

1 Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a
2 fishery vessel outbreak with high attack rate

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19 Running title: SARS-CoV-2 protection in humans

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22

23 **Abstract**

24 The development of vaccines against SARS-CoV-2 would be greatly facilitated by the
25 identification of immunological correlates of protection in humans. However, to date,
26 studies on protective immunity have only been performed in animal models and
27 correlates of protection have not been established in humans. Here, we describe an
28 outbreak of SARS-CoV-2 on a fishing vessel associated with a high attack rate.
29 Predeparture serological and viral RT-PCR testing along with repeat testing after return
30 to shore was available for 120 of the 122 persons on board over a median follow-up of
31 32.5 days (range 18.8 to 50.5 days). A total of 104 individuals had an RT-PCR positive
32 viral test with Ct <35 or seroconverted during the follow-up period, yielding an attack
33 rate on board of 85.2% (104/122 individuals). Metagenomic sequencing of 39 viral
34 genomes suggested the outbreak originated largely from a single viral clade. Only three
35 crewmembers tested seropositive prior to the boat's departure in initial serological
36 screening and also had neutralizing and spike-reactive antibodies in follow-up assays.
37 None of these crewmembers with neutralizing antibody titers showed evidence of bona
38 fide viral infection or experienced any symptoms during the viral outbreak. Therefore,
39 the presence of neutralizing antibodies from prior infection was significantly associated
40 with protection against re-infection (Fisher's exact test, $p=0.002$).
41

42 **Introduction**

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

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

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

63 Given the high number of people exposed to SARS-CoV-2 every day,
64 retrospective analyses of outbreak events may provide insights into the protective

65 nature of neutralizing antibodies. In particular, outbreaks on confined shipping vessels
66 are particularly useful candidates for assessing protection from SARS-CoV-2 infection
67 (16–18). The high population density and large degree of contact between people on
68 ships contributes to a high attack rate. In some cases nearly all passengers will have
69 been exposed (16).

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

75

76 **Methods**

77 *Clinical diagnostic testing*

78 Nasopharyngeal swabs were collected from patients in 3 mL of viral transport media.
79 RT-PCR testing was performed on either the Hologic Panther Fusion, Roche cobas
80 6800, or the University of Washington CDC-based, emergency use authorized
81 laboratory developed test (19). Clinical testing of serum samples was performed using
82 the Abbott Architect SARS-CoV-2 IgG assay (20). Index values associated with the
83 Abbott test are chemiluminescent signal values relative to a calibrator control, and are
84 broadly similar to O.D. values for an ELISA. An index value ≥ 1.40 is qualitatively
85 reported as positive. The case definition for an individual infected on the boat included
86 anyone with a positive RT-PCR with Ct < 35 or seroconversion by the Abbott test during

87 the follow-up period. This study was approved by the University of Washington
88 Institutional Review Board.

89

90 *SARS-CoV-2 whole genome sequencing*

91 RNA was extracted from positive SARS-CoV-2 samples using the Roche MagNA Pure
92 96 (21). Metagenomic sequencing libraries were constructed as previously described
93 (22). Briefly, RNA was DNase-treated using the Turbo DNA-Free Kit (Thermo Fisher).
94 First strand cDNA was synthesized using Superscript IV (Thermo Fisher) and 2.5µM
95 random hexamers (IDT) and second strand synthesis was performed with Sequenase
96 Version 2.0 DNA Polymerase (Thermo Fisher). The resulting double-stranded cDNA
97 was purified using 1.6X volumes of AMPure XP beads (Beckman Coulter). Libraries
98 were constructed using the Nextera DNA Flex Pre-Enrichment kit (Illumina) and cleaned
99 using 0.7X volumes of AMPure XP beads. The resulting libraries were sequenced on a
100 1x75 bp Illumina NextSeq run. A median of 509,551 sequencing reads were obtained
101 for each sample. Sequencing reads are available at NCBI BioProject PRJNA610428
102 and sequence accessions are available in Supplemental Table 1.

