ORIGINAL ARTICLE

SARS-CoV-2 Transmission among Marine Recruits during Quarantine

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ABSTRACT

BACKGROUND

The efficacy of public health measures to control the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has not been well studied in young adults.

METHODS

We investigated SARS-CoV-2 infections among U.S. Marine Corps recruits who underwent a 2-week quarantine at home followed by a second supervised 2-week quarantine at a closed college campus that involved mask wearing, social distancing, and daily temperature and symptom monitoring. Study volunteers were tested for SARS-CoV-2 by means of quantitative polymerase-chain-reaction (qPCR) assay of nares swab specimens obtained between the time of arrival and the second day of supervised quarantine and on days 7 and 14. Recruits who did not volunteer for the study underwent qPCR testing only on day 14, at the end of the quarantine period. We performed phylogenetic analysis of viral genomes obtained from infected study volunteers to identify clusters and to assess the epidemiologic features of infections.

RESULTS

A total of 1848 recruits volunteered to participate in the study; within 2 days after arrival on campus, 16 (0.9%) tested positive for SARS-CoV-2, 15 of whom were asymptomatic. An additional 35 participants (1.9%) tested positive on day 7 or on day 14. Five of the 51 participants (9.8%) who tested positive at any time had symptoms in the week before a positive qPCR test. Of the recruits who declined to participate in the study, 26 (1.7%) of the 1554 recruits with available qPCR results tested positive on day 14. No SARS-CoV-2 infections were identified through clinical qPCR testing performed as a result of daily symptom monitoring. Analysis of 36 SARS-CoV-2 genomes obtained from 32 participants revealed six transmission clusters among 18 participants. Epidemiologic analysis supported multiple local transmission events, including transmission between roommates and among recruits within the same platoon.

CONCLUSIONS

Among Marine Corps recruits, approximately 2% who had previously had negative results for SARS-CoV-2 at the beginning of supervised quarantine, and less than 2% of recruits with unknown previous status, tested positive by day 14. Most recruits who tested positive were asymptomatic, and no infections were detected through daily symptom monitoring. Transmission clusters occurred within platoons. (Funded by the Defense Health Agency and others.)

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ROSPECTIVE STUDIES MAY BE USEFUL TO inform strategies to mitigate the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), particularly in group settings among young adults.1-4 U.S. Department of Defense installations have implemented recommended public health interventions.5 However, confined living spaces, close contact among persons during training regimens and other activities, shared dining facilities, and mixing of persons from across the United States place military populations at risk for contracting contagious respiratory infections such as coronavirus disease 2019 (Covid-19).6-9 The transmission of SARS-CoV-2 and Covid-19 in military settings has not been well studied.

The public health program implemented by the U.S. Marine Corps for all new recruits includes a period of home quarantine followed by a 2-week, strictly supervised quarantine at a closed campus, with the objective of mitigating infection among recruits. To evaluate the effectiveness of these measures, we monitored SARS-CoV-2 infections with serial real-time quantitative polymerase-chain-reaction (qPCR) assays and assessed events of virus transmission by means of phylogenetic analysis of viral genomes obtained from infected participants.

METHODS

STUDY DESIGN AND PARTICIPANTS

To reduce the risk of introducing SARS-CoV-2 into basic training at Marine Corps Recruit Depot, Parris Island, in South Carolina, the Marine Corps established a 14-day supervised quarantine period at a college campus used exclusively for this purpose. Potential recruits were instructed to quarantine at home for 2 weeks immediately before they traveled to campus. At the end of the second, supervised quarantine on campus, all recruits were required to have a negative qPCR result before they could enter Parris Island. Recruits were asked to participate in the COVID-19 Health Action Response for Marines (CHARM) study, which included weekly qPCR testing and blood sampling for IgG antibody assessment.

After potential recruits had completed the 14-day home quarantine, they presented to a local Military Entrance Processing Station, where a medical history was taken and a physical examination was performed. If potential recruits were deemed to be physically and mentally fit for

enlistment, they were instructed to wear masks at all times and maintain social distancing of at least 6 feet during travel to the quarantine campus. Classes of 350 to 450 recruits arrived on campus nearly weekly. New classes were divided into platoons of 50 to 60 recruits, and roommates were assigned independently of participation in the CHARM study. Overlapping classes were housed in different dormitories and had different dining times and training schedules.

