Paired SARS CoV-2 Spike Protein Mutations Observed During Ongoing SARS-CoV-2 Viral Transfer from Humans to Minks and Back to Humans

Scott Burkholz[a], Suman Pokhrel[b], Benjamin R. Kraemer[b] , Daria Mochly-Rosen[b], Richard T. Carback III[a], Tom Hodge[a], Paul Harris[c], Serban Ciotlos[a], Lu Wang[a], CV Herst[a], and Reid Rubsamen[a,d,e,f*]

a Flow Pharma, Inc., Pleasant Hill, CA
b Department of Chemical and Systems Biology, Stanford University School of Medicine, Stanford, CA
c Department of Medicine, College of Physicians and Surgeons, Columbia University, New York City, NY
d Massachusetts General Hospital, Department of Anesthesia, Critical Care and Pain Medicine, Boston, MA
e University Hospitals Cleveland Medical Center, Cleveland, OH
f Case Western Reserve School of Medicine, Cleveland, OH

Abstract
A mutation analysis of a collection of SARS-CoV-2 genomes around the world via sequence, date, geographic location, and species has revealed a large number of variants from the initial reference sequence in Wuhan. It also reveals that humans infected with SARS-CoV-2 have infected mink populations in the Netherlands, Denmark, United States, and Canada. In these animals, a small set of mutations often in combination, in the spike protein receptor binding domain (RBD) has apparently transferred back into humans. The viral genomic mutations in minks observed in the Netherlands and Denmark show the potential for new mutations on the SARS-CoV-2 spike protein RBD to be introduced into humans by zoonotic transfer. Our data suggests that close attention to viral transfer from humans to farm animals and pets will be required to prevent build-up of a viral reservoir for future zoonotic transfer.

Background
Coronaviruses are thought to have ancient origins extending back tens of millions of years with coevolution tied to bats and birds (Wertheim et al., 2013). This subfamily of viruses contains proofreading mechanisms, rare in other RNA viruses, reducing the frequency of mutations that might alter viral fitness (Cyranoski, 2020). The D614G mutation, which lies outside the RBD, is an example of a fitness-enhancing mutation on the spike glycoprotein that is becoming increasingly more prevalent as the virus spreads through human populations (Korber et al., 2020). Another very recent and more infectious mutant D796H paired with ΔH69/V70, also outside the RBD domain, has spread throughout Southeast England in the past few weeks (Kemp et al., 2020). Data from next-generation sequencing has shown that the SARS-CoV-2 viral genome mutates at about half the rate of Influenza and about a quarter of the rate seen for HIV, with about 10 nucleotides of difference between samples (Callaway, 2020). The Global Initiative on Sharing Avian Influenza Data (GISAID) (Elbe & Buckland-Merrett, 2017; Shu & McCauley, 2017), has catalogued over 235,299 SARS-CoV-2 sequences to date, provided by laboratories around the world. Well over 12,000 mutations have been shown to exist, with

potential for non-synonymous substitutions, insertions, or deletions resulting in amino acid changes which could result in structural and functional changes in virus proteins (Callaway, 2020). While Coronaviruses initially developed in animals and transferred to humans, transfer back to animals and then back to humans again has recently been observed in in the *Neovison vison* species of mink*,* currently being raised in farms around the world.

Non-silent mutations in the SARS-CoV-2 spike glycoprotein gene can produce structural and functional changes impacting host receptor binding, and viral entry into the cell. Specifically, a mutation in the RBD region, residues 333-527 (Lan et al., 2020), can potentially either increase or decrease the affinity of spike protein for the ACE2 receptor, directly influencing the ability of the virus to enter a cell and successfully infect the host. The receptor binding motif (RBM), located within the RBD from 438 to 506 (Lan et al., 2020), plays a key role in enabling virus contact with ACE2 and is an optimal target for neutralizing antibodies (Shang et al., 2020). Mutations in this region which also correspond to the complementarity-determining regions (CDR) of a potentially binding immunoglobulin, could affect the antibody's ability to neutralize the RBD (Greaney et al., 2020).

Here we examined the mutations that were associated with the transfer of SARS-CoV-2 from humans to minks and back to humans. This example of zoonotic transfer highlights the importance of paired mutations in the RBD domain and suggests potential challenges for sustained efficacy of neutralizing antibodies focusing on that region.

Methods

Mutation Discovery

All 235,299 available spike glycoprotein sequences available thru December 5th were downloaded from GISAID (Elbe & Buckland-Merrett, 2017; Shu & McCauley, 2017). Sequences thought to be duplicate submissions, not covering the full genome, or containing greater than 5% ambiguous nucleotides were removed from the dataset. Each sequence was individually aligned via MAFFT (Katoh & Standley, 2013) to the WIV04 (MN996528.1) (Zhou et al., 2020) internal reference sequence. Insertions occurring once in the database and containing 30 or more nucleotides compared to the WIV04 reference sequence were also removed. Sequences with shorter insertions were aligned under different parameters for gap penalty and then combined with sequences without insertions. Gene sequences were converted to amino acid sequences based on protein reference sequence positions.

Spike glycoprotein sequences meeting those criteria were re-aligned via MAFFT (Katoh & Standley, 2013) against WIV04 (MN996528.1) (Zhou et al., 2020) with position numbering kept constant. Sequences were split between human (206,591 sequences) and the *Neovison vison* species of mink (332 sequences).

A custom Python (*Python 3*, 2020) script was utilized to compare the variants seen in mink and humans different from the WIV04 reference sequence. Only mutations with at least three

occurrences in minks were kept. After initial examination of the whole genome, all mutations inside of, or within 25 amino acids of, RBD (333-527) (Lan et al., 2020) were also kept for analysis. The script was rerun using only sequences and meta-data matching those criteria. Thirteen human and three mink sequences were removed that were found to contain 25 or more gaps or mutations compared to the WIV04 reference. This was done to avoid the potential for improper mutation identification confounding downstream analysis. After all of these filtering procedures, 782 human and 251 mink sequences remained for analysis.

The statistical package R (R. Core Team, 2017) was utilized to plot mutation frequency by geography, date, and species to reveal patterns indicative of zoonotic transfer. Sequence identifiers, and the respective authors, utilized for results are shown in supplementary table 1.

SARS Alignment Comparisons

The SARS-CoV-2 reference sequence WIV04 (MN996528.1) (Zhou et al., 2020) was aligned against a SARS-CoV-1 reference sequence (NC_004718.3) (He et al., 2004) via MAFFT (Katoh & Standley, 2013). Residue positions were visualized in Jalview (Waterhouse et al., 2009).

3D Structure Visualization

The PDB file, 7A98 (Benton et al., 2020), was downloaded from RCSB.org. Positions of interest were visualized in MOE (ULC, 2020) for figure 1b.

