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1 **Manuscript Number** (if known)

2 **Article Summary Line:** Following widespread infection with SARS-CoV-2 of mink on a farm,
3 all tested animals had seroconverted and the farm was then tested free of infection; however, less
4 than 3 months later, a further round of infection affected more than 75% of tested animals.

5 **Running Title:** Re-infection of mink with SARS-CoV-2

6 **Keywords:** Whole genome sequencing; coronavirus; serology; re-infection; *Neovison vison*

7

8 **Title:** Infection, recovery and re-infection of farmed mink with SARS-CoV-2

9

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23 **Abstract—word count:150**

24 Mink, on a farm with about 15,000 animals, became infected with SARS-CoV-2. Over
25 75% of tested animals were positive for SARS-CoV-2 RNA in throat swabs and 100% of tested
26 animals were seropositive. The virus responsible had a deletion of nucleotides encoding residues
27 H69 and V70 within the spike protein gene. The infected mink recovered and after free-testing of
28 the mink, the animals remained seropositive. During follow-up studies, after a period of more
29 than 2 months without virus detection, over 75% of tested animals scored positive again for
30 SARS-CoV-2 RNA. Whole genome sequencing showed that the virus circulating during this re-
31 infection was most closely related to the virus identified in the first outbreak on this farm but
32 additional sequence changes had occurred. Animals had much higher levels of anti-SARS-CoV-2
33 antibodies after re-infection than at free-testing. Thus, following recovery from an initial
34 infection, seropositive mink rapidly became susceptible to re-infection by SARS-CoV-2.

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36 **Text—word count:** 3296 (limit 3500)

37 **Introduction**

38 The SARS-CoV-2 has caused a pandemic and contributed to the deaths of over 2 million
39 people (1). Farmed mink (*Neovison vison*) are also highly susceptible to infection by SARS-
40 CoV-2 (2, 3). As in humans, the infection in mink can cause respiratory distress and, in some
41 cases, mortality. However, often the proportion of infected mink that show clinical disease is
42 low. Cases of SARS-CoV-2 infection in farmed mink were initially observed in the Netherlands
43 (NL), in April 2020 (3), and then independently in Denmark (DK) in June 2020 (note, different
44 clades of the virus were involved, see (2)). Outbreaks have continued and about 70 farms in the
45 NL have been infected (4) while 290 farms out of about 1200 mink farms in DK were positive

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46 for the virus (5). All mink (>15,000,000) have now been culled in DK (6). Similarly, the
47 termination of mink farming in the NL was brought forward by 3 years from the previously
48 planned date of 1st January 2024 (4). The routes of transmission of the virus between mink farms
49 are not fully understood (5) but it has become apparent that spread of the virus can occur not
50 only from humans to mink but also from mink to humans (2, 7).

51 After the initial cases of SARS-CoV-2 infection in mink in DK, on Farms 1-3 in Northern
52 Jutland (as described in (2)), a regular screening program was established to test dead mink from
53 all Danish mink farms for the presence of SARS-CoV-2, every 3rd week (6). Infection of mink on
54 Farm 4 was identified through this Early Warning (EW) program but, in contrast to Farms 1-3,
55 the mink were not culled and the seropositive animals apparently cleared the infection. This
56 allowed an evaluation of the duration and efficacy of the immune response in mink to protect
57 against re-infection.

58 **Results**

59 *Infection of mink on Farm 4*

60 Farm 4 (with about 2400 adult mink and 12600 kits housed in 24 open sheds), was
61 located near Hjørring (also in Northern Jutland) and was tested as part of the EW screening
62 program. On 20th July 2020, as part of this system, 5 dead mink from this farm were tested for
63 the virus and all were RT-qPCR negative. However, on 11th August, a further 5 dead mink were
64 tested and all were positive in this assay (Table 1). In follow-up testing, on 13th August, 23 of 30
65 live mink tested (16 adults and 14 kits) were positive. A further 7 (of 10) dead mink also tested
66 positive. All live mink tested (30 kits and 30 adults) were also strongly seropositive on 19th
67 August, but a reduced proportion of the mink (13 of these 60 mink tested) were positive for

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68 SARS-CoV-2 RNA. However, throat swab samples from 21 dead mink were all positive for viral
69 RNA. Furthermore, on 31st August, 7 out of 24 dead mink also tested positive by RT-qPCR. The
70 mink on the farm were not culled but closely followed and, from 15th September onwards, no
71 virus was detected by RT-qPCR among the mink. For “free-testing”, 300 animals were tested (in
72 60 pools of 5 samples) on 30th September (Table 1) with negative results. This testing strategy
73 was designed to detect, with 95% confidence, a 1% prevalence of SARS-CoV-2 RNA positive
74 animals. Hence, the infection had apparently disappeared among the mink on this farm.