During the supervised quarantine, public health measures were enforced to suppress SARS-CoV-2 transmission (Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). All recruits wore double-layered cloth masks at all times indoors and outdoors, except when sleeping or eating; practiced social distancing of at least 6 feet; were not allowed to leave campus; did not have access to personal electronics and other items that might contribute to surface transmission; and routinely washed their hands. They slept in double-occupancy rooms with sinks, ate in shared dining facilities, and used shared bathrooms. All recruits cleaned their rooms daily, sanitized bathrooms after each use with bleach wipes, and ate preplated meals in a dining hall that was cleaned with bleach after each platoon had eaten. Most instruction and exercises were conducted outdoors. All movement of recruits was supervised, and unidirectional flow was implemented, with designated building entry and exit points to minimize contact among persons. All recruits, regardless of participation in the study, underwent daily temperature and symptom screening. Six instructors who were assigned to each platoon worked in 8-hour shifts and enforced the quarantine measures. If recruits reported any signs or symptoms consistent with Covid-19, they reported to sick call, underwent rapid qPCR testing for SARS-CoV-2, and were placed in isolation pending the results of testing.

Instructors were also restricted to campus, were required to wear masks, were provided with preplated meals, and underwent daily temperature checks and symptom screening. Instructors who were assigned to a platoon in which a positive case was diagnosed underwent rapid qPCR testing for SARS-CoV-2, and, if the result was positive, the instructor was removed from duty. Recruits and instructors were prohibited from interacting with campus support staff, such as janitorial and food-service personnel. After

each class completed quarantine, a deep bleach cleaning of surfaces was performed in the bathrooms, showers, bedrooms, and hallways in the dormitories, and the dormitory remained unoccupied for at least 72 hours before reoccupancy.

Within 2 days after arrival at the campus, after recruits had received assignments to platoons and roommates, they were offered the opportunity to participate in the longitudinal CHARM study. Recruits were eligible if they were 18 years of age or older and if they would be available for follow-up. The study was approved by the institutional review board of the Naval Medical Research Center and complied with all applicable federal regulations governing the protection of human subjects. All participants provided written informed consent.

PROCEDURES

At the time of enrollment, participants answered a questionnaire regarding demographic characteristics, risk factors for SARS-CoV-2 infection, symptoms within the previous 14 days, and a brief medical history; blood samples and midturbinate nares swab specimens were obtained for qPCR testing to detect SARS-CoV-2. Demographic information included sex, age, ethnic group, race, place of birth, and U.S. state or country of residence; information regarding risk factors included whether participants had used masks, whether they had adhered to self-quarantine before arrival, their recent travel history, their known exposure to someone with Covid-19, whether they had flulike symptoms or other respiratory illness, and whether they had any of 14 specific symptoms characteristic of Covid-19 or any other symptoms associated with an unspecified condition within the previous 14 days.

Study participants were followed up on days 7 and 14, at which time they reported any symptoms that had occurred within the past 7 days. Nares swab specimens for repeat qPCR assays were also obtained. Participants who had positive qPCR results were placed in isolation and were approached for participation in a related but separate study of infected recruits, which involved more frequent testing during isolation. All recruits who did not participate in the current study were tested for SARS-CoV-2 only at the end of the 2-week quarantine, unless clinically indicated (in accordance with the public health procedures of the Marine Corps). Serum specimens obtained at enrollment were tested for

SARS-CoV-2–specific IgG antibodies with the use of the methods described below and in the Supplementary Appendix.

Participants who tested positive on the day of enrollment (day 0) or on day 7 or day 14 were separated from their roommates and were placed in isolation. Otherwise, participants and non-participants were not treated differently: they followed the same safety protocols, were assigned to rooms and platoons regardless of participation in the study, and received the same formal instruction.

LABORATORY METHODS

The qPCR testing of mid-turbinate nares swab specimens for SARS-CoV-2 was performed within 48 hours after collection by Lab24 (Boca Raton, FL) with the use of the TagPath COVID-19 Combo Kit (Thermo Fisher Scientific), which is authorized by the Food and Drug Administration. Specimens obtained from nonparticipants were tested by the Naval Medical Research Center (Silver Spring, MD). Specimens were stored in viral transport medium at 4°C. The presence of IgG antibodies specific to the SARS-CoV-2 receptorbinding (spike) domain in serum specimens was evaluated with the use of an enzyme-linked immunosorbent assay, as previously described,10 with some modifications. At least two positive controls, eight negative controls (serum specimens obtained before July 2019), and four blanks (no serum) were included in every plate. Serum specimens were first screened at a 1:50 dilution. followed by full dilution series if the specimens were initially found to be positive.

