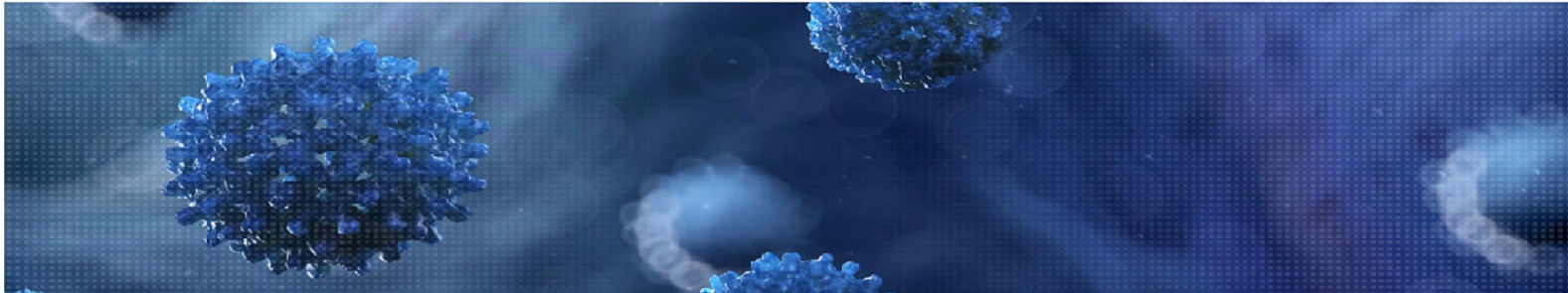




**Viroscience lab**  
WHERE SKILLS MEET TO STUDY & PROTECT

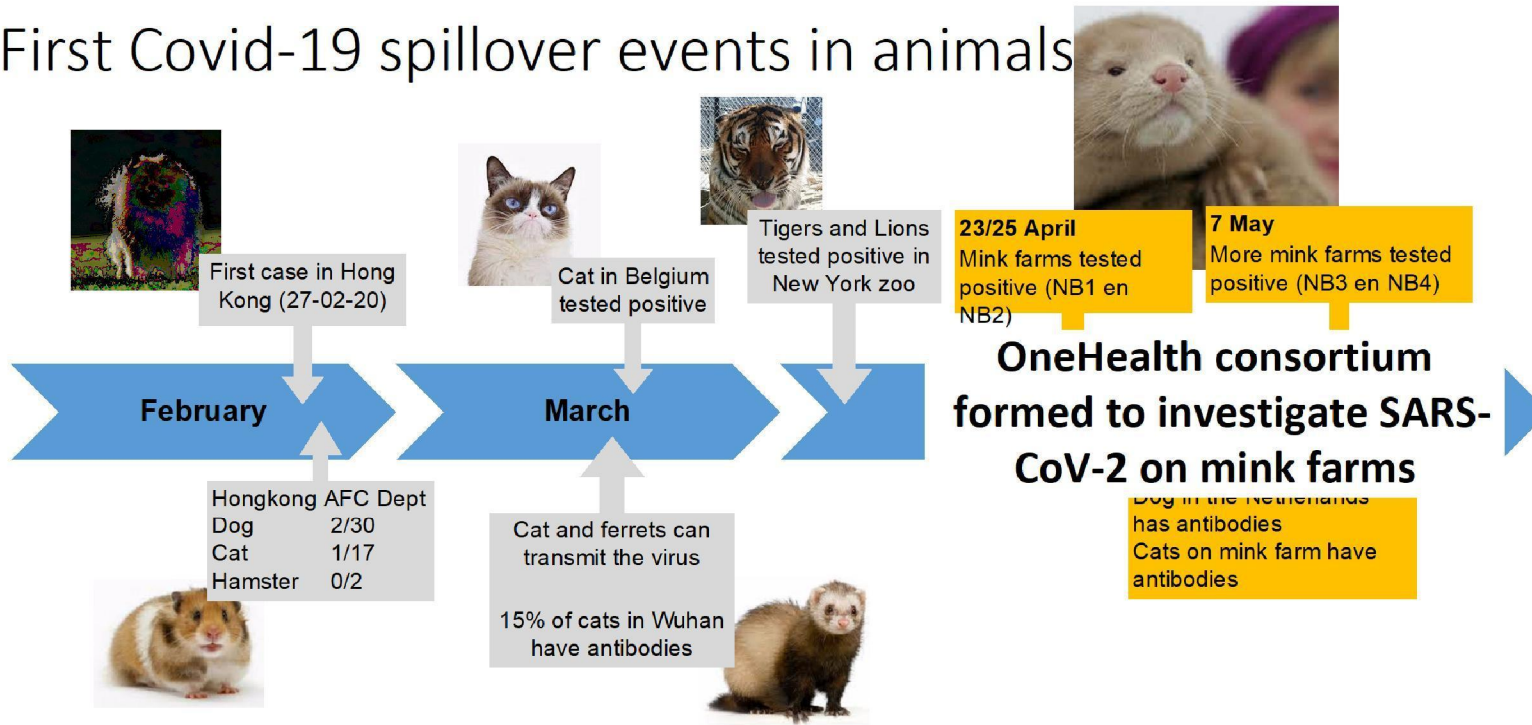


## Whole genome nanopore sequencing in the ongoing SARS-CoV-2 outbreaks in Dutch mink farms

5.1.2e

Erasmus MC, Rotterdam

