




Neutralizing Antibodies Correlate with Protection from SARS-CoV-2 in Humans during a Fishery Vessel Outbreak with a High Attack Rate

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ABSTRACT The development of vaccines against SARS-CoV-2 would be greatly facilitated by the identification of immunological correlates of protection in humans. However, to date, studies on protective immunity have been performed only in animal models and correlates of protection have not been established in humans. Here, we describe an outbreak of SARS-CoV-2 on a fishing vessel associated with a high attack rate. Predeparture serological and viral reverse transcription-PCR (RT-PCR) testing along with repeat testing after return to shore was available for 120 of the 122 persons on board over a median follow-up of 32.5 days (range, 18.8 to 50.5 days). A total of 104 individuals had an RT-PCR-positive viral test with a cycle threshold (C_T) of <35 or seroconverted during the follow-up period, yielding an attack rate on board of 85.2% (104/122 individuals). Metagenomic sequencing of 39 viral genomes suggested that the outbreak originated largely from a single viral clade. Only three crew members tested seropositive prior to the boat's departure in initial serological screening and also had neutralizing and spike-reactive antibodies in follow-up assays. None of the crew members with neutralizing antibody titers showed evidence of bona fide viral infection or experienced any symptoms during the viral outbreak. Therefore, the presence of neutralizing antibodies from prior infection was significantly associated with protection against reinfection (Fisher's exact test, $P = 0.002$).

KEYWORDS COVID-19, SARS-CoV-2, attack rate, boat, correlates, fishing, neutralizing antibodies, protection, vessel

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused tens of millions of infections and hundreds of thousands of deaths worldwide since its emergence in December 2019. Multiple vaccine candidates are currently in phase III trials (1–3). The success of these vaccines could be helped by further insights into the protective nature of neutralizing antibodies in humans.

Neutralizing antibodies have been isolated from individuals previously infected with SARS-CoV-2 (4, 5). These antibodies often target the receptor binding domain (RBD) of the SARS-CoV-2 spike (S) protein and prevent the binding interaction between the spike protein and the host's angiotensin-converting enzyme 2 (ACE2) (4, 5), although neutralizing antibodies that do not inhibit spike's binding to ACE2 have also been identified (6, 7). In animal models, neutralizing antibodies are protective against SARS-CoV-2, although the durability of this protection is unknown (8, 9).

Citation Addetia A, Crawford KHD, Dingens A, Zhu H, Roychoudhury P, Huang M-L, Jerome KR, Bloom JD, Greninger AL. 2020. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with a high attack rate. *J Clin Microbiol* 58:e02107-20. <https://doi.org/10.1128/JCM.02107-20>.

Editor Alexander J. McAdam, Boston Children's Hospital

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Received 12 August 2020

Returned for modification 18 August 2020

Accepted 20 August 2020

Accepted manuscript posted online 21 August 2020

Published 21 October 2020

Vaccines currently in development against SARS-CoV-2 have been shown to elicit levels of neutralizing antibodies comparable to those observed in naturally infected persons (1–3). However, the protective nature of both vaccine- and infection-elicited neutralizing antibodies in humans remains unproven, with animal models being used to make inferences about protection (10, 11). Human challenge trials, which could provide rapid information about the protection conferred by neutralizing antibodies (12, 13), are controversial due to the severity and unknown long-term impacts of SARS-CoV-2 infection and concerns over ethical administration of such trials (14, 15).

Given the high number of people exposed to SARS-CoV-2 every day, retrospective analyses of outbreak events may provide insights into the protective nature of neutralizing antibodies. In particular, outbreaks on confined shipping vessels are particularly useful candidates for assessing protection from SARS-CoV-2 infection (16–18). The high population density and large degree of contact between people on ships contribute to a high attack rate. In some cases, nearly all passengers will have been exposed (16).

Here, we performed a retrospective analysis of a SARS-CoV-2 outbreak on a fishing vessel that departed from Seattle, Washington, in May 2020. Predeparture viral and serological testing was performed on the near entirety of the ship's crew, allowing for testing of how preexisting immunity correlated with subsequent infection during the outbreak.