WHOLE-GENOME SEQUENCING AND ASSEMBLY

SARS-CoV-2 sequencing was performed with the use of two sequencing protocols (an Illumina sequencing protocol and an Ion Torrent sequencing protocol) to increase the likelihood of obtaining complete genome sequences. A custom reference-based analysis pipeline (https://github.com/mjsull/COVID_pipe) was used to assemble SARS-CoV-2 genomes with the use of data from Illumina, Ion Torrent, or both.¹¹

PHYLOGENETIC ANALYSIS

SARS-CoV-2 genomes obtained from patients worldwide and associated metadata were downloaded from the Global Initiative on Sharing All Influenza Data EpiCoV database¹² on August 11, 2020 (79,840 sequences), and a subset of sequence

es was selected from this database with the use of the default subsampling scheme of Nextstrain software¹³ with the aim of maximizing representation of genomes obtained from patients in the United States. Phylogenetic analyses of the specimens obtained from participants were performed with the v1.0-292-ga9de690 Nextstrain build for SARS-CoV-2 genomes with the use of default parameters. Transmission and outbreak events were identified on the basis of clustering of the SARS-CoV-2 genomes obtained from study participants within the Nextstrain phylogenetic tree, visualized with TreeTime.¹⁴ A comparative analysis of mutation profiles relative to the SARS-CoV-2 Wuhan reference genome was performed with the use of Nextclade software, version 0.3.6 (https://clades.nextstrain.org/).

DATA ANALYSIS

The denominator for calculating the percentage of recruits who had a first positive result for SARS-CoV-2 by qPCR assay on each day of testing excluded recruits who had previously tested positive, had dropped out of the study, were administratively separated from the Marine Corps, or had missing data. The denominator for calculating the cumulative positivity rates included all recruits who had undergone testing at previous time points, including those who were no longer participating in the study. Only descriptive numerical results and percentages are reported, with no formal statistical analysis.

RESULTS

STUDY POPULATION

From May 12 to July 15, 2020, a total of 1848 of 3143 eligible recruits (58.8%) across nine recruit classes were enrolled in the CHARM study; 324 recruits were ineligible because they were 17 years of age. A total of 40 study participants (24 participants on day 7 and 16 participants on day 14) did not return for follow-up (Fig. 1). These participants either dropped out of the study, were removed from the quarantine campus for medical or administrative reasons, or were separated from the Marine Corps. Participants were from 45 states, mostly from the eastern United States and particularly from states with larger populations. A total of 133 participants (7.2%) were born outside the United States (in 1 of 64 foreign countries), 1672 (90.5%) were male, 176 (9.5%)

were female, 463 (25.1%) identified as Hispanic, and 271 (14.7%) identified as Black. The mean age of the participants was 19 years (range, 18 to 31), and 1544 (83.5%) were 18 to 20 years of age. Of the 1813 participants who underwent serologic testing at enrollment, 105 (5.8%) had serum specimens that were positive for SARS-CoV-2–specific antibodies.

At the time of enrollment, 16 of 1847 participants (0.9%) tested by means of qPCR were positive for SARS-CoV-2; 5 of these participants also had positive IgG serologic results (Table 1). The 16 participants with positive qPCR results reported that they had self-quarantined at home for 14 days before their arrival, had had no exposure to anyone with flulike symptoms, had had no respiratory distress or known SARS-CoV-2 infection, and had not visited a health care facility during the previous 2 weeks.

POSITIVE RESULTS AND SYMPTOMS

Of the 1801 participants who had negative qPCR results at enrollment, 24 (1.3%) were positive on day 7; of these participants, 4 had positive IgG serologic results on day 0. On day 14, a total of 11 of 1760 (0.6%) of the previously negative participants tested positive; none of these participants were seropositive on day 0. Therefore, 35 participants who had had negative qPCR results within the first 2 days after arrival at the campus became positive during the supervised quarantine. Of the 51 total participants who had at least one positive qPCR test, 22 had positive tests on more than 1 day.

Symptoms in the week before or on the day of the first positive qPCR result were reported in 5 of these 51 (9.8%) positive participants on the formal study questionnaires (Table 1). The symptoms in these 5 participants were runny nose; runny nose, chills, and cough; cough and sore throat; fever and headache; and fever, chills, sore throat, and headache. The viral load at diagnosis, estimated on the basis of the qPCR cycle threshold, was on average approximately 4 times as high in the 5 symptomatic participants as in the 46 participants who were asymptomatic (Table S2). However, some asymptomatic participants had high viral loads estimated on the basis of the cycle threshold (Fig. S1).