Mutation Effect Calculations

Molecular Operating Environment (MOE) (ULC, 2020) software was used with PDB 7A98 (Benton et al., 2020), and prepared with QuickPrep functionality at the default settings, to optimize the H-bond network and perform energy minimization on the system. Affinity calculations were performed using 7A98.A (spike protein monomer) and 7A98.D (ACE-2) chains. Residues in spike protein (7A98.A) within 4.5 Å from ACE2 (7A98.D) were selected and the residue scan application was run by defining ACE2 (7A98.D) as the ligand. Residue scan and change in affinity calculation was also performed on L452. Stability calculations were performed by running the residue scan application using residues in RBD (331-524) of spike protein (7A98.A). Residue scans and change in stability calculations were also performed on Q314. The changes in stability and affinity (kcal/mol) between the variants and the reference sequence were calculated as per MOE's definition (ULC, 2020). The potential effect of variants was predicted using PROVEAN (Choi & Chan, 2015) with R (R. Core Team, 2017) and SIFT (Ng, 2003).

Results

The following six mutations met all of the criteria described in the methods section, and demonstrate the potential for zoonotic transfer between species. Mutations outside of the spike RBD and other proteins were examined, but did not result in significant findings.

Netherlands

F486L

The F486L mutation has a substitution of leucine for phenylalanine occurring within the RBM on the SARS-CoV-2 spike glycoprotein (Figure 1 A, B). These amino acids are similar in physiochemical properties, with an aromatic ring being replaced by an aliphatic chain. This new variant in F486L, conserved across SARS-CoV-1 and SARS-CoV-2, was first seen in a *Rhinolophus affinis* bat sample collected in Yunnan, China during 2013. In 2017, this variant was also found in *Manis javanica,* a species of pangolin. F486L was not present in human sequences from the dataset at the start of the pandemic, but started to appear in minks at the end of April 2020. We found 125 sequences from mink samples with this mutation collected in the Netherlands since that time.

A sample submission date places the first known potential transfer back into humans in August 2020, also in the Netherlands. While sample collection dates are unavailable for these human samples, submission dates show a larger number of human samples with this variant were reported in the Netherlands in October and November 2020. One human sample in Scotland, collected in October 2020 shows that F486L may be viable alone and can occur *de novo*, without evidence of zoonotic transfer. Based on mutations seen with F486L, L452M and Q314K, and considering the potential for sequencing error, this case is likely not linked to the Netherlands sequences (Table 1).

We saw the L452M and Q314K variants almost always appearing concurrently with F486L, with the exception of the first few known mink sequences (Figure 2A). The only other sequences in the database with these variants outside the Netherlands were from two human samples collected in Michigan October 2020, also currently experiencing an outbreak in mink farms (Michigan.gov, 2020). These latter samples with F486L contain a second variant, N501T, which we also saw in four mink samples from the Netherlands. The pairing of some mutations with others (Figure 3) suggests the potential requirement of a specific second mutation, L452M or Q314K, to be present in order for the virus to jump back from minks to humans.

L452M

The L452M spike RBM mutation involves substitution of methionine for leucine at position 452 (Figure 1 A, B). The side chains for these amino acids are quite similar, with the addition of a sulfur. This variant appeared first in mink samples from the dataset dated July 2020, and was always found the L452M variant occurring with F486L in the mink population sequences in the database. We also observed that L452M and F486L always occurred together in human samples from the Netherlands (Table 1), submitted starting August 2020 and continuing through November. The five human samples taken outside of the Netherlands in the database all contain L452M, without being paired with F486L in the sequence database (Figure 2B).

Q314K

Q314K is located close to the RBD, and has lysine is substituted for glutamine at position 314 (Figure 1A) resulting in the addition of a positive charge to the side chain. This new variant in Q314K is found in both SARS-CoV-1 and SARS-CoV-2. The earliest sequences in the database with this mutation were found in five human samples taken in Northern California and one human sample taken in Mexico in May 2020. This variant first appeared in human samples submitted from the Netherlands in October and November 2020 (Figure 2C). The dataset showed that Q314K was first seen in minks starting in August 2020 and was always seen with F486L, but only in the absence of L452M. The ten human samples from outside the Netherlands all had Q314K without the other variants described here (Table 1), suggesting it can occur *de-novo*.

Denmark

Y453F

Y453F is an RBM mutation substituting phenylalanine for tyrosine at position 453 that we observed present in a substantial number of human sequences in the database from samples submitted from around the world since the start of the pandemic (Figure 1A, B). The substituted amino acid is similar to the reference with the change being less polar. This new variant in Y453F is found in both SARS-CoV-1 and SARS-CoV-2. The sequences analyzed for this study show this mutation appearing in minks starting in the Netherlands in April, and Denmark in June 2020. We saw rapid expansion of this mutation, now present in 629 human sample sequences from Denmark, beginning in June 2020 (Figure 2D, Table 1). Of the five samples collected outside the Netherlands or Denmark, one sample came from Utah, one of the top producers of mink fur in the United States and currently experiencing a SARS-CoV-2 outbreak on mink farms (Aleccia, 2020).

United States

N501T

N501T is an RBM mutation, and involves a change from asparagine to threonine at position 501 (Figure 1A, B). This new N501T variant is found in both SARS-CoV-1 and SARS-CoV-2 and was first seen in 2017 in a species of pangolin, where it was seen to co-occur with F486L. N501T was found only sixteen times in humans or minks in the samples we analyzed. Four mink samples with N501T were submitted from the Netherlands between April and June 2020. The twelve human sequences with the N501T variant were seen from March to October 2020, but were not seen in samples collected in the Netherlands or Denmark (Table 1). As mentioned previously, two of the three sequences among the twelve collected outside the Netherlands with F486L contain this variant and were from Michigan, a state with documented mink farm outbreaks. Two of the twelve human sequences with N501T alone were found in Wisconsin on October 3rd. This variant was present in a human sample collected in Taylor county, where a

mink farm was reported on October 8th to have confirmed cases, now with a second farm outbreak and over 5,400 mink deaths from the virus (Kirwan, 2020; Schulte, 2020).

V367F

V367F has a potentially more significant amino acid substitution at (RBD) position 367 (Figure 1 A, B) with addition of an aromatic group (substituting phenylalanine for valine). This new variant in V367F is found in both SARS-CoV-1 and SARS-CoV-2. Our analysis identified this variant in 97 human samples collected around the world, not localized to any one region (Table 1). V367F was present in five minks and ten humans in samples collected in the Netherlands. We observed that this variant can occur *de-novo* without zoonotic transfer. Some cases in humans were seen in samples collected from August to October 2020 in Oregon, another state currently experiencing a mink farm outbreak (Bellware, 2020). We found nine human samples with this variant from Washington State, which has not reported a mink outbreak, spanning February to August and has a small a number of mink farms (USDA, 2020).