MATERIALS AND METHODS

Clinical diagnostic testing. Nasopharyngeal swabs were collected from patients in 3 ml of viral transport medium. Reverse transcription-PCR (RT-PCR) testing was performed using either the Hologic Panther Fusion system, the Roche cobas 6800 system, or the University of Washington CDC-based, emergency use-authorized laboratory-developed test (19). Clinical testing of serum samples was performed using the Abbott Architect SARS-CoV-2 IgG assay (20). Index values associated with the Abbott test are chemiluminescent signal values relative to a calibrator control and are broadly similar to optical density (OD) values for an ELISA. An index value of ≥ 1.40 is qualitatively reported as positive. The case definition for an individual infected on the boat included anyone with a positive RT-PCR with a threshold cycle (C_T) value of < 35 or seroconversion by the Abbott test during the follow-up period. Deidentified clinical testing data are available in Table S1 in the supplemental material. This study was approved by the University of Washington Institutional Review Board.

SARS-CoV-2 whole-genome sequencing. RNA was extracted from positive SARS-CoV-2 samples using the Roche MagNa Pure 96 (21). Metagenomic sequencing libraries were constructed as previously described (22). Briefly, RNA was DNase treated using the Turbo DNA-Free kit (Thermo Fisher). First-strand cDNA was synthesized using Superscript IV (Thermo Fisher) and 2.5 μ M random hexamers (IDT), and second-strand synthesis was performed with Sequenase version 2.0 DNA polymerase (Thermo Fisher). The resulting double-stranded cDNA was purified using 1.6 volumes of AMPure XP beads (Beckman Coulter). Libraries were constructed using the Nextera DNA Flex preenrichment kit (Illumina) and cleaned using 0.7 volumes of AMPure XP beads. The resulting libraries were sequenced on a 1 \times 75 bp Illumina NextSeq run. A median of 509,551 sequencing reads was obtained for each sample.

Consensus genomes were called using a custom SARS-CoV-2 genome calling pipeline (<https://github.com/proychou/hCoV19>). Briefly, sequencing reads were adapter and quality trimmed with BBDuk and mapped to the SARS-CoV-2 reference genome (GenBank accession no. [NC_045512.2](https://www.ncbi.nlm.nih.gov/nuccore/NC_045512.2)) using Bowtie 2 (23). Reads aligning to the SARS-CoV-2 reference genome were filtered using BBDuk and assembled with SPAdes (24). The *de novo* assembled contigs and mapped read assemblies were merged to produce a consensus genome. For samples that did not produce a genome through the automated pipeline, the mapped read assemblies were visualized in Geneious and a consensus genome was called manually.

A phylogenetic analysis was completed using the 39 consensus genomes obtained through metagenomic sequencing and 109 other SARS-CoV-2 isolates downloaded from GISAID (<https://www.gisaid.org/>; accessed 17 July 2020) reflective of the global genomic diversity of SARS-CoV-2. To select 109 SARS-CoV-2 isolates, all global SARS-CoV-2 sequences were downloaded from GISAID. Those composed of $> 5\%$ N's, those with disrupted reading frames, and those with partial genomes were discarded. The strains were then stratified by Pangolin lineage (A or B) (<https://github.com/cov-lineages/pangolin>), and 49 from lineage A and 59 from lineage B were randomly selected along with the Wuhan-Hu-1 reference genome (GenBank accession no. [NC_045512.2](https://www.ncbi.nlm.nih.gov/nuccore/NC_045512.2)) (25). Sequences were aligned with MAFFT v7.453 (26), and a phylogenetic tree was constructed using FastTree (version 2.1.1) (27) with the 5' and 3' untranslated regions (UTRs) masked. The resulting phylogenetic tree was visualized in R (version 3.6.1) using the ggtree package (28). Strains most closely related to the major outbreak clade were identified by searching against a custom BLASTN database containing all SARS-CoV-2 sequences in GISAID (accessed 3 August 2020).

Neutralization assays and anti-spike antibody testing. The presence of anti-spike and neutralizing antibodies was analyzed in predeparture serum samples from individuals that were positive in the

Abbott assay screening by four different methods: spike IgG enzyme-linked immunosorbent assay (ELISA), RBD ELISA, ACE2 blockade of binding ELISA, and pseudovirus neutralization.

