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Letter

Longitudinal Monitoring of SARS-CoV-2 RNA on High-Touch Surfaces in a Community Setting

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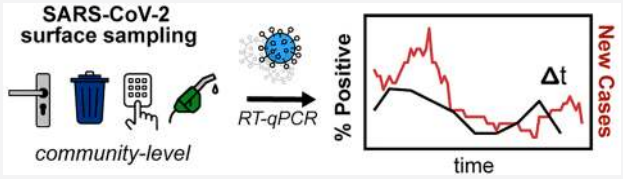
ABSTRACT: Environmental surveillance of surface contamination is an unexplored tool for understanding transmission of SARS-CoV-2 in community settings. We conducted longitudinal swab sampling of high-touch non-porous surfaces in a Massachusetts town during a COVID-19 outbreak from April to June 2020. Twenty-nine of 348 (8.3%) surface samples were positive for SARS-CoV-2 RNA, including crosswalk buttons, trash can handles, and door handles of essential business entrances (grocery store, liquor store, bank, and gas station). The estimated risk of infection from touching a contaminated surface was low (less than 5 in 10,000) by quantitative microbial risk assessment, suggesting fomites play a minimal role in SARS-CoV-2 community transmission. The weekly percentage of positive samples (out of n = 33 unique surfaces per week) best predicted variation in city-level COVID-19 cases with a 7-day lead time. Environmental surveillance of SARS-CoV-2 RNA on high-touch surfaces may be a useful tool to provide early warning of COVID-19 case trends.



INTRODUCTION

SARS-CoV-2, the virus causing the current global COVID-19 pandemic, is believed to be transmitted primarily through droplets and aerosols.[1,2] However, the role of fomites in transmission is unclear.[3] Recent commentaries argue that the risk of transmission via fomites may be low in clinical settings,[4−6] despite common detection of SARS-CoV-2 RNA on surfaces.[7−11] In laboratory experiments, SARS-CoV-2 has been found to remain viable on surfaces for up to 28 days, using large initial viral concentrations and under optimized environmental conditions, with half-lives on plastic and stainless steel ranging from hours to days.[12−17] However, data on the prevalence on high-touch surfaces in community settings are limited,[18−20] and temporal trends during a COVID-19 outbreak have not been measured.

Environmental surveillance is an emerging field for monitoring infectious disease prevalence and trends at the population level. Surveillance of environmental reservoirs has the potential to be less invasive, lower cost, and less biased than sampling individuals, particularly for pathogens with a high proportion of asymptomatic infections. Wastewater sampling (or wastewater-based epidemiology) has successfully been used to track outbreaks that are otherwise difficult to capture through clinical surveillance such as poliovirus and SARS-CoV-2.[21,22] Recent studies have documented that SARS-CoV-2 RNA levels in wastewater track with trends in case numbers in communities.[23−31] However, wastewater epidemiology has not yet been demonstrated to be an early warning system for COVID-19 cases.[29,32]

Environmental surveillance methods that do not rely on shedding in stool, such as fomite or air sampling, may be better situated to provide early warning of spikes in COVID-19 cases. Viral load in the upper respiratory tract peaks within 1 week after symptom onset, whereas viral load in stool has been found to peak 1 to 6 weeks after symptom onset.[33−41] Pre- and asymptomatic patients also shed SARS-CoV-2 in the respiratory tract;[42,43] thus, environmental surveillance may capture trends among total cases.[44] Targeted sampling of high-touch surfaces has the potential to complement other pandemic surveillance strategies by identifying recent locations (e.g., buildings or rooms) of currently infectious individuals, providing insight on fomite transmission pathways and serving as an early warning system of case trends.

