Jumping back and forth: anthropozoonotic and zoonotic transmission of SARS-CoV-2 on mink farms — Source link ↗

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1 **Jumping back and forth: anthropozoonotic and zoonotic**

2 **transmission of SARS-CoV-2 on mink farms**

3

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23

24 **One sentence summary**

25 SARS-CoV-2 transmission on mink farms.

26

Abstract

28   The zoonotic origin of the SARS-CoV-2 pandemic is still unknown. Animal experiments have

29   shown that non-human primates, cats, ferrets, hamsters, rabbits and bats can be infected by

30   SARS-CoV-2. In addition, SARS-CoV-2 RNA has been detected in felids, mink and dogs in the

31   field. Here, we describe an in-depth investigation of outbreaks on 16 mink farms and humans

32   living or working on these farms, using whole genome sequencing. We conclude that the virus

33   was initially introduced from humans and has evolved, most likely reflecting widespread

34   circulation among mink in the beginning of the infection period several weeks prior to

35   detection. At the moment, despite enhanced biosecurity, early warning surveillance and

36   immediate culling of infected farms, there is ongoing transmission between mink farms with

37   three big transmission clusters with unknown modes of transmission. We also describe the

38   first animal to human transmissions of SARS-CoV-2 in mink farms.

39

3

Main text

41 Late December 2019, SARS-CoV-2 was identified as the causative agent in a viral pneumonia

42 outbreak, possibly related to a seafood and a live animal market in Wuhan, China (*1*). Since

43 then, SARS-CoV-2 spread across the world and by August 31rd, over 25,200,000 people had

44 been infected with SARS-CoV-2 resulting in over 840,000 deaths (*2*). In the Netherlands, over

45 72,000 infections have been confirmed, over 6,200 SARS-CoV-2 related deaths have been

46 reported, and drastic measures have been put into place to prevent further spread of SARS-

47 CoV-2 (*3*).

48   In view of the similarities with SARS-CoV-1, a zoonotic origin of the outbreak was

49 suspected by the possible link with the Wuhan market where various animals were sold

50 including fish, shellfish, poultry, wild birds and exotic animals. The finding of cases with onset

51 of illness well before the period observed in the cluster, however, suggests the possibility of

52 other sources (*4*). Although closely related coronaviruses in bats (*5, 6*) and pangolins (*7, 8*)

53 have most sequence identity to SARS-CoV-2, the most likely diversion date of SARS-CoV-2

54 from the most closely related bat sequence is estimated to date back to somewhere between

55 1948-1982 (*9*). Therefore, the animal reservoir(s) of SARS-CoV-2 is (are) yet to be identified.

56   Experimental infections in dogs (*10*), cats (*10, 11*), ferrets (*10, 12*), hamsters (*13, 14*),

57 rhesus macaques (*15*), tree shrew (*16*), cynomolgus macaques (*17*), grivets (*18*), common

58 marmosets (*19*), rabbits (*20*), and fruit bats (*21*) have shown that these species are susceptible

59 to SARS-CoV-2, and experimentally infected cats, tree shrews, hamsters and ferrets could

60 transmit the virus. In contrast, experimental infection of pigs and several poultry species with

61 SARS-CoV-2 proved to be unsuccessful (*10, 21, 22*). SARS-CoV-2 has also sporadically been

62 identified in naturally infected animals. In the USA and in Hong Kong, SARS-CoV-2 RNA has

63 been detected in dogs (*23*). In the Netherlands, France, Hong Kong, Belgium and the USA, cats

64   have tested positive by RT-PCR for SARS-CoV-2 (*24–27*). Furthermore, SARS-CoV-2 has been

65   detected in four tigers and three lions in a zoo in New York (*28*). In Italy, the Netherlands and

66   in Wuhan, antibodies to SARS-CoV-2 have been detected in cats (*29–31*). Recently, detected

67   SARS-CoV-2 was detected in farmed mink (*Neovison vison*) that showed signs of respiratory

68   disease and increased mortality (*29*, *32*).

69   Thereafter, the Dutch national response system for zoonotic diseases was activated,

70   and it was concluded that the public health risk of animal infection with SARS-CoV-2 was low,

71   but that there was a need for increased awareness of possible involvement of animals in the

72   COVID-19 epidemic. Therefore, from May 20th 2020 onwards, mink farmers, veterinarians and

73   laboratories were obliged to report symptoms in mink (family *Mustelidae*) to the Netherlands

74   Food and Consumer Product Safety Authority (NFCPSA) and an extensive surveillance system

75   was set up (*33*).

76   Whole genome sequencing (WGS) can be used to monitor the emergence and spread

77   of pathogens (*34–37*). As part of the surveillance effort in the Netherlands over 1,750 SARS-

78   CoV-2 viruses have been sequenced to date from patients from different parts of the

79   Netherlands (*38*). Here, we describe an in-depth investigation into the SARS-CoV-2 outbreak

80   in mink farms and mink farm employees in the Netherlands, combining epidemiological

81   information, surveillance data and WGS on the human-animal interface.

82

Methods

Outbreak investigation

Following initial detection of SARS-CoV-2 in mink on two farms on April 23rd and April 25th, respectively, as part of routine health monitoring done by the Royal GD Animal Health service and subsequent investigation by Wageningen Bioveterinary Research (WBVR), the national reference laboratory for notifiable animal diseases, a One Health outbreak investigation team was convened (*39, 40*). Subsequently, respiratory signs and increased mortality in mink was made notifiable by the Dutch Ministry of Agriculture, Nature and Food Quality and the farms were quarantined (no movements of animals and manure and visitor restrictions). On May 7th two other mink farms in the same region were confirmed to be infected. By the end of May the Dutch minister of Agriculture decided that all mink on SARS-CoV-2 infected farms had to be culled. Moreover, as the clinical manifestation of the infection was highly variable within and between farms, including asymptomatic infections, weekly testing of dead animals for SARS-CoV-2 infections became compulsory for all mink farms in the Netherlands. Moreover, a nation-wide transport ban of mink and mink manure, and a strict hygiene and visitor protocol was implemented. The first infected mink farms were culled from June 6th onwards. From the 10th infected farm (NB10) onwards, culling took place within 1-3 days after diagnosis. In this manuscript, the data up to June 26th, when a total of 16 mink farms in the Netherlands were found positive for SARS-CoV-2 infections, is presented.

