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# Cross-Sectional Assessment of SARS-CoV-2 Viral Load by Symptom Status in Massachusetts Congregate Living Facilities

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Transmission of coronavirus disease 2019 (COVID-19) from people without symptoms confounds societal mitigation strategies. From April to June 2020, we tested nasopharyngeal swabs by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) from 15 514 staff and 16 966 residents of nursing homes and assisted living facilities in Massachusetts. Cycle threshold (Ct) distributions were very similar between populations with (n = 739) and without (n = 2179) symptoms at the time of sampling (mean Ct, 25.7 vs 26.4; ranges 12–38). However, as local cases waned, those without symptoms shifted towards higher Ct. With such similar viral load distributions, existing testing modalities should perform comparably regardless of symptoms, contingent upon time since infection.

**Keywords.** COVID-19; SARS-CoV-2; molecular diagnostics; viral load; symptoms.

A substantial fraction of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) spread occurs from infected individuals without symptoms of coronavirus disease 2019 (COVID-19) at the time of transmission [1]. We thus sought to understand the relative distributions of viral shedding in patients with and without symptoms. Several small studies found similar SARS-CoV-2 RNA shedding in infected individuals irrespective of symptoms [2–4], although the largest of these quantified viral load only after day 8 of infection, missing the period of maximal viral shedding [3]. Because nasopharyngeal (NP) viral load peaks near the time of symptom onset [1, 5], routine symptom-driven testing often misses this peak, complicating the relationship between measured viral loads and transmissibility or severity.

As the local epidemic neared its peak in April 2020 (Supplementary Figure 1), in response to several outbreaks, the Commonwealth of Massachusetts initiated systematic viral testing of all staff and residents in all skilled nursing and assisted living facilities, along with a binary report of symptom status at the time of sampling. Here we compare estimated viral load distributions between individuals with and without symptoms at the time of testing.

## METHODS

### Study Population

Between 9 April and 9 June 2020, NP swabs were collected from all residents and staff in all skilled nursing and assisted living facilities in Massachusetts, either by onsite staff or by the Massachusetts National Guard. The Broad Institute's Clinical Laboratory Improvement Amendments (CLIA)-certified clinical laboratory received all specimens from 366 facilities; the remaining samples were tested at the Massachusetts Department of Public Health State Public Health Laboratory and are not included in this study. Testing was performed on 32 480 unique individuals at the Broad Institute. For the 6.7% of individuals tested more than once, only data from the first positive test are reported.

### Symptom and Demographic Information

Beginning in the second week of the testing program (17–23 April 2020), a binary judgment of symptom status was reported by a clinician at each facility; if this adjudication was not possible, symptom status was coded as missing. Longitudinal information was not available about whether individuals without symptoms at the time of collection previously had or later developed symptoms, nor about duration or severity in those reporting symptoms. However, because most nursing facilities in Massachusetts during the study period required negative SARS-CoV-2 RT-PCR before accepting patients with known or suspected COVID-19, those testing positive would not include

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many postsymptomatic individuals convalescing from known COVID-19. Personal and demographic information were also collected (Supplementary Table 1).

### Laboratory Testing

NP swabs were placed in barcoded tubes with 3 mL of viral transport medium (VTM) and delivered on ice to the laboratory on the day of collection. RNA was extracted from 50 µL of VTM using the MagMax-96 RNA extraction kit (Thermo Fisher) on a Bravo liquid handler platform (Agilent). One-step real-time reverse transcriptase polymerase chain reaction (RT-qPCR) was performed on a QuantStudio 7 (Applied Biosystems), using a laboratory-developed SARS-CoV-2 Centers for Disease Control and Prevention (CDC) assay protocol run under the Food and Drug Administration (FDA)'s Emergency Use Authorization framework. Cycle threshold (Ct) values were reported for 2 viral probes, the N1 and N2 viral nucleocapsid protein gene regions, and an RNaseP human gene control (RP). The Ct value measures the number of amplification cycles required to detect cDNA produced from viral RNA; a higher Ct value indicates less viral RNA in the sample. Ct values lower than 40 cycles for both N1 and N2 were designated positive (a single positive viral probe was reported as inconclusive). Viral loads (copies/mL) were estimated by interpolation from a standard curve generated by serial dilutions of a synthetic RNA construct (Twist Biosciences) containing the viral N2 target sequence (Supplementary Figure 2).

### Analyses

Ct values for the N1 and N2 probes in positive patients were averaged. One-way ANOVA and pooled *t* tests were performed between subpopulations using SAS JMP software, version 13 (SAS Institute). This work was deemed exempt human subjects research by the Broad Institute Office of Research Subject Protection and approved with waiver of informed consent by the MA Department of Public Health's Institutional Review Board.