We collected longitudinal high-touch surface samples in public locations and essential businesses throughout a COVID-19 outbreak from March 13−June 23, 2020 in Somerville, Massachusetts. Our objectives were to (1) document the types of high-touch non-porous surfaces likely to be contaminated with SARS-CoV-2 RNA during an outbreak, (2) measure the concentration of SARS-CoV-2 RNA on surfaces to estimate risk of infection from contact with fomites in the community setting, and (3) assess the temporal relationship between

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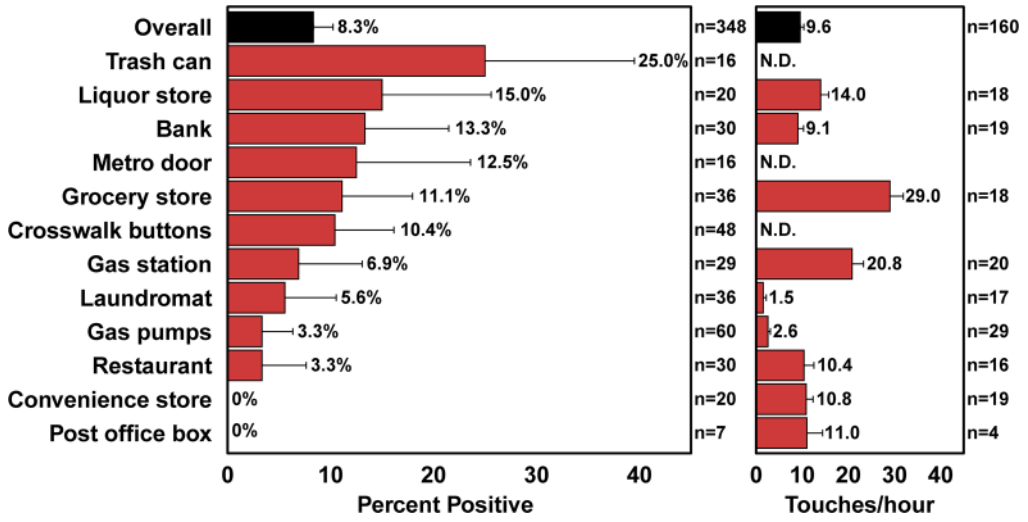

Figure 1. Percentage of positive samples over the duration of the study (left) and mean touches per hour (right) at sampling locations. Error bars show the 90% confidence interval around the mean. For percent positive, n = number of samples collected. For touches/hour, n = number of surfaces observed. N.D. signifies that no observational data were collected at that location.

environmental surface SARS-CoV-2 RNA contamination levels and COVID-19 cases in the community.

MATERIALS AND METHODS

Sample Collection. We collected samples from high-touch surfaces in Somerville, Massachusetts, a city with a population of 81,500, a population density of nearly 20,000 people per square mile, and three zip codes. We collected samples from high-touch surfaces within one zip code in Somerville in two phases: an initial pilot phase where we sampled five unique surfaces twice weekly from March 13−31, 2020 and a full-scale phase where we sampled 33 unique surfaces at 12 locations weekly from April 23−June 23, 2020. At each location, we sampled one to six surfaces, including indoor and outdoor surfaces. Sampling was paused from April 1−22 because of restrictions put into place by Tufts University. During the pilot phase, we sampled three crosswalk buttons, a garbage can handle, and a door handle into a metro station. During the full-scale phase, we expanded sample collection to include essential businesses open throughout the sampling period (grocery store, liquor store, convenience store, gas station, laundromat, bank, and restaurant), in which we sampled door handles, ATM keypads, and gas pump handles (Table S1).

Surfaces were swabbed once per week at a fixed day and time using primarily flocked polypropylene swabs (Puritan Medical Products, Guilford, ME), with the exception of the first 2 weeks of the pilot sampling in March when polypropylene swabs were unavailable and cotton-tipped swabs were substituted. We saturated the swab in 1 mL of 1X phosphate-buffered saline solution (PBS) and then swabbed the entire surface horizontally or vertically, rotating the swab throughout. The swabs were then returned to 1 mL of 1X PBS, stored on ice during sampling, and transport, and stored at −80 °C until further processing. During full-scale sampling, we collected 33 samples and one field blank per week. The field blank consisted of opening a new swab and placing it in PBS in the same manner as the samples.

