Just 2% of SARS-CoV-2–positive individuals carry 90% of the virus circulating in communities

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We analyze data from the fall 2020 pandemic response efforts at the University of Colorado Boulder, where more than 72,500 saliva samples were tested for severe acute respiratory coronavirus 2 (SARS-CoV-2) using qRT-PCR. All samples were collected from individuals who reported no symptoms associated with COVID-19 on the day of collection. From these, 1,405 positive cases were identified. The distribution of viral loads within these asymptomatic individuals was indistinguishable from what has been previously observed in symptomatic individuals. Regardless of symptomatic status, ~50% of individuals who test positive for SARS-CoV-2 seem to be in noninfectious phases of the disease, based on having low viral loads in a range from which live virus has rarely been isolated. We find that, at any given time, just 2% of individuals carry 90% of the virions circulating within communities, serving as viral “super-carriers” and possibly also superspreaders.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus that emerged into the human population in late 2019 (1), presumably from animal reservoirs (2, 3). During the ensuing world-wide pandemic, already more than 3 million lives have been lost due to the virus. Spread of SARS-CoV-2 has thus far been extremely difficult to contain. One key reason for this is that both presymptomatic and asymptomatic infected individuals can transmit the virus to others (4–13). Further, it is becoming clear that certain individuals play a key role in seeding superspreading events (14–17). Here, we analyzed data from a large university surveillance program. Viral loads were measured in saliva, which has proven to be an accessible and reliable biospecimen in which to identify carriers of this respiratory pathogen, and the most likely medium for SARS-CoV-2 transmission (18–20). Our dataset is unique in that all SARS-CoV-2–positive individuals reported no symptoms at the time of saliva collection, and therefore were infected but asymptomatic or presymptomatic. We find that the distribution of SARS-CoV-2 viral loads on our campus is indistinguishable from what has previously been observed in symptomatic and hospitalized individuals. Strikingly, these datasets demonstrate dramatic differences in viral levels between individuals, with a very small minority of the infected individuals harboring the vast majority of the infectious virions.

Results

The University of Colorado Boulder SARS-CoV-2 Screening Operation. We analyzed data resulting from SARS-CoV-2 testing performed on the University of Colorado Boulder campus during the fall academic semester of 2020 (August 27 to December 11, 2020). Residents of dormitories were tested weekly, and several campus testing sites were in operation throughout the semester, offering testing for any campus affiliate. At the time of saliva collection, participants were asked to confirm that symptoms were not present; therefore, any infected persons identified through this surveillance testing were asymptomatic or presymptomatic at the time of saliva collection. It should be noted that all of the samples analyzed herein were collected before the B.1.1.7 (“U.K.”) SARS-CoV-2 variant, and subsequent major variants of concern, were first documented in the United States during the final weeks of 2020 and the beginning of 2021 (21).

During the fall 2020 semester, more than 72,500 saliva samples were screened for SARS-CoV-2. A qRT-PCR assay was used, with the template coming from the direct addition of saliva without RNA purification (22). Three TaqMan primer/probe sets were...

Significance

We analyzed data from saliva-based COVID-19 screening deployed on the University of Colorado Boulder campus. Our dataset is unique in that all SARS-CoV-2–positive individuals reported no symptoms at the time of saliva collection, and therefore were infected but asymptomatic or presymptomatic. We found that 1) the distribution of viral loads observed in our asymptomatic college population was indistinguishable from what has been reported in hospitalized populations; 2) regardless of symptomatic status, approximately 50% of individuals who test positive for SARS-CoV-2 seem to be in noninfectious phases of the infection; and 3) just 2% of infected individuals carry 90% of the virions circulating within communities, serving as viral “supercarriers” and likely also superspreaders.

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Competing interest statement: Some of the authors of this study have financial ties to companies that offer commercial SARS-CoV-2 testing; Q.Y., N.R.M., C.L.P., and S.L.S. are cofounders of Darwin Biosciences; T.K.S., P.K.G., and E.L. are cofounders of TUMI Genomics. R.P. is a cofounder of Faze Medicines, and R.D.D. is a cofounder of Arpeggio Biosciences.

