

1 **SARS-CoV-2 is transmitted via contact and via the air between ferrets.**

2

3 Mathilde Richard<sup>1</sup>, Adinda Kok<sup>1</sup>, Dennis de Meulder<sup>1</sup>, Theo M. Bestebroer<sup>1</sup>, Mart M. Lamers<sup>1</sup>,

4 Nisreen M.A. Okba<sup>1</sup>, Martje Fentener van Vlissingen<sup>2</sup>, Barry Rockx<sup>1</sup>, Bart L. Haagmans<sup>1</sup>, Marion P.G.

5 Koopmans<sup>1</sup>, Ron A.M. Fouchier<sup>1</sup>, Sander Herfst<sup>1\*</sup>.

6

7 <sup>1</sup>Department of Viroscience, Erasmus University Medical Center, Rotterdam, the Netherlands.

8 <sup>2</sup>Erasmus Laboratory Animal Science Center, Erasmus University Medical Center, Rotterdam, the

9 Netherlands.

10 \* Corresponding author: [s.herfst@erasmusmc.nl](mailto:s.herfst@erasmusmc.nl)

11

12 **SARS-CoV-2, a coronavirus that newly emerged in China in late 2019<sup>1,2</sup> and spread rapidly**  
13 **worldwide, caused the first witnessed pandemic sparked by a coronavirus. As the pandemic**  
14 **progresses, information about the modes of transmission of SARS-CoV-2 among humans is critical**  
15 **to apply appropriate infection control measures and to slow its spread. Here we show that SARS-**  
16 **CoV-2 is transmitted efficiently via direct contact and via the air (via respiratory droplets and/or**  
17 **aerosols) between ferrets. Intranasal inoculation of donor ferrets resulted in a productive upper**  
18 **respiratory tract infection and long-term shedding, up to 11 to 19 days post-inoculation. SARS-**  
19 **CoV-2 transmitted to four out of four direct contact ferrets between 1 and 3 days after exposure**  
20 **and via the air to three out of four independent indirect recipient ferrets between 3 and 7 days**  
21 **after exposure. The pattern of virus shedding in the direct contact and indirect recipient ferrets**  
22 **was similar to that of the inoculated ferrets and infectious virus was isolated from all positive**  
23 **animals, showing that ferrets were productively infected via either route. This study provides**  
24 **experimental evidence of robust transmission of SARS-CoV-2 via the air, supporting the**  
25 **implementation of community-level social distancing measures currently applied in many**  
26 **countries in the world and informing decisions on infection control measures in healthcare**  
27 **settings<sup>3</sup>.**