Veterinary and human contact tracing

The Netherlands Food and Consumer Product Safety Authority (NVWA) traced animal related contacts with other mink farms. Backward and forward tracing of possible high-risk contacts was done in the framework of the standard epidemiological investigation by the NVWA (i.e.

107 focused on movement of vehicles, visitors such as veterinary practitioners, (temporary)

108 workers, sharing of equipment between farms and transport and delivery of materials, such

109 as feed, pelts, carcasses and manure). Persons with possible exposure from this investigation,

110 as well as farm owners and resident farm workers were asked to report health complaints to

111 the municipal health service for testing and – in the case of confirmed infections – for health

112 advice and further contact tracing. Farm owners and workers on infected mink farms were

113 informed of potential risks and were given advice on the importance and use of personal

114 protective equipment and hygiene when handling animals (*41*). The contact structure on the

115 farms was assessed through in-depth interviews, to identify additional persons with possible

116 exposure to mink. In order to provide an enhanced set of reference genome sequences,

117 anonymized samples from patients that had been diagnosed with COVID-19 in the area of the

118 same four-digits postal codes as farms NB1-NB4 in March and April 2020 were retrieved from

119 clinical laboratories in the region.

120

121 **SARS-CoV-2 diagnostics and sequencing**

122 The presence of viral RNA in mink samples was determined using a RT-PCR targeting the E

123 gene as previously described (*42*). For the human samples, diagnostic RT-PCR was performed

124 for the E and the RdRp gene (*42*). In addition, serology was performed, using the Wantai Ig

125 total and IgM ELISA, following the manufacturer's instructions(*43*). For all samples with a Ct

126 value <32, sequencing was performed using a SARS-CoV-2 specific multiplex PCR for Nanopore

127 sequencing, as previously described (*3*). The libraries were generated using the native barcode

128 kits from Nanopore (EXP-NBD104 and EXP-NBD114 and SQK-LSK109) and sequenced on a R9.4

129 flow cell multiplexing 24 samples per sequence run. Flow cells were washed and reused until

130 less than 800 pores were active. The resulting raw sequence data was demultiplexed using

7

131 Porechop (https://github.com/rrwick/Porechop). Primers were trimmed after which a

132 reference-based alignment was performed. The consensus genome was extracted and

133 positions with a coverage <30 were replaced with an "N" as described previously (*44*).

134 Mutations in the genome compared to the GISAID sequence EPI_ISL_412973 were confirmed

135 by manually checking the mapped reads and homopolymeric regions were manually checked

136 and resolved by consulting reference genomes. The average SNP difference was determined

137 using snp-dists (https://github.com/tseemann/snp-dists). All sequences generated in this

138 study are available on GISAID.

139

140 **Phylogenetic analysis**

141 All available near full-length Dutch SARS-CoV-2 genomes available on 1st of July were selected

142 (n=1,775) and aligned with the sequences from this study using MUSCLE (*45*). Sequences with

143 >10% "Ns" were excluded. The alignment was manually checked for discrepancies after which

144 IQ-TREE (*46*) was used to perform a maximum likelihood phylogenetic analysis under the

145 GTR+F+I +G4 model as best predicted model using the ultrafast bootstrap option with 1,000

146 replicates. The phylogenetic trees were visualized in Figtree

147 (http://tree.bio.ed.ac.uk/software/figtree/). For clarity reasons all bootstrap values below 80

148 were removed. To look at potential relationships with migrant workers, also all Polish

149 sequences from GISAID (*47*) were included in the alignment (**Supplementary table 1**).

150

151 **Mapping specific mutation patterns on mink farms and in mink farm employees**

152 Amino acid coordinates are described in relation to the Genbank NC_045512.2 reference

153 genome. Open reading frames were extracted from the genome alignment using the genome

154 annotation as supplied with the reference genome. A custom R script was used to distinguish

155 synonymous from non-synonymous mutations and non-synonymous mutations were

156 visualized using a tile map from the ggplot2 package (*48*).

157

158 **Geographical overview of mink farms in the Netherlands and SARS-CoV-2 positive farms**

159 To protect confidentiality, SARS-CoV-2 positive mink farms were aggregated at municipality

160 level. The datasets "*Landbouw; gewassen, dieren en grondgebruik naar gemeente*" and "*Wijk-*

161 *en Buurtkaart 2019*" from Statistics Netherlands (CBS) were used (*49*). Maps were created

162 using R packages sp (*50*), raster (*51*) and rgdal (*52*) and ArcGIS 10.6 software by ESRI.

163

Results

164

165   SARS-CoV-2 was first diagnosed on two mink farms in the Netherlands on April 23rd (NB1) and

166   April 25th (NB2), respectively. After the initial detection of SARS-CoV-2 on these farms an in-

167   depth investigation was started to look for potential transmission routes and to perform an

168   environmental and occupational risk assessment. Here, we describe the results of the

169   outbreak investigation of the first 16 SARS-CoV-2 infected mink farms by combining SARS-CoV-

170   2 diagnostics, WGS and in-depth interviews.

171

Screening of farm workers and contacts

172

173   Farm owners of the 16 SARS-CoV-2 positive mink farms were contacted by the municipal

174   health services to conduct contact investigation and samples were taken for RT-PCR-based

175   and serological SARS-CoV-2 diagnostics. In total, 97 individuals were tested by either

176   serological assays and/or RT-PCR. In total, 43 out of 88 (49%) upper-respiratory tract samples

177   tested positive by RT-PCR while 38 out of 75 (51%) serum samples tested positive for SARS-

178   CoV-2 specific antibodies. In total, 66 of 97 (67%) of the persons tested had evidence for SARS-

179   CoV-2 infection (**table 1**).

180

181   **Table 1**. Overview of human sampling on SARS-CoV-2 positive mink farms.

