

1 **Title: Susceptibility of domestic swine to experimental infection with SARS-CoV-2**

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14

15 **Abstract – 144 words**

16 SARS-CoV-2, the agent responsible for COVID-19 has been shown to infect a number of
17 species. The role of domestic livestock and the risk associated for humans in close contact
18 remains unknown for many production animals. Determination of the susceptibility of pigs to
19 SARS-CoV-2 is critical towards a One Health approach to manage the potential risk of zoonotic
20 transmission. Here, pigs undergoing experimental inoculation are susceptible to SARS-CoV-2 at
21 low levels. Viral RNA was detected in group oral fluids and nasal wash from at least two animals
22 while live virus was isolated from a pig. Further, antibodies could be detected in two animals at
23 11 and 13 days post infection, while oral fluid samples at 6 days post inoculation indicated the
24 presence of secreted antibodies. These data highlight the need for additional livestock assessment
25 to better determine the potential role domestic animals may contribute towards the SARS-CoV-2
26 pandemic.

27

28

29 **Introduction**

30 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the agent of
31 coronavirus disease (COVID-19) was recently identified to cause severe respiratory distress in
32 humans with symptoms ranging from asymptomatic, mild to severe, and sometimes fatal cases
33 (1). Rapidly spreading, this novel virus emerged in Wuhan China, to generate a pandemic as
34 declared by the World Health Organization on March, 11th 2020 (2). Predicted to have
35 originated in bats, SARS-CoV-2 origins are still under intense investigation as reports continue
36 to identify the ability of the virus to infect new animal species (3-8). Detection of natural
37 infections has recently shed light on knowledge gaps in understanding transmission which has
38 raised concerns regarding amplifying or reservoir hosts. In turn, a better understanding of
39 wildlife and domestic animal susceptibility is required to assess the potential roles and present
40 risks to prevent future spread of disease. Domestic swine, one of the most significant and highly
41 produced agricultural species with previous impacts to public health, must be assessed (9-12).
42 The increase in “backyard” small stakeholder animal production in both rural and urban
43 environments provides an important source of high-quality protein and income, but can also
44 serve as a source for zoonotic disease; therefore, it is important to investigate their potential role
45 during SARS-CoV-2 spread (13). Evidence for the involvement of production animals was
46 recently highlighted in The Netherlands where anthroponotic transmission of SARS-CoV-2 from
47 humans to farmed mink with subsequent zoonotic transmission to at least two humans from mink
48 has been proposed, further exemplifying the need to identify the potential role of production
49 animals in disease transmission (14).

50 Angiotensin-converting enzyme 2 (ACE2) has been identified to be the receptor for
51 SARS-CoV-2 (15). A Basic Local Alignment Search Tool (BLAST) query of the protein

52 database using translated nucleotide (BLASTx) from the human ACE2 coding sequence predicts
53 98% coverage and 81% identity for the homologous receptor in swine. Interestingly, using the
54 same search both mink (82%) and feline (85%) show similar identity to the human ACE2 for
55 their cognate receptors. Moreover, both mink and cats have been reported to be susceptible to
56 SARS-CoV-2 and have shown transmission to other animals (5, 16). Work by Zhou *et al.*
57 utilized *in vitro* infectivity studies testing ACE2 receptor from laboratory mice, horseshoe bats,
58 civets and the domestic pig. All of the respective receptors, except mice, were reported to enter
59 HeLa cells indicating a functional target for SARS-CoV-2. Moreover, the authors employed
60 additional known coronavirus receptors including both aminopeptidase N and dipeptidyl
61 peptidase 4 finding neither are used for cell entry outlining the specificity for the ACE2 receptor
62 (17).

63 The work reported here aims to determine whether domestic swine are susceptible to
64 SARS-CoV-2 infection, providing critical information to aid public health risk assessments.
65 Following oronasal inoculation, swine were assessed for: clinical signs and pathology, evidence
66 of virus shedding, viral dissemination within tissues, and seroconversion. The data presented in
67 this study provides evidence live SARS-CoV-2 virus can persist in swine for at least 13 days
68 following experimental inoculation.