A total of 26 of the 1554 nonparticipants (1.7%) were found to be positive on day 14 as a result of qPCR testing at the end of quarantine,

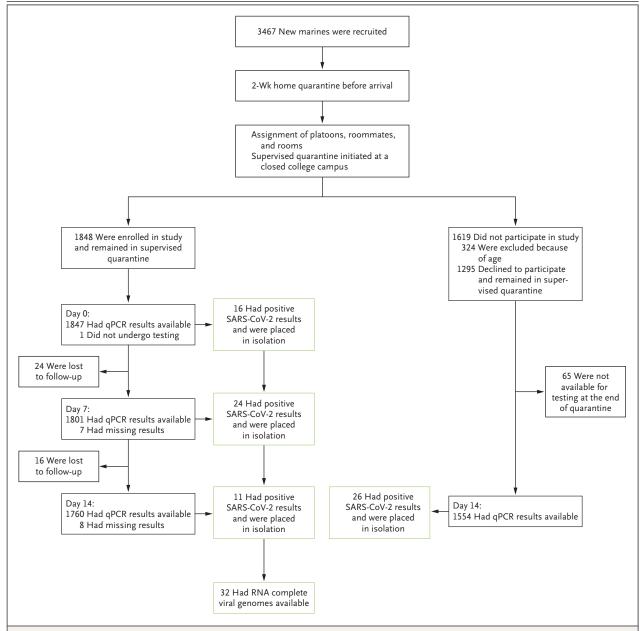


Figure 1. Study Design for SARS-CoV-2 Testing during Quarantine.

Marine Corps recruits entering a strict, supervised 2-week quarantine from May 15 to July 15, 2020, at a closed college campus were recruited for the COVID-19 Health Action Response for Marines (CHARM) longitudinal study for monitoring the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Recruits who were not enrolled in the study were tested by means of quantitative polymerase-chain-reaction (qPCR) assay, as required by the Marine Corps, after 14 days of quarantine.

which was mandated by the Marine Corps. A total of 24 of 77 (31.2%) infected participants and nonparticipants had an infected roommate (Table 1). All study participants and nonparticipants underwent daily screening that included temperature checks and oral reporting of symptoms;

follow-up qPCR testing was performed if indicated by the surveillance check. The results of the mandated symptom screening, which was independent of the study questionnaires regarding symptoms, was not known to the study investigators; however, no recruit with SARS-CoV-2 infec-

Table 1. SARS-CoV-2 Positivity, Presence of Symptoms, and Infected Roommates.*					
Variable	Day 0	Day 7	Day 14		
	number/total number (percent)				
Study participants					
First positive qPCR result†	16/1847 (0.9)	24/1801 (1.3)	11/1760 (0.6)		
Cumulative no. of recruits with positive qPCR results:	16/1847 (0.9)	40/1848 (2.2)	51/1848 (2.8)		
Cumulative no. of symptomatic recruits §	1/16 (6.3)	4/40 (10.0)	5/51 (9.8)		
Nonparticipants¶					
Positive qPCR result	Not tested	Not tested	26/1554 (1.7)		
All recruits, including study participants and nonparticipants					
Cumulative no. of recruits with positive qPCR results	16/1847 (0.9)	40/1848 (2.2)	77/3402 (2.3)		
Cumulative no. of recruits with a positive roommate	0/16 (0.0)	4/40 (10.0)	24/77 (31.2)		

^{*} The abbreviation qPCR denotes quantitative polymerase chain reaction, and SARS-CoV-2 severe acute respiratory syndrome coronavirus 2. † The total number on day 0 does not include 1 participant who was enrolled on day 7. The total number on day 7 includes 1 participant who was first tested on day 7 and excludes participants whose test results were missing (7 participants), who were lost to follow-up (24 participants), or who were previously positive (16 participants). The total number on day 14 includes 1 participant who was first tested on day 7 and excludes participants whose test results were missing (8 participants), who were lost to follow up (40 participants [including 24 who did not return on day 7 or day 14 and an additional 16 participants who did not return on day 14]), or who were previously positive (40 participants). † The cumulative total number includes all recruits who underwent testing up to and including the relevant test day.

§ Symptomatic recruits had a fever or any symptoms within the 7 days before the positive test result. The total number is the cumulative number of study participants with positive qPCR results.