103 Consensus genomes were called using a custom SARS-CoV-2 genome calling
104 pipeline (<https://github.com/proychou/hCoV19>). Briefly, sequencing reads were adapter-
105 and quality-trimmed with BBDuk and mapped to the SARS-CoV-2 reference genome
106 (NC_045512.2) using Bowtie 2 (23). Reads aligning to the SARS-CoV-2 reference
107 genome were filtered using BBDuk and assembled with SPAdes (24). The *de novo*
108 assembled contigs and mapped read assemblies were merged to produce a consensus
109 genome. For samples that did not produce a genome through the automated pipeline,

110 the mapped read assemblies were visualized in Geneious and a consensus genome
111 was called manually.

112 A phylogenetic analysis was completed using the 39 consensus genomes
113 obtained through metagenomic sequencing and 109 other SARS-CoV-2 isolates
114 downloaded from <https://www.gisaid.org/> (accessed July 17, 2020) reflective of the
115 global genomic diversity of SARS-CoV-2. To select 109 SARS-CoV-2 isolates, all global
116 SARS-CoV-2 sequences were downloaded from GISAID. Those composed of >5% Ns,
117 those with disrupted reading frames, and those with partial genomes were discarded.
118 The strains were then stratified by Pangolin lineage (A or B) ([https://github.com/cov-](https://github.com/cov-lineages/pangolin)
119 [lineages/pangolin](https://github.com/cov-lineages/pangolin)) and 49 from lineage A and 59 from lineage B were randomly
120 selected along with the Wuhan-Hu-1 reference genome (NC_045512.2) (25).
121 Sequences were aligned with MAFFT v7.453 (26) and a phylogenetic tree was
122 constructed using FastTree (version 2.1.1) (27) with the 5' and 3'UTRs masked. The
123 resulting phylogenetic tree was visualized in R (version 3.6.1) using the ggtree package
124 (28). Strains most closely related to the major outbreak clade were identified by
125 searching against a custom BLASTN database containing all SARS-CoV-2 sequences
126 in GISAID (accessed August 3, 2020).

127

128 *Neutralization Assays and Anti-Spike Antibody Testing*

129 The presence of anti-Spike and neutralizing antibodies was analyzed in pre-departure
130 sera samples from individuals that were positive in the Abbott assay screening through
131 four different methods: Spike IgG ELISA, RBD ELISA, ACE2 blockade of binding
132 ELISA, and pseudovirus neutralization.

133 RBD and spike protein for the ELISAs were produced as described previously
134 (29). IgG enzyme-linked immunosorbent assays (ELISAs) to spike and RBD were
135 adapted from published protocol (30, 31), with details described previously (32). Spike
136 or RBD was diluted to 2 µg/mL in PBS and 50 µL/well was used to coat 96 well
137 Immulon 2HB plates (Thermo Fisher; 3455) at 4°C overnight. Plates were washed
138 three times the next day with PBS containing 0.1% Tween 20 (PBS-T) using a Tecan
139 HydroFlex plate washer. Plates were blocked for 1 hour with 200 µL/well of 3% non-fat
140 dry milk in PBS-T at room temperature. Sera were diluted 4-fold in PBS-T containing
141 1% non-fat dry milk, starting at a 1:25 dilution. Pooled sera collected from 2017-2018
142 from 75 individuals (Gemini Biosciences, 100-110, lot H86W03J) and CR3022 antibody
143 (starting at 1/ug/mL, also diluted 4-fold) were included as negative and positive controls,
144 respectively. After block was thrown off plates, 100µL diluted sera was added to plates
145 and incubated at room temperature for 2 hours. Plates were again washed three times,
146 and then 50µL of a 1:300 dilution of goat anti-human IgG-Fc horseradish peroxidase
147 (HRP)-conjugated antibody (Bethyl Labs, A80-104P) in PBS-T containing 1% milk was
148 added to each well and incubated for 1 hour at room temperature. Plates were again
149 washed three times with PBS-T. 100µL of TMB/E HRP substrate (Millipore Sigma;
150 ES001) was then added to each well, and after a 5-minute incubation, 100 µL 1N HCl
151 was added to stop the reaction. OD450 values were read immediately on a Tecan
152 infinite M1000Pro plate reader. Area under the titration curve (AUC) was calculated with
153 the dilutions on a log-scale.

