SARS-CoV-2 Seropositivity among US Marine Recruits Attending Basic Training, United States, Spring–Fall 2020

Andrew G. Letizia, Yongchao Ge, Carl W. Goforth, Dawn L. Weir, Rhonda Lizewski, Stephen Lizewski, Alessandra Soares-Schanoski, Sindhu Vangeti, Nada Marjanovic, Stuart C. Sealfon, Irene Ramos

In a study of US Marine recruits, seroprevalence of severe acute respiratory syndrome coronavirus 2 IgG was 9.0%. Hispanic and non-Hispanic Black participants and participants from states affected earlier in the pandemic had higher seropositivity rates. These results suggest the need for targeted public health strategies among young adults at increased risk for infection.

Coronavirus disease (COVID-19) cases are increasing in young adults (*1*). In some instances, prevalence among younger adults exceeds that of older adults (*2*). Younger adults often have a paucisymptomatic or asymptomatic response to infection (*3*). The potential for rapid spread exists within this age group (*4*). Without active serologic surveillance, cases among young adults might not be identified and the cumulative incidence underestimated. Well-defined cohorts are needed to assess the proportion of young adults who have severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies (*5*). We studied the seroprevalence of SARS-CoV-2 IgG among US Marine recruits preparing for basic training at Marine Corps Recruit Depot Parris Island, South Carolina.

The Study

Before beginning basic training, recruits quarantined for 2 weeks at a hotel or college campus as previously described (*6*). Within 48 hours of arriving at the quarantine location, ≈350–500 recruits per week were offered the opportunity to volunteer for the COVID-19 Health Action Response for Marines Study, which included collecting baseline SARS-CoV-2 serologic test results.

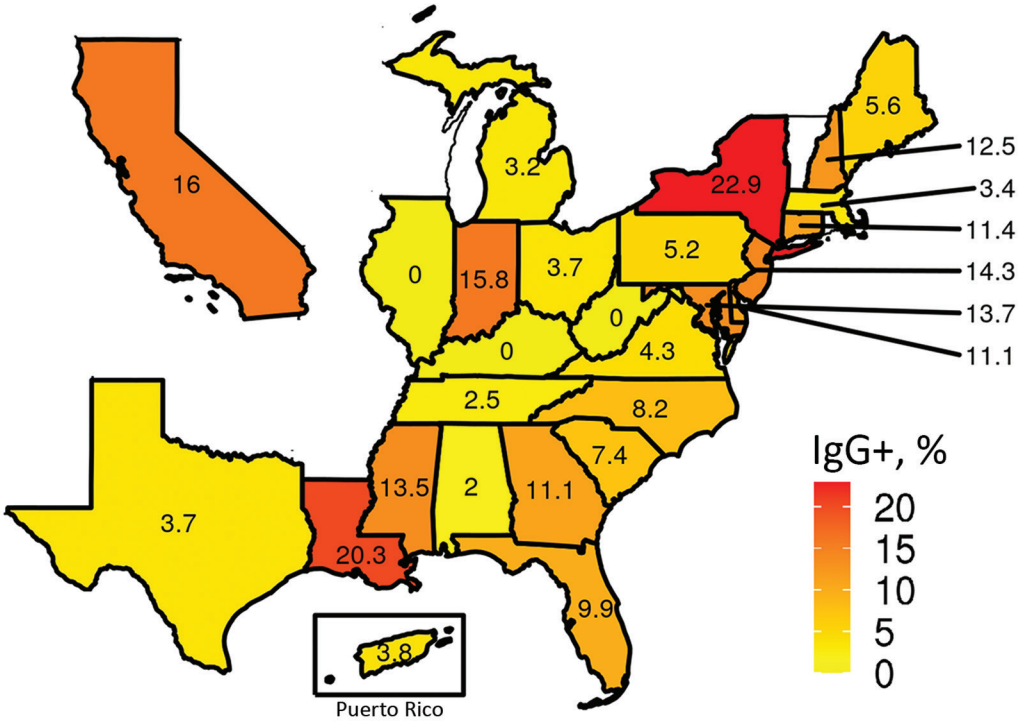
We collected paper questionnaires and assayed serum samples for the presence of SARS-CoV-2 IgG upon participants' arrival at the quarantine location. We tested serum specimens for SARS-CoV-2 IgG by ELISA (*6*) (Appendix, https://wwwnc.cdc.gov/EID/article/27/4/20-4732-App1.pdf). The association between demographics, risk factors, and IgG-positivity variables were analyzed with logistic regression to determine the p value and odds ratio (OR).

The study protocol was approved by the Naval Medical Research Center Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects. All participants provided written informed consent for participation.

During May 11–September 7, 2020, we enrolled 3,249 (69.8%) volunteers out of 4,657 eligible recruits; because the minimum age was 18, 530/5,187 (10.2%) persons who were 17 years of age were ineligible. Valid IgG data were obtained for 3,196/3,249 (98.4%) participants. Most participants were from the Eastern United States or states with larger populations (Figure 1). Study participants had a median age of 19.1 (range 18–31) years, and 257 (8.0%) were women (Table 1). Participants 18–20 years of age (2,748 [86.0%]) were overrepresented in our cohort compared with 3.9% in the general US population according to 2020 Census data. When compared with 2020 Census data for persons 18–20 years of age, our cohort had a similar percentage of Hispanic participants (23.9% compared with 23.9%) and non-Hispanic Black participants (12.04% compared with 15.04%) (*7*).