## RESULTS

### Positive Rates by Symptom Status

Across all facilities, 2654 of 16 966 residents (15.5%) and 624 of 15 514 staff (4.1%) tested positive for SARS-CoV-2. Tested residents were a mean of 82 years old (SD, 13; range, 17–114), 65% female, and 85% white, while tested staff were a mean of 45 years old (SD, 15; range, 16–101), 76% female, and 50% white. Supplementary Table 1 shows aggregate results by demographic group for the resident and staff cohorts. Among 13 341 residents who lacked symptoms at the time of swabbing, 1692 (12.7%) were positive, compared with 487 (3.7%) of 12 724 staff without symptoms. Of 1316 residents with symptoms, 699 (53.1%) tested positive, compared with 40 (18.2%) of 220 staff with symptoms (Supplementary Table 2).

### Comparison of Viral Load Between Individuals With and Without Symptoms at the Time of Testing

Among individuals testing positive, Ct values covered a broad range, from 11.6 to 37.7 cycles in individuals without symptoms and 11.9 to 37 cycles in individuals with symptoms (Figure 1), corresponding to viral loads ranging from 2 billion to 8 copies/mL, respectively. The Ct for the human host probe (RP) was more tightly distributed around a mean of 28.9 (SD, 2.4) and 28.1 (SD, 2.7) cycles for each population (Supplementary Figure 3), indicating reproducible sample collection and handling.

The distributions for the viral level were very similar between individuals with and without symptoms, with a statistically significant but clinically trivial difference in mean Ct of 0.71 cycles (25.7 vs 26.4, $P = .006$) and a slightly different proportion of individuals with Ct $\geq$ 30 cycles (29.2% for individuals with symptoms vs 36% for those without; Figure 1). Similarly, the mean Ct for the human host probe differed by 0.74 cycles ($P = .0001$) between these 2 populations (Supplementary Figure 3). For context, test developers and the FDA typically use a Ct difference of < 3 cycles as an indicator of substantial equivalence between viral testing methods. Furthermore, the observed differences in Ct are less than the typical variability in sampling efficiency, as reflected in the RP probe Ct distributions (SD, 2.4 and 2.7 cycles).

### Variation of Viral Loads Over Time

During the study period, overall COVID-19 burden in the state peaked at >3000 confirmed cases per day on 17 April 2020 (week 2 of the study) and declined thereafter, dropping over 7-fold by the end of the study period (Supplementary Figure 1). When the distribution of viral loads between individuals with and without symptoms was compared over time, on a weekly basis, no difference was observed between the 2 populations, either in mean Ct value or range, during the time period that coincided with the peak outbreak of COVID-19 in Massachusetts (17–23 April 2020; Figure 2 and Supplementary Figure 1). However, as cases waned over the course of the study, a gap emerged, with mean Ct value shifting higher in the population without symptoms while remaining essentially unchanged for symptomatic patients throughout the testing period. Specifically, individuals without symptoms tested during weeks 5 and 6 (7–20 May 2020) had Ct values >3 cycles greater (less virus) than symptomatic individuals ($P = .0013$ and .0007 for weeks 5 and 6, respectively).

### Effect of Age and Other Demographic Variables

Although age dramatically affects COVID-19 severity, viral level did not vary significantly by symptom class in any age group over the entire study period, for either residents or staff (Supplementary Figures 4 and 5). Across other demographic variables (sex, race, ethnicity, resident vs staff), statistically significant but numerically small differences were observed