Observational Data. We observed each sampling location once per week for 30 min at the time of sample collection (time of observations ranged from 10 am−5 pm). During the observation period, we counted the number and type of touches (bare hand, gloved hand, sleeved hand, and other) on the surface and the total number of people observed at each location. For door handles, we counted the total number of people entering and/or exiting. For crosswalks, we counted the people crossing that crosswalk. For locations such as ATMs and gas pumps, the total number of people using the service were counted. Finally, we recorded the proportion of people wearing personal protective equipment (PPE) at each location, including the number wearing face masks, face mask type (N95, cloth, surgical, and other), and number wearing gloves.

COVID-19 Case Data. Local COVID-19 case data were obtained from the City of Somerville by zip code.[45] Cases were reported by date of sample collection. We calculated total daily cases by summing confirmed and probable tests and smoothed data using a 7-day moving mean centered on the date.

RNA Extraction and RT-qPCR. We used the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) to extract RNA from all samples and eluted samples in 80 μL of Buffer AVE (Table S2). We added 10 μL of 10^{-2} dilution of bovine coronavirus vaccine stock (Calf-Guard, Zoetis, Parsippany-Troy Hills, NJ) to each sample prior to RNA extraction as an internal standard. To quantify SARS-CoV-2 RNA in samples, we used the E Sarbeco and CDC N1 assays.[46,47] Each plate consisted of triplicates of standard curve points, 25 samples, and a no template control. We also used the BCoV assay to quantify the bovine coronavirus internal standard (Tables S3 and S4).[48] The limit of detection at which 50% of replicates amplified (LOD_{50}) for each assay was determined through parallel dilutions to quantities between 3 and 10 gene copies (gc)/5 μL.[49] The LOD_{50} was determined to be below 3 gc/5 μL for the E assay and between 5 and 7 gc/5 μL for the N1 assay (Table S5). The limit of quantification (LOQ) was determined based on the lowest point on the standard curve in which all replicates amplified, which was 4.4 gc/5 μL for the E assay and between 4.4 and 44 gc/5 μL for the N1 assay, although we treated the LOQ for N1 as 44 gc/5 μL for these results (Table S6). Standard curves were calculated using a linear mixed effects model on data pooled from all plates to account for batch effects (Table S6).[50] A sample was considered positive if at least one of the triplicates amplified with a Ct below 40 in either the N1 or E assay. This method is

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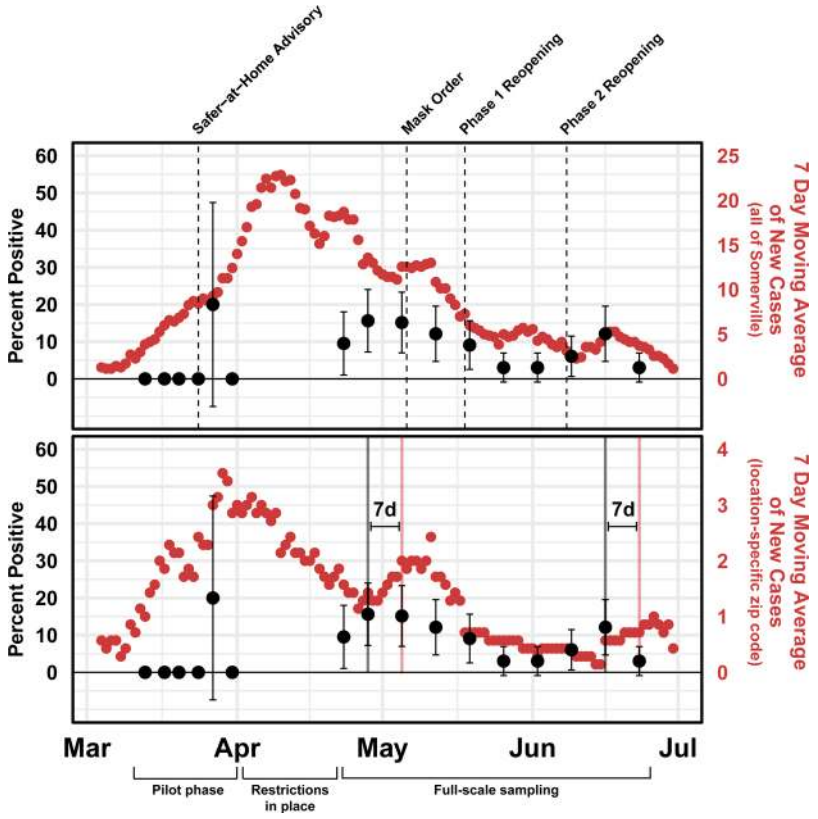

