1   **Pathogenesis, transmission and response to re-exposure of SARS-CoV-2 in domestic cats**

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10

11   **Abstract**

12

13   The pandemic caused by SARS-CoV-2 has reached nearly every country in the world with

14   extraordinary person-to-person transmission. The most likely original source of the virus was

15   spillover from an animal reservoir and subsequent adaptation to humans sometime during the

16   winter of 2019 in Wuhan Province, China. Because of its genetic similarity to SARS-CoV-1, it is

17   likely that this novel virus has a similar host range and receptor specificity. Due to concern for

18   human-pet transmission, we investigated the susceptibility of domestic cats and dogs to infection

19   and potential for infected cats to transmit to naïve cats. We report that cats are highly susceptible

20   to subclinical infection, with a prolonged period of oral and nasal viral shedding that is not

21   accompanied by clinical signs, and are capable of direct contact transmission to other cats. These

22   studies confirm that cats are susceptible to productive SARS-CoV-2 infection, but are unlikely to

23   develop clinical disease. Further, we document that cats develop a robust neutralizing antibody

24   response that prevented re-infection to a second viral challenge. Conversely, we found that dogs

25   do not shed virus following infection, but do mount an anti-viral neutralizing antibody response.

26   There is currently no evidence that cats or dogs play a significant role in human exposure;

27   however, reverse zoonosis is possible if infected owners expose their domestic pets during acute

28   infection. Resistance to re-exposure holds promise that a vaccine strategy may protect cats, and

29   by extension humans, to disease susceptibility.

30

31   **Introduction**

32

33   The COVID-19 pandemic, caused by the SARS-CoV-2 (SARS2) coronavirus, originated in the

34   Wuhan province of China, in late 2019 and within four months spread to nearly every country in

35   the world. Sequence analysis and epidemiological investigations suggest that the virus was of

36   animal-origin, possibly bat, and was first introduced into the human population via an

37   intermediate animal host in the Huanan seafood market in Wuhan, China (Bogoch et al. 2020;

38   Zhou et al. 2020). The virus quickly adapted to humans and human-to-human transmission

39   became the almost immediate source of subsequent infections, with direct contact and aerosol

40   droplets as the primary routes of infection (Li et al. 2020). Early indications suggested that

41   SARS2, much like SARS-CoV-1 (SARS1), infects host cells by binding to the angiotensin-

42   converting enzyme 2 (ACE2), a receptor that is expressed in many animal species, although

43   notably not in mice or rats (Wan et al. 2020). Thus, while humans are almost certainly the sole

44   source of infection to other humans, multiple early studies suggest other animals are susceptible

45   to infection as well.

46 The first report of reverse zoonosis, or transmission from human to animal, was reported

47 from Hong Kong, where a COVID patient's dog tested PCR positive for SARS2 multiple times

48 (Sit et al. 2020). In following weeks, other reports of domestic pets becoming infected following

49 exposure to humans were documented, including another dog in Hong Kong and a cat with

50 clinical disease in Belgium (Chini 2020). Serologic studies so far have failed to identify domestic

51 dogs and cats as a primary source of human infection (Deng et al. 2020). Importantly, a survey of

52 veterinary students with confirmed COVID infection was unable to identify antibodies in their

53 pets (Temmen et al. 2020). Despite the low probability of pet-to-human or human-to-pet

54 transmission, it remains important to clarify what role, if any, that domestic pets play in SARS2

55 transmission.

56 The first published study involving cat experimental infections showed that cats could

57 become infected by SARS2 and could potentially transmit to other cats via aerosols, as defined

58 by PCR positive fecal samples from cats in cages in the same room as directly infected cats. This

59 study also described pathology and mortality in juvenile cats euthanized at 3 and 7 days post-

60 infection (Shi et al. 2020) Additional communications described viral shedding and direct

61 contact transmission in cats as well as seroconversion in cats exposed to infected humans

62 (Halfmann et al. 2020; Zhang et al. 2020). The experiments described herein expand upon

63 existing work by providing shedding kinetics in cats over time, assessing virus neutralization,

64 seroconversion, and exploring transmission. Furthermore, to the author's knowledge, this is the

65 first report of protective immunity against SARS2 following repeated exposure. These studies

66 indicate that cats may serve as a suitable animal model for studying SARS2 infection, and for

67 furthering the development of vaccines and therapeutics for use in both animals and humans. We

68 also confirm an earlier report that dogs do not replicate virus locally (Shi et al. 2020), but

69   document evidence of anti-viral neutralizing activity in post-exposure canine sera. The role of

70   cats in zoonotic transmission remains an open question, but relatively short duration of shedding

71   and resistance to re-exposure suggests risk of this is very low, particularly when cats are kept

72   indoors.