RBD and spike protein for the ELISAs were produced as described previously (29). IgG ELISAs for spike and RBD were adapted from a published protocol (30, 31), with details described previously (32). Spike or RBD was diluted to 2 $\mu\text{g}/\text{ml}$ in phosphate-buffered saline (PBS), and 50 $\mu\text{l}/\text{well}$ was used to coat 96-well Immulon 2HB plates (Thermo Fisher; catalog no. 3455) at 4°C overnight. The plates were washed three times the next day with PBS containing 0.1% Tween 20 (PBS-T) using a Tecan HydroFlex plate washer. The plates were blocked for 1 h with 200 $\mu\text{l}/\text{well}$ of 3% nonfat dry milk in PBS-T at room temperature. Sera were diluted 4-fold in PBS-T containing 1% nonfat dry milk, starting at a 1:25 dilution. Pooled sera collected from 2017 to 2018 from 75 individuals (Gemini Biosciences, item 100-110, lot H86W03J) and CR3022 antibody (starting at 1/ $\mu\text{g}/\text{ml}$, also diluted 4-fold) were included as negative and positive controls, respectively. After the blocking buffer was removed from the plates, 100 μl of diluted sera was added to the plates and incubated at room temperature for 2 h. The plates were again washed three times, and then 50 μl of a 1:300 dilution of goat anti-human IgG-Fc horseradish peroxidase (HRP)-conjugated antibody (Bethyl Labs; catalog no. A80-104P) in PBS-T containing 1% milk was added to each well and incubated for 1 h at room temperature. The plates were again washed three times with PBS-T. One hundred microliters of TMB/E HRP substrate (Millipore Sigma; catalog no. ES001) was then added to each well, and after a 5-min incubation, 100 μl of 1 N HCl was added to stop the reaction. OD values at 450 nm (OD_{450}) were read immediately on a Tecan infinite M1000Pro plate reader. The area under the titration curve (AUC) was calculated with the dilutions on a log scale.

The ACE2 blockade of binding assay was performed using a SARS-CoV-2 surrogate virus neutralization test kit (GenScript). The assay was performed by following the manufacturer's recommendations with 10 μl serum diluted into 90 μl of dilution buffer and read using a D52 microplate reader (Dynex technologies).

Neutralization assays with spike pseudotyped lentiviral particles were performed as described previously (33), with a few modifications. Briefly, cells were seeded in black-walled, clear-bottom, poly-L-lysine-coated 96-well plates (Greiner; catalog no. 655936). About 14 h later, serum samples were diluted in D10 medium (Dulbecco modified Eagle medium [DMEM] with 10% heat-inactivated fetal bovine serum [FBS], 2 mM L-glutamine, 100 U/ml penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin) starting with a 1:20 dilution, followed by six serial 3-fold dilutions. An equal volume of full-length spike-pseudotyped lentiviral particles as diluted serum was added to the serum dilutions and incubated at 37°C for 1 h. One hundred microliters of the virus-plus-serum dilutions was then added to the cells ~16 h after the cells were seeded.

About 52 h postinfection, luciferase activity was measured as described previously (33) except that luciferase activity was read out directly in the assay plates without transferring them to black, opaque-bottom plates. Two "no serum" wells were included in each row of the neutralization plate, and fraction infectivity was calculated by dividing the luciferase readings from the wells with serum by the average of the "no serum" wells in the same row. After calculating the fraction infectivity, we used the neutcurve Python package (<https://jbloomlab.github.io/neutcurve/>) to calculate the serum dilution that inhibited infection by 50% (IC_{50}) and 90% (IC_{90}) by fitting a Hill curve with the bottom fixed at 0 and the top fixed at 1. All serum samples were measured in duplicate. To calibrate our neutralization assays, we also ran them on the NIBSC reference serum sample (product number 20/130) and measured an IC_{50} of 1:2,395. Sera with no neutralizing activity at the lowest titer tested (1:20) were reported as negative.

Data availability. Sequencing reads for the samples examined are available at NCBI BioProject under accession no. [PRJNA610428](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA610428), and sequences determined for isolates in this study have been deposited in the GISAID database under accession numbers EPI_ISL_461450 to EPI_ISL_461477, EPI_ISL_511852 to EPI_ISL_511861, and EPI_ISL_512086 (see Table S2 in the supplemental material).

RESULTS

Predeparture PCR and serology testing. There were a total of 122 people (113 men and 9 women) on the manifest of the ship. Prior to the ship's departure, crew members were screened for active SARS-CoV-2 infection by RT-PCR or for serological evidence of prior or ongoing infection by using the Abbott Architect assay, which detects antibodies against the viral nucleoprotein (N). Predeparture RT-PCR and serology test data were available for 120 crew members. This predeparture screening occurred on day 0 and day 1 prior to the ship's departure on day 2. In this predeparture screening, none of the crew members tested positive for virus by RT-PCR, and six individuals tested seropositive in the Abbott Architect assay (index value, ≥ 1.40) (Fig. 1A).