Figure 2. Sample positivity rate and COVID-19 cases. (Top) Weekly positivity rate of surface samples and 7 day moving average of new cases in Somerville, MA. The percentage of positive samples are displayed in black and COVID-19 cases in red. Error bars represent the 90% confidence interval around the percent positive. Sampling was paused from April 1−22 because of restrictions put into place by Tufts University. (Bottom) Peaks in percent positivity of surface samples precede 7 day moving average of COVID-19 case peaks in the same zip code by 7 days (shown by vertical black and red lines). On March 24, 2020, a Safer-at-Home Advisory was issued in MA recommending residents shelter in place as much as possible, and all nonessential businesses closed. On May 6, a mask order was issued by the City of Somerville requiring all residents to wear a mask in public spaces. The MA Phase 1 Reopening started on May 18 and allowed some businesses to reopen. The MA Phase 2 Reopening started on June 8 and allowed opening of outdoor dining at restaurants, along with more businesses being allowed to reopen. See https://www.mass.gov/info-details/reopening-massachusetts for more details on the MA reopening phases.

consistent with other studies on low abundance pathogen targets in environmental matrices.[51−53] Additional information on extraction and RT-qPCR methods is available in the Supporting Information.

Statistical Analysis. To examine various lag periods for case numbers, we calculated the Pearson correlation coefficient between the percent of positive samples and the 7-day case average, including various lag periods of case numbers by shifting the 7-day moving mean data forward by 0−11 days. The highest r value was considered the optimal lag period between surface SARS-CoV-2 RNA detection and COVID-19 cases data.

QMRA Model. Risks from contacts were estimated using a Quantitative Microbial Risk Assessment (QMRA) framework.[54] Briefly, probability distributions for the model input parameters were obtained from published scientific literature or this article (Table S7). The risk of infection was estimated based on the concentration of SARS-CoV-2 RNA on surfaces adjusted for swab surface recovery efficiencies determined experimentally (Table S8) and assuming a single hand-to-surface contact followed by a single hand-to-face contact. A genome copy to infective virus ratio informed by data on respiratory enveloped viruses was used to convert genome copies to Plaque Forming Units (PFU).[55] The number of viruses transferred from the contaminated surface to the hand upon contact was estimated using the transfer efficiency of viruses between surfaces and hands.[56] Viral dose was calculated using the contact surface area between the hand and the face[57] and the transfer efficiency of the virus from the hand to the mucous membranes.[58] An exponential dose−response model developed elsewhere with pooled data from SARS-CoV and murine hepatitis virus (MHV) was used to calculate the probability of infection.[59,60] When samples were positive in both the N1 and E Sarbeco assays, the higher concentration was used for QMRA. Additional information on the QMRA model can be found in the Supporting Information.

RESULTS AND DISCUSSION

Detection of SARS-CoV-2 RNA on Surfaces. Overall, 29 of 348 (8.3%) total surface samples were positive for SARS-CoV-2 RNA, and we detected SARS-CoV-2 RNA on surfaces in 10 out of 12 locations sampled (Figure 1). We observed a total of 1815 people and 781 bare-hand touches across all sites from April 23 to June 23 (Figure S1). SARS-CoV-2 RNA was detected on surfaces at all locations except for the convenience store and post office box; the percentage of samples positive by location ranged from 0% to 25%. A trash can handle and liquor store door handle were the most frequently contaminated surfaces. Among the 29 samples that were positive by either assay, three amplified in all replicates above the limit of

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Table 1. Risk of Infection from Touching Sampled Surfaces with Quantifiable SARS-CoV-2 RNA Concentrations

Surface	Date	Material	Surface concentration (gc[a]/cm^2)	Infection Risk		
				5th percentile	Median	95th percentile
Grocery store door handle exterior	6/16/20	metal	2.54	1.82×10^{-6}	1.01×10^{-5}	6.57×10^{-5}
Grocery store door handle interior	6/16/20	metal	11.55	8.35×10^{-6}	4.66×10^{-5}	3.04×10^{-4}
Liquor store door handle	5/5/20	metal	102.43	7.27×10^{-5}	4.10×10^{-4}	2.60×10^{-3}

[a]gc: gene copies.

quantification (LOQ): the interior and exterior grocery store door handles and the liquor store door handle. Quantities in these samples ranged from 2.5 to 102 gc/cm^2 (Table S9).