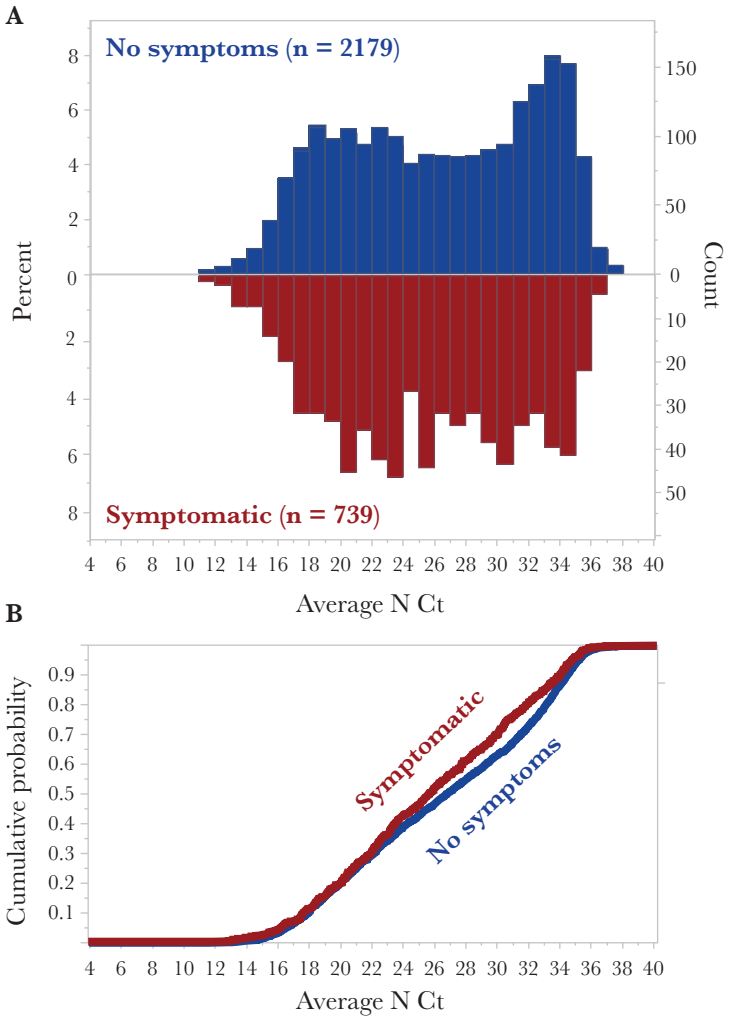


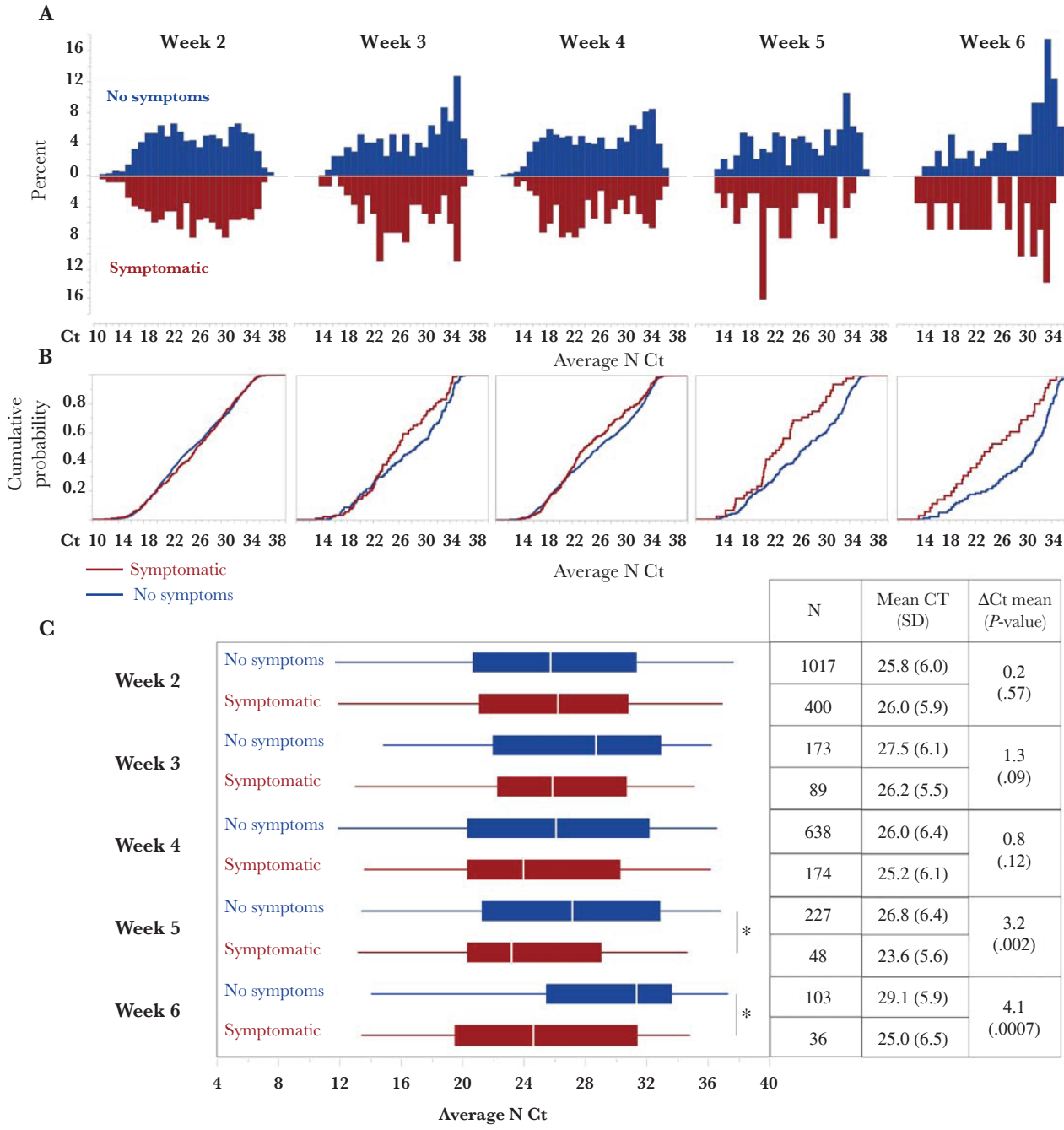

**Figure 1.** Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral load distributions are very similar independent of symptom status at the time of testing. Cycle threshold (Ct) distributions using probes targeting the SARS-CoV-2 N gene (average of N1 and N2 probes) from patients without (gray) or with (black) symptoms at the time of testing are shown as binned histograms (*A*) and cumulative distributions (*B*).

between those with and without symptoms in some categories (ΔCt range 0.8–1.2 cycles among demographic classes with $P < .05$; Supplementary Figure 5).

## DISCUSSION

Because of frequent spread from individuals who do not show symptoms at the time of transmission [1], control measures that aim to substantially mitigate SARS-CoV-2 transmission require diagnosis of infected individuals who do not display symptoms at the time of testing [6]. By screening 16 966 residents and 15 514 staff of residential nursing facilities in Massachusetts, we generated quantitative RT-PCR data from 2179 subjects without and 739 with symptoms, the largest cohort of individuals without symptoms at the time of testing reported to date. The Ct distributions between the 2 populations over the entire study period were remarkably similar, both overall and in each subgroup examined (age, sex, race, and ethnicity).

With or without symptoms, viral loads from NP swabs varied by 250 million-fold, consistent with prior studies [7] (Figure 1). As in other respiratory illnesses [8], it is plausible (but not proven) that infectivity of individuals with SARS-CoV-2 may relate to viral load [9]. Cross-sectional studies only report on viral load at the moment of sampling, whereas transmission may be better predicted