69

70 **Methods**

71 Ethics Statement. Experimental design, including housing conditions, sampling regimen,
72 and humane endpoints, were approved by the Animal Care Committee of the Canadian Science
73 Centre for Human and Animal Health in AUD #C-20-005 and all procedures and housing
74 conditions were in strict accordance with the Canadian Council on Animal Care (CCAC)

75 guidelines. Group housing was carried out in the BSL-3 zoonotic large animal cubicles, and
76 animals were provided with commercial toys for enrichment and access to food and water ad
77 libitum. All invasive procedures, including experimental inoculation and sample collection (nasal
78 washes, rectal swabs, and blood collection) were performed under isoflurane gas anesthesia, and
79 animals were euthanized by intravenous administration of a commercial sodium pentobarbital
80 solution.

81
82 Study design. Nineteen domestic, American Yorkshire crossbred pigs (*Sus scrofa*) (6
83 castrated males and 13 females, age 8 weeks) were locally sourced in Manitoba, Canada and
84 utilized in this study. Sixteen pigs were oronasally challenged with 1×10^6 pfu per animal in a
85 total of 3 mL DMEM under sedation with isoflurane. 1 ml was distributed per nostril and 1 ml
86 placed in the distal pharynx utilizing a sterile, tomcat-style catheter. The challenge dose was
87 confirmed by back-titration of the inoculum on Vero E6 cells. Two naïve pigs were placed in the
88 room with the inoculated pigs at day 10 to serve as in-room transmission controls. One additional
89 uninoculated pig was sampled and necropsied to serve as a “farm control” providing negative
90 control tissues. A physical examination including collection of blood, multiple swabs (rectal,
91 oral, and nasal), and nasal wash with sterile D-PBS was performed at day zero and every other
92 day beginning at three days post-inoculation (DPI) until day 15. The remaining pigs were
93 sampled on 22 and 29 DPI. Group oral fluids from rope chews were collected daily. Necropsies
94 and post-mortem sampling were performed starting at 3 DPI as outlined in Table 1. Animal
95 numbers were not based on power analysis but on the limitations of the containment animal
96 room size and requirements of CCAC guidelines. Group assignment (day of euthanasia and
97 necropsy) was based on randomization at the time of permanent animal identification (ear tag).

98

99 Sampling of animals. Oral, rectal, and nasal swabs were taken from each pig under general
100 anesthesia using isoflurane and placed into sterile D-PBS containing the following antibiotics:
101 streptomycin, vancomycin, nystatin, and gentamycin. Fluid was collected from a bilateral nasal
102 wash using sterile D-PBS. Blood was collected in each of the following via jugular venipuncture:
103 serum, sodium citrate, sodium heparin, and K3 EDTA.

104

105 Hematology, chemistry, and blood gas analyses. Hematology was performed on an HM5
106 analyzer (Abaxis) using K3 EDTA-treated whole blood and the following parameters were
107 evaluated: red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular
108 hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution weight, platelets,
109 mean platelet volume, white blood cells, neutrophil count (absolute (abs) and %), lymphocyte
110 count (abs and %), monocyte count (abs and %), eosinophil count (abs and %), and basophil
111 count (abs and %). Blood chemistries were evaluated on a VetScan 2 (Abaxis) with the
112 Comprehensive Diagnostic Profile rotor (Abaxis) using serum stored at -80°C until tested and the
113 following parameters were evaluated: glucose, blood urea nitrogen, creatinine, calcium, albumin,
114 total protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase,
115 amylase, potassium, sodium, phosphate, chloride, globulin, and total bilirubin. Sodium heparin
116 treated blood was used to analyze venous blood gases, which were performed on an iSTAT
117 Alinity V machine (Abaxis) using a CG4+ cartridge (Abaxis) to measure the following
118 parameters: lactate, pH, total carbon dioxide, partial pressure carbon dioxide, partial pressure
119 oxygen, soluble oxygen, bicarbonate, and base excess. Age-specific values were utilized to
120 establish normal ranges along with the machine reference intervals (18-20).