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used in a multiplex reaction directed against two regions of the SARS-CoV-2 genome (CU-E and CU-N, where CU stands for the University of Colorado) and a host transcript (CU-RNaseP) as control. The multiplex reaction was used to create standard curves to convert Ct value (cycle threshold) of each primer set to viral load (virions per milliliter) in the original saliva sample (SI Appendix, Fig. S1A). To ensure the viral load quantification is accurate for samples with extremely low Ct values (i.e., extremely high viral loads), we performed serial dilution of three saliva samples with among the highest observed viral loads of the semester, and showed that Ct values scale linearly with the dilution factor (SI Appendix, Fig. S1B).

From over 72,500 saliva samples screened, 1,405 SARS-CoV-2–positive samples were identified. The vast majority of these positive samples were from unique individuals, because individuals with positive tests were directed into the health care system for further testing and care. The distribution of the Ct values of these 1,405 individuals, with each of the two primer sets used, is shown in Fig. L4. Overall, the distribution of SARS-CoV-2 viral load fits under a log-normal distribution centered around the mean of $2.1 \times 10^7$ virions per mL (median = $1.1 \times 10^7$ virions per mL) for the CU-E primers or $5.9 \times 10^7$ virions per mL (median = $2.5 \times 10^7$ virions per mL) for the CU-N primers (SI Appendix, Fig. S3). The highest observed viral load was over 6 trillion ($6.1 \times 10^{12}$ virions per mL), which was only observed in one individual. It is remarkable to consider that this individual was on campus and reported no symptoms at our testing site. The lowest viral load detected was eight virions per milliliter. Thus, surveillance testing demonstrates an extremely wide variation in the viral load in infected but seemingly healthy (asymptomatic) individuals.

To verify that these viral load distributions were not influenced by the specific qRT-PCR primers used, we determined the agreement between the CU-N and CU-E primers with regard to the Ct values produced from samples. Different primer sets should encide by the specific qRT-PCR primers used, we determined the agreement between the CU-N and CU-E primers with regard to the Ct values produced from samples. Different primer sets should.

Fig. 1. Saliva viral load distribution within our campus population. (A) Distributions are shown of the viral loads measured in the 1,405 positive samples identified on campus during the fall semester of 2020. Each histogram shows Ct values obtained using TaqMan primer/probe sets targeting either the E gene (“CU-E”) or the N gene (“CU-N”) of SARS-CoV-2. The horizontally axes are labeled with both the Ct values and the corresponding viral load calculated from the standard curve for each primer set (SI Appendix, Fig. S1A). ND denotes no data, as the viral load is below the qRT-PCR detection limit. (B) The Ct values resulting from the two primer sets in A are highly correlated, especially in samples with high viral loads (Ct value lower than 30). Pearson correlation coefficients (PCC) are shown within and beyond the Ct = 30 arbitrary cutoff. (C) For 105 of the SARS-CoV-2–positive saliva samples, we ran qRT-PCR side by side with eight different primer sets commonly used in SARS-CoV-2 diagnostic tests (SI Appendix, Fig. S2). Here, we show the same analysis as in B, except with the CDC primers targeting the E and N genes (see Methods).
A Small Subset of Individuals Carries Most of the Circulating Virions. We next analyzed how virus is distributed between individuals within populations. By summing the viral load across individuals based on the interpolated probability density function representing each population, starting with those with the highest viral loads, we find that just 2% of individuals harbor 90% of the circulating virions (Fig. 3). This is true in both the university (i.e., asymptomatic) and hospitalized (i.e., symptomatic) populations. Further, 99% of community-circulating virions are accounted for by just 10% of the asymptomatic and 14% of the symptomatic population. In both asymptomatic and symptomatic populations, one single individual with the highest saliva viral load carried more than 5% of the total circulating virions. On the other hand, all individuals with saliva viral loads lower than 10^6 virions per mL combined (representing ~50% of the infected individuals) harbor less than 0.02% of the virions in both populations. This can be understood because Ct is a linear representation of logarithmic increases in viral load, so that the viral load increases exponentially as the Ct value decreases (SI Appendix, Fig. S1). Thus, there is a highly asymmetric distribution of viruses within both populations, with just a small number of people carrying the vast majority of the virus. It remains unknown whether these are special individuals capable of harboring extraordinarily high viral loads, or whether many infected individuals pass through a very short time period of extremely high viral load (see further discussion below). Irrespective of mechanism, it is nevertheless true that, at any given moment in time, a small number of people are harboring the vast majority of virions.