Farm:	First diagnosis in animals:	Date(s) of sampling employees and family members:	PCR positive (%)	Serology positive (%)	Employees and family members tested positive (PCR and/or serology)
NB1	24-04-2020	28-04-2020 – 11-05-2020	5/6 (83%)	5/5 (100%)	6/6 (100%)
NB2	25-04-2020	31-03-2020 – 30-04-2020	1/2 (50%)	8/8 (100%)	8/8 (100%)
NB3	07-05-2020	11-05-2020 – 26-05-2020	5/7 (71%)	0/6 (0%)*	5/7 (71%)
NB4	07-05-2020	08-05-2020	1/3 (33%)	2/2 (100%)	2/3 (66%)
NB5	31-05-2020	01-06-2020	2/7 (29%)	3/6 (50%)	3/7 (43%)
NB6	31-05-2020	01-06-2020	1/6 (17%)	4/6 (66%)	4/6 (66%)

NB7	31-05-2020	10-06-2020 – 01-07-2020	8/10 (80%)	NA**	8/10 (80%)
NB8	02-06-2020	03-06-2020	5/10 (50%)	5/9 (56%)	8/10 (80%)
NB9	04-06-2020	07-06-2020	1/7 (14%)	1/7 (14%)	2/7 (29%)
NB10	08-06-2020	11-06-2020	1/8 (13%)	3/8 (38%)	4/8 (50%)
NB11	08-06-2020	11-06-2020	1/3 (33%)	0/2 (0%)	1/3 (33%)
NB12	09-06-2020	11-06-2020	6/9 (66%)	2/8 (25%)	7/9 (78%)
NB13	14-06-2020	11-06-2020 – 18-06-2020	3/3 (33%)	0/2 (0%)	3/3 (33%)
NB14	14-06-2020	14-06-2020	1/3 (100%)	5/6 (83%)	5/6 (83%)
NB15	21-06-2020	10-06-2020 – 30-06-2020	2/2 (100%)	NA**	2/2 (100%)
NB16	21-06-2020	23-06-2020	0/2 (0%)	NA**	0/2 (0%)
Total:			**43/88 (49%)**	**38/75 (51%)**	**66/97 (68%)**

182   * Serology was done approximately one week before the positive PCR test.

183   ** No serology was performed

184

Anthropozoonotic transmission of SARS-CoV-2

186   During the interview on April 28th, four out of five employees from NB1 reported that they

187   had experienced respiratory symptoms before the outbreak was detected in minks, but none

188   of them had been tested for SARS-CoV-2. The first day of symptoms of people working on NB1

189   ranged from April 1st to May 9th. For 16 of the mink, sampled on April 28th, and one farm

190   employee, sampled on May 4th, a WGS was obtained (hCov-

191   19/Netherlands/NoordBrabant_177/2020). The human sequence clusters within the mink

192   sequences although it had 7 nucleotides difference with the closest mink sequence (**Figure 1**

193   and cluster A in **figures 2 and 3**). On farm NB2, SARS-CoV-2 was diagnosed on April 25th.

194   Retrospective analysis showed that one employee from NB2 had been hospitalized with SARS-

195   CoV-2 on March 31st. All samples from the 8 employees taken on April 30th were negative by

196   RT-PCR but tested positive for SARS-CoV-2 antibodies. The virus sequence obtained from

197   animals was distinct from that of farm NB1, indicating a separate introduction (**Figure 2 and**

198   **3**, cluster B).

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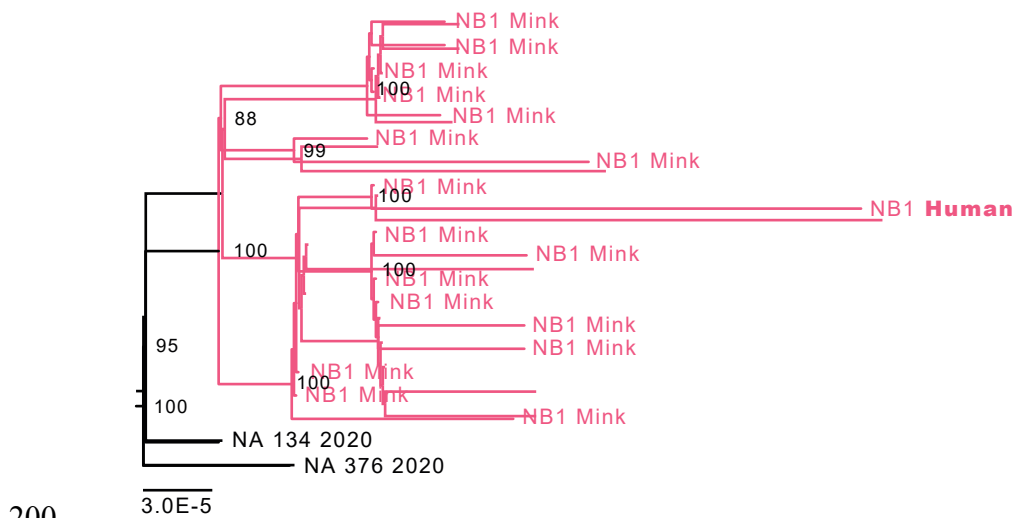
Figure 1: Zoom of the phylogenetic analysis of NB1. A maximum likelihood analysis was performed using all available SARS-CoV-2 Dutch sequences. Sequences from mink on NB1 are depicted in red and from the employee on NB1 in blue. The two sequences in black at the root of the cluster are the closest matching human genome sequences from the national SARS-CoV-2 sequence database. Scale bar represents units of substitutions per site.

Zoonotic transmission of SARS-CoV-2

On mink farm NB3 SARS-CoV-2 infection was diagnosed on May 7th. Initially all seven employees tested negative for SARS-CoV-2, but when retested between May 19th and May 26th after developing COVID-19 related symptoms, 5 out of 7 individuals working or living on the farm tested positive for SARS-CoV-2 RNA. WGS were obtained from these five individuals and the clustering of these sequences with the sequences derived from mink from NB3, together with initial negative test result and the start of the symptoms, indicate that the employees were infected with SARS-CoV-2 after the mink on the farm got infected. Also, an additional infection was observed based on contact-tracing: a close contact of one of the employees – who did not visit the farm – got infected with the SARS-CoV-2 strain found on

12

217   NB3. Animal and human sequences from farm NB3 were related to those from farm NB1, but

218   were both part of cluster A.

219   Similarly, on mink farm NB7 zoonotic transmission from mink to human most likely

220   occurred. On this farm, SARS-CoV-2 infection in mink was diagnosed on May 31st and

221   employees initially tested negative for SARS-CoV-2 but started to develop symptoms at a later

222   stage. Samples were taken between June 10th and July 1st from 10 employees of which 8 tested

223   positive for SARS-CoV-2 RNA. From 2 samples WGS could be generated from the employees

224   which clustered together with the sequences from the animals from this farm.