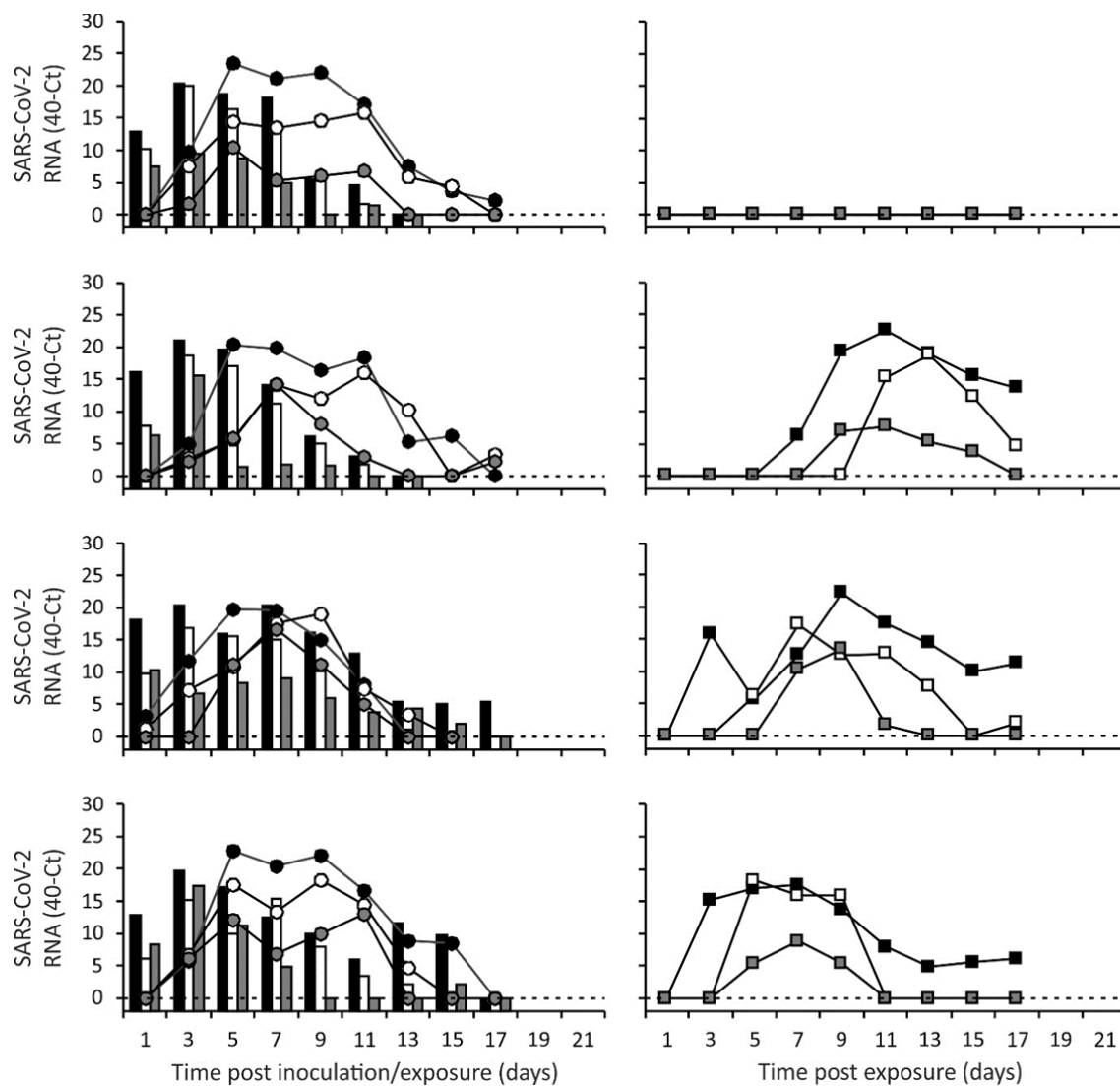
28

29 In late December 2019, clusters of patients in China presenting with pneumonia of unknown etiology  
30 were reported to the World Health Organization (WHO) <sup>1</sup>. The causative agent was rapidly identified  
31 as being a virus from the *Coronaviridae* family, closely related to the severe acute respiratory  
32 syndrome coronavirus (SARS-CoV) <sup>2,4,5</sup>. The SARS-CoV epidemic affected 26 countries and resulted in  
33 more than 8000 cases in 2003. The newly emerging coronavirus, named SARS-CoV-2 <sup>6</sup>, rapidly spread  
34 worldwide and was declared pandemic by the WHO on March 11, 2020 <sup>7</sup>. The first evidence  
35 suggesting human-to-human transmission came from the descriptions of clusters among the early  
36 cases <sup>8,9</sup>. Based on epidemiological data from China before measures were taken to control the  
37 spread of the virus, the reproductive number  $R_0$  (the number of secondary cases directly generated  
38 from each case) was estimated to be between 2 and 3 <sup>10-12</sup>. In order to apply appropriate infection  
39 control measures to reduce the  $R_0$ , the modes of transmission of SARS-CoV-2 need to be elucidated.  
40 Respiratory viruses can be transmitted via direct and indirect contact (via fomites), and through the  
41 air via respiratory droplets and/or aerosols. Transmission via respiratory droplets ( $> 5 \mu\text{m}$ ) is  
42 mediated by expelled particles that have a propensity to settle quickly and is therefore reliant on  
43 close proximity between infected and susceptible individuals, usually within 1 m of the site of  
44 expulsion. Transmission via aerosols ( $< 5 \mu\text{m}$ ) is mediated by expelled particles that are smaller in  
45 size than respiratory droplets and can remain suspended in the air for prolonged periods of time,  
46 allowing infection of susceptible individuals at a greater distance from the site of expulsion <sup>13</sup>.  
47 Current epidemiological data suggest that SARS-CoV-2 is transmitted primarily via respiratory  
48 droplets and contact <sup>8-10,14,15</sup>, which is used as the basis for mitigation of spread through physical and  
49 social distancing measures. However, scientific evidence that SARS-CoV-2 can be efficiently  
50 transmitted via the air is weak.