After becoming aware of the subsequent SARS-CoV-2 outbreak on the ship (see "Testing after ship returned due to outbreak"), we tested residual predeparture serum samples from the six individuals who were seropositive by the Abbott Architect assay to characterize the neutralizing and spike-binding activity of their sera. The sera of three of these six individuals had potent neutralizing activity against SARS-CoV-2 spike pseudotyped lentiviral particles (Table 1; Fig. 1B). The neutralizing titers (1:174, 1:161, and 1:3,082) are in the typical range of titers observed in humans who have been

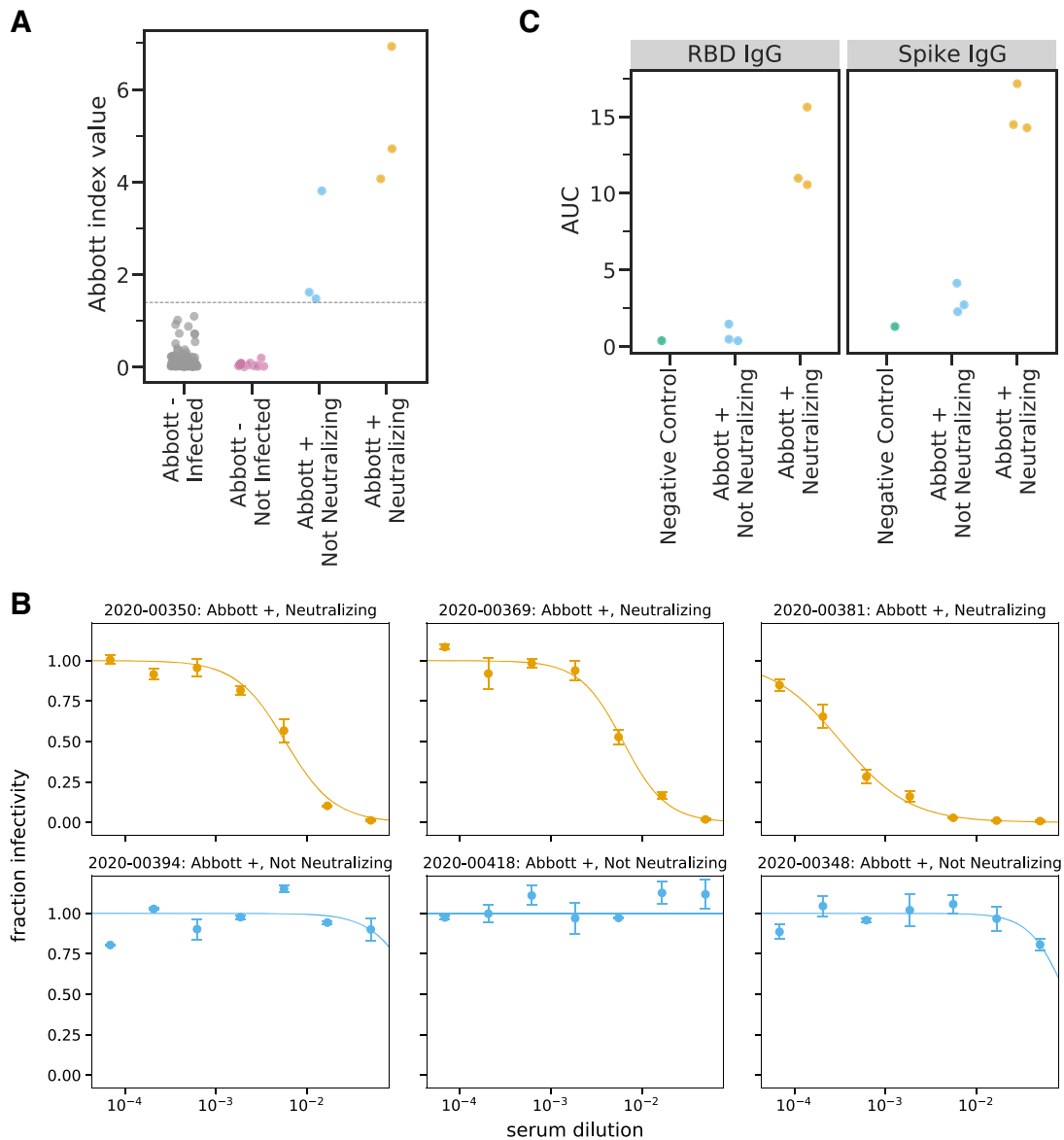


FIG 1 Predeparture serological assays. (A) Abbott Architect index values for all 120 individuals assayed. The gray line indicates the cutoff for a positive Abbott reading (≥ 1.40). Individuals with negative Abbott index values are further classified by whether they subsequently became infected on the ship. Individuals with positive Abbott index values are further characterized by whether their preboarding serum was neutralizing. (B) Neutralization curves for all six preboarding samples that were positive in the Abbott Architect assay. (C) Titers of RBD- or spike-binding IgG antibodies in all six Abbott assay-positive preboarding samples as measured by ELISA. The negative-control sample is pooled sera collected in 2017 to 2018 from 75 individuals (Gemini Biosciences, item 100-110, lot H86W03J).

