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The SARS-COV-2 mink strains and future alarming mutations

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Premise

- The RBD (receptor-binding domain) in the S (spike) protein of SARS-CoV-2 which the virus uses to latch on the ACE2 (angiotensin-converting enzyme 2) receptors, shows a higher affinity and binding capacity to ACE2 of ferrets, cats, minks with high homology of receptors, which explains their susceptibility to infection;
- Despite there are no reports of natural infection in the context of the SARS-CoV-2 pandemic, raccoon dogs due to their experimental susceptibility, whether free-living or held in captivity, might have a role as an intermediate host;
- The potential host range and reservoir species of SARS-CoV-2 can be broad;
- That human-animal-human transmission exists is shown by COVID-19 in mink and in-contact humans on mink-farms in the Netherlands, Denmark, Spain, Greece, Sweden, Italy, Canada, Lithuania, and the United States of America;
- Ferrets akin to minks are susceptible to SARS-CoV-2 through direct contact but also indirect airborne spread;
- There is the potential for the establishment of a non-human reservoir of SARS-CoV-2 in the wild should the virus become established in ferrets and wild mink after escape from farms, as has been reported in Utah, USA;
- Due environmental circulation of SARS-CoV-2, there is also the potential SARS-CoV-2 spillback from infected humans to naive wildlife hosts (eg. bats) that can act as a novel viral reservoir.

The SARS-CoV-2 mink outbreaks

Concerning outbreaks of SARS-CoV-2 began to emerge on mink farms in the Netherlands and Denmark in late spring and early summer 2020.¹ In the Netherlands, with about 125 mink farms, an outbreak of COVID-19 occurred in two of the mink farms in mid-April of 2020 and later in more than forty farms. The genomic and epidemiological investigation demonstrated that the most likely explanation for the infection at the mink farms is the introduction of the virus by humans (reverse zoonosis, human to mink), subsequent transmission mink to mink, and mink to human transmission.² Indeed, the whole-genome sequencing of the human sequences among owners and employees of the 16 SARS-CoV-2 positive mink farms, clustered with the sequences derived from minks, indicated a 'genomic signature' that some of the employees were infected with SARS-CoV-2 (the mink variant) after mink on the farm became infected. This is undoubtedly the first reverse anthroponotic events of SARS-CoV-2 acknowledged so far. Some mink-derived variants accounted for 40% of the total SARS-CoV-2 cases in the Netherlands. The high diversity in the SARS-CoV-2 genomic sequences obtained from a few mink farms indicates a prolonged circulation of the virus among the minks, which might have led to the accumulation of mutations that increased virulence of the virus. Since this mink variant appears to be less lethal and infective compared to those in humans, it was argued that they might be suitable for vaccination for humans, such as in the case of the smallpox virus, which is less infective and toxic to humans.³

Since June 2020 Denmark has experienced an extensive of SARS-CoV-2 outbreaks in mink farms. This country is the world's largest mink fur producer in the world with 1.146 mink farms and a population of 17 million minks. Despite the adoption of hygienic measures and the culling of infected and uninfected mink herds on farms, SARS-CoV-2 spread relentlessly among mink farms across Denmark. As of 1 December 2020, a total of 289 mink farms have been affected, which accounts for approximately 20% of all mink farms in Denmark. A mutated SARS-CoV-2 referred to as mink-related variants, was found circulating in over 200 mink farms, and spilled back from mink farms into the community (mink to human transmission). In early November 2020 Danish authorities reported 214 human cases of COVID-19 infected with SARS-CoV-2 virus variants related to mink.⁴ Some of these cases have no known link to mink farms. The mink variant carries the mutations Y453F and del 69-70. The mutation 69-70delHV is a deletion of a histidine and valine at amino acid positions 69 and 70 in the N-terminal domain of the S1 subunit of S protein. The mutation Y453F is a conservative amino acid substitution located in the RBD in

¹ European Centre for Disease Prevention and Control. Detection of new SARS-CoV-2 variants related to mink. Posted November 12, 2020. Accessed January 3, 2021.