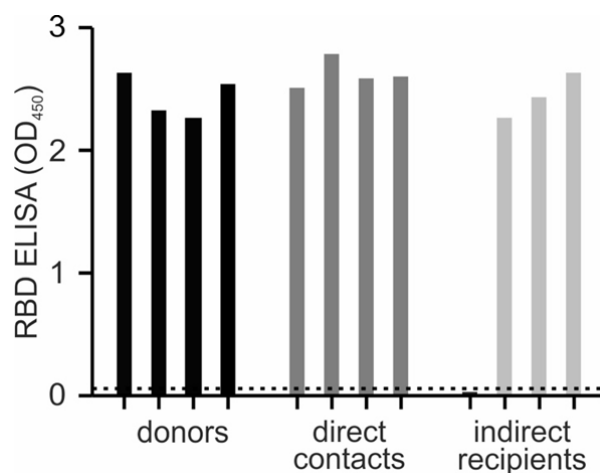
51 Previous studies have shown that ferrets were susceptible to infection with SARS-CoV <sup>16-20</sup>, and that  
52 SARS-CoV was efficiently transmitted to co-housed ferrets via direct contact <sup>16</sup>. Here, we used a  
53 ferret transmission model to assess whether SARS-CoV-2 spreads through direct contact and/or  
54 through the air (via respiratory droplets and/or aerosols). For this purpose, individually housed

55 donor ferrets were inoculated intranasally with a strain of SARS-CoV-2 isolated from a German  
56 traveller returning from China. Six hours post-inoculation (hpi), a direct contact ferret was added to  
57 each of the cages. The next day, indirect recipient ferrets were placed in adjacent cages, separated  
58 from the donor cages by two steel grids, 10 cm apart, allowing viruses to be transmitted only via the  
59 air (Supplementary Figure 1). On alternating days to prevent cross-contamination, throat, nasal and  
60 rectal swabs were collected from each ferret in the inoculated and direct contact groups and from  
61 the indirect recipient group, followed by SARS-CoV-2 detection by RT-qPCR and virus titration.  
62 Ferrets were productively infected by SARS-CoV-2 upon intranasal inoculation, as demonstrated by  
63 the robust and long-term virus shedding from the donor ferrets (Figure 1, Supplementary Figure 2).  
64 SARS-CoV-2 RNA levels peaked at 3 days post-inoculation (dpi) and were detected up to 11 dpi in  
65 two animals and up to 15 and 19 dpi in the other two animals (Figure 1, Supplementary Figure 2).  
66 SARS-CoV-2 was transmitted to direct contact ferrets in four out of four independent experiments  
67 between 1 and 3 days post-exposure (dpe) and viral RNA was detected up to 13 to 15 days (i.e. 13 to  
68 17 dpe) (Figure 1, Supplementary Figure 2). Interestingly, SARS-CoV-2 was also transmitted via the  
69 air to three out of four indirect recipient ferrets. SARS-CoV-2 RNA was detected from 3 to 7 dpe  
70 onwards these indirect recipient ferrets and for 13 to 19 days (Figure 1, Supplementary Figure 2).  
71  
72

73



82 Whereas donor ferrets were inoculated with a high virus dose, direct contact and indirect recipient  
83 ferrets are likely to have received a low infectious dose via direct contact or via the air. In spite of  
84 this, the pattern of virus shedding from the direct contact and indirect recipient ferrets was similar  
85 to that of the inoculated donor ferrets, both in terms of duration and SARS-CoV-2 RNA levels,  
86 corroborating robust replication of SARS-CoV-2 upon transmission via direct contact and via the air,  
87 independent of the infectious dose. In general, higher SARS-CoV-2 RNA levels were detected in the  
88 throat swabs as compared to the nasal swabs. SARS-CoV-2 RNA levels in the rectal swabs were  
89 overall the lowest. From each SARS-CoV-2 RNA positive animal, infectious virus was isolated in  
90 VeroE6 cells from throat and nasal swabs for at least two consecutive days (Supplementary Table 1).  
91 In contrast, no infectious virus was isolated from the rectal swabs. Infectious virus titers ranged from  
92  $10^{0.75}$  to  $10^{2.75}$  TCID<sub>50</sub>/ml (median tissue culture infectious dose per ml) in the donor ferrets, from  
93  $10^{0.75}$  to  $10^{3.5}$  TCID<sub>50</sub>/ml in the direct contact ferrets and from  $10^{0.75}$  to  $10^{4.25}$  TCID<sub>50</sub>/ml in the indirect  
94 recipient ferrets. All SARS-CoV-2 positive ferrets seroconverted 21 dpi/dpe, and the antibody levels  
95 were similar in donor, direct contact and indirect recipient ferrets (Figure 2). The indirect recipient  
96 ferret, in which no SARS-CoV-2 was detected, did not seroconvert as expected.  
97