75 Surveillance of the farm continued and, in early October, 60 live mink were tested and
76 were again all found negative by RT-qPCR but all these mink remained seropositive (Table 1).
77 Thus, no animals tested positive by RT-qPCR in September and October but there was a very
78 high (100%) prevalence of antibodies against anti-SARS-CoV-2. However, unexpectedly, 1 of 2
79 pools of 5 dead mink tested, as part of the continuing EW program, on both 2nd and 4th
80 November were found to be positive by RT-qPCR (Table 1). Consequently, a further 30 animals
81 were tested on 6th November and 23 (77%) were found SARS-CoV-2 RNA positive while 100%
82 of these animals remained seropositive, as observed one month previously. In addition, 3 out of 5
83 additional dead mink were found positive by RT-qPCR. The Ct values for 15 of the 23 samples
84 that were found positive for viral RNA were below 30. No specific clinical signs of respiratory
85 disease were apparent on the farm, however the farmer had noticed reduced feed intake and some
86 cases of diarrhea in the mink.

87 Titration, in the ELISA, of the anti-SARS-CoV-2 antibodies in seropositive serum
88 samples collected from August onwards showed that much higher levels of these antibodies were
89 present in the mink in November, following the second round of infection than in August or
90 October (see Figure 1A) although the seroprevalence in the mink had been high throughout. In

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91 August, the median antibody titre observed (from 16 animals tested) was 800 (with values
92 ranging from 100 to 3200), with just a single animal having the highest titre. In early October (at
93 free-testing), the titres in 15 sera tested were higher (ranging from 800 to 12800, with 4 of the
94 sera having titres of ≥ 6400 , the median titre was 3200). However, in November, following the
95 reappearance of RT-qPCR positive animals, from 22 sera tested, 16 of them had titres ≥ 6400 and
96 12 had titres of 25600, see Figure 1A). Thus, it is clear that the anti-SARS-CoV-2 antibody
97 levels, as measured by the ELISA, in the mink were greatly enhanced following the re-infection.
98 It was apparent that in some individual animals no change in antibody levels were apparent in
99 November, presumably because not all the animals had been re-infected.

100 *Assessment of neutralizing antibodies*

101 To assess whether the anti-SARS-CoV-2 antibodies in the mink that were detected by
102 ELISA were capable of neutralizing virus infectivity, the same serum samples were also tested in
103 virus neutralization assays using a human SARS-CoV-2 isolate that had the same amino acid
104 changes in the spike protein as the viruses identified initially on Farm 4 (as used previously (8)).
105 The results (see Figure 1B) showed a similar pattern of antibody development as observed in the
106 ELISA. All the ELISA positive sera tested had neutralization activity but the levels of these
107 antibodies were greatly elevated after the second round of infection (sera collected in
108 November). There was a high degree of correspondence between the levels of antibodies
109 detected in the two different types of assay (for all samples, the Spearman correlation co-
110 efficient $r = 0.793$, $p < 0.0001$).

111 *Whole genome sequencing of viruses on Farm 4.*

112 The complete genome sequences of the viruses, from multiple samples, from infected
113 mink on Farm 4 in August and in November were determined. The viruses present on Farm 4 in

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114 August were all from clade 20B and were very closely related to the viruses that were identified
115 on Farms 1, 2 and 3 (2) (Table 2 and Figure 2) and appeared to be part of the same transmission
116 chain. In particular, they each had the mutation A22920T in the spike protein coding sequence,
117 resulting in the amino acid substitution Y453F, which is a hallmark of most viruses that have
118 infected mink in DK. This change was also detected on farm NB02, one of four farms initially
119 infected in NL, however, this was within a different virus clade (see (2, 3)). However, in
120 addition to this change, the spike protein gene in the viruses on Farm 4 also had a deletion of 6 nt
121 (Δ 21766-21771). This deletion affects 3 separate codons, changing GCT.ATA.CAT.GTC.TCT to
122 GCT.ATC.TCT, the encoded amino acid sequence is changed from A-I-H⁶⁹-V⁷⁰-S to A-I-S thus
123 residues H69 and V70 in the N-terminal domain (NTD) of the spike protein are lost. This
124 deletion had not been identified previously in mink or in humans in combination with the Y453F
125 substitution (see Table 2) but the deletion of these residues is shared with the SARS CoV-2
126 variant of concern (VOC) 202012/01 (9). Two other deletions in the ORF1a coding sequence
127 (Δ 517-519 and Δ 6510-6512) and two other amino acid substitutions (P3395S in ORF1a and
128 S2430I in ORF1b) were also observed in some of the viruses present in the mink during this
129 initial infection in August. The viruses present on Farm 4 in November were most closely
130 related to those seen previously on Farm 4, over 2 months earlier (Figure 2). It should be noted
131 that, by November 2020, over 200 farms in DK had been identified as having infected mink (5)
132 and a number of different variants had been observed in the animals (6). The viruses on Farms 1-
133 3 were closely related to each other and also to the viruses present in August on Farm 4, but the
134 latter viruses had some additional changes (e.g. the deletion of residues H69 and V70 in the spike
135 protein, see Table 2), which persisted throughout the rest of the outbreaks in farmed mink. Thus,
136 viruses in farms infected after Farm 4 (identified on August 11th) were nearly all derived from