infected with SARS-CoV-2 within the previous few months (29, 34, 35). The sera of the three individuals with neutralizing titers also had high activity in an assay that measures the ability of antibodies to block RBD binding to ACE2, as well as in IgG ELISAs against spike and RBD (Table 1; Fig. 1C). Notably, the sera of the other three individuals who were seropositive in the Abbott Architect assay but did not have neutralizing activity had lower index value readings in the Abbott assay (including two that were close to the cutoff of 1.40) (Fig. 1A) and readings comparable to those from negative controls in the RBD and spike ELISAs (Fig. 1C). Therefore, we speculate that the three individuals without neutralizing activity were false positives in the initial serological screening. However, they might have been in the early stages of active infection, since the Abbott Architect detects antibodies against N while all the other assays we used detect

TABLE 1 Laboratory values for crew members who were predeparture (day 0 to 1) seropositive by the Abbott SARS-CoV-2 IgG assay^a

Sample	RT-PCR result	Abbott IgG index	Neutralization IC ₅₀ ^b	Neutralization IC ₉₀	ACE2 binding blockade (%)	AUC		C _T		Day 28 PCR	Day 25-26 PCR	Day 18-21 PCR	Day 31-36 PCR	Day 31-35 Abbott IgG index	Day 31-35 ACE2 binding blockade (%)
						RBD IgG	Spike IgG	Day 18-21 PCR	Day 25-26 PCR						
						15.62	17.15	Negative	Negative						
2020-00350	Negative	6.93	1:174	1:44	89	15.62	17.15	Negative	Negative	ND	Negative	Negative	Negative	6.40	95
2020-00369	Negative	4.07	1:161	1:48	84	10.98	14.27	Negative	ND	ND	Negative	Negative	Negative	2.93	68
2020-00381	Negative	4.72	1:3,082	1:458	93	10.56	14.48	Negative	37.4	Negative	38.3	Negative	Negative	3.48	90
2020-00394	Negative	1.62	Negative	Negative	-4	1.46	4.13	22.91	ND	ND	27.9	ND	ND	4.29	30
2020-00418	Negative	3.81	Negative	Negative	3	0.47	2.27	22.84	ND	ND	30.4	ND	ND	6.31	93
2020-00348	Negative	1.48	Negative	Negative	0	0.37	2.72	17.57	ND	ND	Negative	Negative	Negative	5.98	35

^aND, not done.

^bThe lowest dilution tested was 1:20.