73

74   **Materials and Methods**

75

76   *Virus*

77   SARS2 virus strain WA1/2020WY96 was obtained from BEI Resources (Manassas, VA, USA),

78   passaged twice in E6 Vero cells and stocks frozen at -80C in Dulbecco's Modified Eagle

79   Medium (DMEM) with 5% fetal bovine serum and antibiotics. Virus stock was titrated on Vero

80   E6 cells using standard double overlay plaque assay (Kropinsky et al. 2008) and plaques counted

81   72 hours post-infection to determine plaque-forming units (pfu) per ml.

82

83   *Animals*

84   Seven adult (1 male, 6 female, 5-8 year old) cats were obtained from a closed breeding colony

85   held at Colorado State University in a pathogen-free environment in an Association for

86   Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accredited

87   animal facility. Cats were screened negative for feline enteric coronavirus antibody prior to

88   transfer. Three dogs (female, 5-6 years old) were obtained from Ridglan Farms (Blue Mounds,

89   WI, USA). Cats and dogs were transferred to the Animal Disease Laboratory, an Animal

90   Biosafety Level-3 (ABSL3) facility at Colorado State University, group housed and fed dry/wet

91   food mix with access to water ad libitum. Animals were allowed several days to acclimate before

92   temperature-sensing microchips (Lifechips, Destron-Fearing) were inserted subcutaneously in

93   the dorsum. Baseline weights, body temperatures, clinical evaluation, and oral swabs were

94   obtained prior to inoculation. All animals were in apparent good health at the onset of the study.

95

96   *Virus challenge*

97   Cats were lightly anesthetized with 30-50 mg subcutaneous ketamine hydrochloride

98   (Zetamine™) and dogs sedated with 1-3 mg xylazine. Virus diluted in phosphate buffered saline

99   (PBS) was administered to both species via pipette into the nares (500ul/nare) for a total volume

100   of 1ml; animals were observed until fully recovered from anesthesia. Virus back-titration was

101   performed on E6 cells immediately following inoculation, confirming that cats received 3.0E5

102   pfu and dogs received 1.4E5 pfu.

103

104   *Sampling*

105   Cat cohort 1 (n=3)

106   Oropharyngeal swabs were collected daily on days 1-5, 7, 10, and 14 post-infection using a

107   polyester tipped swab applicator. Swabs were placed in BA-1 medium (Tris-buffered MEM

108   containing 1% BSA) supplemented with gentamicin, amphotericin B and

109   penicillin/streptomycin. Nasal flushes were performed on 1, 3, 5, 7, 10, and 14 days post-

110   infection (DPI) by instilling 1ml BA-1 dropwise into the nares of awake or lightly anesthetized

111   cats and collecting nasal discharge into a sterile petri dish by allowing the wash fluid to be

112   sneezed out or dripped onto the dish. Blood (5 ml into serum separator tubes) was collected prior

113   to inoculation and on days 7, 14, 21, 28, 35 and 42 post-infection. At 28 DPI, cats were re-

114   challenged with 3e5 pfu of homologous virus. Oronasal sample collection was performed 1, 3,

115   5, 7, 10 and 14 after re-inoculation (days 29, 31, 33, 35, 38 and 42 post initial challenge), at

116   which time cats were euthanized and tissues collected for histopathology.

117

118   Cat cohort 2 (n=4)

119   Two of the four cats were lightly anesthetized, and challenged with SARS2 as for Cohort 1.

120   Forty-eight hours post-infection, two naïve cats were introduced into the room with the infected

121   cats and sampled on the same schedule as before. The two directly challenge cats were

122   euthanized on 5 DPI and the following tissues collected for virus isolation and histopathology:

123   nasal turbinates, trachea, esophagus, mediastinal lymph node, lung, liver, spleen, kidney, small

124   intestine, uterus, and olfactory bulb. Tissues were collected into BA-1 frozen at -80C and

125   homogenized prior to plaque assay. Additional tissues collected for histopathology included

126   heart, colon, pancreas, hemi-lung lobe, and mesenteric lymph nodes. Thoracic radiographs were

127   also obtained for these two cats pre-challenge and just prior to euthanasia. The remaining two

128   cats were euthanized at 30 DPI and necropsied; these cats will be referred to as contact cats

129   hereafter.

130

131   Dogs (n=3)

132   Dogs were sampled at the same frequency, and using the same methods as cats in Cohort 1 for

133   42 days post-infection. Dogs were not re-challenged.

