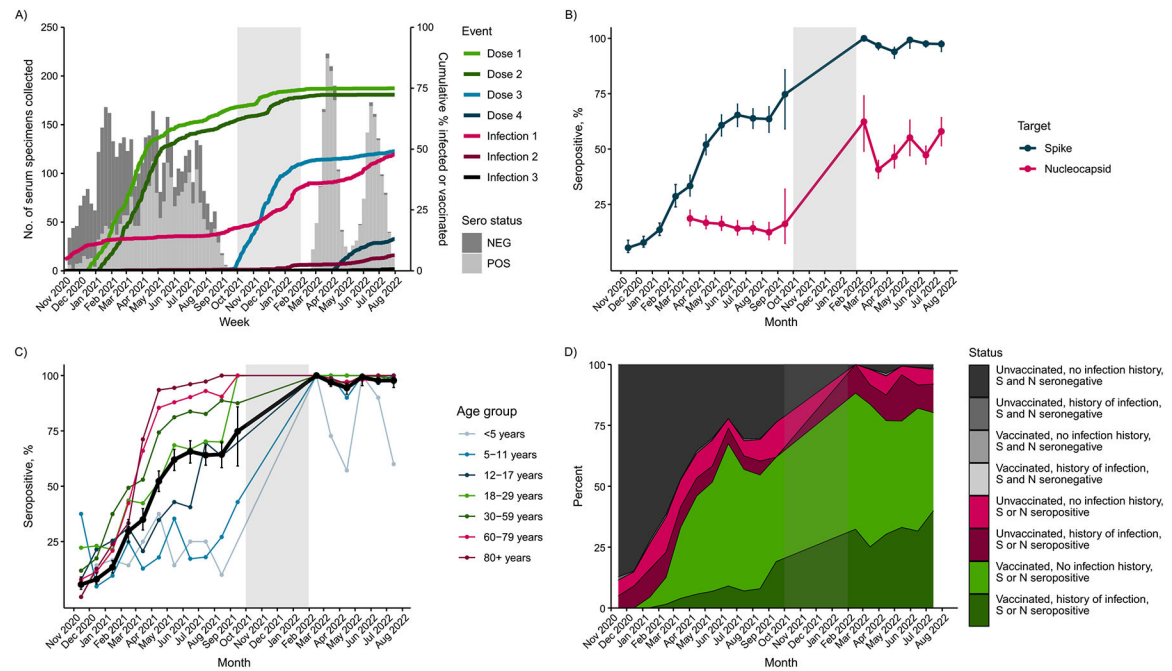


Figure 1.

Time series of infection, vaccination, serum collection, and seroprevalence in the prospective assessment of COVID-19 in a community cohort: November 2020-July 2022. A) Number of serum specimens collected by seropositivity status (positive seropositivity status was defined by spike ELISA optical density measurement ≥ 0.506 or nucleocapsid ELISA optical density measurement ≥ 0.664); cumulative percent infected (SARS-CoV-2 infections identified by study/clinic testing or self-report of a positive test); and cumulative percent vaccinated by study week. B) Age-adjusted spike and nucleocapsid ELISA seroprevalence by month (estimated using least-square mean predictions from age-adjusted generalized estimating equation logit models). C) Monthly spike or nucleocapsid ELISA seroprevalence by age group. Colored lines are observed proportions of individuals who were spike or nucleocapsid ELISA seropositive in each month by age-group. The black line represents overall age-adjusted proportion spike or nucleocapsid ELISA seropositive estimated using least-square mean predictions from age-adjusted generalized estimating equation logistic regression models. D) Monthly proportions of individuals spike or nucleocapsid ELISA seropositive and seronegative by vaccination and infection status. Unvaccinated was defined as no COVID-19 vaccine doses received; vaccinated was defined as ≥ 1 COVID-19 vaccine dose documented in the electronic health record (EHR) or Wisconsin Immunization Registry. No history of infection was defined as no SARS-CoV-2-positive tests identified by research testing, documentation in the electronic health record, or participant self-report; history of infection defined as ≥ 1 SARS-CoV-2 positive test study/clinic identified by research testing, documentation in the EHR, or participant self-report. Seronegative was defined as spike and nucleocapsid protein ELISA seronegative; seropositive was defined as spike or nucleocapsid protein ELISA seropositive. The gray box in each panel represents the months from October 2021 to February 2022, when serum specimens were not routinely collected. Abbreviations: N, nucleocapsid; NEG, negative; POS, positive; S, spike.