98

99 **Figure 2. Antibody responses in donor, direct contact and indirect recipient ferrets at 21 dpi/dpe.**

100 Sera were collected from the donor, direct contact and indirect recipient ferrets at 21 dpi/dpe and  
101 IgG responses were assessed using a SARS-CoV-2 receptor binding domain (RBD) ELISA. The dotted  
102 line indicates the background of the assay.

103

104 SARS-CoV-2 transmission in experimental animal models has recently also been described by others.  
105 SARS-CoV-2 direct contact transmission between ferrets<sup>21</sup> and hamsters<sup>22</sup> was reported, with  
106 similar efficiency as observed in our study. In addition, SARS-CoV-2 was also found to be transmitted  
107 via the air in two out of six ferrets<sup>21</sup>, and in two out of six cats<sup>23</sup>. However, only low levels of SARS-  
108 CoV-2 RNA were detected in nasal washes and feces of the indirect recipient ferrets, and no  
109 infectious virus was isolated<sup>21</sup>. Furthermore, virus shedding was shorter as compared to the donor  
110 animals and only one out of the two SARS-CoV-2 RNA positive indirect recipient ferrets  
111 seroconverted. Similarly, the transmission via the air between cats was not efficient. SARS-CoV-2  
112 RNA was detected in the feces and tissues of one cat at 3 and 11 dpi respectively and in nasal  
113 washes of another cat, but no infectious virus was isolated. Both SARS-CoV-2 RNA positive indirect  
114 recipient cats seroconverted. In contrast, the present study showed that SARS-CoV-2 was efficiently  
115 transmitted via the air between ferrets, as demonstrated by long-term virus shedding and the  
116 presence of infectious virus in the indirect recipient animals, which is comparable to the  
117 transmissibility of pandemic influenza viruses in the ferret model<sup>24</sup>.

118 To date, there is no evidence of fecal-oral transmission of SARS-CoV-2 in humans. However, the  
119 prolonged detection of RNA in consecutive stool samples<sup>25</sup> and the environmental contamination of  
120 sanitary equipment<sup>26</sup> may suggest that the fecal-oral route could be a potential route of  
121 transmission of SARS-CoV-2. Here, no infectious virus was retrieved from any of the rectal swabs.  
122 Despite this, it cannot be fully excluded that SARS-CoV-2 was also transmitted from donors to direct  
123 contact ferrets partly via the fecal-oral route. In the study by Kim *et al.*, ferret fecal material was  
124 used to inoculate ferrets, resulting in a productive infection, indicating that infectious SARS-CoV-2  
125 was shed in fecal specimens<sup>21</sup>.

126 Our experimental system does not allow to assess whether SARS-CoV-2 was transmitted via the air  
127 through respiratory droplets, aerosols or both, as donor and indirect recipient ferret cages are  
128 placed only 10 cm apart from each other. In a recent study, SARS-CoV-2 remained infectious in  
129 aerosols for at least 3h after aerosolization at high titers in a rotating drum, comparable to SARS-CoV  
130<sup>27</sup>. Although it is informative to compare the stability of different respiratory viruses in the air, our  
131 study provides the additional information that infectious SARS-CoV-2 particles can actually be  
132 expelled in the air and subsequently infect recipients. In two other studies, the presence of SARS-  
133 CoV-2 in air samples collected in hospital settings was investigated. However, no SARS-CoV-2 RNA  
134 was detected in the air sampled in three isolation rooms<sup>26</sup>, or 10 cm from a symptomatic patient  
135 who was breathing, coughing or speaking<sup>28</sup>. Nevertheless, RNA was detected on the air exhaust  
136 outlet of one of the isolation rooms in the first study, suggesting that virus-laden droplets may be  
137 displaced by airflows<sup>26</sup>.