121

122 Necropsy. Necropsies were performed after euthanasia via sodium pentobarbital overdose,
123 confirmation of death, and exsanguination by femoral artery laceration. Tissue collection
124 included the following, individually and split between 10% neutral-buffered formalin and fresh
125 tissue: skeletal muscle, abdominal fat, liver, spleen, pancreas, duodenum, jejunum, ileum, spiral
126 colon, kidney, gastrohepatic and mesenteric lymph nodes, right cranial lung lobe, right middle
127 lung lobe, right caudal lung lobe, left cranial lung lobe, left caudal lung lobe, trachea, heart,
128 tracheobronchial lymph nodes, cervical spinal cord, meninges, cerebrum, cerebellum, brainstem,
129 olfactory bulb, nasal turbinates, submandibular lymph nodes, tonsil, trigeminal ganglion, and the
130 entire eye. The reproductive tract (uterus and ovaries) were collected en bloc in female animals.
131 Epiglottis and laryngeal folds were collected from some animals. The following were also
132 collected at necropsy: cerebrospinal fluid, urine (when possible), vitreous, and bronchoalveolar
133 lavage (BAL) utilizing Dulbecco's Modified Eagle's Medium (DMEM).

134

135 Histopathology. Tissues were fixed in 10% neutral phosphate buffered formalin, routinely
136 processed, sectioned at 5 μ m and stained with hematoxylin and eosin (HE) for histopathologic
137 examination.

138

139 In situ hybridization. 5 μ m paraffin-embedded formalin fixed tissue sections were ran
140 according to the user manual for the RNAscope® 2.5HD Detection Reagent – Red kit by
141 Advanced Cell Diagnostics (ACD) using the V-nCoV2019-S probe from ACD. The sections
142 were then counter stained with Gill's 1 hematoxylin, dried and coverslipped.

143

144 Cells and virus. Second passage of SARS-CoV-2 (generously provided by the Public Health
145 Agency of Canada, hCoV-19/Canada/ON-VIDO-01/2020) was propagated on Vero E6 cells in
146 DMEM supplemented with 1% FBS. Virus was titrated by plaque assay and viral isolation was
147 performed as previously described (21, 22).

148

149 Tissue homogenization and virus isolation. Pre-weighed, frozen tissue sections in Precellys
150 bead mill tubes were thawed and D- PBS was added to make 10% w/v tissue homogenates.
151 Tubes were processed using a Bertin Minilys personal tissue homogenizer and clarified by
152 centrifugation at 2000 xg. Clarified homogenates, swabs and fluids collected from experimental
153 animals were inactivated with TriPure Reagent (Roche) and extracted in duplicate as described
154 below. Semi-quantitative real-time RT-PCR (RT-qPCR) positive samples were tested for virus
155 isolation through standard plaque assay on VE6 cells using freshly prepared homogenates of
156 frozen tissue.

157

158 RNA Extraction. Total RNA from cell culture or experimental samples was extracted using
159 the MagMax CORE Nucleic Acid Purification Kit per manufacturer's recommendation with the
160 following modifications. Briefly; sample was diluted in TriPure Reagent at a 1:9 ratio and used
161 in place of the manufacturer's lysis buffer for inactivation. 650 μ L of TriPure-inactivated
162 sample, 30 μ L of binding beads, and 350 μ L of kit-provided CORE binding buffer spiked with
163 Enteroviral armoured RNA was utilized followed by single washes in both Wash 1 and Wash 2
164 buffers, with a final elution volume of 30 μ L of kit-supplied elution buffer using the automated
165 MagMax Express 96 system running the KingFisher-96 Heated Script "MaxMAX_CORE_KF-

166 96” (ThermoFisher Scientific, 2020). The spiked Enteroviral armoured RNA (ARM-ENTERO;
167 Asuragen) was used as an exogenous extraction and reaction control.