134

135   *Clinical observations*

136   Body temperatures were recorded daily for the duration of the study using the thermal

137   microchips. Cats and dogs were observed twice daily for the first seven days post-challenge and

138   at least once daily for the duration of the study. Body weights were obtained weekly. Clinical

139   evaluation included temperament, ocular discharge, nasal discharge, ptyalism,

140   coughing/sneezing, dyspnea, diarrhea, lethargy, anorexia, moribund. None of the animals

141   exhibited clinical signs of disease characterized by any of these symptoms at any time during the

142   study.

143

144   *Viral assays*

145   Virus isolation was performed on all oral swab, nasal flush and 5 DPI tissue samples by double

146   overlay plaque assay on Vero E6 cells as previously described (Kropinsky et al. 2009). Briefly,

147   6-well plates with confluent monolayers of cells were washed once with PBS and inoculated

148   with 100 ul of serial 10-fold dilutions of samples, incubated for 1 hour at $37^{o}C$, and overlaid with

149   a 0.5% agarose in MEM containing 2% fetal bovine serum and antibiotics/antifungal agents. A

150   second overlay with neutral red dye was added at 48 hours and plaques were counted at 72 hours.

151   Viral titers were reported as the $\log_{10}$ plaque-forming units (pfu) per ml.

152

153   Plaque reduction neutralization assays (PRNT) were performed as previously described (Perera

154   et al. 2020). Serum was heat-inactivated for 30 mins at $56^{o}C$, and two-fold dilutions prepared in

155   BA-1 starting at a 1:5 dilution were aliquoted onto 96-well plates. An equal volume of virus was

156   added to the serum dilutions and incubated for 1 hour at $37^{o}C$. Following incubation, serum-virus

157   mixtures were plated onto Vero E6 plates as described for virus isolation assays. Antibody titers

158   were recorded as the reciprocal of the highest dilution in which >90% of virus was neutralized.

159

160   *ELISA*

161   Serum samples from cats were heat inactivated and tested by plaque assay to verify samples

162   were noninfectious prior to conducting ELISA analysis. Positive control antibodies to the

163   receptor-binding domain (RBD) and full-length spike protein were human MAb CR3022

164   antibody (Absolute Antibody, Oxford UK) and human IgG whole molecule (Jackson Immuno

165   Research, West Grove PA, USA). Positive control for the nucleocapsid ELISA was SARS-CoV

166   nucleoprotein rabbit monoclonal antibody (Sino Biological Inc, Beijing, China). Negative

167   controls were reagent grade human sera (compared to Mab CR3022). Cat serum from specific

168   pathogen free, naïve experimental animals, and field isolate bioarchived samples obtained prior

169   to 2019 (Carver et al, 2005, Sprague et al, 2018). ELISA protocols were adapted from protocols

170   for SARS CoV-2 ELISA described by Amanat et al., 2020. ELISA plates (Thermo) were coated

171   at 2ug/ml with spike glycoprotein Receptor Binding Domain (RBD) from SARS-CoV-2,

172   WuHan-Hu-1 recombinant from HEK293T cells (BEI), or Spike glycoprotein (Stabilized) from

173   SARS-CoV-2, Wuhan-Hu-1, recombinant from Baculovirus (BEI). SARS CoV-2 nucleocapsid

174   protein was a gift of Dr. Brian Geiss. Prior to running experimental cat sera, the assay was

175   optimized using positive and negative control sera described above (data not shown.) Samples

176   and controls were diluted 1:50 in ELISA diluent (1X PBS, tween, milk powder) and run in

177   duplicate. Human sera controls were developed using anti-human IgG HRP (Thermo), cat sera

178   was developed using anti-cat IgG HRP (Thermo) or anti-cat IgM (Novus Biologicals) and rabbit

179   mAb SARS-CoV NP was detected by anti-rabbit IgG HRP (Thermo). Secondary antibodies

180   were diluted 1:3000 and SigmaFast OPD was prepared in WFI and added to wells. Plates were

181   read at 490nm using a Multiskan® Spectrum spectrophotometer (Thermo Fisher). The mean of

182   negative control sera OD490 plus three times the standard deviation of the negative control

183   readings were used to determine cut off values for each plate.

184

qRT-PCR

Plaques were picked from culture plates from each cat to confirm SARS2 viral shedding. RNA extractions were performed per the manufacturer's instructions using Qiagen QiaAmp Viral RNA mini kits. RT-PCR was performed as recommended using the E_Sarbeco primer probe sequence as described by Corman and colleagues (2020) and the Superscript III Platinum One-Step qRT-PCR system (Invitrogen), with the following modification; the initial reverse transcription was at $50^{\circ}C$. Standard curves were obtained by serial dilution of stock viral RNA from the original WA1/2020WY96 SARS2 isolate.

Histopathology

Tissues from cats were fixed in 10% buffered formalin for 12 days and transferred to 70% ethanol prior to sectioning for H&E staining. Slides were read by a board certified veterinary pathologist.