<https://www.ecdc.europa.eu/sites/default/files/documents/RRA-SARS-CoV-2-in-mink-12-nov-2020.pdf>.

² Oude Munnink BB, Sikkema RS, Nieuwenhuijse DF, et al. Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. *Science*. 2020;eabe5901.

doi:10.1126/science.abe5901 PubMedGoogle Scholar.

³ <https://www.biorxiv.org/content/10.1101/2020.12.23.424267v1.full>.

⁴ <https://www.who.int/csr/don/06-november-2020-mink-associated-sars-cov2-denmark/en/>.

the S protein at amino acid 453 (tyrosine to phenylalanine), mutation, which is shared with the mink strain sequences from the Netherlands. It is probably an adaptation to mink ACE2 but also increases affinity to human ACE2, which may explain its successful introduction and establishment in humans and replicates as efficiently as the wildtype. However some of the strains from mink in the Netherlands lack Y453F, which demonstrates that it is not a prerequisite for infecting mink, but probably a selected mutation in the mink population.⁵ In Denmark, 4.6% of the sequenced 7108 human samples have shown mink-variants, while 40% of human cases of COVID-19 cases in North Jutland Region are carrying mink-variants.⁶ The SARS-CoV-2 variants detected in these cases were part of at least five closely-related clusters; each cluster was characterised by a specific mink-related variant, identified in humans and animals from infected mink farms.

The Cluster 5

One variant 'Cluster 5' found in twelve cases and reported as early circulating in August and September 2020 raised the alarms and caused particular concern, due to more mutations (I692V, M1229I) in the S protein.⁷ Height of these cases had a link to the mink farming industry, while four were from the surrounding area in the North Jutland Region. The last cluster 5 case was diagnosed on September 14, 2020, and it seems it does not circulate any longer. Based on these few cases there is no evidence that infection with Cluster 5 is associated with more severe symptoms nor changes in transmissibility.⁸ Until now there had been no widespread reports of mink variants in humans outside Denmark.

The mutations in the mink strain in detail

The cluster 5 variant presents a unique and never observed before combination of four mutations (three substitutions and one deletion) in the RBD of S protein in minks and the humans epidemiologically linked to the infected mink farms. They are 69-70del, I692V (a conservative substitution at position 692 that is located seven amino acids downstream of the furin cleavage site), and M1229I (a conservative substitution located within the transmembrane domain). The table below summarizes the mutations of the mink variant. The subsequent table illustrates the 3D positions of the mutations in the S protein.

The mutations of the mink variant
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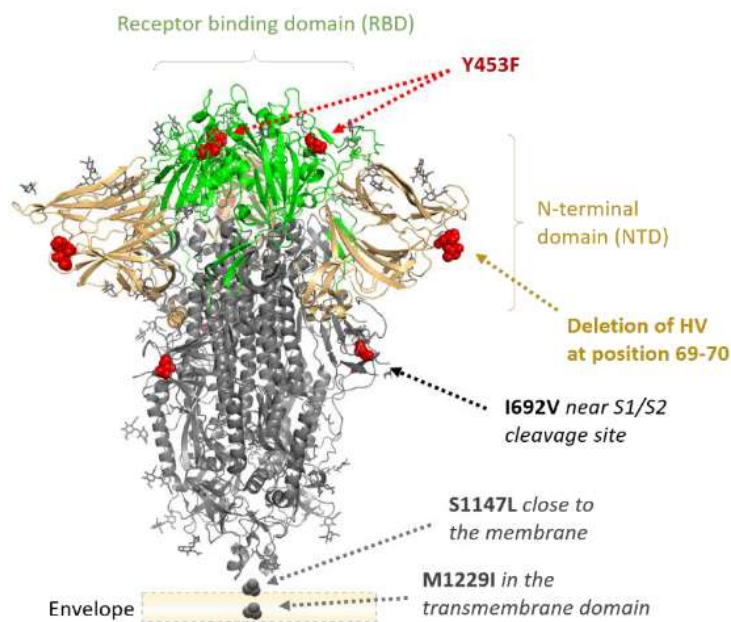
5 https://files.ssi.dk/Mink-cluster-5-short-report_AFO2.