Mutation Effects

The six mutations we studied result from a Single Nucleotide Polymorphism (SNP), and are among those with the least potential consequence on the stability (kcal/mol, supplementary table 2) of the spike glycoprotein. Analysis for ACE2 binding affinity (kcal/mol, supplementary table 3) determined that F486L, Y453F, N501T, and L452M likely cause minimal effects on the affinity of the spike protein to bind ACE2. The calculated difference in affinity score for the Y453F mutation indicates a slightly increased binding potential of this spike variant for ACE2 as compared to the reference amino acid sequence. Predicted effects of all six mutations illustrate neutral and tolerated classifications (supplementary table 4) suggesting that the function of the protein is not drastically changed, and likely remains fit for replication and infection.

Discussion

The mutations outlined for SARS-CoV-2 show a pattern indicative of zoonotic transfer from humans to minks and back to humans.

The presence of F486L paired with either L452M or Q314K in humans and minks illustrates the spread of two separate transfer events. While we observed these mutation pairs in the Netherlands, we did not see them elsewhere in the world (Table 1). The mutation F486L did not make a correlated jump from minks to humans until a second mutation L452M, Q314K, or N501T was simultaneously present (Figure 3). This illustrates the possibility that multiple mutations are required to preserve fitness and facilitate inter-species transfer, particularly in relation to the host's ACE2 protein. Mutations in viruses have been described previously to occur in pairings for function (Altschuh et al., 1987) and furthermore have been shown to have evolutionary relationships based on these coupling of variants (Marks et al., 2011).

In Denmark, Y453F showed a pattern potentially arising from humans to minks and back to humans. In the US, isolated incidences of F486L, N501T, and V367F present evidence for the same type of transfer event, in the same species of mink, but thousands of miles away from Northern Europe. This data supports the interpretation that paired mutations facilitate the zoonotic transfer.

Although some collection dates are lacking from submitted human sequences from the Netherlands, the timeline supports that the spread and transfer from minks to humans is occurring, as suggested by the submitters of the sequences (Oude Munnink et al., 2020), within the GISAID database (Elbe & Buckland-Merrett, 2017; Shu & McCauley, 2017).

Current antibody-based therapeutics, and more under development, are focused on antibody binding to the SARS-CoV-2 spike protein RBD (Wu et al., 2020). Similarly, current vaccines, designed to generate antibody responses to the spike protein, may be dependent on the stability of the primary amino acid sequence in the RBD to maintain their ability to generate neutralizing antibody responses (Poland et al., 2020). It is therefore critical to understand the extent to which SARS-CoV-2 mutations are occurring in regions targeted by antibodies.

The implications of these mutations for the efficacy of vaccines or antibody therapeutic interventions are unknown. The extent to which these mutations in the RBD could have a beneficial or deleterious effect on viral fitness, or on RBD binding affinity to ACE2 potentially increasing or decreasing infectivity is also not understood. The mutation N501T does not appear to be spreading rapidly and may be showing decreased fitness in humans, but the mutation Y453F, now present in 629 human samples from Denmark, beginning in June 2020, suggests an increased fitness of the virus potentially facilitating its spread in human populations. Our calculations also suggest that the N501T variant decreases protein stability and affinity for ACE2, but the change is minimal. The pairing of certain mutations should be tested *in vitro* in the future. A single mutation could have a completely different consequence when paired with another through a combinatory effect. The data suggests that two mutations together may be required for this type of zoonotic transfer to occur. While the number of cases with these pairings is not growing exponentially around the world, a third mutation could occur, further changing binding affinity and stability characteristics.

In Denmark, recent human and mink samples have shown the presence of Y453F in 629 and 85 samples respectively. It has been reported that commercial monoclonal antibodies were not as effective for viruses with Y453F as shown previously (Mallapaty, 2020). The fact that this mutation is showing some potential to escape neutralization by antibodies that were previously shown to neutralize SARS-CoV-2 creates cause for concern. Viral escape could compromise the efficacy of monoclonal antibody cocktails showing promise when used to treat patients with mild to moderate disease.

Gene sequence evidence of SARS-CoV-2 zoonotic transfer as a mechanism capable of increasing mutations needs to be vigilantly followed over the coming months and years. There are multiple species that can be infected by SARS-CoV-2 to assist in this mutation potential (Dhama

et al., 2020). A number of species known to be infected by this virus have not been sequenced to the same extent as minks in the current outbreak. A large number of minks were sequenced in the Netherlands and Denmark. To date, mink sequences from the United States are not publicly available. Tracking mink mutations that might later appear in humans is important. Additionally, transparency of outbreak reporting for animal populations is critical. This has been an ongoing issue in Oregon where the mink farm location has not been disclosed. (Loew, 2020). A new, recent outbreak on mink farms in Canada (Ugen-Csenge, 2020) has provided 4 samples for analysis; collected December 4th. The mutation F486L has shown up in one of the four mink sequences available (not shown in figure 2 or table 1) and will need to be carefully watched for the addition of a second mutation, thought to allow the jump of the SARS-CoV-2 back to humans, as described in figure 3.

Many different species have homologous ACE2, with a highly conserved binding site for the spike protein, demonstrating a wide range of potential hosts for a viral reservoir where new mutations may acquire new infectivity potential for humans (Damas et al., 2020; Melin et al., 2020). A novel artificial intelligence algorithm has shown that minks, along with bats, could be a reservoir of SARS-CoV-2 (Guo et al., 2020). Samples from cats, dogs, ferrets, hamsters, primates, and tree shrews have shown that all of these species have been infected with SARS-CoV-2 (CDC, 2020). Multiple factors contribute to this zoonotic transfer such as ACE2 expression level, and close contact with the same or other species.

While there is significant concern regarding effects of COVID-19 in humans, and the potential of zoonotic transfer to increase mutations and the potential for escape, the extent to which the virus can mutate and solely affect animals will also need to be monitored indefinitely. African swine fever, for example, has been shown to only effect pig populations and has had a profound effect on the worldwide supply of pork (Kedkovid et al., 2020). In addition, critically endangered species such as the European mink (*Mustela lutreola*), known to be infected by SARS-CoV-2, could be at greater risk of extinction because of the virus.