# First Covid-19 spillover events in animals

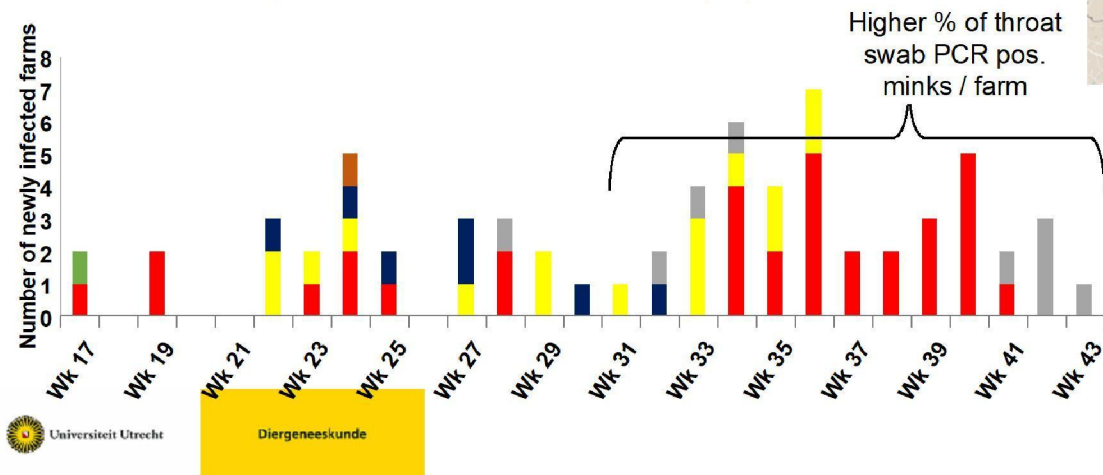
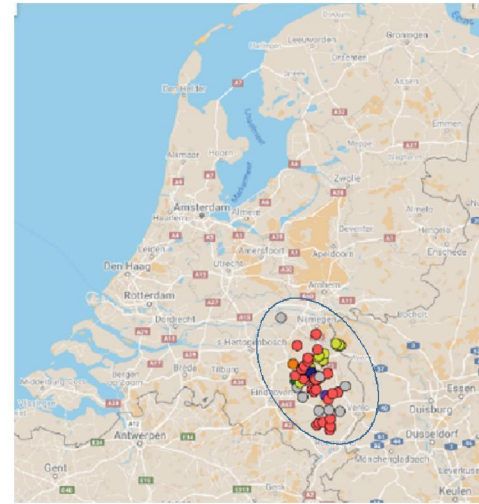


LCI



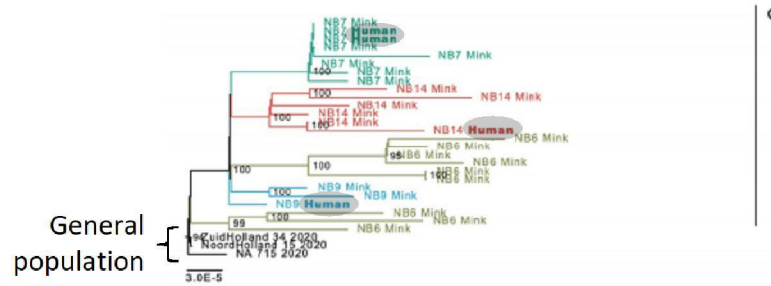
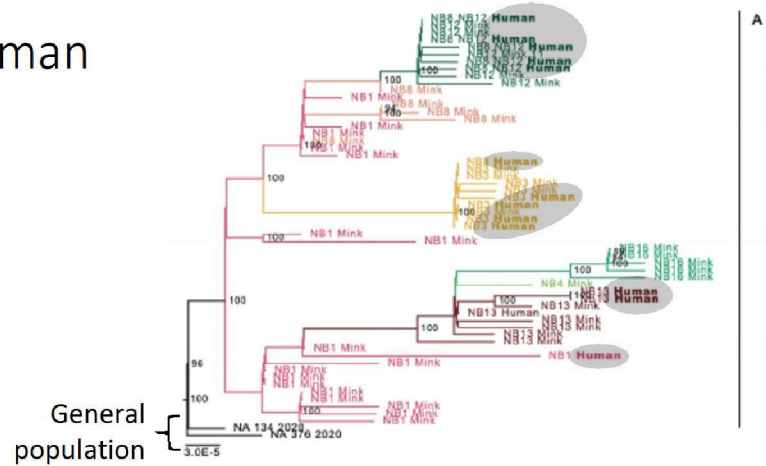
# Current situation of the mink farm outbreaks in the Netherlands