154 The ACE2 blockage of binding assay was performed using the SARS-CoV-2
155 Surrogate Virus Neutralization Test Kit (GenScript). The assay was performed following

156 the manufacturer's recommendations with 10 μ L serum diluted into 90 μ L dilution buffer
157 and read using the DS2 microplate reader (Dynex technologies).

158 Neutralization assays with spike-pseudotyped lentiviral particles were performed
159 as described previously (33), with a few modifications. Briefly, cells were seeded in
160 black-walled, clear bottom, poly-L-lysine coated 96-well plates (Greiner, 655936). About
161 14 hours later, serum samples were diluted in D10 media (DMEM with 10% heat-
162 inactivated FBS, 2 mM l-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin)
163 starting with a 1:20 dilution followed by 6 serial 3-fold dilutions. An equal volume of full-
164 length spike-pseudotyped lentiviral particles as diluted serum was added to the serum
165 dilutions and incubated at 37C for 1 hour. 100 μ L of the virus plus serum dilutions were
166 then added to the cells ~16 hours after the cells were seeded.

167 About 52 hours post-infection, luciferase activity was measured as described
168 previously (33) except luciferase activity was read out directly in the assay plates
169 without transferring to black, opaque bottom plates. Two "no serum" wells were included
170 in each row of the neutralization plate and fraction infectivity was calculated by dividing
171 the luciferase readings from the wells with serum by the average of the "no serum" wells
172 in the same row. After calculating the fraction infectivity, we used the `neutcurve`
173 Python package (<https://jbloomlab.github.io/neutcurve/>) to calculate the serum dilution
174 that inhibited infection by 50% (IC50) by fitting a Hill curve with the bottom fixed at 0 and
175 the top fixed at 1. All serum samples were measured in duplicate. To calibrate our
176 neutralization assays, we also ran them on the NIBSC reference serum sample (product
177 number 20/130) and measured an IC50 of 1:2395.

178

179 **Results**

180 *Predeparture PCR and serology testing*

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

190

Table 1. Laboratory values for crew members who were pre-departure seropositive by Abbott SARS-CoV-2

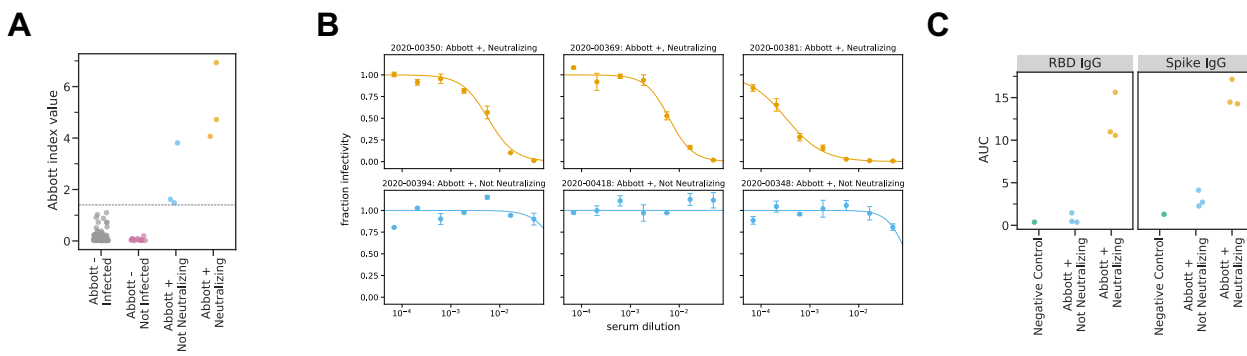
Sample	Day 0-1						Day 18-21 PCR (Ct)	Day 25-26 PCR	Day 28 PCR	Day 31-36 PCR	Day 31-35 Abbott IgG index	Day 31-35 ACE2 BoB
	RT-PCR	Abbott IgG index	Neutralization IC50	ACE2 BoB	RBD IgG AUC	spike IgG AUC						
2020-00350	negative	6.93	1:174	89%	15.62	17.15	negative	negative	n.d.	negative	6.40	95%
2020-00369	negative	4.07	1:161	84%	10.98	14.27	negative	n.d.	n.d.	negative	2.93	68%
2020-00381	negative	4.72	1:3082	93%	10.56	14.48	negative	37.4	negative	38.3	3.48	90%
2020-00394	negative	1.62	>1:20	-4%	1.46	4.13	22.91	n.d.	n.d.	27.9	4.29	30%
2020-00418	negative	3.81	>1:20	3%	0.47	2.27	22.84	n.d.	n.d.	30.4	6.31	93%
2020-00348	negative	1.48	>1:20	0%	0.37	2.72	17.57	n.d.	n.d.	negative	5.98	35%