Author affiliations: Naval Medical Research Center, Silver Spring, Maryland, USA (A.G. Letizia, C.W. Goforth, D.L. Weir); Icahn School of Medicine at Mount Sinai, New York, New York, USA (Y. Ge, A. Soares-Schanoski, S. Vangeti, N. Marjanovic, S.C. Sealfon, I. Ramos); Naval Medical Research Unit 6, Lima, Peru (R. Lizewski, S. Lizewski)

DOI: https://doi.org/10.3201/eid2704.204732



State	No.	F, %	IgG+, %
FL	434	5.3	9.9
NY	292	4.1	22.9
GA	270	4.1	11.1
OH	217	4.1	3.7
PA	192	3.6	5.2
VA	188	5.9	4.3
NC	184	3.8	8.2
MI	156	5.1	3.2
NJ	140	4.3	14.3
TN	120	5.0	2.5
AL	100	3.0	2.0
MD	95	5.3	13.7
SC	94	4.3	7.4
MA	89	4.5	3.4
LA	69	1.4	20.3
KY	65	3.1	0.0
CT	44	4.5	11.4
MS	37	2.7	13.5
WV	29	3.4	0.0
TX	27	81.5	3.7
PR	26	0.0	3.8
CA	25	84.0	16.0
NH	24	0.0	12.5
IN	19	26.3	15.8
DE	18	5.6	11.1
ME	18	11.1	5.6
IL	13	69.2	0.0

Figure 1. Percentage of severe acute respiratory syndrome coronavirus 2 IgG-positive recruits from US states or territories with ≥10 participants in the COVID-19 Health Action Response for Marines Study, May 11–September 7, 2020. The table lists the number of participants and the percentage of women.

Upon arrival at quarantine, 28/3,196 (0.9%) participants were SARS-CoV-2–positive by PCR and 289/3,196 (9.0%) were ELISA-positive for SARS-CoV-2 IgG targeting the receptor-binding domain of the spike protein. A total of 135/768 (17.6%) participants who identified as Hispanic were positive for SARS-CoV-2 IgG (Table 2), higher than the percentage of non-Hispanic White participants (80/1,817 [4.4%]) (OR 3.80, 95% CI 2.82–5.14; p<0.001). Hispanic participants also had higher rates of IgG seropositivity among weekly cohorts throughout the study period, and those rates increased with time (trend p<0.00017); seropositivity rates rose from 12.1% in May and June to 22.3% in July and August. Similarly, non-Hispanic Black participants had higher prevalence of SARS-CoV-2 IgG (62/414 [15.0%]) than non-Hispanic White participants (OR 3.54, 95% CI 2.47–5.05; p<0.001). Seropositivity was also greater in women (32/257, 12.5%) than men (257/2,939, 8.7%) (OR 1.57, 95% CI 1.02–2.33; p = 0.033).

Because participants came from states that were affected by COVID-19 at different times and in variable intensity, we grouped participants' states of origin into 3 categories on the basis of when confirmed COVID-19 cases began to increase in each state (Appendix) (*8*). The groups were early spring, for states in which the outbreak began in March; late spring, for states in which the outbreak began in early June; and summer, for states in which the outbreak began in late June–July (Figure 2, panel A). We plotted weekly IgG-positivity rates during the 17-week study period (Figure 2, panel B) and found that participants from the early spring states had higher IgG seropositivity compared with late spring and summer and maintained a similar rate for the duration of the study. Overall, SARS-CoV-2 IgG seropositivity among participants from summer states (43/994 [4.3%]) and late spring states (126/1,389 [9.1%]) was much lower than in participants from early spring states (110/701 [15.7%]); OR was 0.35 (0.23–0.50; p<0.001) for summer and late spring states and 0.61 (0.46–0.81; p = 0.001) for early spring states. Figure 2, panel C, shows the weekly IgG-positive rate by race and ethnicity.

Conclusions

By using a cross-sectional study design during a 17-week period, the baseline seroprevalence of IgG against SARS-CoV-2 in US Marine recruits primarily from the eastern United States was 9.0%. In the United States, young adults have demonstrated higher levels of SARS-CoV-2–specific antibodies than persons of other ages (*9*). Among persons 18–20 years of age, low adherence to recommendations for social distancing, wearing of masks, and other public health

measures might increase their level of exposure compared with older persons (*10*). The high rate of asymptomatic infection in this age group (*6*) likely leads to underestimates of the cumulative incidence. Subsequent spread could contribute to infections among more vulnerable populations (*11*). Therefore, this age group represents an at-risk population that should be considered for COVID-19 monitoring and other targeted public health measures.

The participants in our study did not come from a cohort of convenience, a group at high risk, or a group receiving medical care; rather, they were selected from a group of young adults for the primary purpose of assessing baseline seropositivity. This process minimized selection bias (*12*), excluding the self-selection that occurred because participants chose to join the US Marine Corps and enroll in our study. Enrollment rate (70%) was high, which increased the likelihood that we studied a representative sample of recruits.