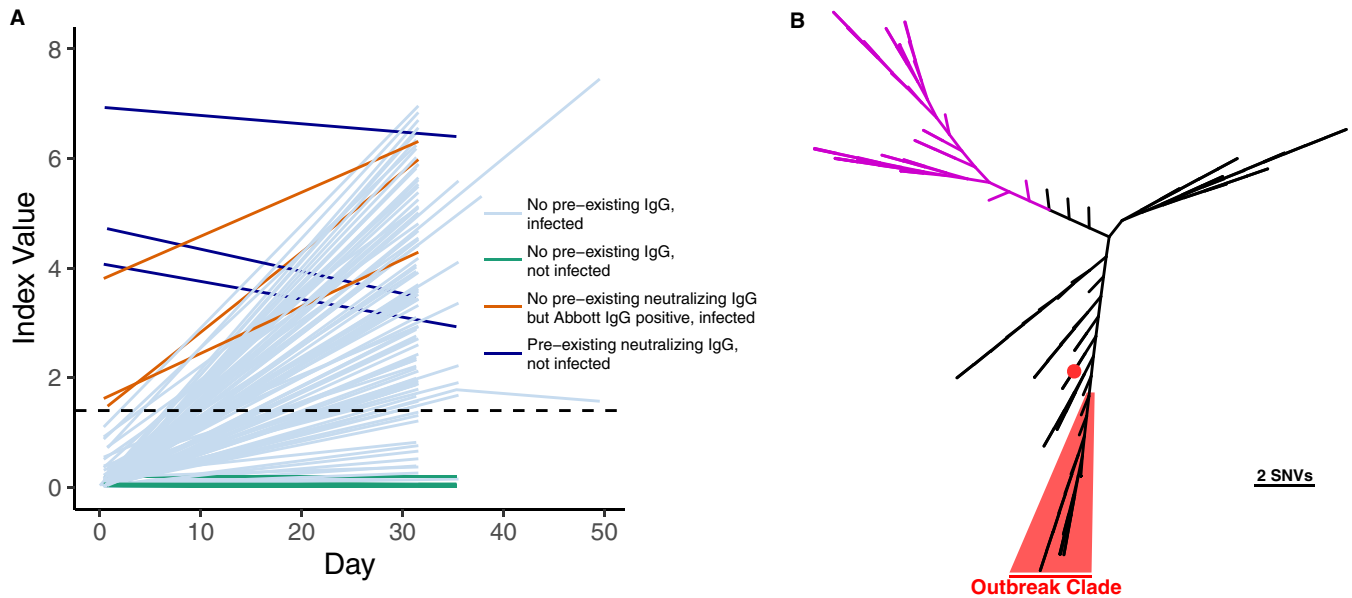


FIG 2 (A) Abbott Architect SARS-CoV-2 index values over time (pre- and postdeparture) are depicted for each individual with at least two serum draws. The dashed line denotes the seropositivity cutoff of the assay (1.40). Individuals who had a positive RT-PCR with a C_T of <35 or who seroconverted during the follow-up period are shown in light blue. Individuals who were not infected by the above case definition criteria are shown in green. Individuals who screened positive by the Abbott Architect SARS-CoV-2 IgG assay but lacked neutralizing antibodies and were infected are shown in brown. Individuals who had preexisting neutralizing antibodies and were not infected are shown in dark blue. (B) SARS-CoV-2 whole-genome sequencing of cases from the fishery vessel confirms an outbreak. SARS-CoV-2 genomes from 39 cases with C_T values of <26 were recovered, and a phylogenetic tree was made using FastTree along with 109 other isolates reflective of global diversity. Thirty-eight cases are highlighted in red with a median pairwise difference of 1 single nucleotide variation, while one outlier case from the boat is indicated by a red dot. Clade A strains associated with early trans-Pacific transmission are shown in purple.

antibodies against spike, and anti-N antibodies appear earlier after infection than anti-spike antibodies (36, 37). Alternatively, they might have experienced mild or asymptomatic infection, which can be associated with transient or low-level seroconversion (38, 39).

Overall, assuming that only individuals who were positive in the initial Abbott Architect assay have neutralizing anti-spike antibodies, then just three of the 120 individuals with predeparture screening data had neutralizing antibodies prior to boarding the ship. We consider this assumption to be well supported by several lines of evidence: large-scale studies have demonstrated that the Abbott Architect assay has close to 100% sensitivity by 2 weeks post-symptom onset (20); several studies (36, 37) have shown that SARS-CoV-2-infected patients usually mount strong and early antibody responses to the N antigen detected by the Abbott Architect assay; and a study (32) using the exact assays described here found that individuals with neutralizing titers to SARS-CoV-2 also had anti-N antibodies.

Testing after ship returned due to outbreak. On day 18, the ship returned to shore after a crew member became sick, tested positive for SARS-CoV-2, and required hospitalization. Testing data after return were available for all 122 crew members for RT-PCR and for 114 crew members for serology using the Abbott assay. RT-PCR and serological testing were performed until day 50, leading to a median follow-up of 32.5 days (range, 18.8 to 50.5 days).

Of the 118 individuals with RT-PCR results from the week of return, 98 tested positive with a C_T of <35 . Three additional crew members tested positive by RT-PCR with a C_T of <35 within the next 10 days. The median of the strongest/minimum C_T for each of these 101 crew members who tested positive with a C_T of <35 was 22.8 (interquartile range [IQR], 19.3 to 26.9). Serological responses among these individuals as measured by the Abbott SARS-CoV-2 IgG index value increased for the majority of these individuals (Fig. 2A).

