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

Introduction

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 (8, 9).

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

3

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,

5

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-

119   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   Immunlon 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 10μL serum diluted into 90μL 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 μg/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. 100μL 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	RT-PCR	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
		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

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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

9

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).
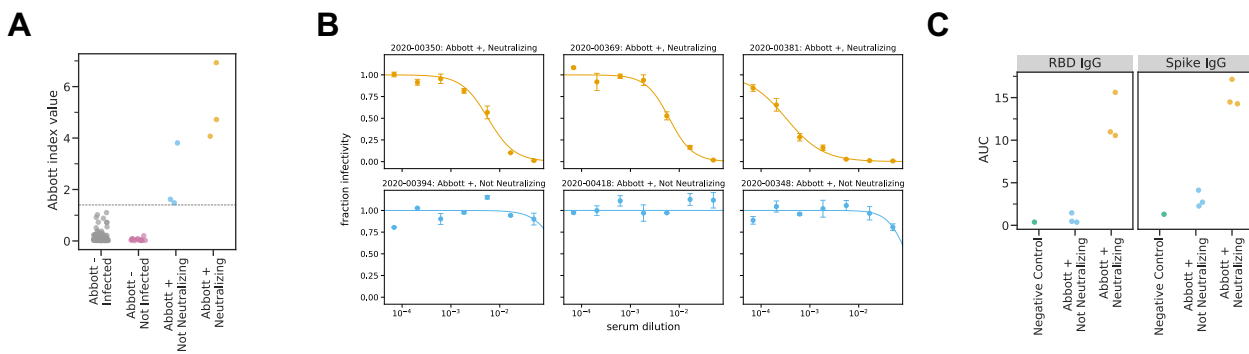
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203

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

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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

11

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

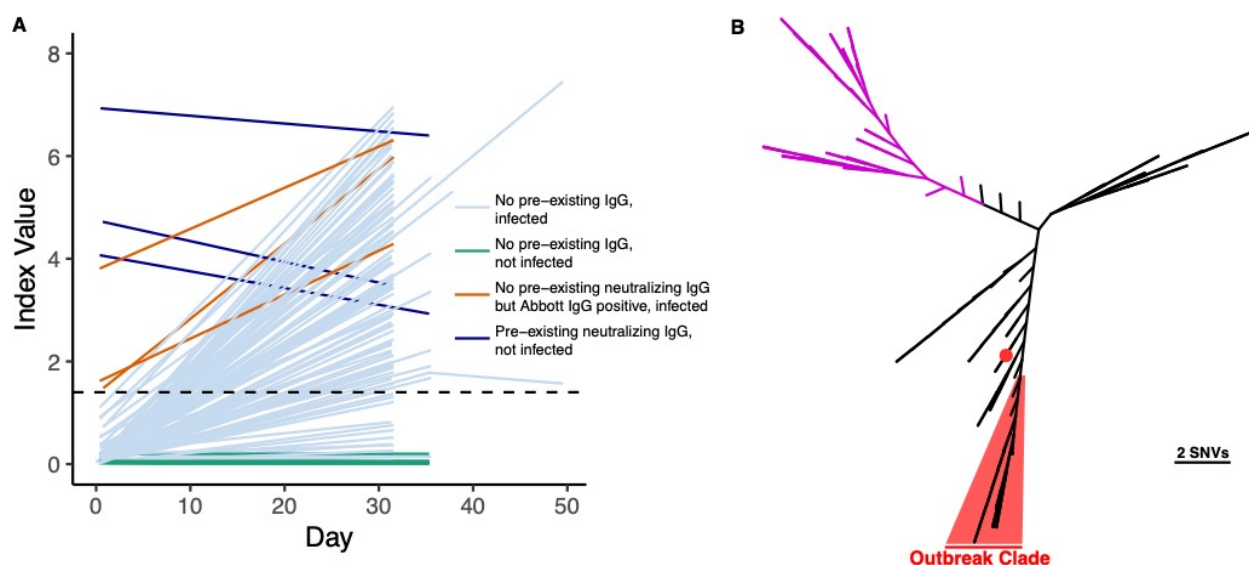247   IgG index value increased for the majority of these individuals (Figure 2A).

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 a
259   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-

13

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

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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

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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 that 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 discrepants, 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

379   **Acknowledgements**

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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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