168

169 Detection of SARS-CoV-2. RT-qPCR was performed on all extracted samples including
170 sodium citrate whole blood using SARS-CoV-2 Envelope (E) gene specific primers and probe
171 (23). RT-qPCR was utilized to detect viral RNA using the following primers and probe:
172 E_SARBECO_F1 FOR: 5'-ACAGGTACGTTAATAGTTAATAGCGT-3', E_SARBECO_R2
173 REV: 5'-ATATTGCAGCAGTACGCACACA-3', and E_SARBECO-P1 Probe: 5'-
174 AACTAGCCATCCTTACTGCGCTTCG-3'. Mastermix for RT-qPCR was prepared using 4X
175 TaqMan® Fast Virus 1-step master mix according to manufacturer’s specifications, using 0.4
176 µM of each E gene primer and 0.2 µM of probe per reaction. Reaction conditions were as
177 follows: 50°C for 5 min, 95°C for 20 sec, and 40 cycles of 95°C for 3s followed by 60°C for 30s.
178 Runs were performed using a 7500 Fast Real-Time PCR System (Thermofisher, ABI) and semi-
179 quantitative results were calculated based on a gBlock (Integrated DNA Technologies, IDT)
180 standard curve for SARS-CoV-2 E gene. SARS-CoV-2 specific primers were used for
181 confirmation targeting both the Spike gene (S gene); SARS2_Spike_Probe:
182 5'TGCCACCTTTGCTCACAGATGAAATGA-3', SARS2_Spike_FOR:5'-
183 TGATTGCCTTGGTGATATTGCT-3', SARS2_Spike_REV:
184 5'CGCTAACAGTGCAGAAGTGTATTGA-3' and the RNA dependent RNA Polymerase gene
185 (RdRp gene) RdRp_SARSr-F 5'-GTGARATGGTCATGTGTGGCGG-3' RdRp_SARSr-R
186 5'CARATGTTAAASACACTATTAGCATA-3' and RdRp_SARSr-P2 5'-
187 CAGGTGGAACCTCATCAGGAGATGC-3'.

188

189 Genome sequencing. Extracted RNA from the Submandibular lymph node of pig 20-06 was
190 processed for high-throughput sequencing with enrichment for sequences for vertebrate viruses
191 according to previously published method and sequenced on an Illumina MiSeq Reagent Kit v3
192 (600-cycle) (1, 24). Sequences were analysed using an in-house nf-villumina (v2.0.0) Nextflow
193 workflow that performed: read quality filtering with fastp; taxonomic classification with
194 Centrifuge and Kraken2 using a Centrifuge index of NCBI nt downloaded 2020-02-04 and a
195 Kraken2 index of NCBI RefSeq sequences of archaea, bacteria, viral and the human genome
196 GRCh38 downloaded and built on 2019-03-22; removal of non-viral reads (NCBI taxonomic id
197 10239) using the Kraken2 and Centrifuge taxonomic classification results; de novo assembly by
198 Shovill, Unicycler and Megahit using the taxonomically filtered reads; nucleotide BLAST search
199 of all assembled contigs against NCBI nucleotide downloaded 2020-04-10. nf-villumina
200 taxonomically filtered reads were mapped against the top viral nucleotide BLAST match (SARS-
201 CoV-2 isolate 2019-nCoV/USA-CA3/2020, MT027062.1) to generate a consensus sequence.