Consistent with other reports (*13*), Hispanic participants had higher IgG seroprevalence (OR 3.80) than non-Hispanic White participants in a multivariable logistic regression. This trend was similar for non-Hispanic Black participants and participants residing in states affected earlier in the pandemic. Our cohort was primarily young adults, many of whom had never held full-time jobs and might not represent essential workers, who have been associated with higher rates of infection among minority groups (*14*). It has been proposed that the higher incidence of SARS-CoV-2 in minority communities is associated with lower socioeconomic status and the associated inability to telecommute, leading to increased workplace exposure (C.T. Rentsch, unpub. data, https://doi.org/10.1101/2020.05.12.20099135). Instead, these data could demonstrate the downstream effects of residing with an essential worker or could reflect intrinsic risk within a community.

Table 1. Demographics of 3,196 COVID-19 Health Action Response for Marines study participants with valid IgG data, United States, May 11–September 7, 2020*

Characteristic	Total
Age, y, mean (SD)	19.1 (1.9)
Sex	
M	2,939 (92.0)
F	257 (8.0)
Race or ethnicity	
Non-Hispanic White	1,817 (56.9)
Non-Hispanic Black	414 (13.0)
Non-Hispanic other†	197 (6.2)
Hispanic	768 (24.0)
IgG	
Negative	2,907 (91.0)
Positive	289 (9.0)
COVID-19 by PCR	
Negative	3,054 (95.6)
Positive	28 (0.9)
Other‡	114 (3.6)
State group§	
Early spring	701 (21.9)
Late spring	1,389 (43.5)
Summer	994 (31.1)
Other‡	112 (3.5)
Resides in a country other than the United States	
No	3,084 (96.5)
Yes	23 (0.7)
Other‡	89 (2.8)
Born in a country other than the United States	
No	2,928 (91.6)
Yes	231 (7.2)
Other‡	37 (1.2)

*Values are no. (%) except as indicated. COVID-19, coronavirus disease.
†Non-Hispanic other also includes participants with missing values.
‡Inconclusive assay or the participant left question blank or answered by marking unknown.
§Defined in Figure 2, panel A, and the Appendix (https://wwwnc.cdc.gov/EID/article/27/4/20-4732-App1.pdf).

Table 2. Association between demographic variables and SARS-CoV-2 IgG results in study of seroprevalence in US Marine recruits, United States, May 11–September 7, 2020*

	IgG result		Univariable analysis		Multivariable analysis	
Characteristic	Negative	Positive	OR (95% CI)	p value	OR (95% CI)	p value
Age, y, mean (SD)	19.1 (1.9)	19.0 (1.7)	0.99 (0.92–1.05)	0.672	0.96 (0.89–1.02)	0.212
Sex						
M	2682 (91.3)	257 (8.7)	Referent		Referent	
F	225 (87.5)	32 (12.5)	1.48 (0.99–2.17)	0.048	1.57 (1.02–2.33)	0.033
Race or ethnicity						
Non-Hispanic White	1737 (95.6)	80 (4.4)	Referent		Referent	
Non-Hispanic Black	352 (85.0)	62 (15.0)	3.82 (2.69–5.42)	<0.001	3.54 (2.47–5.05)	<0.001
Non-Hispanic Other	185 (93.9)	12 (6.1)	1.41 (0.72–2.54)	0.283	1.32 (0.67–2.39)	0.388
Hispanic	633 (82.4)	135 (17.6)	4.63 (3.47–6.22)	0.001	3.80 (2.82–5.14)	<0.001
State group						
Early spring	591 (84.3)	110 (15.7)	Referent		Referent	
Late spring	1263 (90.9)	126 (9.1)	0.54 (0.41–0.71)	<0.001	0.61 (0.46–0.81)	0.001
Summer	951 (95.7)	43 (4.3)	0.24 (0.17–0.35)	<0.001	0.35 (0.23–0.50)	<0.001
Other†	102 (91.1)	10 (8.9)	0.53 (0.25–0.99)	0.065	0.54 (0.25–1.04)	0.085

*Values are no. (%) except as indicated. Univariable odds ratio and p value were computed on the basis of the logistic regression with a single variable only. Multivariable odds ratio and p value were computed by using all 4 variables in the model. OR, odds ratio; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
†Inconclusive assay (not residing in the United States) or participants left residence question blank or answered by marking unknown.

Our study incorporates participants from multiple states and represents a diverse mix of race, ethnicity, and backgrounds, providing a unique assessment relevant to public health concerns among persons 18–20 years of age. Conversely, the study results are not representative of the population as a whole, especially children and older adults. Even among young adults, the results are specific to persons who chose to join the US Marine Corps. Additional limitations include a lack of information regarding exposure, participant risk-taking behavior before enrollment, and lack of confirmation of COVID-19 by PCR before study enrollment.