Results

Clinical Disease

None of the cats in either cohort displayed any clinical signs of disease and remained afebrile throughout the study. Body weights were maintained over time. Radiographs were taken pre-challenge and at 5 DPI just prior to euthanasia for the experimentally inoculated cats in Cohort 2. No evidence of lung involvement or any other radiographically-detectable abnormalities were

206   noted (images not shown). Similarly, dogs inoculated with SARS2 remained clinically normal

207   and afebrile.

208

209   *Viral shedding*

210   In Cohort 1, all three cats shed virus both orally and nasally for up to 5 days post-infection, with

211   peak titers achieved from nasal shedding at day 3. Nasal titers were approximately 1 log higher

212   than oral swabs collected at the same time (Figure 1). There was some variability in titer over the

213   course of infection that is likely attributable to sample collection (i.e. quality of sneezes), but

214   overall the data demonstrates clear presence of infectious virus in both the nasal cavity and the

215   oropharynx for multiple days post-infection. In Cohort 2, the inoculated cats shed virus for 5

216   days post-infection both orally and nasally, with a similar pattern to Cohort 1. The contact cats,

217   however, shed infectious virus orally by 24 hours post-exposure and the duration of shedding

218   was prolonged compared to the inoculated cats, with peak shedding occurring at 7 days post-

219   exposure (Figure 1). Virus was isolated from trachea, nasal turbinates, and esophagus from cats

220   in Cohort 2 necropsied on day 5. Infectious virus was not found in the lung or other organs of

221   either cat. Viral shedding was not detected from any of the dogs at any point post-infection.
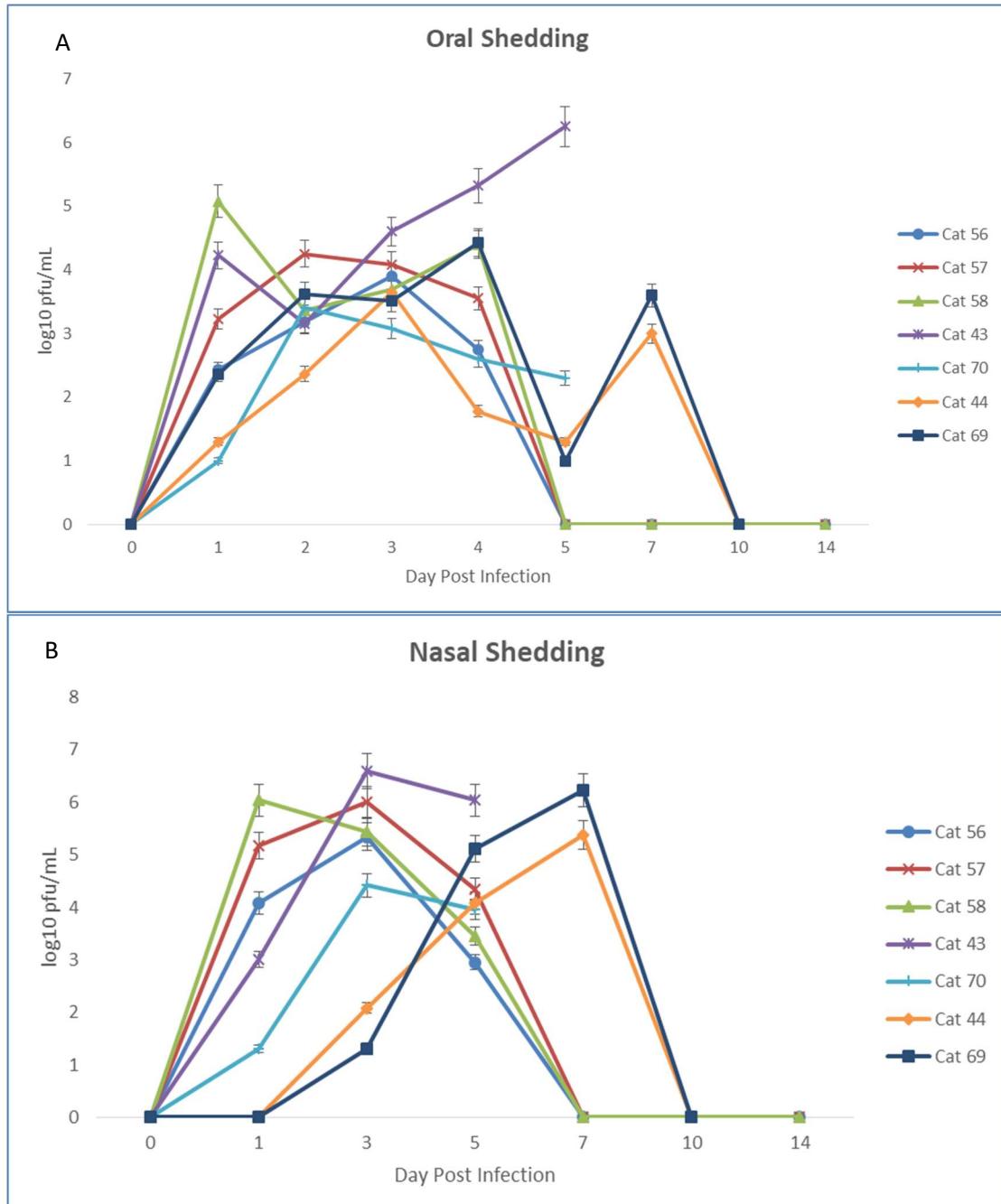
Figure 1: Inoculation and exposure with SARS2 leads to oral and nasal shedding in cats.