202
203 Serum Neutralization Assays. Neutralizing antibody titers in sera were determined via
204 plaque reduction neutralization test against SARS-CoV-2. Serial five-fold dilutions of heat
205 inactivated (30 min at 56°C) sera were incubated with virus for 1 hour at 37°C. Each virus-serum
206 mixture was then added to duplicate wells of Vero E6 cells in a 48-well format, incubated for 1
207 hour at 37°C, and overlaid with 500 µl of 2.0% carboxymethylcellulose in DMEM per well.
208 Plates were then incubated at 37°C for 72 hours, fixed with 10% buffered formalin, and stained
209 with 0.5% crystal violet. Serum dilutions resulting in >70% reduction of plaque counts compared
210 to virus controls were considered positive for virus neutralization.

211

212 Surrogate virus Neutralization Test. Detection and semi-quantitation of neutralizing
213 antibodies was determined using SARS-CoV-2 Surrogate Virus Neutralization Test Kit
214 (Genscript, Cat. No.: L00847). Testing of sera was performed as outlined in the manufacturer's
215 instructions. All sera were assessed from 7 DPI through 29 DPI including archived negative sera
216 and kit-supplied negative controls. Neutralization was determined positive when above the
217 recommended 20% cutoff.

218

219 **Results**

220 Experimental inoculation of sixteen eight week old swine was performed oronasally with
221 1×10^6 pfu of SARS-CoV-2, distributed evenly between both nostrils and the distal pharynx.
222 Starting at 1 day post inoculation (DPI), pigs developed a mild, bilateral ocular discharge and in
223 some cases, this was accompanied by serous nasal secretion. This was observed for only the first
224 three days post inoculation. Temperatures remained normal throughout the study (Table S1).
225 Overall, animals did not develop clinically observable respiratory distress, however one animal
226 (Pig 20-06) presented mild depression at 1 DPI accompanied with a cough which was maintained
227 through 4 DPI. This animal did not display additional clinical signs over the course of the study.

228

229 Viral shedding was evaluated to identify potential transmission which may occur through
230 droplets from coughing, sneezing, oral fluids or gastrointestinal involvement. A sampling
231 schedule was developed with the goal of determining the incidence of viral shedding (Table 1).
232 Every other day starting at 3 DPI to 15 DPI, oral, nasal, and rectal swabs were sampled to
233 evaluate the potential for delayed onset (1). Nucleic acid was extracted from swabs and RT-
234 qPCR was performed to identify SARS-CoV-2 by targeting the envelope gene (E gene). Viral

235 RNA could not be detected in swabs from any animals over the course of the study (Table 2, A).
236 Nasal washes are a sensitive method for detection of pathogens in swine and were routinely
237 sampled using sterile D-PBS to rinse nasal passages. Two pigs (20-10, 20-11) displayed low
238 levels of viral RNA by RT-qPCR at 3 DPI (Table 2, A). Recovery of live virus was attempted for
239 both PCR-positive nasal wash samples, however neither produced cytopathic effect or increased
240 RNA detection via RT-qPCR of the cell culture supernatant. A third method for the detection of
241 viral shedding was performed using a non-invasive, group sampling method. A cotton rope was
242 hung on the pen prior to feeding which allows for deposit of oral fluids. Daily fluids from ropes
243 were processed, with one room (Table 1, Cubicle 1) a weak positive signal for viral RNA at 3
244 DPI by RT-qPCR. Virus isolation was attempted from this sample, but similar to nasal washes,
245 virus was not successfully isolated. It is important to note the positive oral fluid from cubicle 1 is
246 not from the same room as the two positive nasal washes generated from Pigs 20-10 and 20-11
247 as the later were in different animal cubicles. Therefore, at minimum three animals provide
248 evidence of viral nucleic acid in oronasal secretions from two independent animal rooms. Of
249 note, two naïve pigs were introduced to the infected pigs at 10 DPI as transmission contacts,
250 however no indication of viral infection could be detected from these animals at any point during
251 the study.