In our study, the seroprevalence of SARS CoV-2 IgG among a cohort of predominately young men

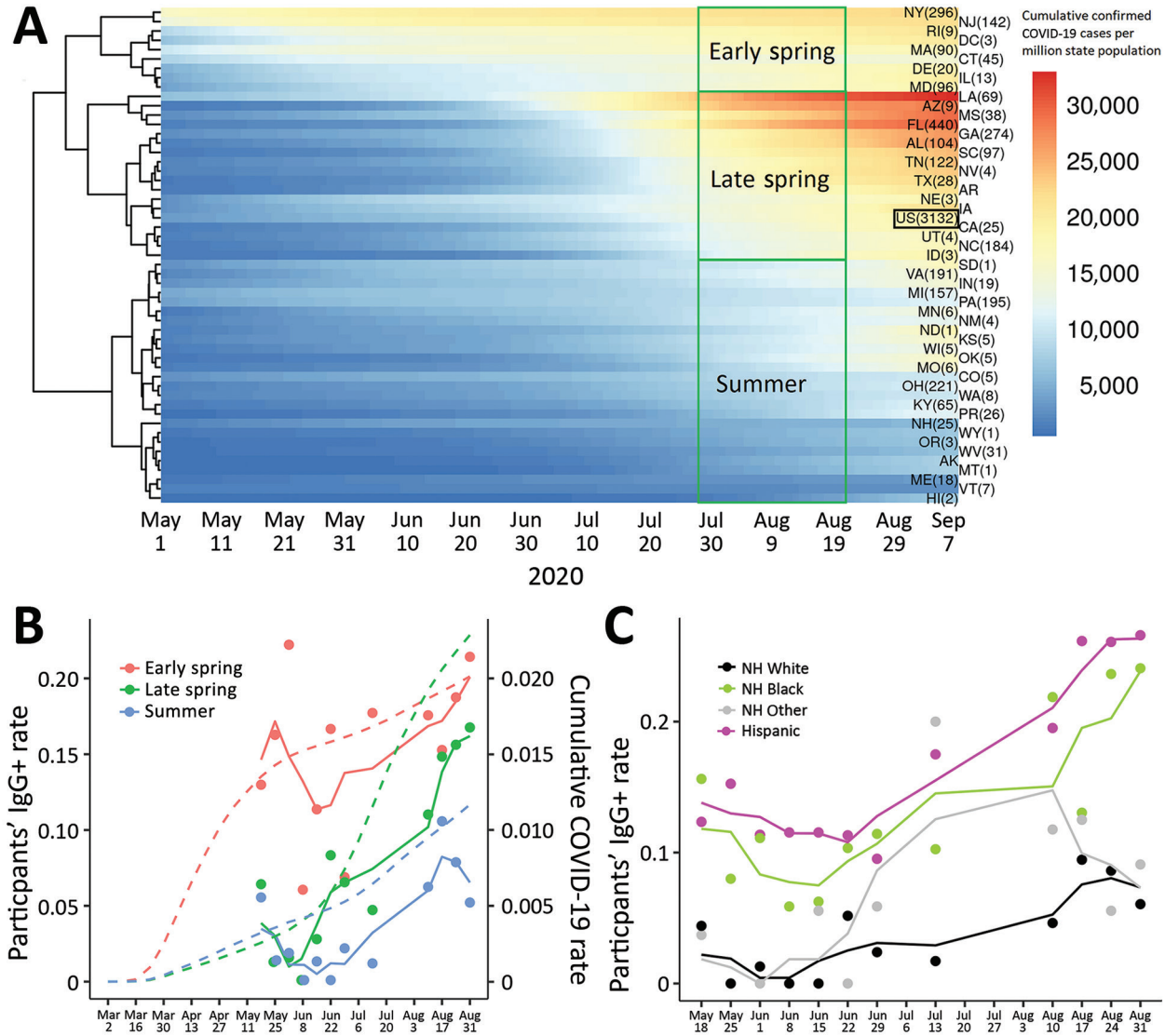


Figure 2. Confirmed COVID-19 and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) IgG-positivity by state, race, and ethnicity, in a study of US Marine recruits, May 11–September 7, 2020. A) Heatmap of cumulated confirmed COVID-19 cases normalized by each state's population. Each row represents 1 state, and number in parentheses indicates number of participants. Color reflects cumulative PCR-confirmed cases per 1 million state population (data obtained from COVID-19 Data Repository by the Center for Systems Science and Engineering at Johns Hopkins University, https://github.com/CSSEGISandData/COVID-19). Each column indicates 1 day during May 1–September 7, 2020. The US in aggregate is shown in the black box. B) SARS-CoV-2 IgG-seropositivity rate by week of enrollment on the basis of state groupings. Colored dots indicate the weekly IgG-positivity rate for study participants grouped by state; colored solid lines show 3-week running means. Dotted lines indicate cumulative PCR-confirmed COVID-19 cases in each state grouping obtained from COVID-19 Data Repository, including data before the study commenced. C) SARS-CoV-2 IgG-positivity by race and ethnicity. Colored dots indicate weekly IgG-positivity rate for study participants; colored solid lines indicate 3-week running means. Because of the relatively small number of participants in the first study week (May 11), they are merged into May 18 data. COVID-19, coronavirus disease; NH, Non-Hispanic.

was 9.0%. Multivariable analysis showed incidence rates were significantly higher in women, Hispanic participants, Non-Hispanic Black participants, and participants from states that were affected earlier in the pandemic. These data can help inform surveillance and management strategies, as well as targeted public health interventions, for this age group.

Acknowledgments

We thank the devoted US Marine Corps recruits who volunteered for this study; Sagie Mofsowitz, Mary Anne Amper, Nitish Seenarine, Mital Vasoya, and Natalia Mendelev for technical assistance; and Russell Tracy for providing pre-COVID-19 serum samples used as negative controls. We also thank Capt. Adam Armstrong for his strategic guidance throughout the study and the many US Navy corpsmen who assisted in logistics and sample acquisition.