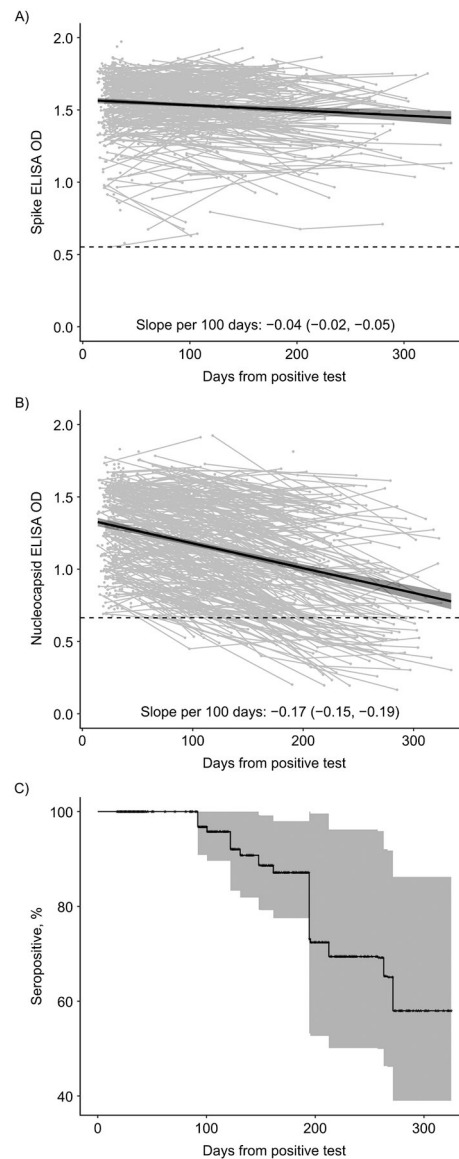


Figure 2.

Persistence of anti-spike and anti-nucleocapsid antibodies. Scatterplot of spike (A) and nucleocapsid (B) ELISA optical density (OD) values by time since SARS-CoV-2 infection among individuals who were seropositive after infection, with linear trend (dark black line) restricted to ≤ 350 days of observation. Observations from the same individual are connected by lines. C) Kaplan-Meier curve of time to nucleocapsid ELISA seronegativity among SARS-CoV-2-infected individuals who were seropositive after infection restricted to ≤ 350 days of observation. Values in parentheses in (A) and (B) are 95% CI of the estimated slope.

Table 1.

Participant characteristics by number of COVID-19 vaccine doses received by the end of follow-up, July 31, 2022, Prospective Assessment of COVID-19 in a Community November 2020-July 2022.

Characteristic	Total (n = 1520)	COVID-19 vaccine doses received by July 31, 2022, ^a no. (%)				P value ^b
		0 (n = 381)	1 (n = 40)	2 (n = 353)	3 (n = 548)	4 (n = 198)
Age group, years						<.001
<5	44 (3)	42 (11)	2 (5)	0 (0)	0 (0)	0 (0)
5–11	206 (14)	128 (34)	4 (10)	53 (15)	21 (4)	0 (0)
12–17	132 (9)	40 (10)	1 (2)	54 (15)	36 (7)	1 (1)
18–29	150 (10)	41 (11)	10 (25)	50 (14)	49 (9)	0 (0)
30–59	482 (32)	89 (23)	15 (38)	131 (37)	219 (40)	28 (14)
60–79	376 (25)	33 (9)	6 (15)	50 (14)	167 (30)	120 (61)
≥80	130 (9)	8 (2)	2 (5)	15 (4)	56 (10)	49 (25)
Sex						.02
Female	874 (57)	202 (53)	16 (40)	210 (59)	335 (61)	111 (56)
Male	646 (42)	179 (47)	24 (60)	143 (41)	213 (39)	87 (44)
Race/ethnicity						.001
Other, unknown, or refused to answer	87 (6)	33 (9%)	4 (10)	24 (7)	24 (4)	2 (1)
Non-Hispanic White	1433 (94)	348 (91)	36 (90)	329 (93)	524 (96)	196 (99)
Chronic conditions ^c						<.001
No	821 (54)	277 (73)	25 (62)	223 (63)	246 (45)	50 (25)
Yes	699 (46)	104 (27)	15 (38)	130 (37)	302 (55)	148 (75)

^aVaccination determined by documentation in the electronic health record or Wisconsin Immunization Registry.

^bAccording to χ^2 test.

^cAt enrollment, participants were asked to report any of the following chronic conditions: asthma, cancer, chronic kidney disease, chronic obstructive pulmonary disease or other chronic lung disease, hypertension, immunocompromised state, cardiac disease (eg, heart failure, coronary artery disease, or cardiomyopathy), diabetes, chronic liver disease, or mental health conditions.