n.d., not done; BoB, blockade of binding

191

192 After becoming aware of the subsequent SARS-CoV-2 outbreak on the ship (see
 193 next section), we tested residual predeparture serum samples from the six individuals
 194 who were seropositive in the Abbott Architect assay to characterize the neutralizing and
 195 spike-binding activity of their sera. The sera of three of these six individuals had potent
 196 neutralizing activity against SARS-CoV-2 spike pseudotyped lentiviral particles (Table 1,
 197 Figure 1B). The neutralizing titers (1:174, 1:161, 1:3082) are in the typical range of titers

198 observed in humans who have been infected with SARS-CoV-2 within the previous few
199 months (29, 34, 35). The sera of the three individuals with neutralizing titers also had
200 high activity in an assay that measure the ability of antibodies to block RBD binding to
201 ACE2, as well as in IgG ELISAs against spike and RBD (Table 1, Figure 1C).
202



203
204 **Figure 1** – Pre-departure serological assays. A) Abbott Architect index values for all 120 individuals
205 assayed. The grey line indicates the cutoff for a positive Abbott reading (≥ 1.40). Individuals with negative
206 Abbott index values are further classified by whether they subsequently became infected on the ship.
207 Individuals with positive Abbott index values are further characterized by whether their pre-boarding
208 serum was neutralizing. B) Neutralization curves for all 6 pre-boarding samples that were positive in the
209 Abbott Architect assay. C) Titers of RBD- or Spike-binding IgG antibodies in all 6 Abbott positive pre-
210 boarding samples as measured by ELISA. The negative control sample is pooled sera collected in 2017-
211 2018 from 75 individuals (Gemini Biosciences, 100-110, lot H86W03J).
212

213 Notably, the sera of the other three individuals who were seropositive in the
214 Abbott Architect assay but did not have neutralizing activity had lower quantitative
215 readings in the Abbott assay (including two that were close to the cutoff of 1.40; Figure
216 1A) and readings comparable to those from negative controls in the RBD and spike
217 ELISA assays (Figure 1C). Therefore, we speculate that the three individuals without
218 neutralizing activity were false positives in the initial serological screening. However,
219 they could have been in the early stages of active infection, since the Abbott Architect
220 detects antibodies against N while all the other assays we used detect antibodies
221 against spike, and anti-N antibodies appear earlier after infection than anti-spike

222 antibodies (36, 37). Alternatively, they could have experienced a mild or asymptomatic
223 infection, which can be associated with transient or low-level seroconversion (38, 39).

224

225 Overall, assuming that only individuals who were positive in the initial Abbott
226 Architect assay have neutralizing anti-spike antibodies, then just three of the 120
227 individuals with pre-departure screening data had neutralizing antibodies prior to
228 boarding the ship. We consider this assumption to be well supported by several lines of
229 evidence: large-scale studies have demonstrated that the Abbott Architect has close to
230 100% sensitivity by two weeks post-symptom onset (20); numerous studies (36, 37)
231 have shown that SARS-CoV-2 infected patients almost invariably mount strong and
232 early antibody responses to the N antigen detected by the Abbott Architect; and a study
233 (32) using the exact assays described here found that only individuals with anti-N
234 antibodies have neutralizing titers to SARS-CoV-2.