138 Here we provide the first experimental evidence that SARS-CoV-2 can be transmitted efficiently via  
139 the air between ferrets, resulting in a productive infection and the detection of infectious virus in  
140 indirect recipients, as a model for human-to-human transmission. Although additional experiments  
141 on the relative contribution of respiratory droplets and aerosols to the transmission of SARS-CoV-2  
142 are warranted, the results of this study corroborate the WHO recommendations about transmission  
143 precautions in health care settings and the social distancing measures implemented in many



144 countries around the globe to mitigate the spread <sup>3</sup>. The ferret transmission model will also be  
145 useful to understand transmission dynamics and the molecular basis of the transmissibility of SARS-  
146 Cov-2 and other betacoronaviruses, which, in the context of the current SARS-CoV-2 pandemic and  
147 future pandemic threats, is clearly of utmost importance.  
148

149 **References**

- 150 1 WHO. *Pneumonia of unknown cause – China*, <[https://www.who.int/csr/don/05-january-](https://www.who.int/csr/don/05-january-2020-pneumonia-of-unknown-cause-china/en/)  
151 [2020-pneumonia-of-unknown-cause-china/en/](https://www.who.int/csr/don/05-january-2020-pneumonia-of-unknown-cause-china/en/)> (2020).
- 152 2 Zhu, N. *et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J*  
153 *Med* **382**, 727-733, doi:10.1056/NEJMoa2001017 (2020).
- 154 3 WHO. *Modes of transmission of virus causing COVID-19: implications for IPC precaution*  
155 *recommendations*, <[https://www.who.int/news-room/commentaries/detail/modes-of-](https://www.who.int/news-room/commentaries/detail/modes-of-transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations)  
156 [transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations](https://www.who.int/news-room/commentaries/detail/modes-of-transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations)>  
157 (2020).
- 158 4 WHO. *Novel Coronavirus – China*, <[https://www.who.int/csr/don/12-january-2020-novel-](https://www.who.int/csr/don/12-january-2020-novel-coronavirus-china/en/)  
159 [coronavirus-china/en/](https://www.who.int/csr/don/12-january-2020-novel-coronavirus-china/en/)> (2020).
- 160 5 Zhou, P. *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat  
161 origin. *Nature* **579**, 270-273, doi:10.1038/s41586-020-2012-7 (2020).
- 162 6 Coronaviridae Study Group of the International Committee on Taxonomy of, V. The species  
163 Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it  
164 SARS-CoV-2. *Nat Microbiol* **5**, 536-544, doi:10.1038/s41564-020-0695-z (2020).
- 165 7 WHO. *WHO Director-General's opening remarks at the media briefing on COVID-19 - 11*  
166 *March 2020*, <[https://www.who.int/dg/speeches/detail/who-director-general-s-opening-](https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020)  
167 [remarks-at-the-media-briefing-on-covid-19---11-march-2020](https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020)> (2020).
- 168 8 Chan, J. F. *et al.* A familial cluster of pneumonia associated with the 2019 novel coronavirus  
169 indicating person-to-person transmission: a study of a family cluster. *Lancet* **395**, 514-523,  
170 doi:10.1016/S0140-6736(20)30154-9 (2020).
- 171 9 Huang, C. *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan,  
172 China. *Lancet* **395**, 497-506, doi:10.1016/S0140-6736(20)30183-5 (2020).
- 173 10 Li, Q. *et al.* Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected  
174 Pneumonia. *N Engl J Med* **382**, 1199-1207, doi:10.1056/NEJMoa2001316 (2020).