Table 2. Incidence of SARS-Cov-2 by pandemic period, type of evidence for infection, age group, and number of infections^a

Type of evidence	Pre-Delta period (March 1, 2020—June 30, 2021)				Delta period (July 1, 2021 -December 19, -July 31, 2022)				Full study period (March 1, 2020—July 31, 2022)			
	No. at risk ^b	No. person-days	Cases (%)	Incidence ^c (95% CI)	No. at risk ^b	No. person-days	Cases (%)	Incidence ^c (95% CI)	No. at risk ^b	No. person-days	Cases (%)	Incidence ^c (95% CI)
Serologic ^d	1520	663 353	408 (26.8)	6.2 (5.6–6.8)	1497	243 268	61 (4.1)	2.5 (2.0–3.2)	1520	954 722	876 (57.6)	9.2 (8.6–9.8)
Self-report ^e	1520	663 353	179 (11.8)	2.7 (2.3–3.1)	1497	243 268	103 (6.9)	4.2 (3.5–5.1)	1520	954 722	392 (25.8)	4.1 (3.7–4.5)
RT-PCR ^f	1520	663 353	178 (11.7)	2.7 (2.3–3.1)	1497	243 268	145 (9.7)	6.0 (5.1–7.0)	1520	954 722	656 (43.2)	6.9 (6.4–7.4)
Any	1520	663 353	428 (28.2)	6.5 (5.9–7.1)	1497	243 268	181 (12.1)	7.4 (6.4–8.6)	1520	954 722	992 (65.3)	10.4 (9.8–11.1)
Age group, years												
<5	44	19 754	10 (22.7)	5.1 (2.8–9.3)	42	6882	7 (16.7)	10.2 (4.9–21.0)	44	28 113	28 (63.6)	10.0 (6.9–14.4)
5–11	206	92 546	54 (26.2)	5.8 (4.5–7.6)	201	31 620	33 (16.4)	10.4 (7.4–14.7)	206	126 091	158 (76.7)	12.5 (10.7–14.6)
12–17	132	57 109	43 (32.6)	7.5 (5.6–10.1)	131	21 132	20 (15.3)	9.5 (6.1–14.6)	132	77 500	103 (78.0)	13.3 (11–16.1)
18–29	150	64 177	43 (28.7)	6.7 (5.0–9.0)	145	23 442	16 (11.0)	6.8 (4.2–11.1)	150	89 330	99 (66.0)	11.1 (9.1–13.5)
30–59	482	205 220	155 (32.2)	7.6 (6.5–8.8)	477	76 763	71 (14.9)	9.2 (7.3–11.7)	482	288 728	353 (73.2)	12.2 (11.0–13.6)
60–79	376	166 969	92 (24.5)	5.5 (4.5–6.8)	373	62 300	22 (5.9)	3.5 (2.3–5.3)	376	256 627	188 (50.0)	7.3 (6.4–8.5)
≥80	130	57 578	31 (23.8)	5.4 (3.8–7.6)	128	21 129	12 (9.4)	5.7 (3.2–9.9)	130	88 333	63 (48.5)	7.1 (5.6–9.1)
Infection no.												
First	1520	663 353	428 (28.2)	6.5 (5.9–7.1)	1074	172 271	157 (14.6)	9.1 (7.8–10.7)	1520	954 722	992 (65.3)	10.4 (9.8–11.1)
Second	428	74 397	10 (2.3)	1.3 (0.7–2.5)	571	79 224	26 (4.6)	3.3 (2.2–4.8)	992	290 652	190 (19.2)	6.5 (5.7–7.5)
Third	10	1165	0 (0.0)	0.0 (0.0–32.9)	35	2552	0 (0.0)	0.0 (0.0–15.0)	190	24 046	14 (7.4)	5.8 (3.5–9.8)

Type of evidence	Pre-Delta period (March 1, 2020—June 30, 2021)				Delta period (July 1, 2021–December 19, -July 31, 2022)				Omicron period (December 20, 2021–July 31, 2022)				Full study period (March 1, 2020—July 31, 2022)			
	No. at risk ^b	No. person-days	Cases (%)	Incidence ^c (95% CI)	No. at risk ^b	No. person-days	Cases (%)	Incidence ^c (95% CI)	No. at risk ^b	No. person-days	Cases (%)	Incidence ^c (95% CI)	No. at risk ^b	No. person-days	Cases (%)	Incidence ^c (95% CI)
Overall weighted to MESA ^g	53	22 936 724	15 928 (30.0)	6.9 (6.3–7.6)	52	8 593 426	8323 (15.7)	9.7 (8.0–11.3)	52	8 780 435	24 203 (45.7)	27.6 (24.6–30.6)	53	32 776 335	40 516 (76.3)	12.4 (11.7–13.1)

^a Prospective Assessment of COVID-19 in a Community Study, November 2020–July 2022. Incidence weighted to the Marshfield Epidemiologic Surveillance Area source population is also provided.