252
253 Detection of SARS-CoV-2 was also attempted from whole blood by RT-qPCR, following
254 the sampling schedule outlined in Table 1. As outlined in Table 2A, viremia, as indicated by the
255 presence of viral RNA in the blood, could not be detected in any animal throughout the study.
256 Blood cell counts, chemistries, and gasses were measured using the Abaxis HM5, VetScan 2, and
257 iSTAT respectively. Although some variation was observed throughout the study, changes were

258 minimal and inconclusive, and profiles consistent with acute viral infection or subsequent organ
259 damage were not observed.

260

261 To identify potential target tissues or gross lesions consistent with SARS-CoV-2 disease,
262 necropsy was performed on two animals starting at 3 DPI and every other day up to day 15; with
263 an additional two pigs necropsied at both 22 and 29 DPI (Table 1). No significant pathology was
264 observed which could be directly attributed to a viral infection. RT-qPCR was performed across
265 all tissues and samples collected at necropsy targeting the E gene of SARS-CoV-2 (Table 2, C).
266 One tissue, the submandibular lymph node tested from Pig 20-06 necropsied at 13 DPI was
267 positive for viral RNA. The tissue sample was repeated in triplicate, on independent days,
268 generating consistent results. Further, RNA was extracted from homogenized tissue and full
269 genome sequence of SARS-CoV-2 was recovered. A 10% homogenate was generated from the
270 submandibular lymph node and used to infect Vero E6 cells. Aliquots were taken from cell
271 culture at 2DPI and 3DPI to monitor viral replication as indicated by an increasing quantity of
272 RNA. Mild CPE was observed by 3DPI in the first passage with an increase in viral RNA
273 measured by RT-qPCR targeting the envelope, spike and RNA dependent RNA polymerase
274 genes. The first passage supernatant was clarified by centrifugation and passaged a second time
275 in Vero E6 cells. At 2DPI of the second passage, significant CPE was exhibited in addition to
276 increasing copies of SARS-CoV-2 viral RNA confirmed by RT-qPCR. Together this
277 demonstrated the presence of live, replication-competent SARS-CoV-2 virus isolated from the
278 submandibular lymph node of Pig 20-06 (Table 2).

279

280 The development of SARS-CoV-2 neutralizing antibodies were monitored over the
281 course of study. Starting at 7 DPI, serum was obtained from individual animals for both virus
282 neutralization test (traditional VNT) and a surrogate virus neutralization test (sVNT; Genscript).
283 Sera was first tested using a traditional VNT, with one pig (20-07) generating neutralizing
284 antibody titers, albeit weak, at a 1:5 dilution with a 70% reduction of plaques at both 13 and 15
285 DPI (Table 3). Consequently, the sVNT assay identified the same animal, Pig 20-07, as antibody
286 positive with 0.188 µg/ml antibody at 15 DPI. A second pig (20-14) was shown to have
287 generated antibody at 11 DPI (0.113 µg/ml) and 13 DPI (0.224 µg/ml). The sVNT was also
288 employed to identify secreted antibody in oral fluids throughout the study. Interestingly, at 6 DPI
289 we detected positive antibody (0.133 µg/ml) from group oral fluid collected from cubicle 1
290 (Table 3).

291

292 **Discussion**

293 The results presented in this study define domestic swine as a susceptible species albeit at
294 low levels to SARS-CoV-2 viral infection. One animal was found to retain live virus, while two
295 additional animals had detectable RNA measured in the nasal wash, and two pigs developed
296 antibodies. In total, of the sixteen animals experimentally inoculated, five displayed some level
297 of exposure or elicited an immune response to the virus, representing roughly 30% of the study
298 cohort. One pig displayed mild, non-specific clinical signs, including coughing and depression in
299 addition to multiple pigs demonstrating mild ocular and nasal discharge. These signs occurred
300 during what could be considered to be the immediate, post-infection period. Over a nine day
301 period, between cessation of clinical signs and post mortem evaluation, the virus was found to be
302 maintained undetected in the submandibular lymph node in this animal. Importantly, of the five

303 animals indicated potential infection, viral RNA was only detected at low levels and no live viral
304 shedding was identified.