The number of recruits indicates the number of positive recruits with a roommate who had a first positive test on or before the given test day, and the total number is the cumulative qPCR positivity for all recruits in this category.

tion was identified as a result of this clinically indicated testing. During the study period, one instructor was found to be positive in a test that was conducted as part of contact tracing related to an infected platoon member.

EPIDEMIOLOGIC ANALYSIS

To assess the epidemiologic features and transmission of SARS-CoV-2 in the context of this study, we obtained more than 95% complete viral genomes from 36 specimens obtained from 32 of 51 participants (62.7%) who had positive qPCR results for SARS-CoV-2; for 3 of these participants, genomes were recovered from samples obtained on more than one test day. Complete genomes could not be recovered from the other samples. Phylogenetic analyses that compared the recovered sequences with those recovered from patients in the United States and in other countries (a sample of 11,434 sequences) showed that most of the clades circulating in the United States were represented in SARS-CoV-2 isolates detected among recruits, a finding consistent

with the geographic diversity of the participants (Fig. S2).

Six independent monophyletic transmission clusters defined by distinct mutations relative to the sampled data from U.S. and global data sets were identified — a result consistent with local transmission during the supervised quarantine. These strains were found in 18 participants; 1 participant had two different cluster strains isolated from samples obtained on different days. Two participants who had had positive qPCR results on day 0 were each infected with different cluster strains (Tables S3 and S4). Epidemiologic data showing infected roommate pairs and the relationship of cluster strains to platoon assignments supported the phylogenetic evidence for transmission of these strains at the supervised quarantine location. Among the participants infected with one of the six cluster strains of SARS-CoV-2, a total of 14 participants shared platoon assignments with other members who were in the same cluster. In addition, 10 infected recruits without sequenced SARS-CoV-2 isolates were as-

[¶] Information regarding the symptoms of nonparticipants is not available. Recruits who did not participate in the study underwent testing only on day 14, as mandated by the Marine Corps. A total of 1619 recruits were categorized as nonparticipants because of ineligibility or because they declined to participate in the study. The total number of 1554 recruits on day 14 includes previous study participants who dropped out of the study but remained at the quarantine site and does not include nonparticipants who were removed from the quarantine site or separated from the Marine Corps; the number of recruits in each of these categories is not known.

Table 2. Phylogenetic Analysis and Epidemiologically Inferred Transmission Clusters.					
Phylogenetic Cluster	Sequenced Strains*	No. of Infected Recruits in the Same Platoon without Sequenced Isolate†	No. of Genomes in Cluster	No. of Infected Recruits with an Infected Roommate	
	no. of strains (no. in same platoon)				
Cluster 1	2 (0)	3	5	0	
Cluster 2	6 (6)	3	9	6	
Cluster 3	2 (2)	0	2	2	
Cluster 4	2 (2)	0	2	0	
Cluster 5	5 (4)	4	9	4	
Cluster 6	2 (NA)‡	0	2	0	
Total	19 (14)	10	29	12	

^{*} A total of 19 genomes belonging to 6 cluster strains were obtained from 18 participants. One participant had 2 different virus strains identified in samples obtained at different times.

signed to the same platoons as the participants in transmission clusters defined by viral sequencing. There were three different clusters among six pairs of infected roommates (Table 2).

The infected participants with sequenced isolates belonging to phylogenetically identified cluster 2 or 5 had room assignments in the same hallway (Fig. 2). Cluster 2 was composed of recruits in platoon F. Cluster 5 was composed of recruits in platoon E, with the exception of a single recruit in platoon F, whose roommate was an infected recruit in platoon E. Aside from this one event, we did not find evidence for transmission events across these platoons, even though recruits in each platoon were staying in rooms in the same hallway and shared a bathroom.

DISCUSSION

We describe the results of a quarantine of nine Marine Corps recruit classes (a population of 3402 recruits) that participated in a public health mitigation program for Covid-19; recruits were under the constant supervision of Marine Corps instructors. Other settings in which young adults congregate are unlikely to reflect similar adherence to measures intended to reduce transmission. At the time of enrollment, after 2 weeks of

home quarantine, approximately 1% of study participants had positive qPCR results, and approximately 2% subsequently became infected during the 2-week supervised quarantine period.