^b Numbers at risk represent the cohort size at the beginning of each period. The cohort sizes at the end of the pre-Delta and Delta periods can be taken as the number at risk in the following period. Further loss to follow-up resulted in a final cohort size of 1159 persons at the end of the Omicron analysis period.

^c SARS-CoV-2 incidence per 10000 person-days. Unless specified, this calculation refers to the rate of first infection in each variant-specific period identified by serology, self-report of a positive SARS-CoV-2 test, positive RT-PCR from study collected specimen, or documentation of SARS-CoV-2 infection in the electronic health record.

^d Seropositive by spike protein ELISA if unvaccinated or seropositive by nucleocapsid protein ELISA regardless of vaccination. Only new serologic evidence of infection was considered in each period in individuals with preinfection-seronegative serum (ie, reinfection was not identified by serology).

^e Participants were periodically asked to report any SARS-CoV-2–positive results from tests performed outside of the study. However, participants may have reported positive results of outside tests for SARS-CoV-2 infections that were also identified by research tests or that were documented in the electronic health record. So, infections identified by self-report and RT-PCR are not expected to be mutually exclusive.

^f Identification of SARS-CoV-2 by real-time, RT-PCR from study collected specimen, or documentation of SARS-CoV-2 infection in the electronic health record.

^g Survey weights were used to estimate incidence in the MESA source population. Weights were computed within cells defined by unique combinations of age group, day within the study period, and number of COVID-19 vaccine doses received to date. Cells for which the Prospective Assessment of COVID-19 in a Community sample size was 0 were not included in the analyses; this is why the estimated population-at-risk and person-day totals are less than those for the total MESA population.

Abbreviations: MESA, Marshfield Epidemiologic Study Area; RT-PCR, reverse transcriptase–polymerase chain reaction.

Table 3.

Number and proportion of individuals^a with SARS-CoV-2 spike and nucleocapsid protein-specific serologic evidence of infection^b after a positive SARS-CoV-2 test by protein-specific, preinfection seropositivity status

Preinfection seropositivity status	Count	Postinfection evidence of infection		
		Spike no. (%)	Nucleocapsid no. (%)	Spike or nucleocapsid no. (%)
Infected in the pre-Delta period				
Spike and nucleocapsid seronegative	29	28 (96.6)	19 (65.5)	28 (96.6)
Spike seropositive only	3	1 (33.3)	0 (0.0)	1 (33.3)
Nucleocapsid seropositive only	3	2 (66.7)	1 (33.3)	2 (66.7)
Spike and nucleocapsid seropositive	2	0 (0.0)	0 (0.0)	0 (0.0)
Infected in the Delta period				
Spike and nucleocapsid seronegative	44	43 (97.7)	39 (88.6)	43 (97.7)
Spike seropositive only	42	2 (4.8)	33 (78.6)	33 (78.6)
Nucleocapsid seropositive only	1	1 (100)	1 (100)	1 (100)
Spike and nucleocapsid seropositive	6	1 (16.7)	1 (16.7)	2 (33.3)
Infected in the omicron period				
Spike and nucleocapsid seropositive	36	36 (100)	27 (75.0)	36 (100)
Spike seropositive only	107	5 (4.7)	95 (88.8)	95 (88.8)
Nucleocapsid seropositive only	0			
Spike and nucleocapsid seropositive	54	1 (1.9)	43 (79.6)	43 (79.6)
Infected in any period				
Spike and nucleocapsid seronegative	109	107 (98.2)	85 (78.0)	107 (98.2)
Spike seropositive only	152	8 (5.3)	128 (84.2)	129 (84.9)
Nucleocapsid seropositive only	4	3 (75.0)	2 (50.0)	3 (75.0)
Spike and nucleocapsid seropositive	62	2 (3.2)	44 (71.0)	45 (72.6)

^aThis analysis included individuals with self-reported or reverse transcriptase–polymerase chain reaction identified infection with spike and nucleocapsid ELISA results from paired specimens bracketing the infection. Infected individuals were excluded if (1) their preinfection specimen was collected > 180 days prior to infection, (2) their postinfection specimen was collected < 14 days or > 180 days after infection, (3) they were vaccinated between the pre- and postinfection collection dates, or (4) they were infected more than once between the pre- and postinfection collection dates.

^bSerologic evidence of infection was defined for nucleocapsid as a seronegative preinfection specimen and a seropositive postinfection specimen or an increase in optical density (OD) value from pre- to postinfection by > 0.256 units if both specimens were seropositive. For spike, evidence of new infection was similarly defined among individuals with no evidence of vaccination between pre- and postinfection specimens using an OD change threshold of 0.49.