305

306 Following the detection of viral RNA in group oral fluids collected by rope chews at 3
307 DPI, the presence of secreted antibody was detected using a surrogate virus neutralization test
308 (sVNT) at 6 DPI in the same sample type. The amount of antibody measured in oral fluids from
309 swine would be considered below a protective cutoff based on comparisons to classical
310 neutralizing titers, however the discovery of secreted antibody in oral fluids may be a useful tool
311 for surveillance efforts. This also demonstrates the possibility that human saliva should be
312 evaluated as a less invasive method to provide accompanying evidence with serosurveillance
313 studies for exposure to SARS-CoV-2.

314

315 The results of this study contradict previous reports indicating swine are not susceptible
316 to SARS-CoV-2 infection (4, 25). RNA was not detected in swabs or organ samples and no
317 seroconversion was measured in these studies. Infectious dose, viral isolate, age, and breed or
318 colony of swine may affect study outcomes. It should be noted in this work, a ten-fold higher
319 viral dose was utilized for experimental infection compared to previous studies. Moreover,
320 animals were obtained from a high health status farm in Manitoba, in contrast to a specific
321 pathogen free colony, for the purpose of determining the risk to Canadian pigs. Altogether, these
322 findings indicate that further investigations into the susceptibility of additional domestic
323 livestock species should be studied to assess their risk. Finally, we emphasize that no cases of
324 domestic livestock have been documented by natural infection to date; however, the results of

325 this study support further investigations in to the role that animals may play in the maintenance
326 and spread of SARS-CoV-2.

327

328

329 **Acknowledgments**

330 We would like to thank the Public Health Agency of Canada for SARS-CoV-2 isolate for
331 this study, in addition the Animal Care and Genomics units for their support during this project.

332 We would also like to thank Dr. Claire Andreasen for her review of the clinical pathology

333 findings. **Funding:** for this project was provided by the Canadian Food Inspection Agency. C.

334 Lewis is funded through the United States Department of Agriculture Animal Plant Health

335 Inspection Service's National Bio- and Agro-defense Facility Scientist Training Program.

336 **Author contributions:** B.P. conceived the research. B.P, G.S., M.M.P., E.M, P.M., and C.E.L.

337 performed the experiments. B.P, G.S., M.M.P., C.E.H and C.E.L. analysed the data. B.P. wrote

338 the manuscript, with input from B.P, G.S., M.M.P., E.M, and C.E.L. All authors discussed the

339 results and reviewed the manuscript. Competing interests: Authors declare no competing

340 interests. Data and materials availability: all data is available in the manuscript or the

341 supplementary materials.

342

343 **Biographical Sketch**

344 Brad Pickering is the Head of the Special Pathogens Unit at the National Centre for Foreign

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346 consequence pathogens including both emerging and re-emerging zoonotic diseases of veterinary

347 importance.

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405

406 **Table 1: Sampling and necropsy schedule**

407

	Pig	0 DPI	3 DPI	5 DPI	7 DPI	9 DPI	11 DPI	13 DPI	15 DPI	22 DPI	29 DPI
cubicle 1	20-01	+	+*	-	-	-	-	-	-	-	-
	20-02	+	+	+*	-	-	-	-	-	-	-
	20-03	+	+	+	+*	-	-	-	-	-	-
	20-04	+	+	+	+	+*	-	-	-	-	-
	20-05	+	+	+	+	+	+*	-	-	-	-
	20-06	+	+	+	+	+	+	+*	-	-	-
	20-07	+	+	+	+	+	+	+	+*	-	-
	20-08	+	+	+	+	+	+	+	+	+*	-
cubicle 2	20-09	+	+*	-	-	-	-	-	-	-	-
	20-10	+	+	+*	-	-	-	-	-	-	-
	20-11	+	+	+	+*	-	-	-	-	-	-
	20-12	+	+	+	+	+*	-	-	-	-	-
	20-13	+	+	+	+	+	+*	-	-	-	-
	20-14	+	+	+	+	+	+	+*	-	-	-
	20-15	+	+	+	+	+	+	+	+*	-	-
	20-16	+	+	+	+	+	+	+	+	+	+*
contact animals	20-17	+	+	+	+	+^	+	+	+	+	+*
	20-18	+	+	+	+	+^	+	+	+	+*	-
	20-19	+	+	+	+	+*	-	-	-	-	-