- 68 farms diagnosed & culled since end of April
- Most detections based on clinical signs or weekly monitoring of dead animals (ratio ~50:50)
- Some after serological screening in May/June & Sept (n=6)
- Most farms in South-East of the Netherlands
- 5 clusters, following probable human introduction, A,C,D show ongoing transmission



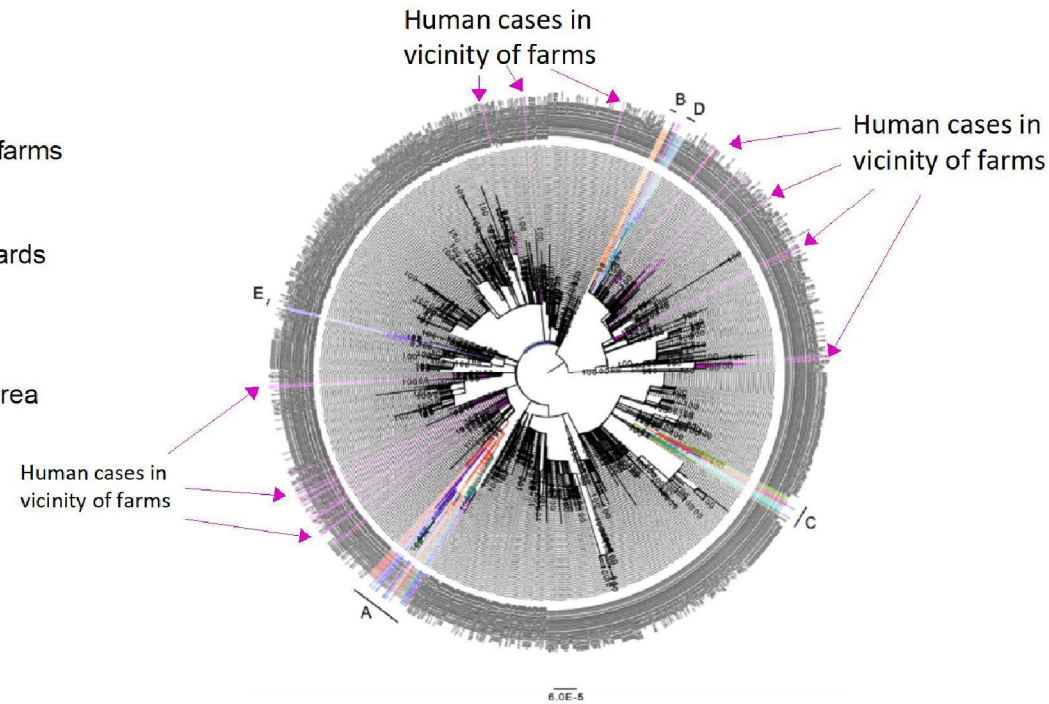
# Evidence for Human-Mink-Human transmission on mink farms

The screenshot shows the bioRxiv preprint page. At the top left is the Cold Spring Harbor Laboratory logo. The main header includes 'bioRxiv' and 'Science'. The title of the preprint is 'Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans'. Below the title, the authors are listed: Bas B. Oude Munnink, Reina S. Sikkema, David F. Nieuwenhuijse, Robert Jan Molenaar, Emmanuelle Mangen, Richard Melnick, Arno van der Spek, Paulien Bakema, Lucreia Boshuizen, Noortje Baumesteele-Vincken, Frank Harders, Renate Hakze-van der Horst, Marjolijn C. A. Wegdam-Blant, Ruth J. Bouwstra, Corine GeurtsvanKessel, Annetiek A. van der Eijk, Francisca C. Velkers, Lidwien A. M. Smit, Arjan Stegeman, Wim H. M. van der Poel, and Marion R. G. Koopmans. The preprint is categorized as a 'REPORT' and includes social media share icons for Facebook, Twitter, LinkedIn, and YouTube. A red banner at the top of the article area says 'Read our COVID-19 research and news.' The URL 'https://doi.org/10.1101/2020.09.16.347162' is visible at the bottom.