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137 those first detected on Farm 4. As indicated above, the November viruses from Farm 4 had the
138 A22920T mutation and the deletions in the S and ORF1a coding sequences. However, the
139 November viruses had additional changes across the genome, both within and outside of the S
140 gene, compared to the viruses in Farms 2 and 3 (Table 2). It is noteworthy that the Farm 4
141 sequences in November had changes at nt 10448 (encoding the substitution P3395S in ORF1a)
142 and 20756 (encoding S2430I in ORF1b) that had only been seen in a subset of the August
143 sequences from Farm 4 (samples Farm4_18_13-08-2020 and Farm4_19_13-08-2020, see Figure
144 2). These changes act as a fingerprint and strongly suggest that it was not an entirely new
145 introduction of virus into the farm from elsewhere. Furthermore, the viruses on Farm 4 in
146 November also all shared changes at nt 3792 (resulting in A1176V), 5167, 10887 (resulting in
147 G3541E), 21727 and 23815 (the latter two silent changes are in the S gene) that were not present
148 in any of the Farm 4 sequences in August (Table 2). The presence of these additional sequence
149 changes indicates that the virus had been replicating in hosts with close connection to this farm
150 between August and November but does not prove that the virus has continued to replicate in
151 mink during this time.

152 Phylogenetic analysis clearly showed that all viruses from Farm 4 were very closely
153 related to each other, including the viruses from both August and November (Figure 2). As
154 described above, two of the early Farm 4 viruses (Farm4_18_13-08-2020 and Farm4_19_13-08-
155 2020) shared additional changes at nt 10448 and 20756 (see Table 2) and the November viruses
156 formed their own distinct branch from these (Figure 2), due to the presence of the further
157 sequence changes (Table 2).

158 **Discussion**

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159 SARS-CoV-2 can readily infect humans and mink. In addition, certain other species, e.g.
160 cats, dogs and ferrets, can also be infected following direct inoculation under experimental
161 conditions (10, 11). Furthermore, some cases of transmission from infected people to their cats
162 and dogs have occurred but it does not seem to happen more generally. Both cellular and
163 humoral immune responses occur within SARS-CoV-2-infected people and animals (12, 13) and
164 it is common for both humans and animals to be both seropositive and RT-qPCR positive
165 simultaneously (see (2, 14)). However, as people and animals recover, the levels of virus subside
166 but antibody levels persist, or increase, at least for some time.

167 Farm 4 was the only Danish mink farm, where the animals were allowed to recover and
168 were tested with the purpose of documenting freedom from SARS-CoV-2 infection. Thus, Farm
169 4 gave a unique opportunity to follow the maintenance of anti-SARS-CoV-2 antibodies over an
170 extended period and the resistance of the animals to reinfection. As observed on other mink
171 farms in DK (5), very widespread infection of the mink on Farm 4 by SARS-CoV-2 occurred in
172 the first wave of infection, with 100 % of the tested animals being seropositive. As indicated
173 above, the mink on Farm 4 were not culled after the detection of infection in August but during
174 the following period of over 2 months, the animals were repeatedly screened and found to be
175 negative by RT-qPCR, while the 100% seroprevalence remained. However, in November, it was
176 observed that the mink had become infected again. A high proportion (>75%) of the animals
177 tested had been re-infected by SARS-CoV-2 (Table 1). The virus responsible for the second
178 round of infection was most closely related to the virus found almost 3 months earlier on this
179 farm, with distinctive differences from the viruses responsible for the initial infections observed
180 in mink on Farms 1-3 (2). Notably, specific deletions were present within the spike protein gene
181 and within the ORF1a gene in the virus responsible for the initial infection in August and in the

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182 later re-infection (Table 2). The virus acquired additional sequence changes during the period
183 between the infections recognized in August and November, indicative of continued replication,
184 rather than simply having been preserved in an infectious form. Since the virus present on Farm
185 4 in November was most closely related to virus present on the same farm in August, it seems
186 most likely that re-infection of the mink from within the farm had occurred. It cannot be
187 established, however, whether the virus had continued to replicate in a small number of mink on
188 the farm, but with very restricted spread, or if it had replicated in an alternative host, linked to
189 the farm, during this time and had then been re-introduced into the seropositive mink. It has been
190 demonstrated, in both DK and the NL, that transmission between humans and mink can occur in
191 both directions (2, 6). Transmission to, and from, other hosts is theoretically possible (but not
192 described previously; some cats were found to be infected on mink farms in DK and in the NL
193 but they do not seem to spread the virus). It has been found that there was a cluster of
194 occurrences of SARS-CoV-2 with the Δ H69/N70 and Y453F changes (as in Farm 4) in the local
195 human population in August. Furthermore, a virus containing these changes plus the additional
196 mutations (i.e. C3792T, C5167T, G10887A, C21727T and T23815C, see Table 2), which were
197 present in the mink viruses from Farm 4 in November, was found in one person in the first half
198 of November (data not shown). It seems likely that these human cases were infections derived
199 from the mink.

200 A high proportion of the sequence changes observed in mink (see Table 2), which
201 occurred in the viruses from Farm 4 between August and November (and also between the clade
202 20B viruses and the Wuhan virus, see (2)), involved C to T changes (in cDNA) that correspond
203 to C to U changes in the viral RNA. Several of these nt changes are synonymous, i.e., they do not
204 result in amino acid sequence changes. It has been suggested that such changes reflect host

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205 immune pressure via RNA editing systems (e.g. by APOBEC) rather than selection for increased
206 transmissibility in particular hosts (15, 16, 17). However, this process of RNA editing is not
207 relevant to the key mutation in the S gene (A22920T), which seems to be an adaptation that
208 occurred during the initial infection of mink (2), or to the generation of deletions. The loss of
209 residues H69 and V70 in the spike protein, seen in mink for the first time on Farm 4, and in
210 certain variants from people, has been reported to double the infectivity of pseudoviruses
211 displaying the mutant spike protein compared to the wild type particles (18).