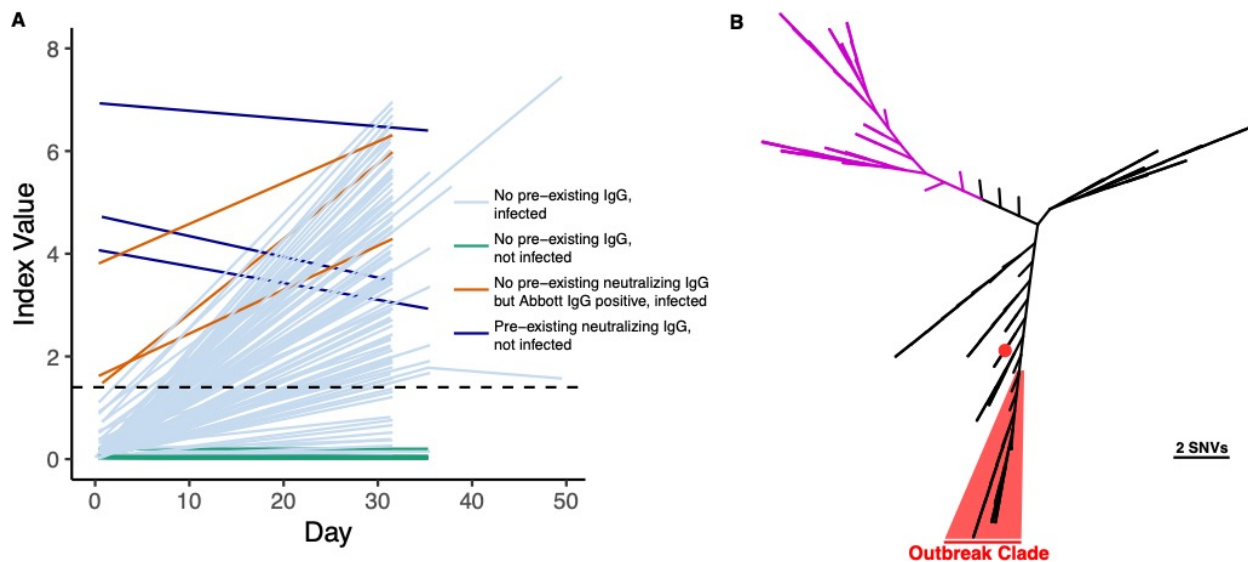
235

236 *Testing after ship returned due to outbreak*

237 On Day 18, the ship returned to shore after a crewmember became sick, tested positive
238 for SARS-CoV-2, and required hospitalization. Testing data after return was available
239 for all 122 crewmembers for RT-PCR and 114 crewmembers for serology using the
240 Abbott assay. RT-PCR and serological testing was performed until day 50, leading to a
241 median follow-up of 32.5 days (range 18.8 to 50.5 days).

242 Of the 118 individuals with RT-PCR results from the week of return, 98 tested
243 positive with a Ct < 35. Three additional crewmembers tested positive by RT-PCR with
244 a Ct < 35 within the next 10 days. The median of the strongest/minimum Ct for each of

245 these 101 crewmembers who tested positive with Ct < 35 was 22.8 (IQR 19.3 – 26.9).
246 Serological responses among these individuals as measured by Abbott SARS-CoV-2
247 IgG index value increased for the majority of these individuals (Figure 2A).