- 175 11 Riou, J. & Althaus, C. L. Pattern of early human-to-human transmission of Wuhan 2019 novel  
176 coronavirus (2019-nCoV), December 2019 to January 2020. *Euro Surveill* **25**,  
177 doi:10.2807/1560-7917.ES.2020.25.4.2000058 (2020).
- 178 12 Wu, J. T., Leung, K. & Leung, G. M. Nowcasting and forecasting the potential domestic and  
179 international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling  
180 study. *Lancet* **395**, 689-697, doi:10.1016/S0140-6736(20)30260-9 (2020).
- 181 13 Kutter, J. S., Spronken, M. I., Fraaij, P. L., Fouchier, R. A. & Herfst, S. Transmission routes of  
182 respiratory viruses among humans. *Curr Opin Virol* **28**, 142-151,  
183 doi:10.1016/j.coviro.2018.01.001 (2018).
- 184 14 Liu, J. *et al.* Community Transmission of Severe Acute Respiratory Syndrome Coronavirus 2,  
185 Shenzhen, China, 2020. *Emerg Infect Dis* **26**, doi:10.3201/eid2606.200239 (2020).
- 186 15 Burke, R. M. *et al.* Active Monitoring of Persons Exposed to Patients with Confirmed COVID-  
187 19 - United States, January-February 2020. *MMWR Morb Mortal Wkly Rep* **69**, 245-246,  
188 doi:10.15585/mmwr.mm6909e1 (2020).
- 189 16 Martina, B. E. *et al.* Virology: SARS virus infection of cats and ferrets. *Nature* **425**, 915,  
190 doi:10.1038/425915a (2003).
- 191 17 ter Meulen, J. *et al.* Human monoclonal antibody as prophylaxis for SARS coronavirus  
192 infection in ferrets. *Lancet* **363**, 2139-2141, doi:10.1016/S0140-6736(04)16506-9 (2004).
- 193 18 Weingartl, H. *et al.* Immunization with modified vaccinia virus Ankara-based recombinant  
194 vaccine against severe acute respiratory syndrome is associated with enhanced hepatitis in  
195 ferrets. *J Virol* **78**, 12672-12676, doi:10.1128/JVI.78.22.12672-12676.2004 (2004).
- 196 19 Chu, Y. K. *et al.* The SARS-CoV ferret model in an infection-challenge study. *Virology* **374**,  
197 151-163, doi:10.1016/j.virol.2007.12.032 (2008).
- 198 20 van den Brand, J. M. *et al.* Pathology of experimental SARS coronavirus infection in cats and  
199 ferrets. *Vet Pathol* **45**, 551-562, doi:10.1354/vp.45-4-551 (2008).

- 200 21 Kim, Y. I. *et al.* Infection and Rapid Transmission of SARS-CoV-2 in Ferrets. *Cell Host Microbe*,  
201 doi:10.1016/j.chom.2020.03.023 (2020).
- 202 22 Chan, J. F. *et al.* Simulation of the clinical and pathological manifestations of Coronavirus  
203 Disease 2019 (COVID-19) in golden Syrian hamster model: implications for disease  
204 pathogenesis and transmissibility. *Clin Infect Dis*, doi:10.1093/cid/ciaa325 (2020).
- 205 23 Shi, J. *et al.* Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-  
206 coronavirus 2. *Science*, doi:<https://doi.org/10.1126/science.abb7015> (2020).
- 207 24 Munster, V. J. *et al.* Pathogenesis and transmission of swine-origin 2009 A(H1N1) influenza  
208 virus in ferrets. *Science* **325**, 481-483, doi:10.1126/science.1177127 (2009).
- 209 25 Wu, Y. *et al.* Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet*  
210 *Gastroenterol Hepatol*, doi:10.1016/S2468-1253(20)30083-2 (2020).
- 211 26 Ong, S. W. X. *et al.* Air, Surface Environmental, and Personal Protective Equipment  
212 Contamination by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) From a  
213 Symptomatic Patient. *JAMA*, doi:10.1001/jama.2020.3227 (2020).
- 214 27 van Doremalen, N. *et al.* Aerosol and Surface Stability of SARS-CoV-2 as Compared with  
215 SARS-CoV-1. *N Engl J Med*, doi:10.1056/NEJMc2004973 (2020).
- 216 28 Cheng, V. C. C. *et al.* Escalating infection control response to the rapidly evolving  
217 epidemiology of the Coronavirus disease 2019 (COVID-19) due to SARS-CoV-2 in Hong Kong.  
218 *Infect Control Hosp Epidemiol*, 1-24, doi:10.1017/ice.2020.58 (2020).
- 219 29 van Doornum, G. J., Schutten, M., Voermans, J., Guldemeester, G. J. & Niesters, H. G.  
220 Development and implementation of real-time nucleic acid amplification for the detection of  
221 enterovirus infections in comparison to rapid culture of various clinical specimens. *J Med*  
222 *Virol* **79**, 1868-1876, doi:10.1002/jmv.21031 (2007).
- 223 30 Corman, V. M. *et al.* Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR.  
224 *Euro Surveill* **25**, doi:10.2807/1560-7917.ES.2020.25.3.2000045 (2020).