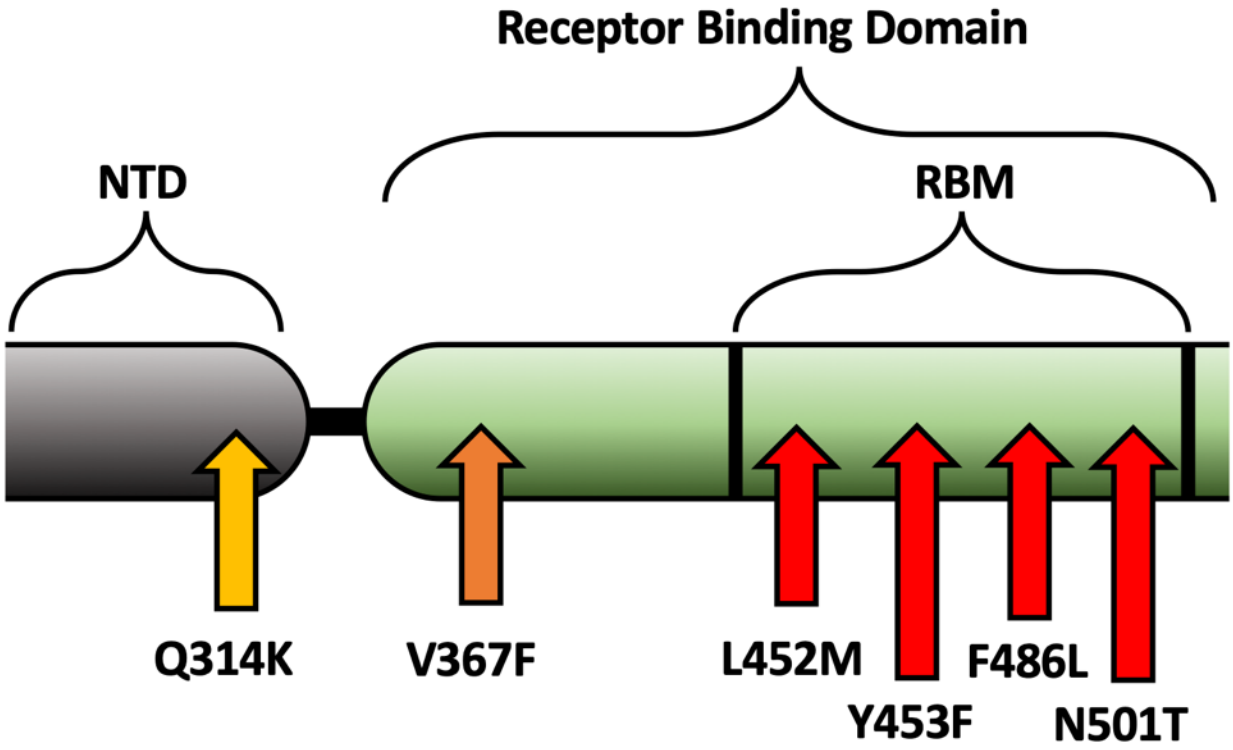
3D simulations can be used to predict which mutations are most likely to occur. Stability (expressed in terms of energy as kcal/mol) can be used to estimate the extent to which amino acid mutations could cause a change in energy within two proteins such as the RBD and ACE2 or RBD and an antibody. Affinity between the RBD and amino acid residues in close contact with ACE2 (at a range of about 4.5 Å), can characterize the potential positive or negative effects of a mutation on this crucial binding interaction. Additionally, prediction algorithms can determine the extent to which a single mutation or a combination of mutations could positively or negatively alter the function of a protein. Together, these methods can be utilized to identify optimal targets for vaccines and antibody therapeutics. Other computational methods, similar to the ones described here, have been demonstrated previously to assist in rapid vaccine design (Cunha-Neto et al., 2017).

Changes in the efficacy of vaccines and monoclonal antibody therapies targeting SARS-CoV-2 spike need to also be followed closely, especially as selection pressure from the widespread use of these interventions increases the potential for virus escape (Greaney et al., 2020; Haynes et

al., 2020). In addition, a vaccine or antibody therapy that previously was shown to have a neutralizing effect on the virus, could become non-neutralizing after a mutation matches the antibody CDR, which may also lead to Antibody Disease Enhancement (ADE) (Smatti et al., 2018; Yong et al., 2019).

Conclusion

SARS-CoV-2 has shown mutation potential in the RBD for zoonotic transfer starting with the pandemic in China, and has continued most recently with minks in the Netherlands, Denmark, United States, and Canada. Whether these mutations become widespread and affect the efficacy of vaccines and monoclonal antibody cocktail therapeutics remains to be seen, but the potential exists for future mutations arising due to close contact between a wide range of species. There is significant cause for concern that SARS-CoV-2 may evolve further with zoonotic transfer, as it did at the origin of the outbreak, involving various species. This has implications for facilitating human viral escape, potentially reducing the efficacy of antibody based vaccines and therapies. Worldwide sequencing of samples from many species correlated with human sequences will need to be performed indefinitely to monitor mutations, and combinations of mutations. This will serve as an early warning system for potential viral escape in humans that may lead to loss of efficacy for vaccines and therapies critical to mitigating the effects of the SARS-CoV-2 pandemic.



B

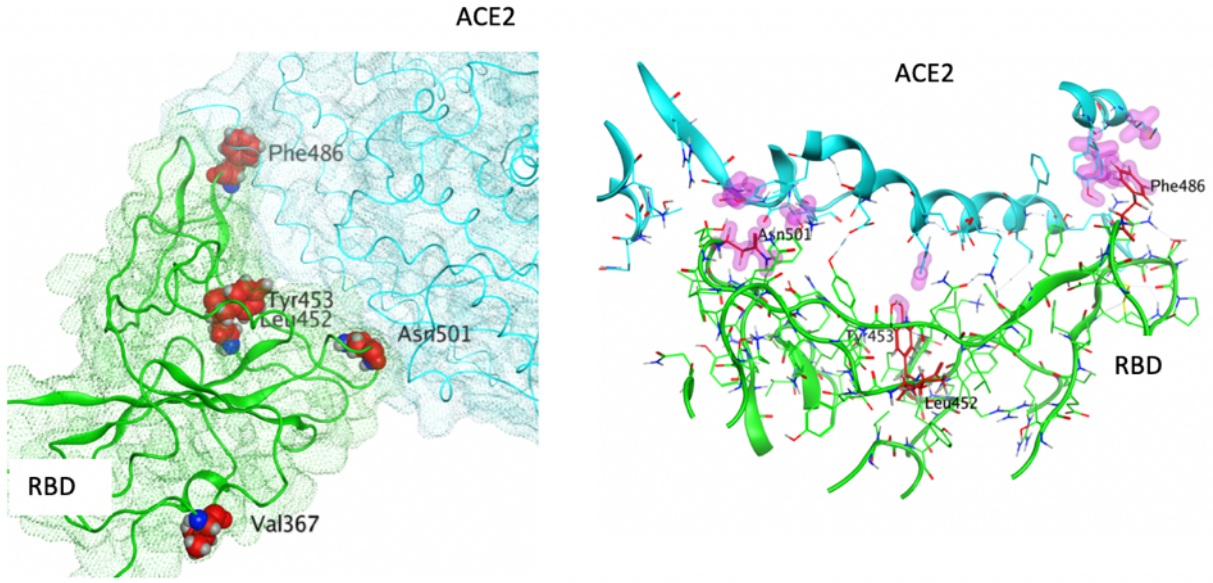


Figure 1 A: Illustration of positions of mutation variants described on the SARS-CoV-2 spike glycoprotein for the N-terminal domain (NTD), receptor binding domain (RBD), and receptor binding motif (RBM). B: Left: Crystal Structure (PDB ID: 7A98) of SARS-CoV-2 receptor binding domain (green) in complex with ACE2 (teal) with residues highlighted in red. Right: Interaction of highlighted residues (red) with ACE2 (teal). Interaction denoted with magenta clouds.

