1 Zoonotic spillover of SARS-CoV-2: mink-adapted virus in humans

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ABSTRACT

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The COVID-19 pandemic caused by SARS-CoV-2 started in fall 2019. A range of different
mammalian species, including farmed mink, have been confirmed as susceptible to infection
with this virus. We report here the spillover of mink-adapted SARS-CoV-2 from farmed
mink to humans after extensive adaptation that lasted at least 3 months. We found the
presence of four mutations in the S gene (that gave rise to variant: G75V, M177T, Y453F and
C1247F) and others in an isolate obtained from SARS-CoV-2 positive patient.

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Keywords: SARS-CoV-2; Bat coronavirus; mink; transmission; spillover; zoonoses;
Expanding host range; jumping the species barrier

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Conflict of interest: None declared

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48 **1. Introduction**

49 Coronaviruses are known as potential zoonotic pathogens, and severe acute respiratory
50 syndrome coronavirus 2 (SARS-CoV-2) is the third highly pathogenic member of this family
51 to have emerged in the 21st century (1). While mass vaccinations are currently underway, the
52 question of the fate of the virus remains open. The concept of herd immunity and eradication
53 of the virus is somewhat unrealistic when considering the prevalence, genetic diversity, and
54 the existing animal reservoirs. Thus far, SARS-CoV-2 infections have been reported in
55 different mammalian species worldwide, including dogs, cats, tigers, lions, ferrets, minks,
56 felines, and deer (2,3). SARS-CoV-2 infections in farmed mink have recently been confirmed
57 in European countries (4). Transmission of the virus from infected mink to humans has been
58 reported in Denmark and the Netherlands (5–7). After Denmark, Poland is the second-largest
59 producer of mink pelts in Europe, with 354 active Polish mink farms harbouring
60 approximately 6.3 million mink in total (8).

61 **2. Material and methods**

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63 This study was approved by the Independent Bioethical Committee for Scientific Research at
64 the Medical University of Gdansk, Gdansk, Poland (Statement no. NKBBN/183/2020).

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66 SARS-CoV-2 genome sequencing was performed at the University of Gdansk, Poland using
67 sample containing RNA isolated from positive swab (amplification of two target genes in
68 RT-PCR). ARTICv3 amplicon generation followed by Oxford Nanopore Technology
69 MinION run was performed (Quick 2020). Reads were basecalled, debarcoded and trimmed
70 to delete adapter, barcode and PCR primer sequences. ARCTIC pipeline software were used
71 to generate SARS-CoV-2 genome. Phylogenetic analysis was performed using the procedure
72 recommended by Nextstrain.org (Hadfield et al., 2018)

73 **3. Results and Discussion**

74 The capacity of coronaviruses to transfer between mammalian species is not surprising. First,
 75 the transfer of highly pathogenic variants from bats usually occurs via intermediate hosts.
 76 Infection of dromedary camels and palm civets and later transfer to humans has been
 77 described for MERS-CoV and SARS-CoV, respectively (9). A more comprehensive analysis
 78 of existing strains suggests that this was not an exception. The human coronavirus OC43
 79 virus (HCoV-OC43) is a betacoronavirus described for the first time in the 1960s. This
 80 pathogen is associated with upper and lower respiratory tract disease in humans (10).
 81 Interestingly, closely related species are found in cattle (bovine coronavirus) and dogs
 82 (canine respiratory coronavirus). While the exact transfer route between different species
 83 remains unknown, it is conceivable that as in SARS-CoV-2, the virus has jumped between
 84 humans, and companion and farmed animals in the past (11).

85 Recently, we and others have detected SARS-CoV-2 infection in farmed minks in Northern
 86 Poland (12,13). The first report identified SARS-CoV-2 in samples collected from mink in
 87 mid-November 2020. While the prevalence of the virus was low and, most likely, only
 88 isolated cases were present, we have sequenced the isolates and have shown that the positive
 89 signal did not originate from contamination. The most recent data collected and deposited by
 90 the laboratory of the National Veterinary Research Institute in Poland has indicated isolation
 91 of the SARS-CoV-2 variants from animals at the same farm. Phylogenetic analysis of the
 92 data have indicated that the virus belongs to the B.1.1.279 lineage (Pangolin classification),
 93 which is not surprising considering the prevalence of this variant in Europe. Genome analysis
 94 shows that the new isolates carry the combination of mutations typical of viruses isolated
 95 already in November 2020 from mink (listed in Figure 1 bottom left radial tree), but
 96 additional new changes have accumulated since then. These include the Y453F mutation,

97 which was previously reported to have emerged in minks during serial passages (e.g., in
98 Denmark and recently Lithuania), and a novel mutation not present in any global SARS-
99 CoV-2 isolate that truncate ORF 7b at position L22. With all the available data taken into
100 account, we speculate that the virus was present already in the mink population by November
101 2020, presumably after a single introduction during the late summer or autumn of that year.