225

226   **Comparison with national reference database and enhanced regional sampling**

227   The sequences generated from mink farms and from mink farm employees were compared

228   with the national database consisting of around 1,775 WGS. In addition, to discriminate

229   between locally acquired infections and mink farm related SARS-CoV-2 infection, and to

230   determine the potential risk for people living close to mink farms, WGS was also performed

231   on 34 SARS-CoV-2 positive samples from individuals who live in the same four-digit postal

232   code area compared to the first four mink farms. These local sequences reflected the general

233   diversity seen in the Netherlands and were not related to the clusters of mink sequences

234   found on the mink farms, thereby also giving no indication of spill-over to people living in close

235   proximity to mink farms (sequences shown in magenta, **Figure 2**). The sequences from the

236   mink farm investigation were also compared to sequences from Poland (n=65), since many of

237   the mink farm workers were seasonal migrants from Poland, but the these were not related.

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6.0E-5

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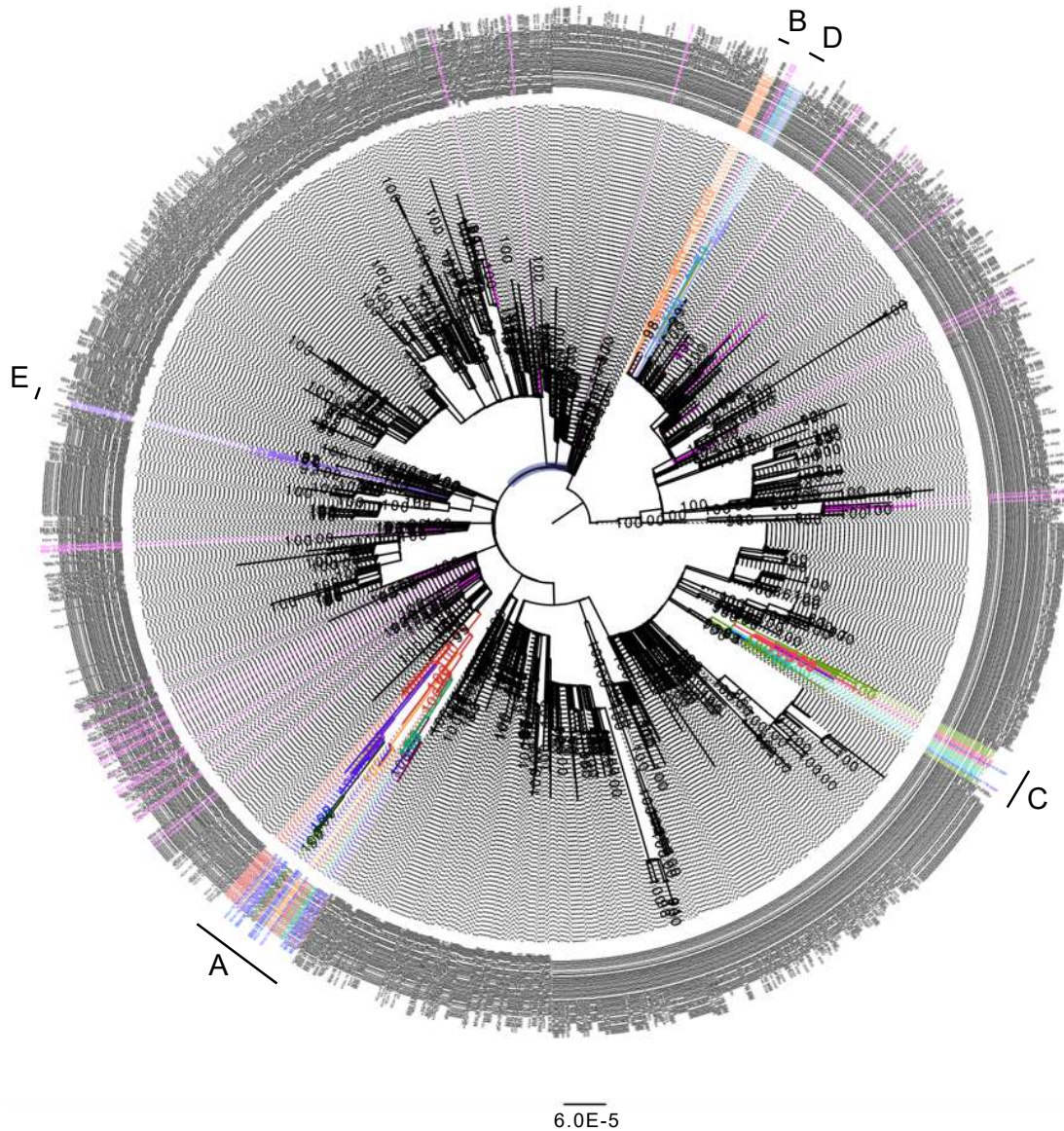
Figure 2: **Maximum likelihood analysis of all SARS-CoV-2 Dutch sequences**. The sequences derived

from minks from different farms are indicated with different colors, human sequences related to the

mink farms in blue and samples from similar 4-digit postal code are indicated in magenta. Scale bar

represents units of substitutions per site.

244

Mink farm related sequence clusters

Phylogenetic analysis of the mink SARS-CoV-2 genomes showed that mink sequences of 16

farms grouped into 5 different clusters (**Figure 2 and 3**). Viruses from farms NB1, NB3, NB4,

14

248   NB8, NB12, NB13 and NB16 belonged to cluster A, sequences from NB2 were a separate

249   cluster (B), those from farms NB6, NB7, NB9 and NB14 grouped together in cluster C, NB5,

250   NB8, NB10 and NB15 grouped to cluster D, and NB11 had sequences designated as cluster E.

251   On farm NB8, SARS-CoV-2 viruses could be found from both cluster A and cluster D. A detailed

252   inventory of possible common characteristics, like farm owner, shared personnel, feed

253   supplier and veterinary service provider, was made. In some cases, a link was observed with

254   the same owners of several farms, for instance for cluster A for NB1 and NB4, and for NB8 and

255   NB12. Although NB7, NB11 and NB15 were also linked to the same owner, viruses from these

256   farms belonged to cluster C, D and E respectively. No common factor could be identified for

257   most farms and clustering could also not be explained by geographic distances as multiple

258   clusters were detected in different farms located close to each other (**Table 2 and figure 4**).