248
249 **Figure 2** – Return to shore testing. A) Abbott Architect SARS-CoV-2 index values over time (pre- and
250 post-departure) are depicted for each individual with at least 2 serum draws. The dashed line denotes the
251 seropositivity cutoff of the assay (1.40). Individuals who had a positive RT-PCR with Ct < 35 or who
252 seroconverted during the follow-up period are shown in light blue. Individuals who were not infected by
253 the above case definition criteria are shown in green. Individuals who screened positive by the Abbott
254 Architect SARS-CoV-2 IgG assay but lacked neutralizing antibodies and were infected are shown in
255 brown. Individuals who had pre-existing neutralizing antibodies and were not infected are shown in blue.
256 B) SARS-CoV-2 whole genome sequencing of cases from the fishery vessel confirms outbreak. SARS-
257 CoV-2 genomes from 39 cases with Ct < 26 were recovered and a phylogenetic tree was made using
258 FastTree along with 109 other isolates reflective of global diversity. 38 cases are highlighted in red with
259 a median pairwise difference of 1 single nucleotide variant, while one outlier case from the boat is shown
260 with a red dot. Clade A strains associated with early trans-Pacific transmission are shown in purple.
261

262
263 Among the 21 crewmembers who never had a positive RT-PCR test with Ct < 35,
264 three individuals seroconverted based on Abbott Architect index value during the follow-
265 up period. Two of these three crewmembers had positive RT-PCR values with Ct values
266 > 35, while RT-PCR data was not available for the third until Day 49. These three
267 individuals were considered infected on the vessel. In addition, three of the 21
268 crewmembers without a positive RT-PCR result with Ct < 35 were not tested by

269 serology after returning to shore, though two of the three crewmembers tested negative
270 3 and 4 times, respectively, by RT-PCR over three weeks after returning.

271

272 *Confirmation of outbreak with whole genome sequencing*

273 Metagenomic recovery of 39 SARS-CoV-2 whole genomes from the outbreak indicated
274 a major single outbreak clade (FastTree support value: 1.00) covering 38 isolates that
275 differed by a median of one nucleotide across the genome (range 0-5) (Figure 2B).
276 Sixteen of these isolates shared completely identical sequence. The closest SARS-
277 CoV-2 whole genome sequences in GISAID (August 3, 2020) to the major outbreak
278 clade were strains from Virginia (USA/VA-DCLS-0561/2020), New York City (USA/NY-
279 NYUMC650, NYUMC624, NYNYUMC474, NYUMC426/2020), Minnesota (USA/MN-
280 MDH-1288/2020), or Michigan (USA/MI-MDHHS-SC20223/2020) at 2 SNVs apart.

281

282 *The three crewmembers with neutralizing antibodies were protected from infection*

283 We can assess the effects of pre-existing neutralizing antibodies on infection during the
284 outbreak using the pre-departure serological screening (available for 120 of 122
285 individuals) and the subsequent testing of all 122 individuals for infection. None of the
286 three individuals who had neutralizing antibodies prior to departure were infected during
287 the subsequent outbreak using our case definition of a positive RT-PCR test with Ct <
288 35 or seroconversion, and none reported any symptoms upon return to shore. In
289 contrast, among the other 117 of 120 individuals with pre-departure serological data
290 who were seronegative or lacked spike-reactive antibodies prior to departure, 103 of
291 117 were infected using the same case definition (of the 2 individuals without pre-

292 departure serological screening, one tested positive and one tested negative by RT-
293 PCR on return). Therefore, the overall rate of infection was 0 of 3 among individuals
294 with neutralizing antibodies, and 103 of 117 among individuals without such antibodies.
295 This difference is statistically significant (Table 2, Fisher's exact test $P = 0.002$),
296 indicating that pre-existing neutralizing antibodies are significantly associated with
297 protection against SARS-CoV-2 infection.
298

Table 2. Summary table of infection status of crew members for which pre-departure serology testing was performed.

		Pre-departure	
		Neutralizing Ab (+)	Neutralizing Ab (-)
On boat	Infected	0	103
	Not Infected	3	14

p=0.0024

299
300
301 The three crewmembers who were seropositive for anti-N antibodies by Abbott but did
302 not have neutralizing antibodies were all infected during follow-up, with minimum Cts of
303 17.6, 22.8, and 22.9 and increases in Abbott index values (Table 1). Sex did not differ
304 between uninfected and infected, with females composing 5.6% (1 of 18) and 7.7% (8 of
305 104) of these two groups, respectively (Fisher's exact test, $p=1$).