SARS2 virus is detected by plaque assay from (A) oral and (B) nasal secretions of cats 1-5 days post infection. Viral titers expressed as log_{10}pfu/ml. Cats 56, 57, and 58 represent Cohort 1. Cats 43, 70, 44, and 69 represent Cohort 2. Cats 43 and 70 were euthanized on 5 DPI. Cats 44 and 69 were introduced to the infected cats in Cohort 2 on 2 DPI.

229

230   *Pathology*

231   Gross lesions were not observed in any of the necropsied cats. Histologically, in both cats

232   sacrificed at 5 DPI from Cohort 1, moderate ulcerative, suppurative lymphoplasmacytic rhinitis

233   was observed in the nasal turbinates along with mild lymphoplasmacytic tracheitis. These cats

234   also had minimal alveolar histiocytosis with edema and one of the cats had rare type II

235   pneumocyte hyperplasia (Figure 2). All three cats from Cohort 1 sacrificed 42 DPI had mild lung

236   changes, including mild interstitial lymphocytic pneumonia with peribronchiolar and

237   perivascular lymphocytic cuffing and alveolar histiocytosis. Two of these cats also had minimal

238   tracheitis, but largely the lesions in the upper respiratory tract appear decreased in comparison to

239   the early timepoint cats, while lung pathology was more evident in these animals compared to

240   those sacrificed during acute infection. Dogs were not euthanized at the time of this report.
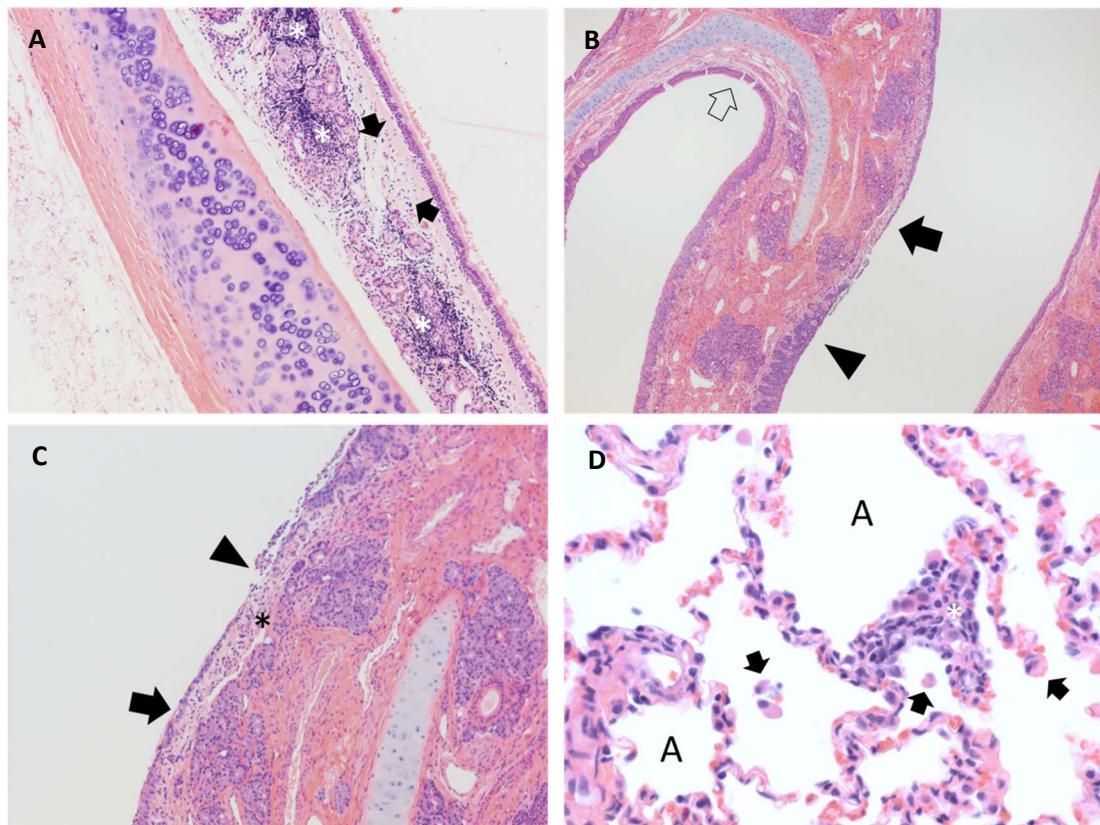