259

260   **Table 2.** Overview of the clusters detected on the different farms.

Farm:	Date of diagnosis:	Sequence cluster:	Same owner:	Feed supplier:	Vet**:	Number of sequences (human):	Sequence diversity (average):	Mink population size:	Detection***:
NB1	24-04-20	A	NB1, NB4	1	I	17 (1)	0-9 (3.9)	75,711	Notification
NB2	25-04-20	B		1	II	8	0-8 (3.6)	50,473	Notification
NB3	07-05-20	A		2	III	5 (5)	0-2 (0.6)	12,400	Notification
NB4	07-05-20	A	NB1, NB4	1	I	1	NA	67,945	Contact tracing NB1
NB5	31-05-20	D		1	IV	1	NA	38,936	EWS-Ser+PM-1st
NB6	31-05-20	C		3	V	9	0-12 (6.8)	54,515	EWS-Ser+PM-1st
NB7	31-05-20	C	NB7, NB11, NB15	3	II	6 (2)	0-4 (1.4)	79,355	EWS-PM-1st
NB8	02-06-20	A/D	NB8, NB12*	3	V	6 (5)	0-6 (2.6)	39,144	EWS-Ser+PM-1st
NB9	04-06-20	C		2	V	2 (1)	0-3 (1.5)	32,557	EWS-Ser+PM-2nd
NB10	08-06-20	D		3	II	4	0-3 (1.1)	26,824	EWS-Ser+PM-2nd

| NB11	08-06-20	E	NB7, NB11, NB15	3	II	4	0-4 (2.2)	38,745	EWS-PM-2nd
NB12	09-06-20	A	NB8, NB12*	3	II	5	0-3 (1.2)	55,352	Notification
NB13	14-06-20	A		3	V	5 (3)	0-5 (3.2)	20,366	EWS-PM-5th
NB14	14-06-20	C		3	II	5 (1)	0-7 (3.7)	28,375	EWS-PM-5th
NB15	21-06-20	D	NB7, NB11, NB15	3	II	5	0-2 (0.6)	35,928	EWS-PM-6th
NB16	21-06-20	A		3	II	5	0-4 (1.6)	66,920	EWS-PM-6th

261   * There was exchange of personnel in these two locations.

262   ** Veterinarian II and V were both from the same veterinary practice.

263   *** Notification: based on reporting of clinical signs which was obligated from 26 April onwards; EWS-Ser-

264   Detection based on a one-off nation-wide compulsory serological screening of all mink farms at the end of

265   May/early June by GD Animal Health; EWS-PM-Detection based on the early warning monitoring system

266   for which carcasses of animals that died of natural causes were submitted weekly for PCR testing by GD

267   Animal Health from the end of May onwards in a weekly cycle (EWS-PM 1st to 6th post mortem screening).

268

269   In total 18 sequences from mink farm employees or close contacts were generated from seven

270   different farms. In most cases, these human sequences were near-identical to the mink

271   sequences from the same farm. For NB1 the situation was different and the human sequence

272   clusters deeply within the sequences derived from mink (Figure 1), with 7 nucleotides

273   difference with the closest related mink sequence. This was also the case on farm NB14, with

274   4 nucleotides difference with the closest related mink sequence. Employees sampled at mink

275   farm NB8 clustered with animals from NB12 which can be explained by the exchange of

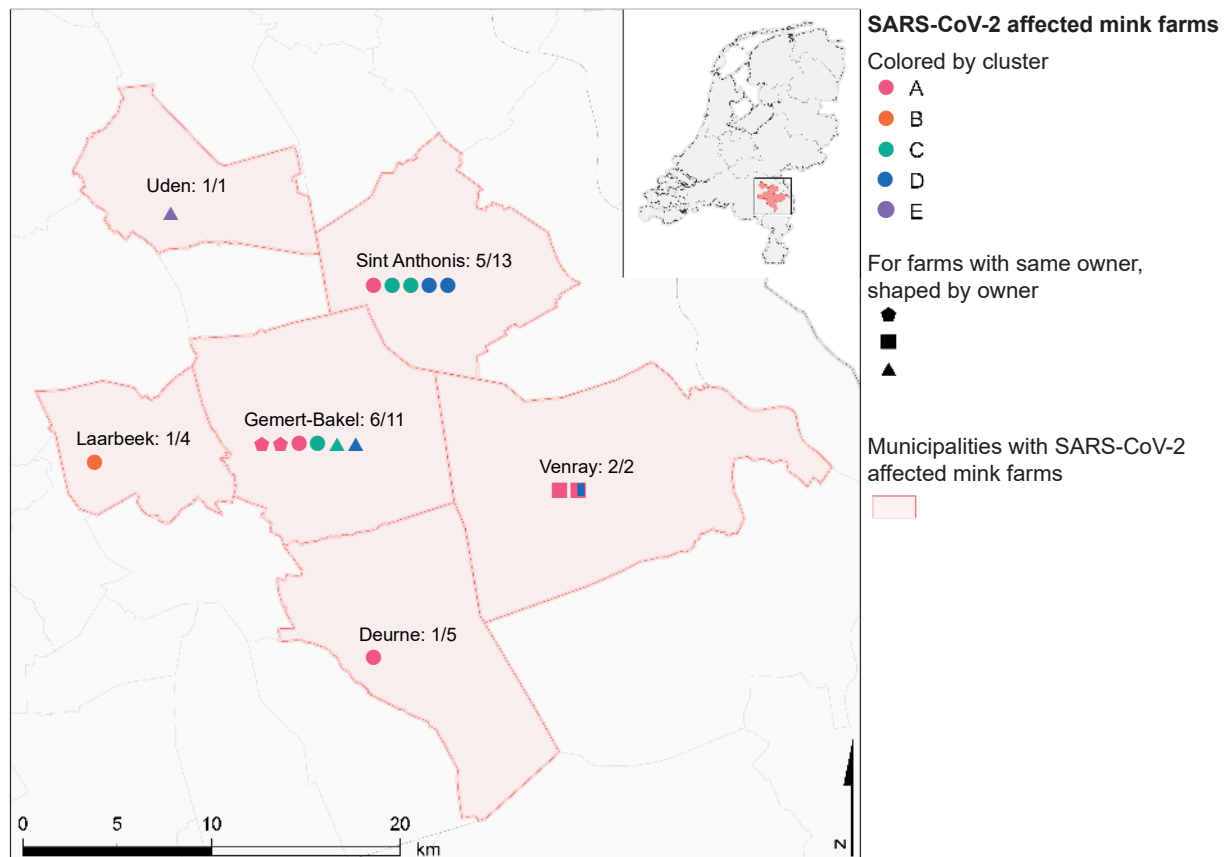276   personnel between these two farms.