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* indicates necropsy

412 **Table 2: Detection of SARS-CoV-2 by real-time RT-PCR**

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414 **A**

	0 DPI	3 DPI	5 DPI	5 DPI	9 DPI	11 DPI	13 DPI	15 DPI	22 DPI	29 DPI
Swabs	0\57	0\57	0\51	0\45	0\39	0\30	0\24	0\18	0\12	0\6
Nasal Wash	0\16	2\16	0\14	0\12	0\12	0\10	0\8	0\6	0\4	0\2
Blood	0\16	0\16	0\14	0\12	0\12	0\10	0\8	0\6	0\4	0\2

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416 **B**

	0 DPI	1 DPI	2 DPI	3 DPI	4 DPI	5 DPI	6 DPI	7 DPI	8 DPI	9 DPI	10 DPI	11 DPI	12 DPI	13 DPI	14 DPI
Oral fluids	0\2	0\2	0\2	1\2	0\2	0\2	0\2	0\2	0\2	0\2	0\2	0\2	0\2	0\2	0\2
	15 DPI	16 DPI	17 DPI	18 DPI	19 DPI	20 DPI	21 DPI	22 DPI	23 DPI	24 DPI	25 DPI	26 DPI	27 DPI	28 DPI	29 DPI
Oral fluids	0\2	0\2	0\2	0\2	0\2	0\2	0\2	0\1	0\1	0\1	0\1	0\1	0\1	0\1	0\1

417

418 **C**

	3 DPI		5 DPI		7 DPI		9 DPI		11 DPI		13 DPI		15 DPI		22 DPI		29 DPI	
Pig ID	20-01	20-09	20-02	20-10	20-03	20-11	20-04	20-12	20-05	20-13	20-06	20-14	20-07	20-15	20-08	20-18	20-16	20-17
no. tissues	0\35	0\35	0\36	0\35	0\35	0\35	0\35	0\34	0\35	0\36	1\35	0\35	0\36	0\35	0\36	0\35	0\35	0\35

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420 *** grey cells indicate positive RT-qPCR**

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Table 3: Neutralizing antibody development

Pig	0 DPI		7 DPI		9 DPI		11 DPI		13 DPI		15 DPI		22 DPI		29 DPI	
	VNT (1:5)	sVNT (µg/ml)	VNT (1:5)	sVNT (µg/ml)	VNT (1:5)	sVNT (µg/ml)	VNT (1:5)	sVNT (µg/ml)	VNT (1:5)	sVNT (µg/ml)	VNT (1:5)	sVNT (µg/ml)	VNT (1:5)	sVNT (µg/ml)	VNT (1:5)	sVNT (µg/ml)
20-01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-07	-	-	-	-	-	-	-	-	+	-	+	0.188	-	-	-	-
20-08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-14	-	-	-	-	-	-	-	0.113	-	0.224	-	-	-	-	-	-
20-15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
sVNT (µg/ml)																
	0 DPI	1 DPI	2 DPI	3 DPI	4 DPI	5 DPI	6 DPI	7 DPI	8 DPI	9 DPI	10 DPI	11 DPI	12 DPI	13 DPI	14 DPI	15 DPI
Oral Fluid	-	-	-	-	-	-	0.113	-	-	-	-	-	-	-	-	-

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