This work was supported by a grant (9700130) from the Defense Health Agency through the Naval Medical Research Center and by the Defense Advanced Research Projects Agency (contract no. N6600119C4022). The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, or the US Government.

A.G.L., C.W.G., D.L.W., R.L., and S.L. are military Service members or employees of the US Government. This work was prepared as part of official duties. Title 17, U.S.C., §105 provides that copyright protection under this title is not available for any work of the US Government. Title 17, U.S.C., §101 defines a US Government work as a work prepared by a military Service member or employee of the US Government as part of that person's official duties.

About the Author

Dr. Andrew Letizia is the head of the Emerging Infectious Disease Department and Deputy Director of the Infectious Disease Directorate at the Naval Medical Research Center in Silver Spring, Maryland, USA. He is a board-certified infectious disease physician on active duty in the US Navy.

References:

1. Cunningham JW, Vaduganathan M, Claggett BL, Jering KS, Bhatt AS, Rosenthal N, et al. Clinical outcomes in young US adults hospitalized with COVID-19. JAMA Intern Med. 2020 Sep 9 [Epub ahead of print]. https://doi.org/10.1001/jamainternmed.2020.5313
2. Amorim Filho L, Szwarcwald CL, Mateos SOG, Leon ACMP, Medronho RA, Veloso VG, et al.; Grupo Hemorio de Pesquisa em Covid-19. Seroprevalence of anti-SARS-CoV-2 among blood donors in Rio de Janeiro, Brazil. Rev Saude Publica. 2020;54:69. https://doi.org/10.11606/s1518-8787.2020054002643
3. Payne DC, Smith-Jeffcoat SE, Nowak G, Chukwuma U, Geibe JR, Hawkins RJ, et al.; CDC COVID-19 Surge Laboratory Group. SARS-CoV-2 infections and serologic responses from a sample of U.S. Navy service members—USS Theodore Roosevelt, April 2020. MMWR Morb Mortal Wkly Rep. 2020;69:714–21. https://doi.org/10.15585/mmwr.mm6923e4
4. Huang L, Zhang X, Zhang X, Wei Z, Zhang L, Xu J, et al. Rapid asymptomatic transmission of COVID-19 during the incubation period demonstrating strong infectivity in a cluster of youngsters aged 16-23 years outside Wuhan and characteristics of young patients with COVID-19: a prospective contact-tracing study. J Infect. 2020;80:e1–13. https://doi.org/10.1016/j.jinf.2020.03.006
5. Lipsitch M, Swerdlow DL, Finelli L. Defining the epidemiology of Covid-19—studies needed. N Engl J Med. 2020;382:1194–6. https://doi.org/10.1056/NEJMp2002125
6. Letizia AG, Ramos I, Obla A, Goforth C, Weir DL, Ge Y, et al. SARS-CoV-2 transmission among Marine recruits during quarantine. N Engl J Med. 2020;383:2407–16. https://doi.org/10.1056/NEJMoa2029717
7. United States Census Bureau. National demographic analysis tables: 2020. 2020 [cited 2020 Dec 20]. https://www.census.gov/data/tables/2020/demo/popest/2020-demographic-analysis-tables.html
8. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis. 2020;20:533–4. https://doi.org/10.1016/S1473-3099(20)30120-1
9. Bajema KL, Wiegand RE, Cuffe K, Patel SV, Iachan R, Lim T, et al. Estimated SARS-CoV-2 seroprevalence in the US as of September 2020. JAMA Intern Med. 2020 Nov 24 [Epub ahead of print]. https://doi.org/10.1001/jamainternmed.2020.7976
10. Nivette A, Ribeaud D, Murray A, Steinhoff A, Bechtiger L, Hepp U, et al. Non-compliance with COVID-19-related public health measures among young adults in Switzerland: insights from a longitudinal cohort study. Soc Sci Med. 2021;268:113370. https://doi.org/10.1016/j.socscimed.2020.113370
11. Gao Z, Xu Y, Sun C, Wang X, Guo Y, Qiu S, et al. A systematic review of asymptomatic infections with COVID-19. J Microbiol Immunol Infect. 2020 May 15 [Epub ahead of print]. https://doi.org/10.1016/j.jmii.2020.05.001
12. Anand S, Montez-Rath M, Han J, Bozeman J, Kerschmann R, Beyer P, et al. Prevalence of SARS-CoV-2 antibodies in a large nationwide sample of patients on dialysis in the USA: a cross-sectional study. Lancet. 2020;396:1335–44. https://doi.org/10.1016/S0140-6736(20)32009-2
13. Martinez DA, Hinson JS, Klein EY, Irvin NA, Saheed M, Page KR, et al. SARS-CoV-2 positivity rate for Latinos in the Baltimore-Washington, DC region. JAMA. 2020;324:392–5. https://doi.org/10.1001/jama.2020.11374
14. Williams JC, Anderson N, Holloway T, Samford E III, Eugene J, Isom J. Reopening the United States: Black and Hispanic workers are essential and expendable again. Am J Public Health. 2020;110:1506–8. https://doi.org/10.2105/AJPH.2020.305879

Address for correspondence: Andrew G. Letizia, Naval Medical Research Center, 503 Robert Grant Ave, Silver Spring, MD 20910, USA; email: Andrew.G.Letizia.mil@mail.mil; Irene Ramos, Icahn School of Medicine at Mount Sinai, Annenberg 14-83, 1 Gustave L. Levy Pl, New York, NY 10029, USA; email: irene.ramos-lopez@mssm.edu