Figure 1. Phylogenetic analysis of SARS-CoV-2 lineage B.1.1.279 combined with inferred time (bottom of each radial time tree) of fixing mutations (upper right of each radial time tree) in all isolates form a group that leads to the generation of the mink variants. Yellow colour represents new variants, A - November 2020 mink isolates, B - January 2021 mink isolates and single human isolate, C - nearest neighbours human isolates that share a common ancestor with A-B: Norway/4235/2020, Germany/NW-HHU-340/2020, Iceland/4563/2021.

In the current study, we have identified a case of infection with a mink-adapted variant in humans. Following the identification of SARS-CoV-2 cases in farmed animals, the exposed staff were tested for SARS-CoV-2 (nasopharyngeal swabs) using RT-qPCR. A single positive case was detected in a sample collected on 1st February 2021 and was sequenced using the ARTIC Nanopore technology protocol. The resulting sequence has been deposited in GISAID under the accession number EPI_ISL_1034274. The host was asymptomatic. Phylogenetic analysis (Figure 1) shows that the virus clusters closely with viruses isolated from mink (group B on bottom radial trees). Further, mutations characteristic of the mink-adapted variant were present in the virus's genomic sequence (Table 1), confirming that it was most likely contracted by the infected subject from animals.

Table 1. Representation of mutations in mink originated SARS-CoV-2 isolates. Group A - November 2020 mink isolates, Group B - January 2021 mink isolates and single human isolate, C - nearest neighbours in lineage B.1.1.279 human isolates. The yellow colour represents mutations fixed in November 2020. The red colour represents mutations acquired during three months of passage at the mink farm.

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nt position	aa variant	Reference	Group A	Group B	Group C
1218	nsp2: S138L	C	T	T	T
1630	nsp2: Q275H	A	T	T	T
2426	nsp2: V541I	G	A	A	A
3373	nsp3: D218E	C	A	A	A
5326	nsp3: E869D	G	T	T	T
12795	nsp9: G37E	G	G	A	G
12854	nsp9: P57S	C	C	T	C
14408	nsp12: P323L	C	T	T	T
18186	nsp14: M49I	G	T	T	G
18859	nsp14: A274S	G	T	T	T
20273	nsp15: M218T	T	C	C	C
21786	S: G75V	G	T	T	T
22092	S: M177T	T	T	C	T
22920	S: Y453F	A	A	T	A
25302	S: C1247F	G	T	T	G
27820	ORF 7b: L22*	T	T	A	T
28881/2	N: R203K	GG	AA	AA	AA
28884	N: G204L	G	T	T	G
29300	N: D343Y	G	T	T	G

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126 The emerging variant clearly reflects adaptation of the virus to the animal host, with some
127 point mutations fixed early during transmission between animals and additional changes
128 accumulating over time (Figure 1, bottom right). While the mutations' exact role is to be
129 determined, they may provide increased fitness in the new host (14). It remains unknown
130 whether these features alter the course of disease transmissibility, or immunogenicity in
131 humans (15). However, the variant should be tracked in the general population. Our results
132 confirm the need for country-scale epizootiological monitoring and careful analysis of SARS-
133 CoV-2 positive patients.

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188 **Data availability**

189 The complete genome sequences of SARS-CoV-2 isolated from the patient was deposited in
190 GISAID under the accession number: EPI_ISL_1034274

191 **Authors' contributions:**

192 The study was conceived and designed by LR and MG. Data handling: LR, MK, MG, KP.
193 Material handling and laboratory work: LR, MK, NMP. Statistical and phylogenetic analysis
194 was carried by LR. Interpretation of data: LR, KP, MK. Data visualisation: LR. Supervision:
195 MG, KP, TS. The manuscript was written by LR, MG, KP, RK, TS, BS, KBS in consultation
196 with all co-authors. MG, KP, LR, TS, RK revised the manuscript. All authors accepted the
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