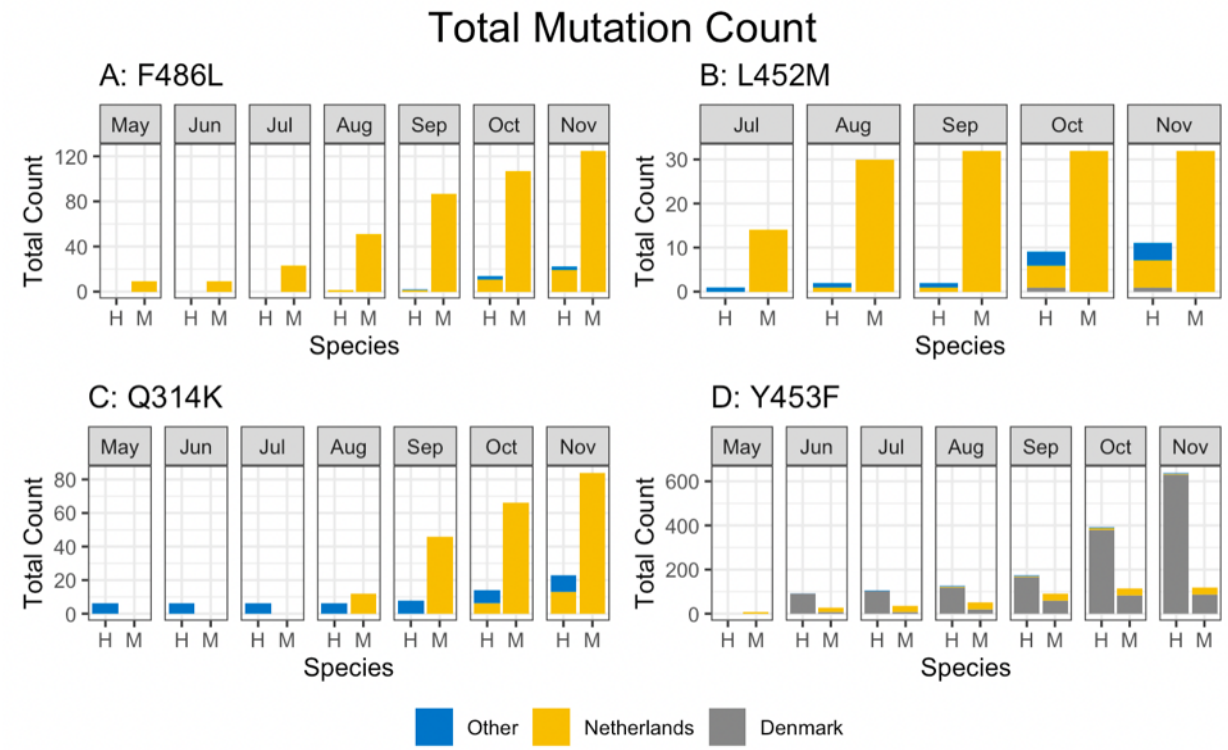
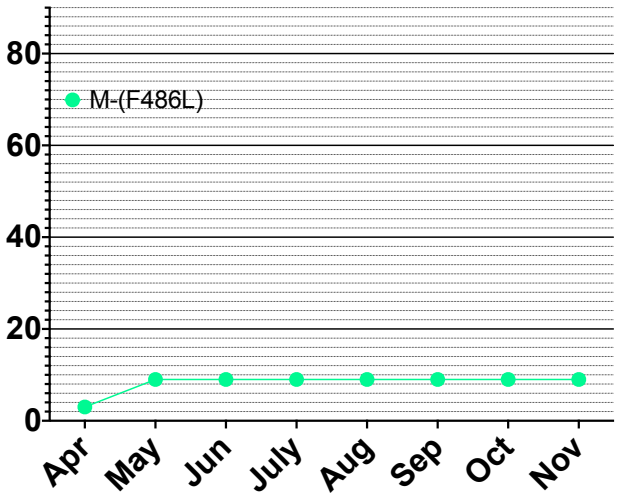
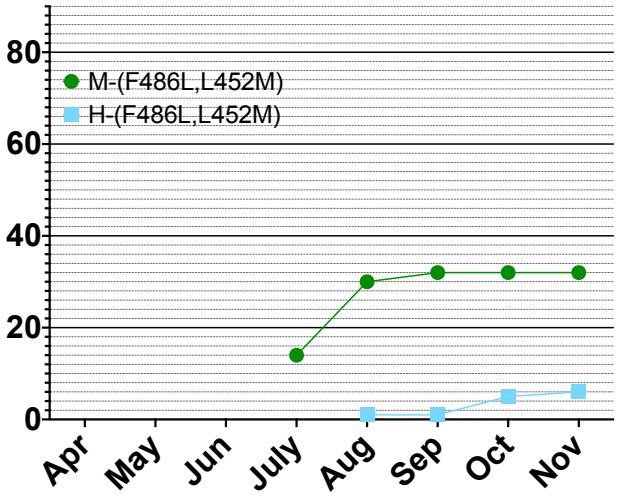


Figure 2: Total mutation counts stratified by mutation, location, and species (human and mink). Dates reported for human samples from the Netherlands are predominately submission dates, in contract to the reported collection dates seen in meta data associated with sequences submitted from other countries.


80
60
40
20
0
M-(F486L)
Apr
May
Jun
July
Aug
Sep
Oct
Nov


80
60
40
20
0
M-(F486L,L452M)
H-(F486L,L452M)
Apr
May
Jun
July
Aug
Sep
Oct
Nov

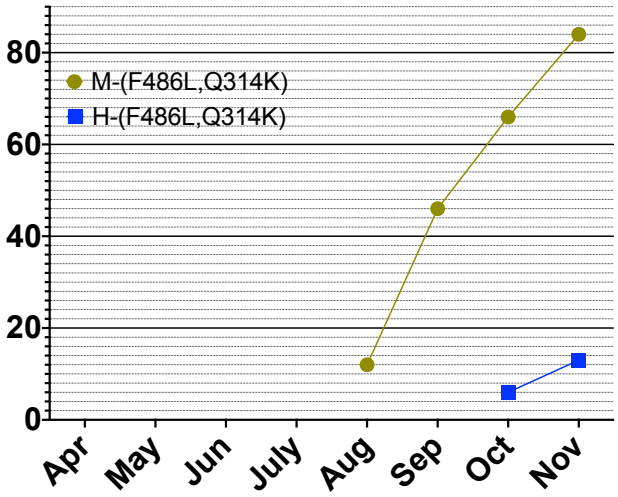

80
60
40
20
0
M-(F486L,Q314K)
H-(F486L,Q314K)
Apr
May
Jun
July
Aug
Sep
Oct
Nov

Figure 3: Cumulative counts for mutation F486L individually and in pairs with L452M or Q314K in the Netherlands.