Article DOI: https://doi.org/10.3201/eid2704.204732

SARS-CoV-2 Seropositivity among US Marine Recruits Attending Basic Training, United States, Spring–Fall 2020

Appendix

Methods

Study Design and Participants

After recruits chose to join the United States Marine Corps, they attended basic training at 1 of 2 locations: Marine Corps Recruit Depot San Diego in California or Marine Corps Recruit Depot Parris Island (MCRDPI) in South Carolina. The location a recruit attended was primarily determined by the geography of the recruit's state of residence; states East of the Mississippi River, in general, go to MCRDPI and those to the West attend San Diego. Exceptions were made for administrative reasons with regard to recruit training class size. However, all female recruits attended MCRDPI. These procedures help to explain the large proportion of study participants from the Eastern United States and the high prevalence of women from Western states that have larger populations.

Once a recruit was assigned a training date and location, they were instructed to quarantine at home for 14 days. A recruiter, wearing a mask and maintaining maximum possible distance, would transport the recruit, who was also masked, in a vehicle to a local Military Entrance Processing Station where a provider performed a history and physical examination on the recruit. If deemed physically and mentally fit for Marine Corps enlistment, the recruit traveled by bus or plane to the quarantine campus or hotel. Recruits were instructed to wear masks at all times and maintain social distancing of $\geq$6 feet and avoid interactions with others while traveling. Once a recruit arrived at the local airport or bus station, they were picked up by van or bus and transported to the supervised quarantine location, where they observed the same COVID-19 mitigation strategies for an additional 14 days. The quarantine settings were selected for the specific purpose of strictly enforced public health measures implemented for the entire 2

weeks. The recruits and staff were forbidden to leave and no visitors, other than persons delivering supplies and food, local essential workers, and study staff, were allowed onto the premises. All of these measures were enforced by Marines at all times. Specific public health measures have been previously described (*1*).

Within 48 hours of arriving at the quarantine location, ≈350–500 recruits per week were offered the opportunity to volunteer for the COVID-19 Health Action Response for Marines (CHARM) Study, which included collecting baseline SARS-CoV-2 serologic test results. Recruits were eligible if they were ≥18 years and could complete follow-up encounters. Recruits 17 years of age were ineligible. Study enrollment occurred after recruits had been in-processed and had personal effects (including cell phones) secured, rooms assigned, and gear issued. The recruits attended a group consent brief of 50–100 participants using an ombudsman who explained the study, exactly what was being asked of participants, risks, benefits, and the state of COVID-19 in the recruit setting. Since recruits are a vulnerable population and at risk for coercion, special measures were undertaken including study briefers, who are active duty Navy personnel, wearing civilian clothes, not disclosing military ranks, not having members in the recruit's chain of command present, and ensuring that participation would not affect a recruit's medical care or influence the grading of a recruit's military performance.

Institutional Review Board approval was obtained from the Naval Medical Research Center (protocol no. NMRC.2020.0006) in compliance with all applicable federal regulations governing the protection of human subjects. All participants provided written informed consent for study participation.

Procedures

Recruits consented to undergo a mid-turbinate nares swab for SARS-CoV-2 qPCR testing and blood draw upon enrollment that included serum. We collected paper questionnaires (Appendix Figure) to identify demographics, risk factors, and symptoms, and assayed serum for the presence of SARS-CoV-2 IgG upon arrival at the quarantine location. Data was first recorded in Microsoft Excel spreadsheets before automated integration with the statistical programming language R 3.6.3 (*2*). The data collected included sex, age, ethnicity, race, place of birth, state or country of residence, medical history including smoking or vaping or exposure to secondhand smoke, and risk factors including use of masks, practicing self-quarantine before arrival, recent

travel, known exposure to persons with COVID-19, and exposure to someone with flu-like or other respiratory illness.

Laboratory Methodology

Presence of SARS-CoV2 IgG in serum was evaluated using ELISA with some modifications from Amanat et al. (*3*), as previously described (*1*). Briefly, 384-well Immulon 4 HBX (Thermofisher, https://www.thermofisher.com) plates were coated overnight at 4°C with recombinant His-tagged Spike (S) receptor-binding domain (RBD) (SinoBiological, https://www.sinobiological.com) at a concentration of 2 µg/ml in phosphate-buffered saline (PBS). Plates were washed 3 times with 0.1% Tween-20 (Fisher Scientific) PBS (PBS-T) using an automated ELISA plate washer (Aquamax 4000, Molecular devices), and blocked for 1 h at room temperature (RT) with 3% milk PBS-T. Blocking solution was removed, and serum samples diluted in 1% milk PBS-T were dispensed in the wells. At least 2 positive controls (serum samples with known SARS-CoV-2 IgG presence), 8 negative controls (serum samples collected before July 2019) and 4 blanks (no serum) were included in every plate. Plates were incubated for 2 h at room temperature and washed 3 times with PBT-T. Next, peroxidase conjugated goat F(ab')2 Anti-Human IgG (abcam) were added at a dilution 1:5,000–1:10,000 dilutions (determined after optimization for each antibody lot) in 1% milk PBS-T, and plates were incubated for 1 h at RT. Plates were washed 6 times with PBS-T, developed by using SIGMAFAST OPD (Sigma-Aldrich, https://www.sigmaaldrich.com), and the reaction was stopped after 10 min with 3M HCl. Optical density (OD) at 492 nm was measured by using a Spectramax M2 microplate reader (Molecular Devices, https://www.moleculardevices.com). All serum samples were screened at a 1:50 dilution. Those samples with an OD 492 nm value higher than the average of the negative controls plus 3 times their SD in the screening underwent titration assay (6 serial 1:3 serum dilutions starting at 1:50). Serum samples were considered positive when at least 2 consecutive dilutions showed higher OD 492 nm than the average of the negative controls plus 3 times their SD at the correspondent dilution or 0.15 OD 492 nM.