225 31 Okba, N. M. A. *et al.* Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody  
226 Responses in Coronavirus Disease 2019 Patients. *Emerg Infect Dis* **26**,  
227 doi:10.3201/eid2607.200841 (2020).  
228  
229

230 **Methods**

231 **Virus and cells**

232 SARS-CoV-2 (isolate BetaCoV/Munich/BavPat1/2020; kindly provided by Prof. Dr. C. Drosten) was  
233 propagated to passage 3 on VeroE6 cells (ATCC) in Opti-MEM I (1X) + GlutaMAX (Gibco),  
234 supplemented with penicillin (10,000 IU mL<sup>-1</sup>, Lonza) and streptomycin (10,000 IU mL<sup>-1</sup>, Lonza) at  
235 37°C in a humidified CO<sub>2</sub> incubator. VeroE6 cells were inoculated at an moi of 0.01. Supernatant was  
236 harvested 72 hpi, cleared by centrifugation and stored at -80°C. VeroE6 cells were maintained in  
237 DMEM (Gibco) supplemented with 10% foetal calf serum (Greiner), 2 mM of L-glutamine (Gibco),  
238 10 mM Hepes (Lonza), 1.5 mg ml<sup>-1</sup> sodium bicarbonate (NaHCO<sub>3</sub>, Lonza), penicillin (10,000 IU/mL)  
239 and streptomycin (10,000 IU/mL) at 37°C in a humidified CO<sub>2</sub> incubator. All work was performed in a  
240 Class II Biosafety Cabinet under BSL-3 conditions at the Erasmus Medical Center.

241 **Ferret transmission experiment**

242 All relevant ethical regulations for animal testing have been complied with. Animals were housed  
243 and experiments were performed in strict compliance with the Dutch legislation for the protection  
244 of animals used for scientific purposes (2014, implementing EU Directive 2010/63). Influenza virus,  
245 SARS-CoV-2 and Aleutian Disease Virus seronegative 6 month-old female ferrets (*Mustela putorius*  
246 *furo*), weighing 700–1000 g, were obtained from a commercial breeder (TripleF (USA)). Research was  
247 conducted under a project license from the Dutch competent authority (license number  
248 AVD1010020174312) and the study protocol was approved by the institutional Animal Welfare Body  
249 (Erasmus MC permit number 17-4312-02). Animal welfare was monitored on a daily basis. Virus  
250 inoculation of ferrets was performed under anesthesia with a mixture of ketamine/medetomidine  
251 (10 and 0.05 mg kg<sup>-1</sup> respectively) antagonized by atipamezole (0.25 mg kg<sup>-1</sup>). Swabs were taken  
252 under light anesthesia using ketamine to minimize animal discomfort.

253 Four donor ferrets were inoculated intranasally with  $6 \cdot 10^5$  TCID<sub>50</sub> of SARS-CoV-2 virus diluted in 500  
254  $\mu$ l of phosphate-buffered saline (PBS) (250  $\mu$ l instilled dropwise in each nostril) and were housed  
255 individually in a cage. Six hpi, direct contact ferrets were placed in the same cage as the donor  
256 ferrets. One day later, indirect recipient ferrets were placed in an opposite cage separated by two  
257 steel grids, 10 cm apart, to avoid contact transmission (Figure S1). Throat, nasal and rectal swabs  
258 were collected from the animals every other day, to prevent cross-contamination, until they were  
259 negative for SARS-CoV-2 RNA or maximum for 21 dpi/dpe by determined by real-time RT-qPCR as  
260 described below. Swabs were stored at  $-80$  °C in transport medium (Minimum Essential Medium  
261 Eagle with Hank's BSS (Lonza), 5 g L<sup>-1</sup> lactalbumine enzymatic hydrolysate, 10% glycerol (Sigma-  
262 Aldrich), 200 U ml<sup>-1</sup> of penicillin, 200 mg ml<sup>-1</sup> of streptomycin, 100 U ml<sup>-1</sup> of polymyxin B sulfate  
263 (Sigma-Aldrich), and 250 mg ml<sup>-1</sup> of gentamicin (Life Technologies)) for end-point titration in VeroE6  
264 cells as described below. Ferrets were euthanized at 21 dpi/dpe by heart puncture under  
265 anaesthesia. Blood was collected in serum-separating tubes (Greiner) and processed according to  
266 the manufacturer's instructions. Sera were heated for 1h at 60 °C and used for the detection of  
267 specific antibodies against SARS-CoV-2 as described below. [All animal experiments were performed](#)  
268 [in class III isolators in a negatively pressurized ABSL3+ facility.](#)