Infectious virions have rarely been isolated from clinical samples of individuals with viral load less than 10^6 virions per mL. (28, 30–35). One hypothesis is that people in this low range of viral load may simply be shedding viral genomes from damaged tissue that is undergoing repair, and, for this reason, they may not pose a substantial risk of infecting others. Our distributions suggest that approximately half of the people who test positive may not be infectious to others (Fig. 3), based on this line of reasoning.

Discussion

An important finding herein is that the vast majority of circulating virions in communities are found within the bodies of a small number of individuals. These findings corroborate similar trends observed elsewhere (14–17, 25). Although it remains to be seen exactly how transmission probability relates to viral load, a strong implication is that these individuals who are viral supercarriers may also be superspreaders. Higher viral loads have been shown to increase the probability of transmission to others in China (36), in Spain (37), and between pairs of roommates on our university campus (38). A higher rate of spread by viral supercarriers would be consistent with recent contact tracing analyses suggesting that 80 to 90% of infections are caused by 10 to 20% of infected individuals (14–17). A higher rate of spread by viral supercarriers would also be consistent with the surprisingly low transmission rates being reported between roommates (38), schoolmates (39, 40), and household members (41), which could be explained if only a small fraction of infected individuals have high enough viral loads to facilitate active transmission.

One potential explanation for the differences in viral loads between individuals is that individuals were simply tested at different stages of otherwise similar viral infections. However, longitudinal analyses of individual infections show that peak viral loads vary dramatically between individuals (42–44). Thus, the parsimonious explanation is that individuals produce different levels of virus. Whether this is due to variation in the immune response, variation in host factors supporting virus replication like ACE2, the specific viral variant infecting, or initial infection site or dose remains to be determined (45–48). To look at this further, we compared the viral load distributions analyzed herein to a theoretical normal distribution using quantile–quantile plots (SI Appendix, Fig. S3). The data deviate from the normal distribution at the extreme ends, including in the part of the population with the highest viral loads. This is consistent with the hypothesis that a small percentage of individuals represent a unique

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Fig. 2. Viral load distributions are similar in asymptomatic and symptomatic populations. (A) A histogram of saliva viral loads in our asymptomatic campus population (n = 1,405, blue) compared to the same histogram of saliva viral loads from symptomatic (n = 404, red) individuals. The latter represents data compiled from the 10 studies in SI Appendix, Table S1. A log-normal probability density function is fitted onto the two distributions given the population mean and standard deviation. (B) Empirical cumulative distribution functions (ECDFs) of saliva viral load in the asymptomatic (n = 1,405, blue) and symptomatic (n = 404, red) populations. The similarity of the two ECDFs was assessed with the Kolmogorov–Smirnov test, which resulted in D statistic = 0.03, and P value = 0.97.
The concentration of a majority of the virus in a small fraction of the population at a given time is a critical observation with actionable conclusions. Community screening to identify viral supercarriers within presymptomatic and asymptomatic stages of disease will be important, since these individuals will continue to sustain and drive the epidemic if not located. Finding viral supercarriers will have a disproportionately large impact on curbing new COVID-19 infections, yet individuals without symptoms don’t tend to seek out testing, so screening will need to target healthy populations. Modeling approaches show that one of the most important factors in screening for SARS-CoV-2 will be the speed with which infected people receive their test results (also referred to as turnaround time) (49). The longer it takes for people to receive their results, the more time goes by where they might unwittingly infect others. Therefore, it is imperative that we find virus supercarriers, and inform them of their infection status in a way that is fast, easy, and accessible. Although detection limits vary between current monitoring and diagnostic paradigms, all are more than capable of finding the majority of infected individuals and the vast majority of circulating virions (Fig. 3) (50–52).

**Methods**

**Collection of University Samples.** For sample collection conducted at our university, individuals were asked to fill out a questionnaire (https://www.colorado.edu/daily-health-form) to confirm that they did not present any symptoms consistent with COVID-19, and to collect no less than 0.5 mL of saliva into a 5-mL screw-top collection tube. Saliva samples were heated at 95 °C for 30 min on site to inactivate the viral particles for safer handling, and then placed on ice or at 4 °C before being transported to the testing laboratory for qRT-PCR analysis on the same day.