by peak viral load; this peak is difficult to capture, particularly because it occurs very near the time of symptom onset [1, 5]. Only careful prospective longitudinal studies have reliably captured this peak, but these have been small and in specialized populations [10]. Cross-sectional studies capture the distribution of viral loads at one moment in time that should include this peak in a subset of subjects, whereas convenience samples, such as those from symptomatic or hospitalized patients, often miss this peak entirely but have been the basis for most comparisons of viral load to date [11, 12].




| | N | Mean CT (SD) | ΔCt mean (*P*-value) |
|---|---|---|---|
| Week 2 | 1017 | 25.8 (6.0) | 0.2 (.57) |
| | 400 | 26.0 (5.9) | |
| Week 3 | 173 | 27.5 (6.1) | 1.3 (.09) |
| | 89 | 26.2 (5.5) | |
| Week 4 | 638 | 26.0 (6.4) | 0.8 (.12) |
| | 174 | 25.2 (6.1) | |
| Week 5 | 227 | 26.8 (6.4) | 3.2 (.002) |
| | 48 | 23.6 (5.6) | |
| Week 6 | 103 | 29.1 (5.9) | 4.1 (.0007) |
| | 36 | 25.0 (6.5) | |

**Figure 2.** Distributions of cycle thresholds diverged as the local epidemic waned, with individuals without symptoms shifting to lower viral loads (higher cycle thresholds [Cts]). *A*, Ct distribution by symptom class by week of study (gray, no symptoms; black, with symptoms). Weeks with fewer than 20 data points in either category are not shown. Week 1 data are omitted, as symptom class was not captured. *B*, Cumulative distribution plots of the data from (*A*). *C*, Box-plots of the average viral N probe (N1 and N2) Ct by week and symptom class, with vertical line at median, shaded boxes at interquartile range, and whiskers showing full range. *$P < .05$ within a subcategory. The sample size, mean Ct, SD, ΔCt between symptom classes, and associated *P* value are shown.