6 Krammer F. SARS-CoV-2 vaccines in development. *Nature*. 2020 Oct;586(7830):516–527.

7 <https://www.who.int/csr/don/06-november-2020-mink-associated-sars-cov2-denmark/en/>.

8 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7678454/#bib7>.

	Definition (amino acid changes)	Potential public health impact of variant	Geographical spread
Danish mink variant	del 69-70, Y453F	Transmission from mink to humans and community spread confirmed, no changes in transmissibility reported.	Prevalent in Denmark. Not detected elsewhere
Danish mink cluster 5	del 69-70, Y453F, I692V, M1229I	Preliminary report of moderate reduction of neutralisation by convalescent sera.	Denmark, not observed since September 2020.



The mink-associated mutations in the SARS-CoV-2 spike protein.

Since the RBD of S protein is a major target for immune response and vaccines, such mutations could, in theory, have implications for viral fitness (ability to infect humans and animals), transmissibility, and antigenicity (antibody-mediated immunity provided by SARS-CoV-2 infection or vaccines in humans). Preliminary findings of an initial investigation of nice human convalescent serum samples indicate that the mutations in the S protein might lead to a modest and variably statistically significant reduction in neutralization activity against cluster 5 viruses. However, scientific and laboratory-based studies are needed to clarify the extent of the possible implications of this new variant in terms of SARS-CoV-2 treatment, diagnostic tests and virus antigenicity. ⁹

⁹ https://files.ssi.dk/Mink-cluster-5-short-report_AFO2...

How the Danish Government reacted?

Due to accumulated mutations of SARS-CoV-2 found circulating in over 200 mink farms, on 5 November 2020, the Ministry of Environment and Food of Denmark announced the culling of all mink in the country, estimated to approximately 17 million animals, including the breeding stock, and the long-term shutdown of the Danish mink industry.¹⁰ Likewise, the Government locked down seven municipalities in North Jutland including restricting movement between these and testing all 280.000 inhabitants and sequencing all positive samples. The public health reaction consisting of a generalized depopulation and the killing of healthy animals spurred harsh criticisms and distrusts on the too rushed Government's decision.

Why did that happen at what is next?

SARS-CoV-2 has accumulated mutations since its emergence in the human population in 2019, with a typical rate of only two single-letter mutations per month in its genome. It is known that when a virus switches host species an increased mutation rate can occur due to the virus adapting to its new host. The rapid circulation of SARS-CoV-2 in the mink population likely triggered a strong antibody reaction among the infected minks, which might have exerted a selective pressure on the virus, which began to mutate to quell the antibodies. The high circulation and the mink outbreaks are most likely caused by the husbandry conditions, with the overcrowding of thousands of individuals in cages made of wire netting, allowing free airflow and contact between animals. Therefore it is not surprising that rapid animal-to-animal transmission accelerated the evolution of SARS-CoV-2. This made possible the adaptation of SARS-CoV-2 to the mink ACE 2 receptor, thereby changing its S protein and evolving into a mink-coronavirus variant, an antigenic drift well known for influenza virus. The apparent adaptation of SARS-CoV-2 to mink is concerning because continued evolution and mutations of the virus in an animal reservoir could potentially lead to recurrent spill-over events of novel SARS-CoV-2 from mink to humans and other mammals.¹¹ This theoretical risk prompted many countries to increase surveillance efforts, and in some cases as a precautionary measure, implement large-scale culls (ie. selective slaughter) of mink on farms. However, there is also a chance that the mink variant may lose the ability as it evolves as may not transmit well to human.

What other mutations might emerge?

The epidemiological evolution of COVID-19 pandemic looks like a dog (vaccine) trying to catch a hare (the virus), that is running alarmingly faster. Given the relentless massive circulation of SARS-CoV-2 in the human population, dangerous mutations are constantly emerging (eg.