241

242 **Figure 2: SARS2 exposure results in acute upper respiratory inflammation and mild lung**

243 **infiltrates during later courses of infection.**

244 Panel A: **Cat 43, trachea 5 DPI.** The submucosa is expanded by edema (arrow) and abundant

245 lymphocytic inflammatory infiltrates (asterisks) which dissect and disrupt submucosal glands.

246 Hematoxylin & eosin stain. 100x magnification.

247 Panel B: **Cat 70, nasal turbinates 5 DPI.** Normal thickness respiratory mucosa is present in the

248 section (open arrow). Nasal respiratory epithelium ranges from hyperplastic (filled black arrow)

249 to ulcerated (arrowhead). The submucosa in regions of ulceration is edematous and infiltrated by

250 scattered neutrophils and mononuclear cells. Hematoxylin & eosin stain. 40x magnification.

251 Panel C: **Cat 70, nasal turbinates, 5 DPI.** Nasal respiratory epithelium ranges from attenuated

252 (arrow) to ulcerated (arrowhead) with overlying remnant cellular debris. The submucosa

253 (asterisk) in regions of ulceration is edematous and infiltrated by scattered neutrophils and

254 mononuclear cells. Hematoxylin & eosin stain. 100x magnification.

255 Panel D: **Cat 56, lung, 42 DPI.** Alveolar spaces (A) contain scattered mononuclear cells (arrow).

256 The alveolar wall is expanded by mixtures of mononuclear cells and occasional neutrophils

257 (asterisk). Hematoxylin & eosin stain. 400x magnification.

258

259 *Seroconversion*

260 Cats in both Cohort 1 and the direct contact cats developed neutralizing activity as measured by

261 PRNT as early as 7 DPI. Neutralizing titers in all cats reached or exceeded 1:2560 by 14 DPI and

262 either maintained or increased in titer between 28 and 42 DPI. Cats re-challenged at 28 DPI

263 displayed a moderate increase in PRNT titer in the 14 days following exposure (Table 1). Dogs

264   developed neutralizing antibodies by 14 DPI and peaked at 21 DPI with titers between 1:40-1:80

265   (Table 1).

266

267   **Table 1: PRNT90 values for cats and dogs**

Animal	0 DPI	7 DPI	14 DPI	21 DPI	28 DPI	42 DPI
Cat 56 (Cohort 1)	<50%	320	5120	2560	2560	10240
Cat 57 (Cohort 1)	<50%	80	5120	2560	2560	5120
Cat 58 (Cohort 1)	<50%	80	2560	2560	1280	5120
Cat 43 (Cohort 2)	<50%					
Cat 70 (Cohort 2)	<50%					
Cat 44 (Cohort 2)	<50%	NT	2560	5120	5120	
Cat 69 (Cohort 2)	<50%	NT	2560	1280	10240	
Dog 46	<50%	<10	10	40	40	80
Dog 47	<50%	10	80	20	20	40
Dog 48	<50%	<10	20	40	20	20

268

269   IgG antibodies responses exceeding OD490 cut off values were detected at day 7 PI against both

270   the complete spike glycoprotein and RBD in all experimentally inoculated cats, and

271   seroconversion against NP was detected in 2 of 3 cats at this time. By day 14 all five cats had

272   OD values that neared upper limit of detection in the Spike ELISA; RBD and NP OD saturation

273   was obtained by day 21 and did not increase following re-exposure (Figure 3). Rates of

274   seroconversion and absorbance levels were similar between contact cats and experimentally

275   infected cats. Seroconversion to spike protein was most rapid and robust, and the specificity of

276   response to RBD exceeded that of NP. Seroconverted cat OD values for all three antigens

277   exceeded absorbances of SPF or field domestic cats, and background was highest for NP. IgM

278   antibodies against RBD were detected at days 7 and 14 but not at day 28. IgG responses were