When grouped by location or by week of collection, we found no association between the percentage of positive samples and the number of touches on a surface or number of people per location (Table S10). At the locations with the highest number of touches and visitors per hour (the gas station and grocery store), the percentage of positive samples were not significantly different from the overall positivity rate.

The percent of positive samples per week was inversely associated with daily maximum temperature (Pearson's $r = -0.68$, $p = 0.03$) and absolute humidity ($r = -0.71$, $p = 0.02$), while no relationship was found with relative humidity ($r = -0.44$, $p = 0.21$) (Figure S2). SARS-CoV-2 survival times on surfaces are known to decrease with rising temperatures.[13,16]

Our findings contribute to a growing literature of detectable but low-level SARS-CoV-2 RNA contamination on public surfaces. Previously reported concentrations of SARS-CoV-2 RNA detected on surfaces in Brazil have ranged from less than 0.1 to 40 gc/cm^2 (compared to our 2.5−102 gc/cm^2) with the fraction of quantifiable to positive samples of 12% (compared to our 10%).[18]

Longitudinal Surface Positivity and COVID-19 Case Trends. The percent of positive samples per week during full-scale sample collection varied from 0% to 16%, with peaks occurring on April 28, 2020 and June 16, 2020 (Figure 2). We explored lead periods of 0−11 days for the association between sample positivity rate and the 7-day moving average of case numbers and found that the weekly percentage of positive samples was most strongly associated with COVID-19 cases 7 days later (Figure S3, Table S11). Using the 7-day lead time, the weekly surface positivity rate explained 68.9% of the variation in COVID-19 cases within the same zip code ($r = 0.83$, $r^2 = 0.689$, $p = 0.003$) and 54.8% of the variation in COVID-19 cases in all of Somerville ($r = 0.74$, $r^2 = 0.548$, $p = 0.02$; Table S11). Notably, both peaks in surface positivity preceded corresponding peaks in COVID-19 cases within the same zip code by approximately 7 days (Figure 2).

Our findings demonstrate the potential for environmental surveillance of high-touch surfaces to inform disease dynamics during the COVID-19 pandemic and motivate future longitudinal studies in additional settings to confirm its utility as an early warning monitoring tool. This study was conducted in a densely populated urban setting with universal access to water and sanitation. The results of our study could be impacted by other local conditions, including residents' behavior as well as government ordinances. Although we did not find associations between observed behaviors and percent positivity, unobserved location-specific behaviors such as how often surfaces were disinfected and availability of hand sanitizer in public areas likely impacted the presence of SARS-CoV-2 RNA on surfaces and the strength of the association with COVID-19 cases.

High-touch surface monitoring could be especially useful at finer spatial scales such as within buildings, when regular widespread human testing is not possible. Surface sampling within buildings could inform the locations of currently infectious individuals and enable early identification of potential COVID-19 cases when individuals are most infectious, including when they are presymptomatic or asymptomatic.[42,43] This may be especially useful within schools or universities, as door entrances and surfaces within each classroom could be tested, and if positive, classrooms could be quarantined before COVID-19 has the chance to spread. An additional advantage of surface surveillance is that swab samples can be analyzed using the same sample protocols and biosafety precautions as human nasal swab specimens. As lower cost and rapid human diagnostic assays are being developed during the pandemic, surface sampling could benefit from these advances as well.[61−65]

Infection Risk. Infection risks ranged from 2 in 10,000,000 to 4 in 10,000 (mean = 6.5×10^{-5}, median = 2.2×10^{-6}). The majority of our positive samples were not quantifiable by qPCR and were therefore treated as the theoretical qPCR limit of detection (LOD) for this analysis. Samples below the limit of quantification had a low risk of infection (2.4×10^{-7} to 3.5×10^{-4}), which varied based on object surface area and material (Table S9). Of the three quantifiable samples (a door handle at the liquor store and two grocery store door handles), the risk ranged from 1 in 100,000 to 4 in 10,000 (Table 1).