212 The sampling of the mink on Farm 4 tested, at most, 300 animals on any particular date,
213 out of a population of about 15,000 animals. The free-testing strategy was designed to detect 1%
214 prevalence with high (95%) confidence. It is clearly possible that a small number of infected
215 animals were missed although the repeated follow-up screening makes this unlikely. However,
216 the level of seroprevalence prior to the second round of infection had remained very high (100%)
217 in the animals tested. Thus, it is not clear why so many animals (77% of 30 animals tested) were
218 susceptible to a second round of infection. It has been considered whether the seropositivity
219 detected in kits in August may be a consequence of maternally derived antibodies that could
220 potentially decline more rapidly than antibodies generated from the infection in each animal.
221 However, it seems difficult to reconcile this with the fact that >80% of throat swabs from mink
222 kits tested clearly positive by RT-qPCR in August, which indicated a high level of infection
223 amongst the kits in the first wave also.

224 The measurements of antibody responses were made using an ELISA that targets the
225 receptor binding domain (RBD) of the spike protein. Antibodies to the SARS-CoV-2 spike
226 protein were present in up to 100% of the infected mink. The antibody titres, measured in this
227 assay, increased to very high levels during the period of re-infection (see Figure 1A). In studies

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228 on human sera, samples testing clearly positive (10 x cut-off) in this ELISA all had neutralizing
229 antibodies (19). Indeed, assessment of the same samples of mink sera as tested by ELISA in
230 virus neutralization tests indicated a high correspondence between these two types of assay.
231 Thus, the ELISA positive mink sera neutralized the virus and, furthermore, the sera collected in
232 November, after reinfection, had much higher levels of anti-SARS-CoV-2 antibodies as
233 measured in each assay (Figure 1B).

234 It appears that the virus responsible for the infections in November was not antigenically
235 distinct from the virus in August since there were no non-synonymous changes within the spike
236 protein gene during this time, although some silent sequence changes (i.e. C21727T and
237 T23815C) had occurred, as well as changes elsewhere, within the virus genome, as usually
238 occurs.

239 The most plausible conclusion is that infection of farmed mink with SARS-CoV-2 does
240 not induce long-term protection against the virus. This should be compared with the situation in
241 rhesus macaques where primary infection did protect against reinfection at about 1 month post-
242 initial infection (13, 20) and in humans where protection from reinfection may last at least eight
243 months (12, 21). However, some cases of re-infection have been reported in health care workers
244 in Brazil (22), although this seems to have occurred in people who only developed a weak
245 immune response during the initial infection. Furthermore, only about 50% of people in DK,
246 who were over 65 years of age and had been infected with SARS-CoV-2, were found to be
247 protected against re-infection (23). On a mink farm, a large number of animals live in close
248 proximity to each other and, potentially, once the infection occurs in some animals then there can
249 be a rapid increase in virus production and a strong challenge to neighbouring animals. Perhaps
250 this is sufficient to overcome the immune response. It is notable that greatly enhanced levels of

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251 anti-SARS-CoV-2 antibodies were detected in the mink following the second round of infection
252 (Figure 1), but this was also observed following challenge of previously infected rhesus
253 macaques which did not become re-infected (13, 20). Currently, there are no “correlates of
254 protection” that can be used to evaluate the immune responses in mink.

255 **Methods**

256 Blood and throat-swab samples were collected from mink (adults and kits) as indicated in
257 Table 1. The presence of SARS-CoV-2 RNA was determined by RT-qPCR (2). The SARS-CoV-
258 2 Ab ELISA (Beijing Wantai Biological Pharmacy Enterprise, Beijing, China) was performed as
259 described by the manufacturer, with the addition of an extra titration of positive samples.
260 Antibody titres are presented as the reciprocal of the highest dilution of the serum giving a
261 positive result. Neutralising antibody titres were determined as described previously (8). SARS-
262 CoV-2 positive RNA samples were sequenced as described (2) and SARS-CoV-2 sequences
263 were aligned using MAFFT (24). Phylogenetic analysis was performed using the Maximum
264 Likelihood method with the General-Time-Reversible model (25).

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270 **Author Bio**

271 Dr. Thomas Bruun Rasmussen, a senior researcher at the Statens Serum Institut in Copenhagen,
272 is a veterinary virologist with special interest and expertise in molecular methods applied to
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287

288

289 **References**

290 1. Johns Hopkins University coronavirus resource center, <https://coronavirus.jhu.edu/>

291

292 2. Hammer AS, Quaade ML, Rasmussen TB, Fonager J, Rasmussen M, Mundbjerg K, et
293 al. SARS-CoV-2 Transmission between Mink (*Neovison vison*) and Humans, Denmark.
294 Emerg Infect Dis. 2021;27(2):547-51. doi: 10.3201/eid2702.203794.