Country	Mutations	Human Count	Mink Count
F486L			
Netherlands	F486L, L452M	6	32
Netherlands	F486L, Q314K	13	84
Other (Michigan)	F486L, N501T	2	N/A
Other	F486L	1	N/A
Netherlands	F486L	0	9
L452M			
Netherlands	L452M, F486L	6	32
Denmark	L452M	1	0
Other	L452M	4	N/A
Q314K			
Netherlands	Q314K, F486L	13	84
Other	Q314K	10	N/A
Y453F			
Denmark	Y453F	629	85
Netherlands	Y453F	4	32
Netherlands	Y453F, V367F	1	1
Other	Y453F	5	N/A
N501T			
Other (Michigan)	N501T, F486L	2	N/A
Other	N501T	10	N/A
Netherlands	N501T	0	4
V367F			
Netherlands	V367F, Y453F	1	1
Netherlands	V367F	9	4
Denmark	V367F	2	0
Other	V367F	85	N/A

Table 1: Mutations counted by species, location, and linkage with other mutations. Highlighted mutations illustrate inheritance patterns.

Supplementary Table 1:
GISAID sequence citations will be available after the database authors review the paper and provide a table of labs and authors.

Supplementary Table 2:

F486L			L452M			Y453F			N501T			V367F			Q314K		
mutation	dstability	SNP	mutation	dstability	SNP	mutation	dstability	SNP	mutation	dstability	SNP	mutation	dstability	SNP	mutation	dStability	SNP
1:F486F	0.000	1	1:L452L	0.000	1	1:Y453Y	0.000	1	1:N501N	0.000	1	1:V367V	0.000	1	1:Q314Q	0.000	1
1:F486L	**0.716**	**1**	1:L452F	0.875	1	**1:Y453F**	**0.695**	**1**	**1:N501T**	**0.915**	**1**	1:V367I	0.331	1	1:Q314L	0.081	1
1:F486I	0.746	1	1:L452R	1.014	1	1:Y453H	1.902	1	1:N501D	1.081	1	**1:V367F**	**0.336**	**1**	1:Q314R	0.649	1
1:F486Y	0.996	1	**1:L452M**	**1.116**	**1**	1:Y453D	2.484	1	1:N501S	1.124	1	1:V367M	0.449	1	1:Q314H	1.049	1
1:F486V	1.208	1	1:L452Q	1.329	1	1:Y453N	2.500	1	1:N501H	1.687	1	1:V367L	0.785	1	1:Q314E	1.199	1
1:F486C	1.941	1	1:L452W	1.337	1	1:Y453C	2.523	1	1:N501I	3.430	1	1:V367A	1.214	1	**1:Q314K**	**1.262**	**1**
1:F486S	2.152	1	1:L452S	1.462	1	1:Y453S	2.794	1	1:N501K	3.648	1	1:V367E	1.278	1	1:Q314P	1.640	1
1:F486M	0.866	0	1:L452V	1.465	1	1:Y453M	1.479	0	1:N501Y	33.473	1	1:V367G	1.365	1	1:Q314I	0.161	0
1:F486W	1.442	0	1:L452H	1.590	1	1:Y453W	1.738	0	1:N501L	0.482	0	1:V367D	1.771	1	1:Q314F	0.377	0
1:F486T	1.628	0	1:L452I	1.778	1	1:Y453Q	2.118	0	1:N501C	0.898	0	1:V367R	0.615	0	1:Q314M	0.401	0
1:F486Q	1.655	0	1:L452P	3.124	1	1:Y453T	2.597	0	1:N501A	0.967	0	1:V367Y	0.663	0	1:Q314V	0.522	0
1:F486E	1.797	0	1:L452Y	0.931	0	1:Y453A	2.658	0	1:N501M	1.493	0	1:V367W	0.863	0	1:Q314Y	0.546	0
1:F486R	1.940	0	1:L452A	1.245	0	1:Y453E	2.964	0	1:N501G	1.872	0	1:V367K	0.945	0	1:Q314W	0.775	0
1:F486H	1.942	0	1:L452C	1.326	0	1:Y453V	3.565	0	1:N501Q	2.025	0	1:V367Q	1.053	0	1:Q314T	0.777	0
1:F486A	1.955	0	1:L452N	1.408	0	1:Y453G	3.577	0	1:N501E	2.866	0	1:V367C	1.169	0	1:Q314A	0.934	0
1:F486N	2.001	0	1:L452E	1.475	0	1:Y453R	5.515	0	1:N501V	3.857	0	1:V367T	1.288	0	1:Q314G	1.008	0
1:F486K	2.120	0	1:L452K	1.517	0	1:Y453I	7.582	0	1:N501R	7.724	0	1:V367S	1.399	0	1:Q314C	1.015	0
1:F486D	2.288	0	1:L452D	1.668	0	1:Y453P	10.805	0	1:N501F	12.759	0	1:V367N	1.507	0	1:Q314N	1.041	0
1:F486G	2.371	0	1:L452G	1.847	0	1:Y453K	11.958	0	1:N501P	102.865	0	1:V367H	1.705	0	1:Q314S	1.169	0
1:F486P	2.518	0	1:L452T	2.451	0	1:Y453L	13.639	0	1:N501W	105.796	0	1:V367P	3.039	0	1:Q314D	1.212	0

Supplementary Table 3:

F486L			Y453F			N501T			L452M		
mutation	daffinity	SNP	mutation	daffinity	SNP	mutation	daffinity	SNP	mutation	dAffinity	SNP
1:F486Y	-0.578	1	**1:Y453F**	**-0.080**	**1**	1:N501H	-0.250	1	1:L452H	-0.082	1
1:F486F	0.000	1	1:Y453Y	0.000	1	1:N501N	0.000	1	**1:L452M**	**-0.038**	**1**
1:F486L	**2.796**	**1**	1:Y453N	0.559	1	1:N501D	0.278	1	1:L452P	-0.010	1
1:F486I	2.912	1	1:Y453S	0.588	1	**1:N501T**	**0.872**	**1**	1:L452F	-0.005	1
1:F486V	4.245	1	1:Y453H	0.598	1	1:N501S	2.281	1	1:L452Q	-0.004	1
1:F486C	4.583	1	1:Y453C	0.606	1	1:N501Y	2.286	1	1:L452S	-0.003	1
1:F486S	5.042	1	1:Y453D	0.687	1	1:N501I	13.551	1	1:L452L	0.000	1
1:F486W	-0.296	0	1:Y453W	-0.429	0	1:N501K	15.768	1	1:L452I	0.006	1
1:F486M	0.776	0	1:Y453K	-0.411	0	1:N501Q	-1.315	0	1:L452V	0.014	1
1:F486Q	0.856	0	1:Y453M	0.293	0	1:N501E	-0.826	0	1:L452W	0.040	1
1:F486H	1.433	0	1:Y453E	0.375	0	1:N501M	0.797	0	1:L452R	0.195	1
1:F486E	1.989	0	1:Y453Q	0.388	0	1:N501V	1.205	0	1:L452E	-0.165	0
1:F486R	2.077	0	1:Y453I	0.496	0	1:N501C	1.229	0	1:L452D	-0.013	0
1:F486K	3.285	0	1:Y453L	0.512	0	1:N501L	2.066	0	1:L452G	-0.010	0
1:F486N	3.786	0	1:Y453P	0.532	0	1:N501P	2.123	0	1:L452A	-0.004	0
1:F486T	4.312	0	1:Y453T	0.538	0	1:N501A	2.721	0	1:L452N	-0.003	0
1:F486D	4.431	0	1:Y453V	0.561	0	1:N501G	3.323	0	1:L452T	-0.001	0
1:F486P	5.072	0	1:Y453G	0.593	0	1:N501F	49.293	0	1:L452Y	0.016	0
1:F486A	5.334	0	1:Y453A	0.600	0	1:N501R	64.572	0	1:L452C	0.027	0
1:F486G	5.827	0	1:Y453R	25.426	0	1:N501W	306.442	0	1:L452K	0.062	0