279   much more robust than IgM (Figure 3). ELISA assays were not performed on dog sera.
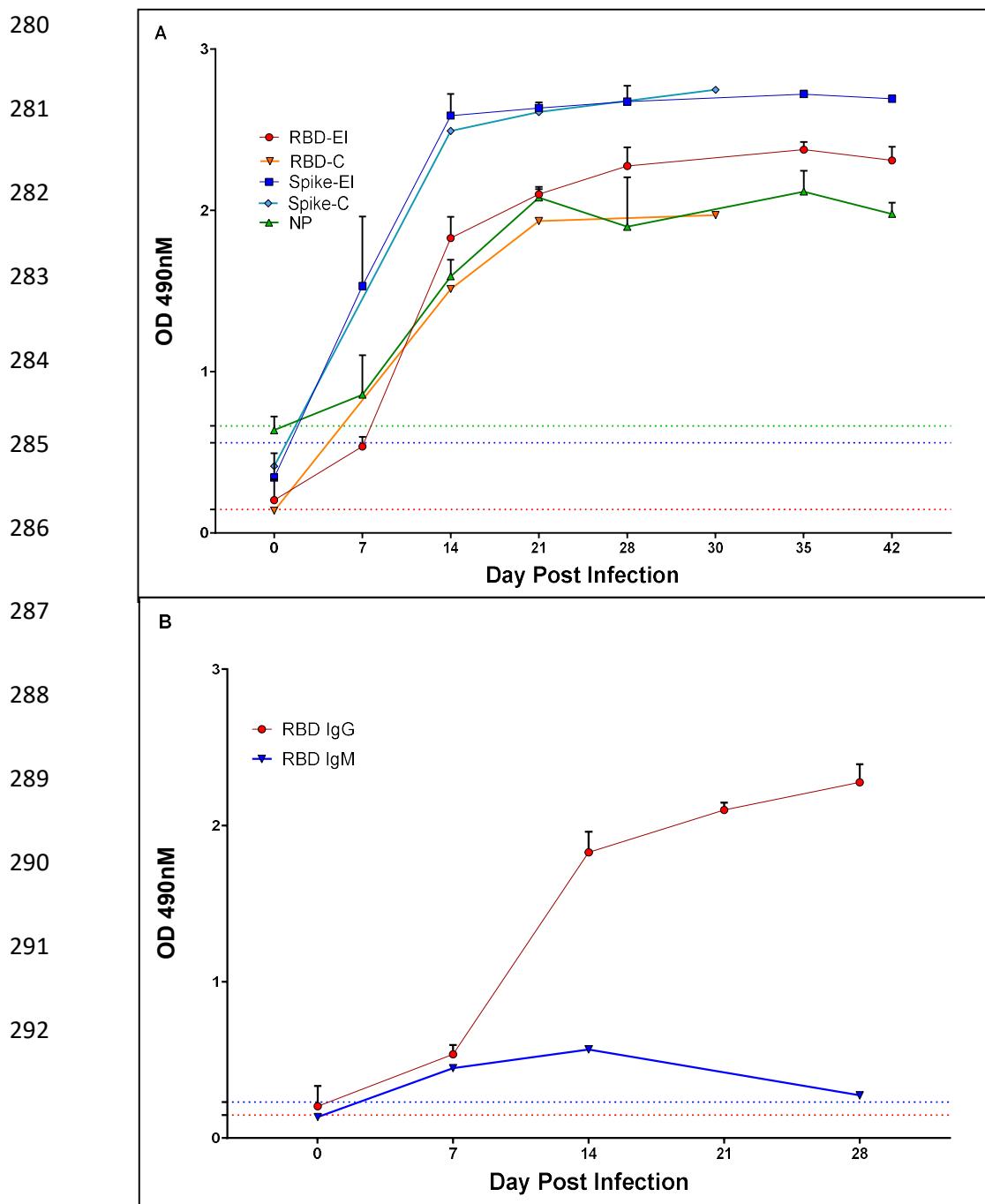
293 **Figure 3. Cats infected with SARS CoV-1 rapidly develop antibodies against viral**

294 **antigens**. (A) Sera from cats with intranasal inoculation of SARS CoV-2 (n=3, 'EI') or exposed

295 to inoculated cats (n=2, 'C') were evaluated for seroreactivity to receptor binding domain

296 (RBD), Spike, or nucleocapsid protein (NP) for 30-42 days post exposure. IgG Reactivity to

297 Spike and RBD was evident at day 7, and all animals had clearly seroconverted by day 14. (B)

298 IgM against RBD was transiently detected at low levels relative to IgG on days 7 and 14 post

299 exposure (experimentally inoculated animals, n=3). Bars represent 1 SE of the mean.