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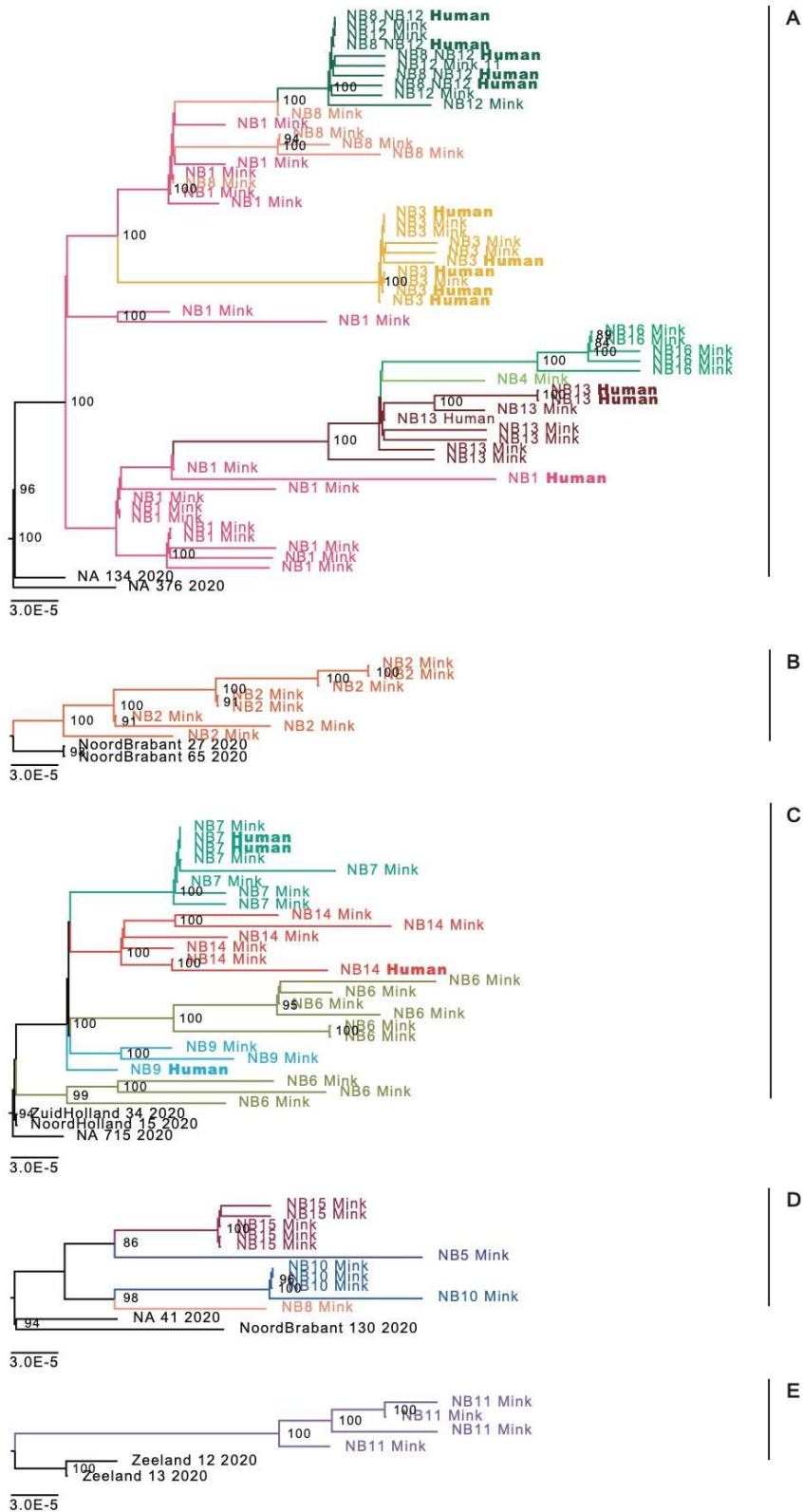
Figure 3: Phylogenetic analysis of SARS-CoV-2 strains detected in the 5 mink farm clusters. The sequences derived from different farms are depicted in different colors. Scale bar represents units of substitutions per site.

17

Figure 4. Geographical overview of SARS-CoV-2 positive mink farms per municipality affected. The proportion of SARS-CoV-2 positive mink farms over the total number of mink farms (CBS, 2019) is indicated. Symbols for positive farms are colored by cluster and shapes indicate farms with a same owner.

Within farm diversity

SARS-CoV-2 was detected on mink farm NB1-NB4 after reports of respiratory symptoms and increased mortality in mink. The sequences from farm NB1 had between 0 and 9 nucleotides differences (average 3.9 nucleotides) and from NB2 between 0 and 8 nucleotides differences (average of 3.6), which is much more than what has been observed in outbreaks in human settings. The sequences from NB3 had 0 to 2 nucleotides difference suggesting that the virus was recently introduced, in line with the observed disease in humans, which occurred in the weeks post diagnosis of the infection in mink. After the initial detection of SARS-CoV-2 on mink farms, farms were screened weekly. The first, second, fifth and sixth weekly screening yielded new positives. The sequences of mink at NB6 had between 0 and 12 nucleotides differences, whereas diversity was lower for the subsequent farm sequences (Table 2).

Several non-synonymous mutations were identified among the mink sequences compared to the Wuhan reference sequence NC_045512.2. However, no particular amino acid substitutions were found in all mink samples (**Figure 5**). Of note, three of the clusters had the position 614G variant (clusters A, C and E), and 2 had the original variant. There were no obvious differences in the presentation of disease in animals or humans between clusters based on the data available at this stage, but further data collection and analysis, also for cases after NB16, are ongoing to investigate this further. The observed mutations can also be found in the general population and the same mutations also were found in human cases which were related to the mink farms.