Statistical Analyses

Analyses, figures, and tables were generated by using R 3.6.3 (*2*). Associations between demographics, risk factors, and IgG-positivity variables were analyzed with logistic regression to compute the p value and the odds ratio. None of the risk factor data (Appendix Figure) was statistically significant and is not displayed. Significance was a priori established at <0.05.

The logistic regression is analyzed with 2 approaches: a) single variable approach: $\log\frac{p}{1-p} = \beta_0 + \beta x$ and b) multivariate approach: $\log\frac{p}{1-p} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_p x_p$. Note that when variable x_i is a categorical variable with L_i possible values coded from 1 to L_i and the code 1 is for the reference group, $\beta_i x_i$ should be understood as $\sum_{j=2}^{L_i} I(x_i = j)\beta_{i,j}$.

The collinearity for the variables in the multivariable logistic regression was assessed by using GVIF (generalized variance-inflation factors) (*4*). $GVIF^{\frac{1}{2*df}}$ (df is the degree of freedom of the variable) is computed for all variables in this paper. All variables were less than 1.06, indicating collinearity did not impact the analysis or violate assumptions. The collinearity is also assessed by the conditioner number which is ≈12, less than the 30, also indicating weak collinearity.

The trend test for the weekly IgG-positive rate of participants of Hispanic ethnicity is based on the Cochran-Armitage test. Because of the relatively small number of participants in the first study week (May 11), the participants' weekly IgG-positive rates have been smoothed with a 3-week running mean.

Race and ethnicity were categorized as non-Hispanic White, non-Hispanic Black, non-Hispanic Other, and Hispanic. A total of 18/3,196 (0.6%) participants did not supply any information on race or ethnicity and were grouped into the non-Hispanic Other category.

The 2020 US census data was downloaded from https://www.census.gov/data/tables/2020/demo/popest/2020-demographic-analysis-tables.html on December 20, 2020. The data contain information regarding the percentage of the US population that identifies as Black or Hispanic for each age year, but subcategories of race for the non-Hispanic population are still unavailable. For this reason, we compared data for the Black category, which was available in the census data, with data for non-Hispanic Black participants within our study. Specifically, we focused on the percentage of Black and Hispanic persons 18–20 years of age in the general population compared with our non-Hispanic Black and Hispanic study participants within the same age range.

Daily COVID-19 cases confirmed by viral tests during January 22–September 7 were downloaded from the COVID-19 Data Repository of the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (*5*). The heatmap in Figure 2, panel A

(https://wwwnc.cdc.gov/EID/article/27/4/20-4732-F2) represents the cumulative confirmed COVID-19 cases per 1 million of the state population. In the heatmap, states are clustered by temporary profiles of cumulative confirmed COVID-19 cases, as indicated by the dendrogram, which separates the states into 3 major groups. The groups were Early Spring, for states in which the first outbreak began in March; Late Spring, for states in which the outbreak began in early June; and Summer, for states in which the outbreak began in late June–July. The overall profile of the whole country (labeled US on 1 row) is in the Late Spring group and is placed in a black box. We used the aggregated data of each state group to compute the cumulated rate (dotted lines in Figure 2, panel B, right axis); the first outbreak is identified by the first local maximum slope. Our study had 701 (21.9%) participants from Early Spring states, 1,389 (43.5%) from Late Spring states, and 994 (31.1%) from Summer states. A total of 112 (3.5%) participants were not included in the analysis since they resided in a foreign country or did not provide a residence.

Specificity and Sensitivity of SARS-CoV-2 S-RBD IgG Serologic Test

To determine the specificity of the S-RBD IgG ELISA assay, we used 70 commercial serum samples drawn before July 2019 (44 purchased from BioChemed Services and 26 provided by Dr. Russell Tracy, Larner College of Medicine, University of Vermont, Burlington, Vermont, USA). To determine the sensitivity of the assay, we used 51 serum samples from subjects that had been previously confirmed as SARS-CoV-2–positive by PCR ≥14 days before serum sample collection (all of them were <90 days from PCR-positive test). All samples were screened at a 1:50 dilution, and those identified as positive were titered using 6 serial 1:3 serum dilutions (starting at 1:50). Those with at least 2 positive consecutive dilutions in the titration step (titer of 1:150) were considered seropositive. This assay was shown to have a 97.14% specificity (95% CI 93.24–100.00) and 96.08 sensitivity (95% CI 90.75–100.00).