295

296 3. Oreshkova N, Molenaar RJ, Vreman S, Harders F, Munnink BBO, Hakze-van der
297 Honing RW et al. SARS-CoV-2 infection in farmed minks, the Netherlands, April and
298 May 2020. Euro Surveill. 2020;25:2001005. [https://doi.org/10.2807/1560-](https://doi.org/10.2807/1560-7917.ES.2020.25.23.2001005)
299 [7917.ES.2020.25.23.2001005](https://doi.org/10.2807/1560-7917.ES.2020.25.23.2001005)

300

301 4. <https://promedmail.org/promed-posts/id=20201204.7994061>

302

Reserved space. Do not place any text in this section. Include the mandatory author checklist or your manuscript will be returned. Use *continuous* line numbering in your manuscript.

- 303 5. Boklund A, Hammer AS, Quaade ML, Rasmussen TB, Lohse L, Strandbygaard B, et al.
304 SARS-CoV-2 in Danish Mink Farms: Course of the Epidemic and a Descriptive Analysis
305 of the Outbreaks in 2020. *Animals (Basel)*. 2021;11(1):164. [doi: 10.3390/ani11010164](https://doi.org/10.3390/ani11010164).
306
- 307 6. Larsen HD, Fonager J, Lomholt FK, Dalby T, Benedetti G, Kristensen B, et al.
308 Preliminary report of an outbreak of SARS-CoV-2 in mink and mink farmers associated
309 with community spread, Denmark, June-November 2020. *Euro Surveill*. 2021;26(5). [doi:](https://doi.org/10.2807/1560-7917.ES.2021.26.5.210009)
310 [10.2807/1560-7917.ES.2021.26.5.210009](https://doi.org/10.2807/1560-7917.ES.2021.26.5.210009)
311
- 312 7. Oude Munnink BB, Sikkema RS, Nieuwenhuijse DF, Molenaar RJ, Munger E,
313 Molenkamp R, et al. Transmission of SARS-CoV-2 on mink farms between humans and
314 mink and back to humans. *Science*. 2020 Nov 10:eabe5901. [doi:](https://doi.org/10.1126/science.abe5901)
315 [10.1126/science.abe5901](https://doi.org/10.1126/science.abe5901).
316
- 317 8. Lassaunière, R, Fonager, J, Rasmussen, M, Frische, A, Polacek, C, Rasmussen, TB, et al.
318 *In vitro* characterization of fitness and convalescent antibody neutralisation of SARS-
319 CoV-2 Cluster 5 variant emerging in mink at Danish farms. (submitted).
320
- 321 9. Bal A, Destras G, Gaymard A, Stefic K, Marlet J, Eymieux S, et al. Two-step strategy for
322 the identification of SARS-CoV-2 variant of concern 202012/01 and other variants with
323 spike deletion H69-V70, France, August to December 2020. *Euro Surveill*. 2021
324 Jan;26(3):2100008. [doi: 10.2807/1560-7917.ES.2021.26.3.2100008](https://doi.org/10.2807/1560-7917.ES.2021.26.3.2100008).
325

Reserved space. Do not place any text in this section. Include the mandatory author checklist or your manuscript will be returned. Use *continuous* line numbering in your manuscript.

- 326 10. Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, et al. Susceptibility of ferrets, cats,
327 dogs, and other domesticated animals to SARS–coronavirus 2. *Science*. 2020;368:1016-
328 20. <https://doi.org/10.1126/science.abb7015>
329
- 330 11. Schlottau K, Rissmann M, Graaf A, Schön J, Sehl J, Wylezich C, et al. SARS-CoV-2 in
331 fruit bats, ferrets, pigs, and chickens: an experimental transmission study. *The Lancet*
332 *Microbe*. 2020; 1(5):e218-25. [https://doi.org/10.1016/S2666-5247\(20\)30089-6](https://doi.org/10.1016/S2666-5247(20)30089-6)
333
- 334 12. Hartley GE, Edwards ESJ, Aui PM, Varese N, Stojanovic S, McMahon J, et al.. Rapid
335 generation of durable B cell memory to SARS-CoV-2 spike and nucleocapsid proteins in
336 COVID-19 and convalescence. *Sci Immunol*. 2020;5(54):eabf8891. [doi:
337 10.1126/sciimmunol.abf8891.](https://doi.org/10.1126/sciimmunol.abf8891)
338
- 339 13. Chandrashekar A, Liu J, Martinot AJ, McMahan K, Mercado NB, Peter L, et al. SARS-
340 CoV-2 infection protects against rechallenge in rhesus macaques. *Science*. 2020
341 369(6505):812-17. [doi: 10.1126/science.abc4776.](https://doi.org/10.1126/science.abc4776)
342
- 343 14. Hung IF, Cheng VC, Li X, Tam AR, Hung DL, Chiu KH, et al. SARS-CoV-2 shedding
344 and seroconversion among passengers quarantined after disembarking a cruise ship: a
345 case series. *Lancet Infect Dis*. 2020;20(9):1051-60. [doi: 10.1016/S1473-3099\(20\)30364-
346 9.](https://doi.org/10.1016/S1473-3099(20)30364-9)
347

Reserved space. Do not place any text in this section. Include the mandatory author checklist or your manuscript will be returned. Use *continuous* line numbering in your manuscript.