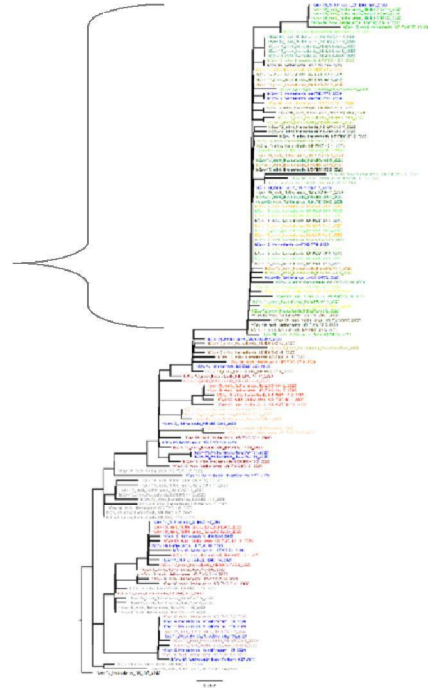
## No mink related strains in surrounding Dutch population

- 66/97 (66%) persons on the first 16 farms PCR and/or serological positive
- 22 people PCR pos from NB17 onwards (no active surveillance, no serology)
- No related cases based on genome sequences from same postal code area



## Sorting by virus cluster to evaluate links with feed company and owners/personnel

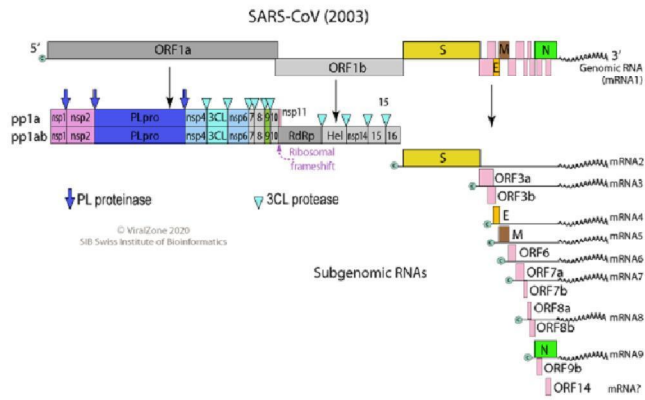
Farm	Wk	CI	Feed	Shared owner/people
NB1	17	A	F	NB1A, NB1B, NB4
NB3	19	A	P	
NB4	19	A	F	NB1A, NB1B, NB4
NB12	24	A	K	NB8, NB12, NB34
NB13	24	A	K	
NB16	25	A	K	
NB20	28	A	P	NB20, NB22
NB21	28	A	K	Owned by same company (>15 farms)
NB27	32	A	K	Owned by same company (>15 farms)
NB33	34	A	K	Owned by same company (>15 farms)
NB35	34	A	K	Owned by same company (>15 farms)
NB36	34	A	K	Owned by same company (>15 farms)
NB38	34	A	K	Owned by same company (>15 farms)
NB40	35	A		NB25, NB40
NB42	35	A	K	Owned by same company (>15 farms)
NB43	36	A	F	NB63
NB44	36	A		
NB47	36	A	K	Owned by same company (>15 farms)
NB48	36	A		
NB49	36	A	K	
NB50	37	A	K	
NB51	37	A	K	Owned by same company (>15 farms)
NB52	38	A		
NB53	38	A	P	
NB54	39	A	F	
NB55	39	A	F	
NB56	39	A	P	
NB57	40	A	K	Shared personnel large company?
NB58	40	A	K	Shared personnel large company?
NB59	40	A	K	Shared personnel large company?
NB60	40	A	K	Shared personnel large company?
NB61	40	A	P	Family relation Large company (>15)
NB62	41	A	P	Family relation Large company (>15)
NB8	23	A/A	K	NB8, NB12, NB34



- Not always same cluster for linked farms with same owners or shared personnel
- No clear links related to feed company, veterinarian, etc.
- Close links based on sequencing data of ErasmusMC
- Farms with related sequences are further investigated in tracking & tracing documents to find epidemiological links