References

<jrn>1. Letizia AG, Ramos I, Obla A, Goforth C, Weir DL, Ge Y, et al. SARS-CoV-2 Transmission among Marine recruits during quarantine. N Engl J Med. 2020;383:2407–16. https://doi.org/10.1056/NEJMoa2029717 PubMed</jrn>

<eref>2. R Project for Statistical Computing. R: A language and environment for statistical computing. 2020 [cited 2020 Feb 29]. http://www.R-project.org/</eref>

<jrn>3. Amanat F, Stadlbauer D, Strohmeier S, Nguyen THO, Chromikova V, McMahon M, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. Nat Med. 2020;26:1033–6. https://doi.org/10.1038/s41591-020-0913-5 PubMed</jrn>

<jrn>4. Fox J, Monette G. Generalized collinearity diagnostics. J Am Stat Assoc. 1992;87:178–83. https://doi.org/10.1080/01621459.1992.10475190</jrn>

<jrn>5. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis. 2020;20:533–4. https://doi.org/10.1016/S1473-3099(20)30120-1 PubMed</jrn>

Initial Questionnaire Version 1 14APRIL2020
COVID-19 Health Action Response for Marines (CHARM) Study

Completed by Study Personnel
Participant ID: PI-_____-_____ Visit Number _____
Sample ID (PI-XXXX): PI-_____ Samples Collected: ☐ Sputum ☐ Nares Swab ☐ Blood
(PI=Parris Island; Initials=First Middle Last; XXXX= sample number)

Date: ____/____/_____ Name: _____ SSN Last 4:_____
Last, First, Middle Initial

Phone Number:_____ Email:_____

Drill Instructor Name:_____ Company/Platoon:_____/_____

Demographics

Age:_____(years) Sex: ☐ Male ☐ Female Ethnicity: ☐ Hispanic/Latino ☐ Non-Hispanic/Latino ☐ Not specified

Race: ☐ Asian ☐ American Indian/Alaska Native
☐ Black ☐ Native Hawaiian/Other Pacific Islander
☐ White ☐ Other, specify:_____

Birthplace:_____ Region of primary residence:_____

Have you ever resided outside of the US for greater than 1 month? ☐ Yes ☐ No
If yes, specify:_____

Marine Corps Recruit Depot, Parris Island (PI) Information

Date arrived:___/___/_____ Recruit class:_____

Have you been exposed to anyone with flu-like illness since arriving? ☐ Yes ☐ No ☐ Unk
If yes, specifiy when and where:_____

List all locations you visited 14 days prior to arrival:_____
_____

Did you practice self-quarantine or isolation at home prior to arrival? ☐ Yes ☐ No

Barracks location: _____

How far away do you sleep from someone else? ☐ <6 feet ☐ >6 feet ☐ No one else in the room

What type of personal protection are you using? (check all that apply)
☐ None ☐ Surgical mask ☐ Cloth mask ☐ Other, specify:_____

Pre-existing medical conditions?

History of asthma	☐ Yes	☐ No	
Current smoker (including vaping)	☐ Yes	☐ No	If yes, for how many years: _____
Former smoker (including vaping)	☐ Yes	☐ No	If yes, date quit:___/___/_____
Family smoking history/second-hand exposure	☐ Yes	☐ No	If yes, specify:_____

Other, specify: _____

NAVY MEDICINE HRPP
HRPP#: NMRC . 2020 . 0006
Approval Date: 03 May 2020
Expiration Date: 30 April 2021
Verified By: TRG

Initial Questionnaire Version 1 14APRIL2020
COVID-19 Health Action Response for Marines (CHARM) Study

Within the last 14 days have you had any of the following exposures (check all that apply):

- ☐ Travel to mainland China or other non-US country Specify:_____
- ☐ Known contact with a lab-confirmed COVID-19 case-patient?
- ☐ Hospital, clinic, or other medical facility
- ☐ Other, specify:_____
- ☐ Exposure to anyone with severe acute lower respiratory distress (difficulty breathing)?
- ☐ Exposure to anyone with flu-like symptoms?
- ☐ Contact with animals. Specify: _____
- ☐ Unknown

Have you experienced any of the following flu-like symptoms within the last 14 days?			
Fever >100.4F (38C)	☐ Yes	☐ No	☐ Unk
Subjective fever (felt feverish)	☐ Yes	☐ No	☐ Unk
Chills	☐ Yes	☐ No	☐ Unk
Muscle aches	☐ Yes	☐ No	☐ Unk
Fatigue	☐ Yes	☐ No	☐ Unk
Runny nose	☐ Yes	☐ No	☐ Unk
Sore throat	☐ Yes	☐ No	☐ Unk
Cough (new onset or worsening of chronic cough)	☐ Yes	☐ No	☐ Unk
Shortness of breath	☐ Yes	☐ No	☐ Unk
Nausea or vomiting	☐ Yes	☐ No	☐ Unk
Headache	☐ Yes	☐ No	☐ Unk
Decreased ability to taste or smell	☐ Yes	☐ No	☐ Unk
Abdominal pain	☐ Yes	☐ No	☐ Unk
Diarrhea (≥3 loose/looser than normal stools/24hr period)	☐ Yes	☐ No	☐ Unk

Other, specify: _____

Page 2

NAVY MEDICINE HRPP
HRPP#: NMRC . 2020 . 0006
Approval Date: 03 May 2020
Expiration Date: 30 April 2021
Verified By: TRE

Adapted from CDC PUI Screening Tool

Page 1

Appendix Figure. Questionnaire administered to participants in COVID-19 Health Action Response for Marines study, May 11–September 7, 2020.