- 348 15. Simmonds P. Rampant C→U hypermutation in the genomes of SARS-CoV-2 and other
349 coronaviruses: causes and consequences for their short- and long-term evolutionary
350 trajectories. *mSphere* 5, e00408-20 (2020).
351
- 352 16. Di Giorgio S, Martignano F, Torcia MG, Mattiuz G, Conticello SG. Evidence for host-
353 dependent RNA editing in the transcriptome of SARS-CoV-2. *Sci Adv* 2020.
354 [doi:10.1126/sciadv.abb5813](https://doi.org/10.1126/sciadv.abb5813).
355
- 356 17. van Dorp L, Richard D, Tan CCS, Shaw LP, Acman M, Balloux F. No evidence for
357 increased transmissibility from recurrent mutations in SARS-CoV-2. *Nat*
358 *Commun* 11, 5986 (2020). <https://doi.org/10.1038/s41467-020-19818-2>
359
- 360 18. Kemp SA, Collier DA, Datir RP, Ferreira IATM, Gayed S, Jahun A, et al., SARS-CoV-2
361 evolution during treatment of chronic infection. *Nature*. 2021 Feb 5. doi:
362 10.1038/s41586-021-03291-y.
363
- 364 19. GeurtsvanKessel, CH, Okba N, Igloi Z, Bogers S, Embregts C, Laksono BM, et al. An
365 evaluation of COVID-19 serological assays informs future diagnostics and exposure
366 assessment. *Nature Communications*. 2020; 11(1), 3436. [https://doi.org/10.1038/s41467-](https://doi.org/10.1038/s41467-020-17317-y)
367 [020-17317-y](https://doi.org/10.1038/s41467-020-17317-y)
368

Reserved space. Do not place any text in this section. Include the mandatory author checklist or your manuscript will be returned. Use *continuous* line numbering in your manuscript.

- 369 20. Deng W, Bao L, Liu J, Xiao C, Liu J, Xue J, et al. Primary exposure to SARS-CoV-2
370 protects against reinfection in rhesus macaques. *Science*. 2020; 369(6505):818-823. [doi:](https://doi.org/10.1126/science.abc5343)
371 [10.1126/science.abc5343](https://doi.org/10.1126/science.abc5343).
372
- 373 21. Choe PG, Kim K-H, Kang CK, Suh HJ, Kang E, Lee SY, et al. Antibody responses 8
374 months after asymptomatic or mild SARS-CoV-2 infection. *Emerg Infect Dis*.
375 2021. Epub <https://doi.org/10.3201/eid2703.204543>
376
- 377 22. Adrielle Dos Santos L, Filho PGG, Silva AMF, Santos JVG, Santos DS, Aquino MM et
378 al. Recurrent COVID-19 including evidence of reinfection and enhanced severity in thirty
379 Brazilian healthcare workers. *J Infect*. 2021:S0163-4453(21)00043-8. doi:
380 10.1016/j.jinf.2021.01.020.
381
- 382 23. Hansen CH, Michlmayr D, Gubbels SM, Mølbak K, Ethelberg S. Assessment of
383 protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals
384 in Denmark in 2020: a population-level observational study. *Lancet*. 2021:S0140-
385 6736(21)00575-4. doi: 10.1016/S0140-6736(21)00575-4
386
- 387 24. Katoh K, Standley DM. MAFFT Multiple Sequence Alignment Software Version 7:
388 Improvements in Performance and Usability. *Molecular Biology and Evolution*. 2013;
389 30(4):772–780. doi:10.1093/molbev/mst010
390

Reserved space. Do not place any text in this section. Include the mandatory author checklist or your manuscript will be returned. Use *continuous* line numbering in your manuscript.

391 25. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New
392 Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the
393 Performance of PhyML 3.0, *Systematic Biology*. 2010; 59(3):307–321,
394 doi:10.1093/sysbio/syq010

395

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401 Table 1. Summary of laboratory analysis of mink sampling from Farm 4.

| Sample origin | ELISA | | RT-qPCR | | Date of sample collection |
|-----------------|------------------------|-----|--------------------------------|-----|---------------------------|
| | Sera (positive/tested) | % | Throat swabs (positive/tested) | % | |
| Dead mink (EW) | n.d. | | 0/5 | 0 | 20-07-2020 |
| Dead mink (EW) | n.d. | | 5/5 | 100 | 11-08-2020 ¹ |
| Live adult mink | n.d. | | 11/16 | 69 | 13-08-2020 |
| Live mink kits | n.d. | | 12/14 | 86 | 13-08-2020 |
| Dead mink | n.d. | | 7/10 | 70 | 13-08-2020 |
| Live adult mink | 30/30 | 100 | 4/30 | 13 | 19-08-2020 |
| Live mink kits | 30/30 | 100 | 9/30 | 30 | 19-08-2020 |
| Dead mink | n.d. | | 21/21 | 100 | 19-08-2020 |
| Dead mink | n.d. | | 7/24 | 29 | 31-08-2020 |
| Dead mink | n.d. | | 0/31 | 0 | 15-09-2020 |
| Dead mink | n.d. | | 0/25 | 0 | 28-09-2020 |
| Live mink | n.d. | | 0/60* | 0 | 30-09-2020 |
| Live mink | 60/60 | 100 | 0/60 | 0 | 05-10-2020 |
| Dead mink (EW) | n.d. | | 1/2** | 50 | 02-11-2020 |
| Dead mink (EW) | n.d. | | 1/2** | 50 | 04-11-2020 |
| Live mink | 30/30 | 100 | 23/30 | 77 | 06-11-2020 |
| Dead mink | n.d. | | 3/5 | 60 | 06-11-2020 |

402 n.d. : not done

403 1: Samples were received at SSI on this date.