#### 269 **RNA isolation and RT-qPCR**

270 RNA was isolated using an in-housed developed high-throughput method in a 96-well format. Sixty  
271  $\mu$ l of sample were added to 90  $\mu$ l of MagNA Pure 96 External Lysis Buffer (Roche). A known  
272 concentration of phocine distemper virus (PDV) was added to the sample as internal control for the  
273 RNA extraction<sup>29</sup>. The 150  $\mu$ l of sample/lysis buffer was added to a well of a 96-well plate containing  
274 50  $\mu$ l of magnetic beads (AMPure XP, Beckman Coulter). After thorough mixing by pipetting up and  
275 down at least 10 times, the plate was incubated for 15 minutes (min) at room temperature. The  
276 plate was then placed on a magnetic block (DynaMag™-96 Side Skirted Magnet  
277 (ThermoFisher Scientific)) and incubated for 3 min to allow the displacement of the beads towards  
278 the side of the magnet. Supernatants were carefully removed without touching the beads and beads

279 were washed three times for 30 seconds (sec) at room temperature with 200  $\mu$ l/well of 70% ethanol.  
280 After the last wash, a 10  $\mu$ l multi-channel pipet was used to remove residual ethanol. Plates were  
281 air-dried for 6 min at room temperature. Plates were removed from the magnetic block and 30  $\mu$ l of  
282 PCR grade water was added to each well and mixed by pipetting up and down 10 times. Plates were  
283 incubated for 5 min at room temperature and then placed back on the magnetic block for 2 min to  
284 allow separation of the beads. Supernatants were pipetted in a new plate and RNA was kept at 4°C.  
285 The RNA was directly used for RT-qPCR using primers and probes targeting the E gene of SARS-CoV-2  
286 as previously described<sup>30</sup>. The primers and probe for PDV detection were described previously<sup>29</sup>.

### 287 **Virus titrations**

288 Throat, nasal and rectal swabs were titrated in quadruplicates in VeroE6 cells. Briefly, confluent  
289 VeroE6 cells were inoculated with 10-fold serial dilutions of sample in Opti-MEM I (1X) + GlutaMAX,  
290 supplemented with penicillin (10,000 IU mL<sup>-1</sup>), streptomycin (10,000 IU mL<sup>-1</sup>). At one hpi, the first  
291 three dilutions were washed twice with media and fresh media was subsequently added to the  
292 whole plate. At six dpi, virus positivity was assessed by reading out cytopathic effects. Infectious  
293 virus titers (TCID<sub>50</sub>/ml) were calculated from four replicates of each throat, nasal and rectal swabs  
294 and from 24 replicates of the virus stock using the Spearman-Kärber method.

### 295 **Serology**

296 Sera were tested for SARS-CoV-2 antibodies using a receptor binding domain (RBD) enzyme-linked  
297 immunosorbent assay (ELISA) as described previously, with some modifications<sup>31</sup>. Briefly, ELISA  
298 plates were coated overnight with either SARS-CoV-2 RBD. After blocking, sera were added and  
299 incubated for 1h at 37°C. Bound antibodies were detected using horseradish peroxidase (HRP)-  
300 labelled goat anti-ferret IgG (Abcam) and 3,3',5,5'-Tetramethylbenzidine (TMB, Life Technologies) as  
301 a substrate. The absorbance of each sample was measured at 450 nm.



302 **Data availability**

303 All data are available from the corresponding author (S.H.) on reasonable request.

304 No custom software was used in this study.

305

306 **Acknowledgements**

307 We thank Prof. Dr. Christian Drosten (Charité – Universitätsmedizin Berlin) for providing the SARS-

308 CoV-2 isolate used in this study and Drs Rik de Swart and Mathieu Sommers for their help with

309 animal ethics and study approval. This work was supported by European Union's *Horizon 2020*

310 research and innovation program *VetBioNet* (grant agreement No *731014*) and NIH/NIAID (contract

311 number HHSN272201400008C). S.H. was funded in part by an NWO VIDI grant (contract number

312 91715372).

313

314 **Author Contributions**

315 M.R. and S.H. conceived, designed, analysed and performed the work. M.R. and S.H. wrote the

316 manuscript. A.K., D.M., T.B., M.L., N.O. helped with performing the work. M.F.V., B.R., B.H., M.K.,

317 R.A.M.F. helped with the design of the work, interpretation of the data and manuscript revision. All

318 authors read and approved the final manuscript.

319

320 The authors declare no competing interests.

321

322 **Additional information**

323 Supplementary Information is available for this paper.

324 Correspondence and requests for materials should be addressed to Sander Herfst:

325 [s.herfst@erasmusmc.nl](mailto:s.herfst@erasmusmc.nl)