**Saliva qRT-PCR Used for Screening Saliva Samples on the University of Colorado Boulder Campus.** For qRT-PCR analysis, the university testing team transferred 75 μL of saliva into one well of a 96-well plate where each well had been preloaded with 75 μL of 2x Tris borate-ethylenediaminetetraacetic acid (TBE) buffer supplemented with 1% Tween-20. Of this diluted sample, 5 μL was then added to one well of a separate 96-well plate where each well had been preloaded with 15 μL of reaction mix composed of TaqPath 1-Step Multiplex Master Mix (Thermo Fisher A28523), nuclease-free water, and triplex primer mix consisting of CU-E, CU-N, and CU-RNaseP primer and probe sets (Table 1; conditions changed slightly during the semester). The reagents were mixed, spun down, and loaded onto a Bio-Rad CFX96 or CFX384 qPCR machine. The qRT-PCR was run using the standard mode, consisting of a hold stage (25 °C for 2 min, 50 °C for 15 min, and 95 °C for 2 min) followed by 44 cycles of a PCR stage (95 °C for 3 s, 55 °C for 30 s, with a 1.6 °C/s ramp-up and ramp-down rate). Ct values from all campus testing efforts were communicated to us as deidentified data.
Focused Analysis of 105 SARS-CoV-2—Positive Samples. For a smaller subset of 105 samples, as described herein, we did a side-by-side comparison of three different qRT-PCR multiplex assays commonly used in SARS-CoV-2 diagnostics. We thawed 105 frozen, deidentified saliva samples which had previously tested positive for SARS-CoV-2 in the campus screening operation and performed all of the following qRT-PCR analyses side by side on the day of sample thawing.

First, 25 μL of thawed, previously heated saliva was transferred into one well of a 96-well plate where each well had been preloaded with 25 μL of 2× TBE buffer supplemented with 1% Tween-20. Next, 5 μL of the diluted sample was added to separate 96-well plates where each well had been preloaded with 15 μL of reaction mix composed of TaqPath 1-Step Multiplex Master Mix (Thermo Fisher A28523), nuclease-free water, and US Centers for Disease Control’s (CDC) triplex primer mix or CU triplex primer mix (Table 1). The reagents were mixed, spun down, and loaded onto a Bio-Rad CFX96 qPCR machine. The qRT-PCR was run using the standard mode, consisting of a hold stage (25 °C for 2 min, 50 °C for 1 min, and 95 °C for 2 min) followed by 44 cycles of a PCR stage (95 °C for 3 s, 55 °C for 30 s, with a 1.6 °C/s ramp-up and ramp-down rate). Each plate also contained two wells of negative control template (5 μL of nuclease-free water diluted 1:1 with 2× TBE supplemented with 1% Tween-20) and two wells of positive control template (5 μL of synthetic SARS-CoV-2 RNA [Twist Biosciences 102024] diluted to 1,000 genome copies per μL, and 5 μL of total human reference RNA [Agilent 750500] diluted to 10 ng/μL in nuclease-free water).

We also performed the SalivaDirect TaqMan qRT-PCR analysis (20) on each of these samples; 75 μL of each saliva specimen was combined with 9.4 μL of Proteinase K (20 mg/mL, New England Biolabs, P81075). Samples were incubated at ambient temperature for 15 min and then heated to 95 °C for 5 min to inactivate the Proteinase K. Next, 5 μL of saliva was used as template in a 20-μL reaction that also contained 1× TaqPath 1-Step Multiplex Master Mix, nuclease-free water, and primer and probe sets at concentrations described below. The qRT-PCR was run on the BioRad CFX96 qPCR machine using the same program described for the CU assays (20).