Among the 21 crew members who never had a positive RT-PCR test with a C_T of <35 , three individuals seroconverted based on the Abbott Architect index value during

TABLE 2 Summary of infection status of crew members for which predeparture serology testing was performed

Status on boat	No. of crew members predeparture ^a		P value
	Neutralizing Ab(+)	Neutralizing Ab(-)	
Infected	0	103	0.0024
Not infected	3	14	

^aAb(+), antibody positive; Ab(-), antibody negative.

the follow-up period. Two of these three crew members had positive RT-PCR values with C_T values of >35 , while RT-PCR data were not available for the third crew member until day 49. These three individuals were considered infected on the vessel. In addition, 3 of the 21 crew members without a positive RT-PCR result with a C_T of <35 were not tested by serology after returning to shore, although two of the three crew members tested negative 3 and 4 times, respectively, by RT-PCR over 3 weeks after returning.

Confirmation of outbreak with whole-genome sequencing. Metagenomic recovery of 39 SARS-CoV-2 whole genomes from the outbreak indicated a major single outbreak clade (FastTree support value, 1.00) covering 38 isolates that differed by a median of 1 nucleotide across the genome (range, 0 to 5 nucleotides) (Fig. 2B). Sixteen of these isolates shared completely identical sequences. The closest SARS-CoV-2 whole-genome sequences in GISAID (3 August 2020) to the major outbreak clade were strains from Virginia (USA/VA-DCLS-0561/2020), New York City (USA/NY-NYUMC650, NYUMC624, NYNYUMC474, NYUMC426/2020), Minnesota (USA/MN-MDH-1288/2020), and Michigan (USA/MI-MDHHS-SC20223/2020) at 2 single nucleotide variations (SNVs) apart.

The three crew members with neutralizing antibodies were protected from infection. We can assess the effects of preexisting neutralizing antibodies on infection during the outbreak by using the predeparture serological screening (available for 120 of 122 individuals) and the subsequent testing of all 122 individuals for infection. None of the three individuals who had neutralizing antibodies prior to departure were infected during the subsequent outbreak based on our case definition of a positive RT-PCR test with a C_T of <35 or seroconversion, and none reported any symptoms upon return to shore. In contrast, among the other 117 of 120 individuals with predeparture serological data who were seronegative or lacked spike-reactive antibodies prior to departure, 103 of 117 were infected using the same case definition (of the two individuals without predeparture serological screening, one tested positive and one tested negative by RT-PCR on return). Therefore, the overall rate of infection was 0 of 3 among individuals with neutralizing antibodies and 103 of 117 among individuals without such antibodies. This difference is statistically significant (Table 2, Fisher's exact test, $P = 0.002$), indicating that preexisting neutralizing antibodies are significantly associated with protection against SARS-CoV-2 infection. The three crew members who were seropositive for anti-N antibodies by the Abbott assay but did not have neutralizing antibodies were all infected during follow-up, with minimum C_T s of 17.6, 22.8, and 22.9 and increases in Abbott index values (Table 1). Sex did not differ between uninfected and infected, with females composing 5.6% (1 of 18) and 7.7% (8 of 104) of these two groups, respectively (Fisher's exact test, $P = 1$).

We also looked in detail at the viral testing results of the three crew members who were positive for neutralizing antibodies to assess the strength of the evidence that they were not reinfected during this ship outbreak. Two tested fully negative by RT-PCR on three or more occasions, with negative tests on days 18, 25, 35, and 36 for one and on days 18, 35, and 36 for the other. The third individual tested negative on the Roche cobas system on day 21 and day 28 and positive only by the E-gene primer/probe set ($C_T = 37.4$) and negative by the orf1ab primer set on the Roche cobas system on day 25. This individual also tested positive ($C_T = 38.3$) on day 31 on the Hologic Panther Fusion system. By our case definition (which required a positive RT-PCR test with C_T of <35), these results are not consistent with being infected on the boat. The sporadic

high C_T results might be consistent with intermittent, low-level shedding associated with recent past infection, as low levels of SARS-CoV-2 have been detected in nasal passages for more than 80 days (40). Of note, only two other crew members had a minimum C_T of >35 in the postdeparture follow-up period, and both of these individuals were considered infected due to seroconversion during the follow-up period. In contrast, Abbott assay index values decreased for all three of the crew members with predeparture neutralizing antibodies during the follow-up period.

DISCUSSION

Here, we report an outbreak of SARS-CoV-2 on a fishing vessel with an attack rate greater than 85%. Screening with the Abbott Architect anti-nucleocapsid IgG antibody test, followed by confirmation of positives with multiple anti-spike protein antibody tests, including neutralization assays, demonstrated the protective nature of neutralizing antibodies. In particular, none of the three individuals with preexisting neutralizing antibodies were infected, whereas the vast majority of other individuals were infected. These findings are consistent with data from animal models, in which the elicitation of high titers of neutralizing antibodies was protective against rechallenge with SARS-CoV-2 (8, 10, 41). In addition, the high attack rate suggests that any preexisting cross-reactive immunity caused by prior infection with other seasonal coronaviruses (e.g., cross-reactive T-cells [42]) provides limited protection against SARS-CoV-2 infection.