Supplementary Table 4:

Variant	Within 4.5Å of ACE2?	PROVEAN	SIFT
F486L	**Yes**	Neutral	Tolerated
L452M	No	Neutral	Tolerated
Q314K	No	Neutral	Tolerated
Y453F	**Yes**	Neutral	Tolerated
N501T	**Yes**	Neutral	Tolerated
V367F	No	Neutral	Tolerated

Citations:

Aleccia, J. (2020). Thousands of Minks Dead as COVID Outbreak Escalates on Utah Farms. *KHN.Org*.

Altschuh, D., Lesk, A. M., Bloomer, A. C., & Klug, A. (1987). Correlation of co-ordinated amino acid substitutions with function in viruses related to tobacco mosaic virus. *Journal of Molecular Biology*, *193*(4), 693–707. https://doi.org/10.1016/0022-2836(87)90352-4

Bellware, K. (2020). Fourth state confirms mink farm coronavirus outbreaks as U.S. looks to avoid Denmark's disaster. *The Washington Post*.

Benton, D. J., Wrobel, A. G., Rosenthal, P. B., & Gamblin, S. J. (2020). *SARS-CoV-2 Spike Glycoprotein with 3 ACE2 Bound*. Worldwide Protein Data Bank. https://doi.org/10.2210/pdb7a98/pdb

Callaway, E. (2020). The coronavirus is mutating—Does it matter? *Nature*, *585*(7824), 174–177. https://doi.org/10.1038/d41586-020-02544-6

CDC. (2020). COVID-19 and Animals. *Centers for Disease Control*. https://www.cdc.gov/coronavirus/2019-ncov/daily-life-coping/animals.html

Choi, Y., & Chan, A. P. (2015). PROVEAN web server: A tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics*, *31*(16), 2745–2747. https://doi.org/10.1093/bioinformatics/btv195

Cunha-Neto, E., Rosa, D. S., Harris, P. E., Olson, T., Morrow, A., Ciotlos, S., Herst, C. V., & Rubsamen, R. M. (2017). An Approach for a Synthetic CTL Vaccine Design against Zika Flavivirus Using Class I and Class II Epitopes Identified by Computer Modeling. *Frontiers in Immunology*, *8*. https://doi.org/10.3389/fimmu.2017.00640

Cyranoski, D. (2020). Profile of a killer: The complex biology powering the coronavirus pandemic. *Nature*, *581*(7806), 22–26. https://doi.org/10.1038/d41586-020-01315-7

Damas, J., Hughes, G. M., Keough, K. C., Painter, C. A., Persky, N., Corbo, M., Hiller, M., Koepfli, K.-P., Pfenning, A., Zhao, H., Genereux, D. P., Swofford, R., Pollard, K. S., Ryder, O. A., Nweeia, M. T., Lindblad-Toh, K., Teeling, E. C., Karlsson, E. K., & Lewin, H. A. (2020). Broad host range of SARS-CoV-2 predicted by comparative and structural analysis of ACE2 in vertebrates. *PNAS; Proceedings of the National Academy of Sciences*, *117*(36), 22311–22322.

Dhama, K., Patel, S. K., Sharun, K., Pathak, M., Tiwari, R., Yatoo, M. I., Malik, Y. S., Sah, R., Rabaan, A. A., Panwar, P. K., Singh, K. P., Michalak, I., Chaicumpa, W., Martinez-Pulgarin, D. F., Bonilla-Aldana, D. K., & Rodriguez-Morales, A. J. (2020). SARS-CoV-2 jumping the

species barrier: Zoonotic lessons from SARS, MERS and recent advances to combat this pandemic virus. *Travel Medicine and Infectious Disease*, *37*, 101830. https://doi.org/10.1016/j.tmaid.2020.101830

Elbe, S., & Buckland-Merrett, G. (2017). Data, disease and diplomacy: GISAID\textquotesingles innovative contribution to global health. *Global Challenges*, *1*(1), 33–46. https://doi.org/10.1002/gch2.1018

Greaney, A. J., Starr, T. N., Gilchuk, P., Zost, S. J., Binshtein, E., Loes, A. N., Hilton, S. K., Huddleston, J., Eguia, R., Crawford, K. H. D., Dingens, A. S., Nargi, R. S., Sutton, R. E., Suryadevara, N., Rothlauf, P. W., Liu, Z., Whelan, S. P. J., Carnahan, R. H., Crowe, J. E., & Bloom, J. D. (2020). Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition. *Cell Host & Microbe*. https://doi.org/10.1016/j.chom.2020.11.007

Guo, Q., Li, M., Wang, C., Wang, P., Fang, Z., tan, J., Wu, S., Xiao, Y., & Zhu, H. (2020). Host and infectivity prediction of Wuhan 2019 novel coronavirus using deep learning algorithm. *BioRxiv*.

Haynes, WinstonA., Kamath, K., Bozekowski, J., Baum-Jones, E., Campbell, M., Casanovas-Massana, A., Daugherty, P. S., Dela+Cruz, C. S., Dhal, A., Farhadian, S. F., Fitzgibbons, L., Fournier, J., Jhatro, M., Jordan, G., Kessler, D., Klein, J., Lucas, C., Luchsinger, L. L., Martinez, B., … Shon, J. C. (2020). High-resolution mapping and characterization of epitopes in COVID-19 patients. *MedRxiv*.