404 *300 animals were tested in pools of 5, i.e. in 60 assays.

405 ** two pools of 5 samples test

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406

407 Table 2 is in a separate file (landscape orientation)

408

409 Figure 1. Panel A. Anti-SARS-CoV-2 antibody titres measured by ELISA. Selected positive sera
410 from mink collected at the time of initial diagnosis (blue circles), at free-testing (grey circles)
411 and following re-infection (red circles), on 19-08-20, 02-10-20 and 06-11-20 respectively, were
412 titrated and assayed by ELISA. The reciprocals of the highest dilution yielding a positive signal
413 are plotted. Mean (+/- SEM) values are indicated by horizontal black lines. Panel B. The same
414 serum samples were also assayed in virus neutralization assays and the calculated antibody titres
415 are plotted using the same colour scheme.

416

417 Figure 2. Phylogenetic tree showing the relationships between the full genome sequences of
418 SARS-CoV-2 samples from Farms 1-4. Sequences from the re-infection (collected in November)
419 are indicated in red while samples collected in August are indicated in blue.

420

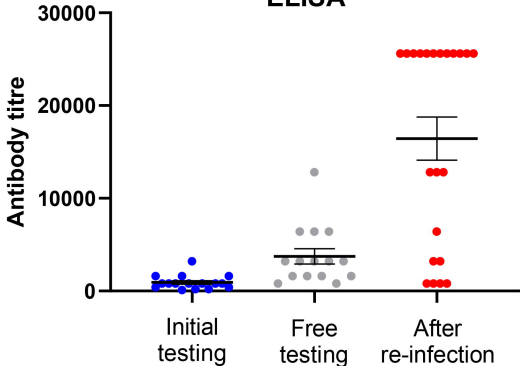
421

| Location | 5'-UTR | ORF1a | | | | ORF1b | | | S | | | ORF3a | N | |
|--------------------|--------|-------|------|--------|-------|-------|-------|--------|--------------|-------|-------|-------|-------|---|
| Nt | 241 | 3037 | 5144 | 10448 | 11776 | 14408 | 15656 | 20756 | Δ21766-21771 | 22920 | 23403 | 25936 | 28854 | other |
| Virus | | | | | | | | | | | | | | |
| Wuhan | C | C | C | C | C | C | C | G | - | A | A | C | C | |
| EPI_ISL_455326 20B | T | T | C | C | C | T | C | G | - | A | G | C | C | |
| Farm 1 | T | T | C | C | C | T | T | G | - | T/A | G | T | C | |
| Farm 2 | T | T | C | C | C | T | T | G | - | T | G | T | C | |
| Farm 3 | T | T | C | C | C | T | T | G | - | T | G | T | C | |
| Aug 2020 | | | | | | | | | | | | | | |
| Farm4_5 | T | T | T | C | T | T | T | G | + | T | G | T | T | |
| Farm4_6 | T | T | T | C | T | T | T | G | + | T | G | T | T | G488A |
| Farm4_8 | T | T | T | C | T | T | T | G | + | T | G | T | T | Δ21984-21995 |
| Farm4_18 | T | T | T | T | T | T | T | T | + | T | G | T | T | |
| Farm4_19 | T | T | T | T | T | T | T | T | + | T | G | T | T | |
| Farm4_21 | T | T | T | C | T | T | T | G | + | T | G | T | T | A652C (K129N) ¹ |
| Farm4_35 | T | T | T | C | T | T | T | G | + | T | G | T | T | Δ27982-28030 |
| Farm4_37 | T | T | T | C | T | T | T | G | + | T | G | T | T | T1873C,G2035T(L590F) |
| Nov 2020 | | | | | | | | | | | | | | |
| Farm4_1 | T | T | T | T | T | T | T | T | + | T | G | T | T | C1913T (R550C), C3792T (A1176V), C5167T, G10887A (G3541E), C21727T, T23815C |
| Farm4_14 | T | T | T | T | T | T | T | T | + | T | G | T | T | A3303G, C3792T (A1176V), C5167T, G10887A (G3541E), C21727T, T23815C |
| Farm4_15 | T | T | T | T | T | T | T | T | + | T | G | T | T | A3303G, C3792T (A1176V), C5167T, G10887A (G3541E), C21727T, T23815C |
| AA change | - | - | - | P3395S | - | P314L | T730I | S2430I | ΔH69-V70 | Y453F | D614G | H182Y | S194L | |

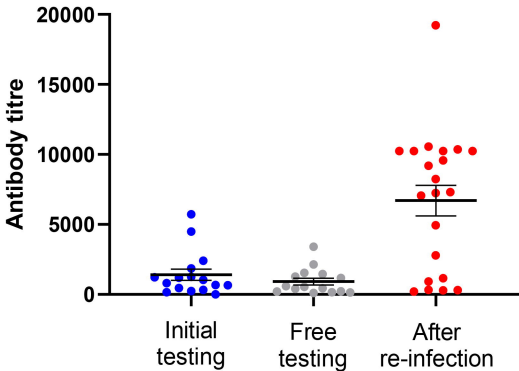
Table 2. Sequence changes within SARS-CoV-2 in mink on Farm 4.