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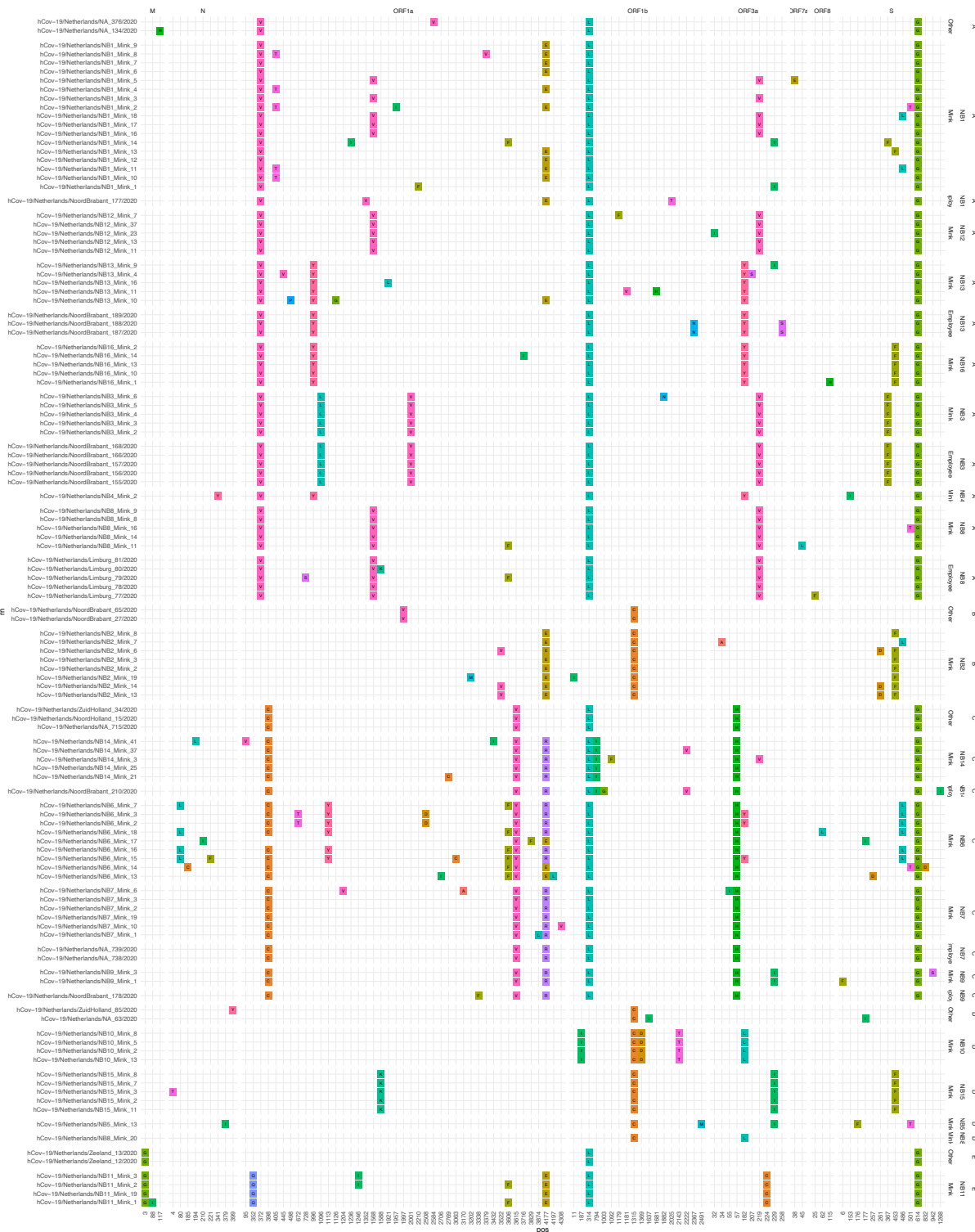
Figure 5. Overview of the specific amino acid mutations found in mink farms. Above the x-axis the open reading frames (ORF) are indicated and on the x-axis the amino acid position within each ORF is indicated. On the y-axis the sequence names are indicated and on the right side of the

311   graph the cluster numbers and specific farm identifiers and the type of host are used to group

312   the samples.

313

314   **Discussion**

315   Here we show ongoing SARS-CoV-2 transmission in mink farms and spill-over events to

316   humans. To the best of our knowledge, these are the first animal to human SARS-CoV-2

317   transmission events documented. More research in minks and other mustelid species, to

318   demonstrate if these species can be a true reservoir of SARS-CoV-2 although from our

319   observations we consider this likely. After the detection of SARS-CoV-2 on mink farms, 68% of

320   the tested farm workers and/or relatives or contacts were shown to be infected with SARS-

321   CoV-2, indicating that contact with SARS-CoV-2 infected mink is a risk factor for contracting

322   COVID-19.

323   A high diversity in the sequences from some mink farms was observed which most

324   likely can be explained by many generations of infected animals before an increase in

325   mortality was observed. The current estimates are that the substitution rate of SARS-CoV-2 is

326   around $1.16*10^{-3}$ substitutions/site/year (*53*), which corresponds to around one mutation

327   per two weeks. This could mean that the virus was already circulating in mink farms for some

328   time. However, there was also a relatively high sequence diversity observed in farms which

329   still tested negative one week prior, hinting towards a faster evolution of the virus in the mink

330   population. This can indicate that the virus might replicate more efficiently in mink or might

331   have acquired mutations which makes the virus more virulent. However, no specific mutations

332   were found in all mink samples, making increased virulence less likely. In addition, mink farms

333   have large populations of animals which could lead to very efficient virus transmission.

334   Generation intervals for SARS-CoV-2 in humans have been estimated to be around 4-5

335 days(*54*), but with high dose exposure in a high-density farm could potentially be shorter.

336 Recently, a specific mutation in the spike protein (D614G) was shown to result in an increased

337 virulence *in vitro* (*55*), while it was not associated an increased growth rate for cluster nor an

338 increased mortality (*56*). This mutation was present in farm clusters A, C and E, but no obvious

339 differences in clinical presentation, disease severity, or rate of transmission to humans was

340 observed.