Study participants completed a detailed symptom questionnaire on each day of the scheduled qPCR testing. Approximately 10% of the infected study participants reported that they had had symptoms during the week before a positive qPCR result or on the day that testing occurred. Independent of the study, all participants and nonparticipants underwent a daily temperature check and brief symptom screening, as mandated by the Marine Corps; follow-up clinical qPCR testing was performed only if indicated by this screening. During the supervised quarantine period, no SARS-CoV-2 infections were identified as a result of clinical testing performed because of symptom screening. All cases of infection in recruits were diagnosed as a result of the scheduled qPCR testing performed on days 0, 7, and 14 (in study participants) and on day 14 (in nonparticipants).

Viral genomes were recovered from almost two thirds of infected study participants. Phylogenetic analysis of these genomes identified six independent monophyletic transmission clusters indicative of local transmission during the super-

[†] Values indicate the number of infected study participants or nonparticipants who were assigned to the platoon most associated with the phylogenetically defined transmission cluster. The transmission cluster was defined according to the SARS-CoV-2 strain sequence. Infected recruits in each platoon with the highest proportion of participants infected with a sequenced cluster strain were tentatively assigned to that cluster if no sequenced isolate was obtained from that recruit.

[‡]The platoon assignments of these participants were not available.

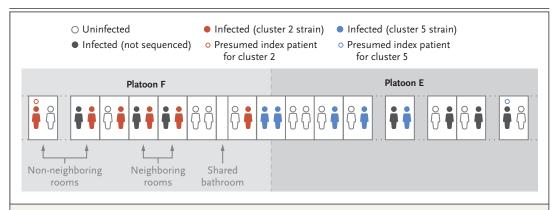


Figure 2. Local Transmission of SARS-CoV-2 during Quarantine.

Participants who were associated with the two largest transmission clusters (clusters 2 and 5) were identified by means of sequencing and were either roommates or members of the same platoons, which indicates that double-occupancy rooming and shared platoon membership were important contributors to transmission. Other infected members of these platoons whose samples were not sequenced may have been infected with the same cluster strains. One recruit in each platoon was found to be infected at the beginning of quarantine and represents the potential source of each cluster strain.

vised quarantine. Most clusters predominantly included members of the same platoon, and many infected recruits had an infected roommate. The two largest sequence-defined clusters occurred in the same class of recruits, and each cluster occurred within a platoon, with the exception of one recruit, who was roomed with an infected recruit from another platoon and was infected with a strain that belonged to the same cluster as that found in other members of that platoon. Although many infected recruits in both clusters had nearby room assignments and shared a bathroom, the epidemiologic analysis suggests that platoon membership and double-occupancy rooming were risk factors for infection, but room proximity and shared bathrooms were not (Fig. 2).

The index patient for each cluster strain could have been a study participant, a nonparticipant among the 26 found to be infected when tested at the end of quarantine, a nonparticipant who was infected at the time of arrival on campus but cleared the virus by the end of quarantine, or other personnel. During the study, only one instructor tested positive after rapid qPCR SARS-CoV-2 testing, indicating that instructors were an unlikely source of infection. Although campus service workers cannot be excluded as sources for virus introduction, they were separated from the recruits and instructors. Overall, the recruits are the most likely source of introduction and transmission of the cluster strains.

Two recruits who had positive qPCR results on day 0 may have been the index patients for the strains involved in the two largest clusters that spread among members of their platoons. A third recruit who may have been the index patient for a cluster had a positive qPCR result on day 0, and his roommate, infected with the same strain, received a diagnosis on day 14. None of the three potential index patients reported symptoms, which is consistent with asymptomatic transmission. We could not reconstruct the chain of infection for each cluster because complete viral genomes could not be recovered from all study participants, and samples from infected nonparticipants were unavailable for analysis. A limitation of this study is that the infection rate during the supervised quarantine period could not be estimated accurately because of possible false negative qPCR tests and because infection may have been acquired during the first self-quarantine at home or during travel to the campus but was not yet detectable on day 0 by means of qPCR

Our study showed that in a group of predominantly young male military recruits, approximately 2% became positive for SARS-CoV-2, as determined by qPCR assay, during a 2-week, strictly enforced quarantine. Multiple, independent virus strain transmission clusters were identified. Shared rooms and shared platoon membership were risk factors for transmission. Most

study participants with positive qPCR tests were asymptomatic, and all cases among participants and nonparticipants were identified as the result of scheduled testing rather than clinical qPCR testing performed as a result of daily screening.

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the U.S. Navy, the Department of Defense, the U.S. government, or the institutions affiliated with the authors.

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APPENDIX

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