He, R., Dobie, F., Ballantine, M., Leeson, A., Li, Y., Bastien, N., Cutts, T., Andonov, A., Cao, J., Booth, T. F., Plummer, F. A., Tyler, S., Baker, L., & Li, X. (2004). Analysis of

multimerization of the SARS coronavirus nucleocapsid protein. *Biochemical and Biophysical Research Communications*, *316*(2), 476–483. https://doi.org/10.1016/j.bbrc.2004.02.074

Katoh, K., & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution*, *30*(4), 772–780. https://doi.org/10.1093/molbev/mst010

Kedkovid, R., Sirisereewan, C., & Thanawongnuwech, R. (2020). Major swine viral diseases: An Asian perspective after the African swine fever introduction. *Porcine Health Management*, *6*(1). https://doi.org/10.1186/s40813-020-00159-x

Kemp, S. A., Collier, D. A., Datir, R., Gayed, S., Jahun, A., Hosmillo, M., Ferreira, I. A., Rees-Spear, C., Mlcochova, P., Lumb, I. U., Roberts, D., Chandra, A., Temperton, N., Sharrocks, K., Blane, E., Briggs, J., +, +, Smith, K. G., … Gupta, +Ravindra+K. (2020). Neutralising antibodies drive Spike mediated SARS-CoV-2 evasion. *MedRxiv*.

Kirwan, H. (2020). More Than 3K Mink Dead From Coronavirus At Taylor County Mink Farm. *Wisconsin Public Radio*.

Korber, B., Fischer, W. M., Gnanakaran, S., Yoon, H., Theiler, J., Abfalterer, W., Hengartner, N., Giorgi, E. E., Bhattacharya, T., Foley, B., Hastie, K. M., Parker, M. D., Partridge, D. G., Evans, C. M., Freeman, T. M., Silva, T. I. de, McDanal, C., Perez, L. G., Tang, H., … Wyles, M. D. (2020). Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. *Cell*, *182*(4), 812-827.e19. https://doi.org/10.1016/j.cell.2020.06.043

Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., Wang, Q., Zhang, L., & Wang, X. (2020). Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature*, *581*(7807), 215–220. https://doi.org/10.1038/s41586-020-2180-5

Loew, T. (2020). Gov. Brown, agencies condemned for secrecy surrounding Oregon COVID-19 mink farm outbreak. *Statesman Journal*.

Mallapaty, S. (2020). COVID mink analysis shows mutations are not dangerous—Yet. *Nature*, *587*(7834), 340–341. https://doi.org/10.1038/d41586-020-03218-z

Marks, D. S., Colwell, L. J., Sheridan, R., Hopf, T. A., Pagnani, A., Zecchina, R., & Sander, C. (2011). Protein 3D Structure Computed from Evolutionary Sequence Variation. *PLoS ONE*, *6*(12), e28766. https://doi.org/10.1371/journal.pone.0028766

Melin, A. D., Janiak, M. C., Marrone, F., Arora, P. S., & Higham, J. P. (2020). Comparative ACE2 variation and primate COVID-19 risk. *Communications Biology*, *3*(1). https://doi.org/10.1038/s42003-020-01370-w

Michigan.gov. (2020). Mink Farm Tests Positive with SARS-CoV-2. *Michigan.Gov*.

Ng, P. C. (2003). SIFT: predicting amino acid changes that affect protein function. *Nucleic Acids Research*, *31*(13), 3812–3814. https://doi.org/10.1093/nar/gkg509

Oude Munnink, B. B., Sikkema, R. S., Nieuwenhuijse, D. F., Molenaar, R. J., Munger, E., Molenkamp, R., van der Spek, A., Tolsma, P., Rietveld, A., Brouwer, M., Bouwmeester-Vincken, N., Harders, F., der Honing, R. H., Wegdam-Blans, M. C. A., Bouwstra, R. J., GeurtsvanKessel, C., van der Eijk, A. A., Velkers, F. C., Smit, L. A. M., … Koopmans, M. P. G. (2020). Jumping back and forth: Anthropozoonotic and zoonotic transmission of SARS-CoV-2 on mink farms. *BioRxiv*.

Poland, G. A., Ovsyannikova, I. G., & Kennedy, R. B. (2020). SARS-CoV-2 immunity: Review and applications to phase 3 vaccine candidates. *The Lancet*, *396*(10262), 1595–1606. https://doi.org/10.1016/S0140-6736(20)32137-1

Python 3. (2020). https://www.python.org

R. Core Team. (2017). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing.

Schulte, L. (2020). Two Taylor County mink farms under quarantine after more than 5,000 animals died from COVID-19. *Milwaukee Journal Sentinel*.

Shang, J., Ye, G., Shi, K., Wan, Y., Luo, C., Aihara, H., Geng, Q., Auerbach, A., & Li, F. (2020). Structural basis of receptor recognition by SARS-CoV-2. *Nature*, *581*(7807), 221–224. https://doi.org/10.1038/s41586-020-2179-y

Shu, Y., & McCauley, J. (2017). GISAID: Global initiative on sharing all influenza data – from vision to reality. *Eurosurveillance*, *22*(13). https://doi.org/10.2807/1560-7917.es.2017.22.13.30494

Smatti, M. K., Thani, A. A. A., & Yassine, H. M. (2018). Viral-Induced Enhanced Disease Illness. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.02991

Ugen-Csenge, E. (2020). COVID-19 outbreak declared at mink farm in B.C.'s Fraser Valley. *CBC News*.

ULC, C. C. G. (2020). *Molecular Operating Environment (MOE)*.

USDA. (2020). Mink Production (2018-2020). *National Agricultural Statistics Service (NASS), Agricultural Statistics Board, United States Department of Agriculture (USDA).*

Waterhouse, A. M., Procter, J. B., Martin, D. M. A., Clamp, M., & Barton, G. J. (2009). Jalview Version 2–a multiple sequence alignment editor and analysis workbench. *Bioinformatics*, *25*(9), 1189–1191. https://doi.org/10.1093/bioinformatics/btp033

Wertheim, J. O., Chu, D. K. W., Peiris, J. S. M., Kosakovsky Pond, S. L., & Poon, L. L. M. (2013). A Case for the Ancient Origin of Coronaviruses. *Journal of Virology*, *87*(12), 7039–7045.

Wu, Y., Wang, F., Shen, C., Peng, W., Li, D., Zhao, C., Li, Z., Li, S., Bi, Y., Yang, Y., Gong, Y., Xiao, H., Fan, Z., Tan, S., Wu, G., Tan, W., Lu, X., Fan, C., Wang, Q., … Liu, L. (2020). A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2. *Science*, *368*(6496), 1274–1278.

Yong, C. Y., Ong, H. K., Yeap, S. K., Ho, K. L., & Tan, W. S. (2019). Recent Advances in the Vaccine Development Against Middle East Respiratory Syndrome-Coronavirus. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.01781

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., Chen, H.-D., Chen, J., Luo, Y., Guo, H., Jiang, R.-D., Liu, M.-Q., Chen, Y., Shen, X.-R., Wang, X., … Shi, Z.-L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, *579*(7798), 270–273. https://doi.org/10.1038/s41586-020-2012-7