300

301 *Reinfection*

302 Re-challenged cats in Cohort 1 were sampled for oral and nasal shedding for 7 days post-

303 exposure by viral isolation, and shedding was not detected in any cat at any timepoint following

304 rechallenge.

305

306 **Discussion**

307   The COVID19 pandemic represents the first global pandemic of an emerging zoonotic

308 disease in this century. The SARS2 virus is one of three emergent zoonotic coronaviruses

309 capable of causing significant disease in humans in the last two decades, following SARS1 and

310 MERS (Guarner 2020). The overall trend of disease emergence favors viral spillover from

311 animals to human, and land use and wildlife encroachment are just two of the factors

312 contributing to this phenomenon (Olival et al. 2017). The continued presence of live animal

313 markets provides optimal conditions for emergence of zoonoses (Wang and Eaton, 2007). As

314 with SARS1 and MERS, SARS2 is of probable bat origin based on phylogenetic analysis (Zhou

315 et al. 2020), but unlike its predecessors, SARS2 has rapidly evolved for highly efficient human-

316   to-human transmission (Chan et al et al. 2020). While animals, including domestic animals and

317   pets, are frequently implicated as the source of emerging pathogens, reverse zoonosis of SARS2

318   is more probable, as human cases are far more prevalent than domestic animals. Similar results

319   were seen with SARS1, where domestic cats exposed to the virus by infected humans became

320   infected, and cats experimentally infected shed virus for several days (Martina et al. 2003, van

321   den Brand et al. 2008) There have been several cases of pets becoming infected by SARS2

322   following exposure to infected humans in New York, Hong Kong, Belgium, Germany, Spain,

323   France and Russia (Sit et al. 2020; as communicated by ProMed). Other animal exposures from

324   infected humans include farmed mink, which appear to display respiratory symptoms following

325   infection (ProMed). In several of these cases, including nondomestic felids at the Bronx Zoo and

326   pet cats in New York and Europe, animals displayed signs of respiratory disease and/or

327   conjunctivitis. None of the cats or dogs in this study exhibited any clinical signs of disease, but

328   individual animal health status, age and comorbidities may be responsible for this variability.

329   Pathological changes in cats suggest that mild subclinical disease in otherwise healthy animals

330   occurs but is not recognizable symptomatically. This is not altogether different from human

331   infections, where the majority of cases are relatively mild but more severe disease tends to occur

332   in older patients with significant comorbidities (Nikolich-Zugich et al. 2020). In a recent

333   serosurvey of cats in Wuhan, China, nearly 14.7% of sampled animals were seropositive for

334   SARS2 by RBD ELISA, suggesting that the cat population in areas with high human

335   transmission is also likely to be exposed to the virus (Zhang et al. 2020) Considering that the

336   number of human infections have reached the millions and yet only a handful of animals have

337   tested PCR positive, it seems unlikely that domestic pets are a significant source of infection or

338   are at serious risk for developing severe disease. Importantly, infected cats shed for no more than

339   5 days following exposure, suggesting that cats, if exposed to infected humans, will develop and

340   clear infection rapidly. In comparison, humans typically have an incubation period of

341   approximately 5 days and can shed virus for more than three weeks (Lauer et al. 2020, Noh et al.

342   2020). Thus, if symptomatic humans follow appropriate quarantine procedures and stay home

343   with their pets, there is minimal risk of a potentially exposed cat infecting another human.

344   Infected pet cats should not be allowed outdoors to prevent potential risk of spreading infection

345   to other outdoor cats. More research into the susceptibility of wildlife species and potential for

346   establishment of infection in outdoor cat populations is necessary to identify risk factors and

347   mitigation strategies to prevent establishment of reservoir infections in feral cats.

348   The development of animal models for studying SARS-2 is an important step in research

349   methodologies. Rhesus macaques, hamsters, and ferrets are all suitable models for replicating

350   asymptomatic or mildly clinical disease, and, while not often used as a traditional animal model,

351   this work demonstrates that cats may serve as an alternative model (Kim et al. 2020, Chan et al.

352   2020, Munster et al. 2020) The cats in this study developed subclinical pathological changes in

353   the upper respiratory tract early in the course of infection with more lower respiratory tract

354   pathology later following viral clearance, which suggests that, while subclinical, viral infection

355   of cats is not completely benign, and may make their utility as an animal model more relevant to

356   mild human disease. Additionally, the relatively high-titer viral shedding produced by cats and

357   the rapidity of transmission may make them an ideal model for simulation of aerosols. As such,

358   cat models may be quite useful for understanding the shed/spread kinetics of SARS2. Perhaps

359   most importantly, cats develop significant neutralizing antibody titers, and are resistant to re-

360   infection, which could prove a useful measurement for subsequent vaccine trials for both human

361   and animal vaccine candidates.

362

382

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