The mean and distribution of Ct values for individuals with and without symptoms were nearly identical at first, shortly after a surge in statewide cases (Supplementary Figure 1), before diverging in subsequent weeks as local prevalence subsided, when the mean viral load in individuals without symptoms declined (Figure 2). Because viral load peaks early during infection before waning slowly [1], viral loads at a given point in time depend on the distribution of time since infection for the population tested. Symptoms are typically displayed within a limited time period early in infection, close to the peak of viral shedding [1]. As a consequence, even if the distribution of viral levels over time is identical between individuals with and without symptoms, the set of individuals with symptoms at any given time is selected for more recent infections and thus

higher viral levels compared to individuals without symptoms. This dependence on epidemic dynamics of the relationship between symptom status and Ct may explain conflicting results seen in other cross-sectional studies [13]. During the rapid initial growth phase of a local epidemic, this skew in time since infection between those with and without symptoms is modest because the vast majority of infections are recent. Because the expected skew in time since infection is minimized in the rapid initial growth phase of a local epidemic, this period may provide a better representation of the prospective distribution of viral loads across individuals infected at roughly the same time, compared with a stable or declining local epidemic. As a local epidemic stabilizes or declines, this skew will increase, as symptomatic cases are still selected to reflect recent infection, while those without symptoms will on average be later since infection. This property of Ct distributions from a random cross-sectional sampling might even be used to estimate recent trends in disease incidence in a population [14].

This finding also has important implications for interpreting Ct distributions from cross-sectional or convenience samples of newly discovered variants or of vaccinated individuals who become infected. A variant that is increasing in frequency in a population will have proportionally more recent cases than the wild-type strain it is displacing, which would tend to artifactually increase the average measured viral load, because variant cases would on average be newer. Conversely, soon after a vaccination rollout, cases in vaccinated individuals will decline rapidly over time, meaning cases found in vaccinated individuals will be more likely to have been acquired remotely than recently, whereas this constraint will not occur in unvaccinated individuals in the same population (until population immunity thresholds are approached). This discrepancy would tend to artifactually increase Ct (reduce observed viral load) in vaccinated populations simply due to a systematic difference in the time of sampling relative to peak shedding between the 2 populations. While altered peak viral load may be a plausible mechanism by which variants may increase [15] or vaccines may reduce transmissibility [16], caution must be taken to disentangle these systematic sampling biases driven by epidemic dynamics [14], which are one important determinant among many that may affect observed Ct distributions.

The majority of positive tests from both residents (1692 of 2391, 70.8%) and staff (487 of 527, 92.4%) of all ages came from individuals without symptoms at the time of testing (Supplementary Figure 5). However, each group of individuals would have been depleted for symptomatic COVID-19 (residents with severe symptoms may have been transferred to hospitals, while most symptomatic staff would likely have stayed home), explaining the higher percentage compared with smaller-scale cross-sectional studies [17]. Modeling studies suggest that a substantial fraction of transmission occurs from people who are not symptomatic at the time, whether asymptomatic or presymptomatic [1], which is reinforced by contact-tracing studies [2, 17]. Our finding that infected individuals without symptoms shed as much SARS-CoV-2 as those with symptoms underscores the need to expand beyond symptom-based screening as a sole tactic for detecting infected individuals and preventing transmission.

This study should be interpreted with certain caveats. First, without longitudinal follow-up, we cannot distinguish infected individuals who are permanently asymptomatic from those who are presymptomatic. However, both classes likely carry risk for transmitting the virus unwittingly [1, 2, 17], even while differing in their implications for contact tracing and COVID-19 pathogenesis. Second, with only a binary point-prevalence assessment of symptoms at the time of testing, we cannot resolve the relationship between viral load and concurrent or future symptom incidence or severity in this population. These are important avenues for future exploration in longitudinal studies. Third, nursing home residents and staff may differ with respect to stages or disease severity from other populations, such as severely symptomatic individuals presenting to an acute setting for testing or requiring hospitalization [12], or asymptomatic individuals in different settings [2, 3]. Nonetheless, these data represent Ct values for nonhospitalized individuals who did not seek acute testing, which represents the majority of COVID-19 cases and the vast majority of those at risk for ongoing transmission. Finally, RNA levels from NP swabs may not reflect viral loads in other body sites and cannot distinguish live virus from inactive or killed virus [9].

In summary, individuals with and without symptoms showed very similar distributions of SARS-CoV-2 viral loads, particularly early in the study during the peak of the local epidemic. Testing of asymptomatic individuals is under consideration in many settings, including contact tracing by public health departments and screening in workplaces or schools. While optimal implementation strategies and cost-effectiveness must be carefully considered, these findings build confidence in the technical feasibility of identifying asymptomatic individuals harboring SARS-CoV-2 by standard RT-PCR assays or other viral-directed diagnostics such as antigen testing, once timing of infection is considered, which may be less certain in those without symptoms.

## Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

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