An assumption of our analysis is that the only individuals who had preexisting neutralizing and anti-spike antibodies were those who tested seropositive in the initial predeparture Abbott Architect anti-N serological screening, since only individuals positive in that screening were subjected to additional serological assays for anti-spike and neutralizing antibodies. However, this assumption is well supported by the validated high sensitivity of the Abbott Architect assay (20), plus the well-established fact that anti-N antibodies appear earlier than anti-spike antibodies (36, 37). Additionally, our four anti-spike antibody tests showed a high level of consistency among seropositive samples, and prior work using the exact same assays has found neutralizing antibodies only among individuals who were positive by the Abbott Architect assay (32). As shown by others, the RBD ELISA and neutralizing antibody assays were highly consistent (43, 44). The ACE2 blockade of binding functional ELISA showed excellent consistency with the more laborious pseudovirus neutralizing antibody assay (45).

It is intriguing that one individual who had predeparture neutralizing antibodies and was classified as uninfected by our case definition nonetheless had a sporadic very weak signal in viral testing on two different RT-PCR platforms. It is well established that SARS-CoV-2 can be detected for multiple weeks in the nasopharyngeal tract, well after the resolution of symptoms and elicitation of an antiviral immune response (46, 47). However, it is unclear at this time whether immunity to SARS-CoV-2 will be sterilizing (10, 48), and it is possible that the sporadic weak signal in viral testing for this individual was the result of reexposure to virus on the boat.

In prior studies, the Abbott SARS-CoV-2 IgG assay has shown excellent performance characteristics with high specificity (99.1 to 99.9%) for prior infection with SARS-CoV-2 (20, 49, 50). Curiously, the positive predictive value for the Abbott SARS-CoV-2 IgG assay for neutralizing antibodies or protection in our population was only 50% (3/6 crew members). It is difficult to conclusively determine whether these represented false positives or just anti-N/anti-spike discrepant results, particularly given that anti-N antibodies tend to appear before anti-spike antibodies (36, 37). All three of the individuals who were Abbott IgG positive prior to departure but lacked neutralizing and anti-spike antibodies and were RT-PCR positive upon return showed strong increases in index value. In addition, two of these three individuals had predeparture Abbott index values that were close to the positivity cutoff. Unfortunately, we did not have sufficient residual predeparture serum to run on a separate anti-N platform such as the Roche Elecsys anti-SARS-CoV-2 test (51).

This study is limited by a lack of information of clinical symptoms for the majority

of crew members on the vessel and of direct knowledge of contacts on the boat. We cannot also necessarily know that the three individuals with neutralizing antibodies prior to departure were exposed directly to SARS-CoV-2 on the vessel. We were unable to test everyone on the vessel for neutralizing or anti-spike antibodies, since negative serology samples are not stored very long in our laboratory. In addition, our study shows only that neutralizing antibodies are a correlate of protection: we cannot be sure that protection comes from neutralizing antibodies *per se* rather than some other immune response with which they correlate, such as T cells. The study is also limited by the low seroprevalence in the predeparture cohort, which is consistent with the approximate seroprevalence in May 2020 in the Seattle area but means that there were only three individuals with preexisting neutralizing antibodies. Nonetheless, with an overall attack rate of >85%, the lack of infection in the three individuals with neutralizing antibodies was statistically significant in comparison to the rest of the boat's crew. Overall, our results provide the first direct evidence that anti-SARS-CoV-2 neutralizing antibodies are protective against SARS-CoV-2 infection in humans.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, XLS file, 0.2 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.01 MB.

ACKNOWLEDGMENTS

We thank Nicole Lieberman for helpful comments, Nathan Breit for data pulls, and Ann Jarris and Mark Wener for protocol development. We also thank Brooke Fiala, Samuel Wrenn, Deleah Pettie, and Neil P. King at the Institute for Protein Design for sharing reagents for ELISA assays.

Work performed in the clinical laboratory was supported by the Department of Laboratory Medicine and Pathology. This research was supported by the following grants from the NIAID of the NIH: R01AI141707 (to J.D.B.) and F30AI149928 (to K.H.D.C.). J.D.B. is an Investigator of the Howard Hughes Medical Institute.

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