341   While we found sequences matching with the animal sequences on several farms, not

342 all of these can be considered direct zoonotic transmissions. For instance, the two employees

343 from mink farm NB3 were most likely infected while working at the mink farm given the

344 specific clustering in the phylogenetic tree and the timing of infection. Subsequent human

345 infections may have originated from additional zoonotic infections, or from human to human

346 transmission within their household. Further proof that animals were the most likely source

347 of infection was provided by the clear phylogenetic separation between farm related human

348 cases and animal cases, from sequences from cases within the same 4-digit postal code area.

349 Spill-back into the community living in the same 4-digit postal code area was not observed

350 using sequence data, but cannot be entirely ruled out as the testing strategy during April and

351 May was focusing on health care workers, persons with more severe symptoms, and persons

352 at risk for complications, rather than monitoring community transmission and milder cases.

353   While the number of SARS-CoV-2 infected individuals was decreasing in the

354 Netherlands in May and June, an increase in detection of SARS-CoV-2 in mink farms was

355 observed. Based on WGS these sequences are part of multiple individual transmission chains

356 linked to the mink farms and are not a reflection of the situation in the human population

357 during this time. In some cases, the farms had the same owner but in other cases no

358 epidemiological link could be established. People coming to the different farms might be a

22

359   source but also semi-wild cats roaming around the farms or wildlife might play a role (*27*). So

360   far, the investigation failed to identify common factors that might explain farm to farm spread.

361   During interviews, it became clear that farms had occasionally hired temporary workers that

362   had not been included in the testing and were lost to follow-up, stressing the need for vigorous

363   biosecurity and occupational health guidance. Since our observation, SARS-CoV-2 infections

364   have also been described in mink farms in Denmark, Spain and the USA (*57–59*), and mink

365   farming is common in other regions of the world as well, also in China where around 26 million

366   mink pelts are produced on a yearly basis (*60*). The population size and the structure of mink

367   farms is such that it is conceivable that SARS-CoV-2 – once introduced – could continue to

368   circulate. Therefore, continued monitoring and cooperation between human and animal

369   health services is crucial to prevent the animals serving as a reservoir for continued infection

370   in humans.

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643   Authors declare no competing interests.

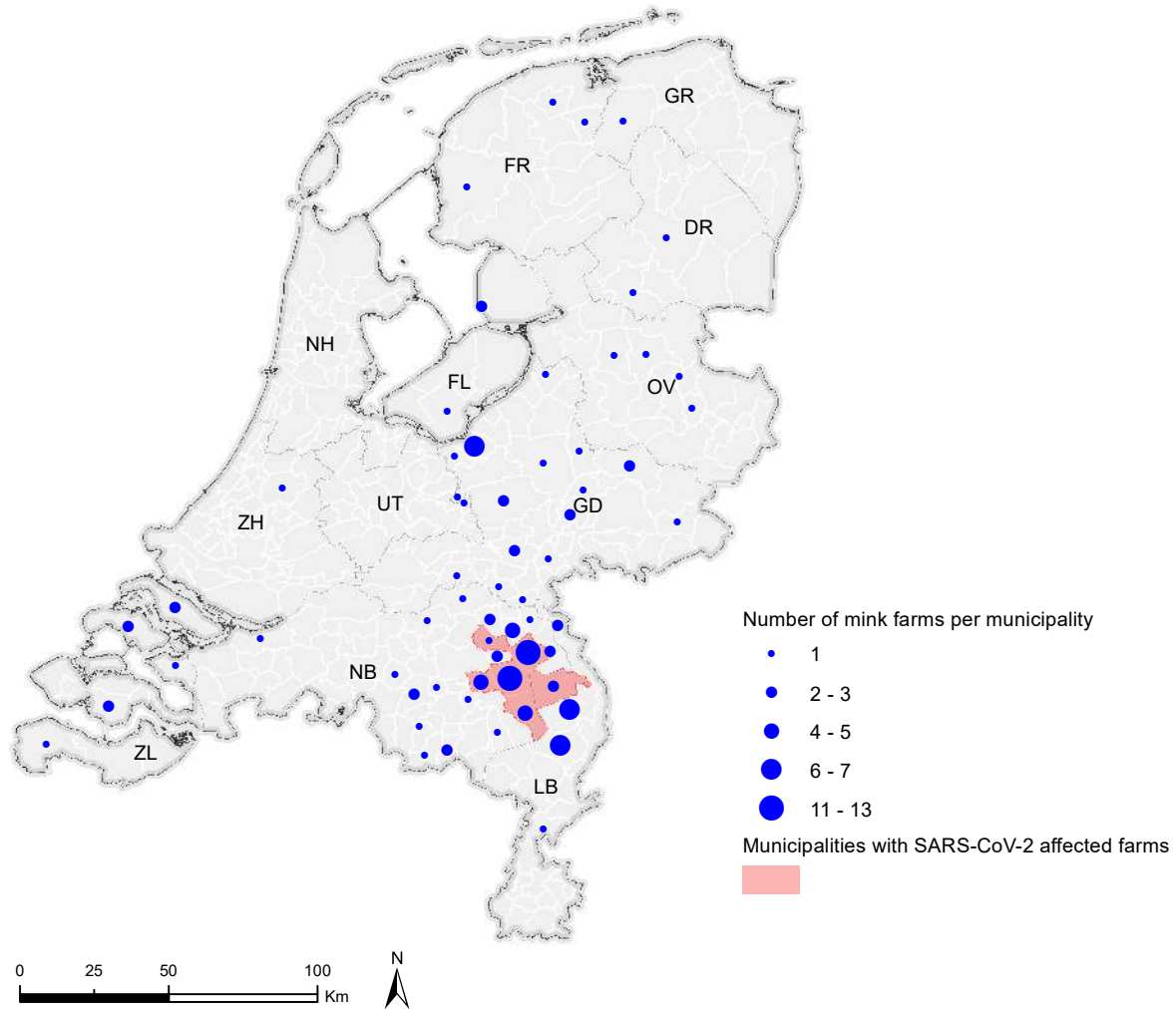644

Data and material availability:

646   All data, code and materials used described in this manuscript are publicly available.

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648   **Supplements**

649

650   **Supplementary Figure 1: Number of mink farms per municipality in the Netherlands.**

651   Overview of the total number of mink farms per municipality (CBS, 2019). Municipalities

652   with SARS-CoV-2 affected farms by June 21st 2020 are shown in red.

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654   **Supplementary table 1:** GISAID acknowledgement table.