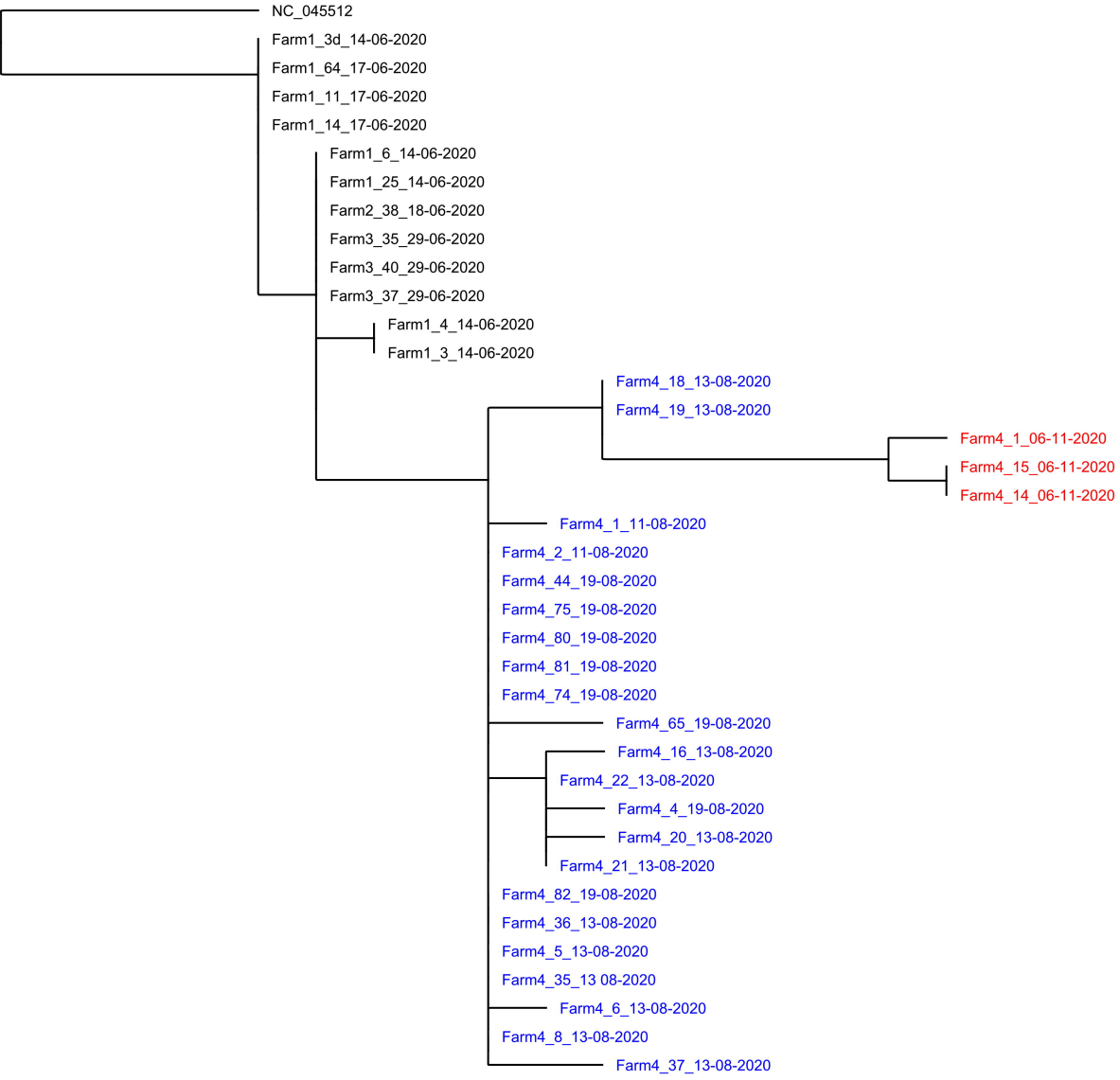
1: Note the same additional sequence change was also present in 4 other samples (Farm4_16_13-08-2020, Farm4_20_13-08-2020, Farm4_22_13-08-2020 and Farm4_4_19-08-2020). N.B. All the mink viruses, together with the EPI_ISL455326 clade 20B representative sequence, shown here were from clade 20B and had G28881A, G28882A and G28883C changes compared to the Wuhan strain. In addition, the mink viruses from Farm 4 also lacked nt 517-519 and nt 6510-6512. Other nt changes from the Wuhan reference sequence are highlighted in yellow while nt changes from the representative clade 20B virus are shown in red type. Shared additional changes that occurred in viruses on Farm 4 between August and November 2020 are indicated with colour codes, encoded amino acid changes, where applicable, are shown in parenthesis.

ELISA



VNT





7.0E-5