306 We also looked in detail at the viral testing results of the three crewmembers who
307 were positive for neutralizing antibodies to assess the strength of the evidence that they
308 were not re-infected during this ship outbreak. Two tested fully negative by RT-PCR on
309 3+ occasions, with negative tests on Days 18, 25, 35, and 36 and Days 18, 35, and 36.
310 The third individual tested negative on the Roche cobas on Day 21 and Day 28, and
311 positive only by the E-gene primers/probe set (Ct 37.4) and negative by the orf1ab

312 primer set on the Roche cobas on Day 25. This individual also tested positive (Ct 38.3)
313 on Day 31 on the Hologic Panther Fusion. By our case definition (which required a
314 positive RT-PCR test with Ct < 35), these results are not consistent with being infected
315 on the boat. The sporadic high-Ct results could be consistent with intermittent, low-level
316 shedding associated with recent past infection, as low levels of SARS-CoV-2 have been
317 detected in nasal passages for more than 80 days (40). Of note, only two other
318 crewmembers had a minimum Ct > 35 in the post-departure follow-up period and both
319 of these individuals were considered infected due to seroconversion during the follow-
320 up period. In contrast, Abbott index values decreased for all three of the crewmembers
321 with predeparture neutralizing antibodies during the follow-up period.

322

323 **Discussion**

324 Here, we report an outbreak of SARS-CoV-2 on a fishing vessel with an attack
325 rate greater than 85%. Screening with the Abbott Architect anti-nucleocapsid IgG
326 antibody test followed by confirmation of positives with multiple anti-spike protein
327 antibody tests including neutralization assays demonstrated the protective nature of
328 neutralizing antibodies. In particular, none of the three individuals with pre-existing
329 neutralizing antibodies were infected, whereas the vast majority of other individuals
330 were infected. These findings are consistent with data from animal models, in which the
331 elicitation of high titers of neutralizing antibodies was protective against re-challenge
332 with SARS-CoV-2 (8, 10, 41).

333 An assumption of our analysis is that the only individuals who had pre-existing
334 neutralizing and anti-spike antibodies were those who tested seropositive in the initial

335 pre-departure Abbot Architect anti-N serological screening, since only individuals
336 positive in that screening were subjected to additional serological assays for anti-spike
337 and neutralizing antibodies. However, this assumption is well supported by the validated
338 high sensitivity of the Abbott Architect assay (20), plus the well-established fact that
339 anti-N antibodies appear earlier than anti-spike antibodies (36, 37). Additionally, our four
340 anti-spike antibody tests showed a high level of consistency among seropositive
341 samples, and prior work using the exact same assays has found neutralizing antibodies
342 only among individuals who were positive in the Abbott Architect assay (32). As shown
343 by others, the RBD ELISA and neutralizing antibody assays were highly consistent (42,
344 43). The ACE2 blockade of binding functional ELISA assay showed excellent
345 consistency with the more laborious pseudovirus neutralizing antibody assay (44).

346 It is intriguing that one individual who had predeparture neutralizing antibodies
347 and was classified as uninfected by our case definition nonetheless had a sporadic very
348 weak signal in viral testing on two different RT-PCR platforms. It is well-established that
349 SARS-CoV-2 can be detected for multiple weeks in the nasopharyngeal tract, well after
350 the resolution of symptoms and elicitation of an antiviral immune response (45, 46).
351 However, it is unclear at this time whether immunity to SARS-CoV-2 will be sterilizing
352 (10, 47), and it is possible that the sporadic weak signal in viral testing for this individual
353 was the result of re-exposure to virus on the boat.

354 In prior studies, the Abbott SARS-CoV-2 IgG assay has shown excellent
355 performance characteristics with high specificity (99.1-99.9%) for prior infection with
356 SARS-CoV-2 (20, 48, 49). Curiously, the positive predictive value for the Abbott SARS-
357 CoV-2 IgG assay for neutralizing antibodies or protection in our population was only

358 50% (3/6 crewmembers). It is difficult to conclusively determine whether these
359 represented false positives or just anti-N/anti-spike discrepant, particularly given that
360 anti-N antibodies tend to appear before anti-spike antibodies (36, 37). All three of the
361 individuals who were Abbott IgG positive prior to departure but lacked neutralizing and
362 anti-spike antibodies and were RT-PCR positive upon return showed strong increases
363 in index value. In addition, two of these three individuals had pre-departure Abbott index
364 values that were close to the positivity cut-off. Unfortunately, we did not have sufficient
365 residual pre-departure serum to run on a separate anti-N platform such as the Roche
366 Elecsys anti-SARS-CoV-2 (50).