Table 1. The qRT-PCR TaqMan primer/probe sets used for university screening and focused analysis

| Assay name | Primer/probe set target and designation | Primer or probe name | 1x concentration, nM | Sequence (5’ to 3’)* |
|------------|----------------------------------------|----------------------|---------------------|----------------------|
| CU SARS-CoV-2 E gene “CU-E” | E_Sarbeco_F (IDT 10006888) | 400 | ACAGTGTCGTAAATAGTGAATCGT |
| | E_Sarbeco_R2 (IDT 10006890) | 400 | ATATTGCGAGCTACGAGACACAA |
| | E_Sarbeco_P1 (IDT Custom) | 200 | TexRd-ACACTAGCTTACCTGCGCCGCTTGC- IABkFQ |
| SARS-CoV-2 N gene “CU-N” | nCOV_N1_F (IDT 10006830) | 500 | GACCCCCAAATCAGCGGAAT |
| | nCOV_N1_R (IDT 10006831) | 500 | TCTGTGTAATGCGATTTGAATCTG |
| | nCOV_N1_P (IDT Custom) | 250 | HEX-ACCCCGCAT-ZEN-TACGGTTTGGGACC- IABkFQ |
| Human RNase P “CU-RNaseP” | RNaseP_F (IDT 10006836) | 50 | AGATTGGAGCTGCGAGCC |
| | RNaseP_R (IDT 10006837) | 50 | GACGGCGGTGTCTTACCAAA |
| | RNase_P_P (IDT 10006838) | 50 | FAM-TTCTGACTZEN-GAAGGGCTTGGCGG- IABkFQ |
| CDC SARS-CoV-2 E gene “CDC-E” | E_Sarbeco_F1 (IDT 10006888) | 400 | ACAGTGTCGTAAATAGTGAATCGT |
| | E_Sarbeco_F2 (IDT 10006890) | 400 | ATATTGCGAGCTACGAGACACAA |
| | E_Sarbeco_P1 (IDT 10006893) | 200 | FAM-ACACTAGCTTACCTGCGCCGCTTGC- IABkFQ |
| SARS-CoV-2 N gene “CDC-N” | nCOV_N2_F (IDT 10006833) | 600 | TTAAACACATGGGGGCGGAA |
| | nCOV_N2_R (IDT 10006834) | 600 | GGCACGATACTCGAGAA |
| | nCOV_N2_P (IDT 10007049) | 300 | SUN-ACAATTTGCCCCCAGGCCTTCAG- IABkFQ |
| Human RNase P “CDC-RNaseP” | RNaseP_F (IDT 10006836) | 50 | AGATTGGAGCTGCGAGCC |
| | RNaseP_R (IDT 10006837) | 50 | GACGGCGGTGTCTTACCAAA |
| | RNase_P_P (IDT 10007062) | 50 | ATTO647-TTCTGACTZEN-GAAGGGCTTGGCGG- IABkFQ |
| SalivaDirect (20) SARS-CoV-2 N gene “SalivaDirect-N” | nCOV_N1_F (IDT 10006830) | 400 | GACCCCCAAATCAGCGGAAT |
| | nCOV_N1_R (IDT 10006831) | 400 | TCTGTGTAATGCGATTTGAATCTG |
| | nCOV_N1_P (IDT 10006832) | 200 | FAM-ACCCCGCATATTGCTTTTGGGACC- IABkFQ |
| Human RNase P “SalivaDirect-RNaseP” | RNaseP_F (IDT 10006836) | 150 | AGATTGGAGCTGCGAGCC |
| | RNaseP_R (IDT 10006837) | 150 | GACGGCGGTGTCTTACCAAA |
| | RNase_P_P (IDT 10007062) | 200 | ATTO647-TTCTGACTZEN-GAAGGGCTTGGCGG- IABkFQ |

*Explanation for some of the TaqMan fluorophores and quenchers used: IAbRQSp, Iowa Black Dark Quenchers RQ; IABkFQ, Iowa Black Dark Quenchers FQ; ZEN, internal quencher; TexRd, Texas Red; HEX, Hexachloro-fluorescein; FAM, fluorescein.
Ethics Statements. Viral load data on university participants were deidentified and aggregated from the University of Colorado Boulder operational screening for SARS-CoV-2. This activity does not meet the definition of human subject research described in the United States Health and Human Services 45 Code of Federal Regulations Part 46. Deidentified saliva samples (n = 105) used for the cross-comparison of primers were collected under protocol 20-0662, approved by the University of Colorado Boulder Institutional Review Board.

Data Availability. All study data are included in the article and SI Appendix.

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