Notably, we estimated risks for a single touch on a single surface. While the risk of an individual transmission event is low, we observed a median of six touches per surface per hour, which extrapolates to 336 touches per week (assuming similar touch rates 9 am−5 pm daily, 7 days per week). Therefore, disinfection of frequently touched surfaces, such as door handles to essential businesses, is likely still useful to prevent possible cases of fomite transmission. Hand disinfection after touching public surfaces could further reduce transmission risk.[54] Nevertheless, the low infection risk estimated in this study supports prioritizing COVID-19 pandemic response resources to focus on reducing spread via aerosols and droplets (e.g., wearing masks) and by close contacts (e.g., social distancing).

Estimated risk of infection from exposure to the contaminated surfaces here is lower than estimates for inhalation exposure to SARS-CoV-2 and lower than fomite transmission risk of other respiratory pathogens. The median risk of infection in our study was lower than the median estimate of infection risk of COVID-19 via aerosols in a seafood market in South China (2.23×10^{-5}) with only one infected person present.[66] Compared to other viruses, the risk of fomite-mediated infection in this study is lower than risk of fomite-mediated infection of influenza (median risk = 1.25×10^{-4}), which is thought to spread primarily via droplets and aerosols,[67,68] and much lower than risk of norovirus infection

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(mean risk = 2.7×10^{-3}), where fomites have been found to play a role in spread.[69−71]

Overall, our results are consistent with fomite-mediated transmission of COVID-19 being possible but likely a secondary pathway. Uncertainty in key QMRA model parameters could lead to an overestimate of risk. Our QMRA model was highly sensitive to the ratio of RNA to infectious virus on surfaces, which has not yet been definitively determined. A study that measured both viable virus and viral RNA in patient samples found virus could not be isolated after 8 days despite measuring high viral loads (approximately 10^5 RNA copies/mL).[72,73] In addition, the ratio of RNA to viable virus in clinical samples may be different than this ratio in environmental surface samples. We did not attempt to culture live virus from any of our surface samples and therefore cannot determine the viability or infectivity of the SARS-CoV-2 detected in our samples. Future work is needed to confirm the relationship between SARS-CoV-2 RNA concentrations and viable virus on surfaces (which substantially influences the estimated probability of infection) and to determine if infective SARS-CoV-2 can be recovered from fomites in community settings.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.0c00875.

> Methods for RNA extraction and RT-qPCR, swab recovery efficiency experiments, RT-qPCR inhibition testing, the QMRA model, observational data, temperature and humidity associations with weekly percent positive samples, QMRA model sensitivity, and associations between differing lag periods of our weekly percent positive samples and local COVID-19 cases (PDF)

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Author Contributions

[▽]A.P.H. and E.R.F. contributed equally to this work.

Notes

The Somerville COVID-19 dashboard can be accessed at https://public.tableau.com/profile/cityofsomerville#!/vizhome/SomervilleCOVID-19Dashboard_v5/Dashboard3?publish=yes. The sample positivity and COVID-19 case data, as well as R code to replicate our analysis, can be accessed at https://github.com/abharv52/COVID19_longitudinalsampling. A version of this manuscript is available at Abigail P. Harvey, Erica P. Fuhrmeister, Molly E. Cantrell, Ana K. Pitol, Jenna M. Swarthout, Julie E. Powers, Maya L. Nadimpalli, Timothy R. Julian, and Amy J. Pickering. Longitudinal monitoring of SARS-CoV-2 RNA on high-touch surfaces in a community setting. medRxiv, 2020. https://www.medrxiv.org/content/10.1101/2020.10.27.20220905v1 (accessed December 4, 2020).

The authors declare no competing financial interest.

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