¹⁰ Ministry of Environment and Food of Denmark. COVID-19: all mink in Denmark must be culled. 2020. <https://en.mfvm.dk/news/news/nyhed/covid-19-all-mink-in-denmark-must-be-culled/>

¹¹ <https://www.theguardian.com/environment/2020/nov/05/danish-covid-19-mink-variant-could-spark-new-pandemic-scientists-warn>.

mutations N501Y and E484K in South Africa, Brazil, UK). It won't be surprising if the virus takes another step forward. To mimick the natural evolution, a recent study using yeast displaying ACE methods, lab-accelerated the evolution of the RBD.¹² The initial results gave rise to the N501Y and E484K mutations, which is consistent with what is happening in the real world. After a few more rounds of evolution, the researchers found the Q498R mutation, which by virtue of a synergistic effect with N501Y and E484K, increases the binding capacity of RBD to the ACE2 receptor by 50 times and confers resistance to antibodies. An even faster evolution has resulted in an RBD hosting nine mutations that bind to the ACE2 receptor 600 times greater in intensity. However, the tighter binding of the virus to cell receptors does not always reflect biological fitness (i.e., the virus's ability to replicate efficiently). At times, overly tight binding may hinder the subsequent viral fusion with the cell membrane. Both receptor binding and viral fusion are required to complete infection.¹³ So, it is clear that these results do not always translate accurately in the real world and more tests are needed to determine what's the clinical significance of the Q498R. However, since RBD evolved to bind the ACE2 receptor so strongly, the complete blockage of viral replication makes the decoded and purified RBD mutant a promising drug candidate to prevent SARS-CoV-2 infection.

Risk assessment: what is the risk to human health posed by SARS-CoV-2 mink related variants?

ECDC has published a rapid risk assessment of the human health risk posed by COVID-19 outbreaks in mink and.¹⁴ Here are the conclusions.

Transmissibility

The variants can circulate rapidly in mink farms and the human communities close to the farms, however, they do not appear to be more transmissible than other circulating SARS-CoV-2 variants.

Severity

The current impact of COVID-19 on disease severity in patients infected with any mink-related variant appears to be similar to those infected with non-mink-related variants.

Immunity, reinfection, vaccination and treatment

Of all mink-related variants analysed so far, only the Cluster 5 variant has raised specific concern due to its effect on antigenicity. Further investigations are needed to assess whether this may have any impact on the risk of reinfection, reduced vaccine efficacy or reduced benefit of

12 <https://www.biorxiv.org/content/10.1101/2021.01.06.425392v3>.

13 <https://royalsocietypublishing.org/doi/10.1098/rsif.2013.1083#d3e1991>

14 <https://www.ecdc.europa.eu/en/publications-data/detection-new-sars-cov-2-variants-mink..>

treatment with plasma from convalescent patients or with monoclonal antibodies. S-gene deletion at genomic positions 21765-21770, corresponding to residues 69-70 in the S protein in the mink-related variants from Denmark, as well as variant VOC 202012/01 and other variants carrying this mutation, may cause some RT-PCR assays targeting the S-gene to produce a negative result (S-gene drop-out). If the SARS-CoV-2 spread among mink is not controlled and there is recurrent spill-over into the human population, variants that provide a selective advantage in mink populations could become widespread in humans, even without any selective advantage in the human population

Risk management (response strategies)

Close collaboration between animal and public health authorities under the One Health approach is crucial for the early detection of SARS-CoV-2 infection outbreaks at mink farms and human cases related to mink farms, to allow timely response and control measures. To decrease the risk posed to public health, national health authorities should consider implementing the following measures aimed at mink farms, mink farm workers and communities in contact with mink farms:

- **human testing with sequencing and characterisation** of antigenic properties and virus infectivity; (Early Warning and Response System -EWRS) and the international community.)
- **infection prevention and control measures** for mink farm workers and visitors;
- **animal testing and prevention of spread from animals** (intensively-reared animal species, susceptible to SARS-COV-2, such as mink may act as an amplifier of the virus, leading to increased virus biomass in the environment, and a greater risk of virus mutations and transmission within farmed mink and between mink and humans.)
- **development of 'One Health' preparedness and response strategies**, with enhanced coordination between the agricultural, animal health, and human health (including occupational health and safety) sectors.

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