367 This study is limited by lack of information on clinical symptoms for the majority of
368 crewmembers on the vessel and direct knowledge of contacts on the boat. We cannot
369 also necessarily know that the three individuals with neutralizing antibodies prior to
370 departure were exposed directly to SARS-CoV-2 on the vessel. The study is also limited
371 by the low seroprevalence in the predeparture cohort--which is consistent with the
372 approximate seroprevalence in May 2020 in the Seattle area, but means that there were
373 only three individuals with pre-existing neutralizing antibodies. Nonetheless, with an
374 overall attack rate of >85%, the lack of infection in the three individuals with neutralizing
375 antibodies was statistically significant compared to the rest of the boat's crew. Overall,
376 our results provide the first direct evidence anti-SARS-CoV-2 neutralizing antibodies are
377 protective against SARS-CoV-2 infection in humans.

378

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387

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617 **Supplemental Table 1 – SARS-CoV-2 isolates and accessions sequenced in this study.**

Isolate	GISAID Accession Number
USA/WA-UW-10027/2020	EPI_ISL_461450
USA/WA-UW-10028/2020	EPI_ISL_461451
USA/WA-UW-10029/2020	EPI_ISL_461452
USA/WA-UW-10030/2020	EPI_ISL_511852
USA/WA-UW-10031/2020	EPI_ISL_461453
USA/WA-UW-10034/2020	EPI_ISL_511853
USA/WA-UW-10036/2020	EPI_ISL_461454
USA/WA-UW-10038/2020	EPI_ISL_511854
USA/WA-UW-10039/2020	EPI_ISL_461455
USA/WA-UW-10040/2020	EPI_ISL_461456
USA/WA-UW-10042/2020	EPI_ISL_461457
USA/WA-UW-10088/2020	EPI_ISL_461458
USA/WA-UW-10089/2020	EPI_ISL_461459
USA/WA-UW-10090/2020	EPI_ISL_461460
USA/WA-UW-10091/2020	EPI_ISL_461461
USA/WA-UW-10093/2020	EPI_ISL_461462
USA/WA-UW-10094/2020	EPI_ISL_461463
USA/WA-UW-10101/2020	EPI_ISL_511855
USA/WA-UW-10102/2020	EPI_ISL_461464
USA/WA-UW-10105/2020	EPI_ISL_511856
USA/WA-UW-10106/2020	EPI_ISL_461465
USA/WA-UW-10107/2020	EPI_ISL_461466
USA/WA-UW-10108/2020	EPI_ISL_461467
USA/WA-UW-10113/2020	EPI_ISL_511857
USA/WA-UW-10114/2020	EPI_ISL_461468
USA/WA-UW-10115/2020	EPI_ISL_511858
USA/WA-UW-10116/2020	EPI_ISL_512086
USA/WA-UW-10117/2020	EPI_ISL_461469
USA/WA-UW-10118/2020	EPI_ISL_461470
USA/WA-UW-10124/2020	EPI_ISL_511859
USA/WA-UW-10126/2020	EPI_ISL_511860
USA/WA-UW-10127/2020	EPI_ISL_461471
USA/WA-UW-10128/2020	EPI_ISL_461472
USA/WA-UW-10129/2020	EPI_ISL_461473
USA/WA-UW-10130/2020	EPI_ISL_461474
USA/WA-UW-10131/2020	EPI_ISL_461475
USA/WA-UW-10133/2020	EPI_ISL_511861
USA/WA-UW-10136/2020	EPI_ISL_461476
USA/WA-UW-10138/2020	EPI_ISL_461477

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