# What amino acid variations in mink clusters?

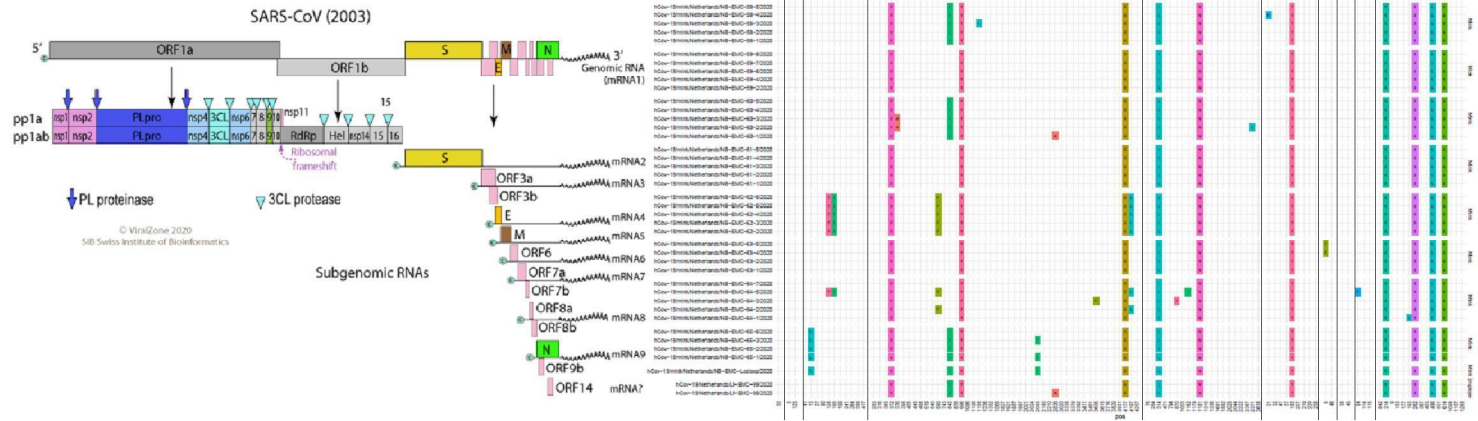
- Sequence analysis of 65 mink farms
- Variations vs Wuhan-Hu-1 strain



Latest cases in Cluster A:

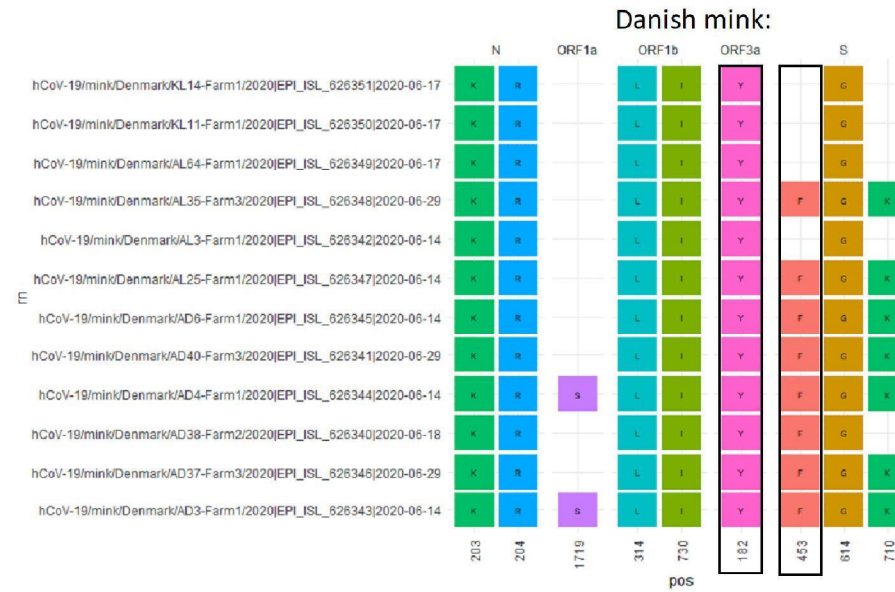
# What amino acid variations in mink clusters?

- Sequence analysis of 65 mink farms
- Variations vs Wuhan-Hu-1 strain

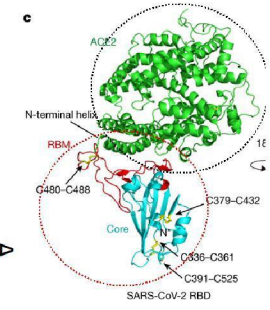
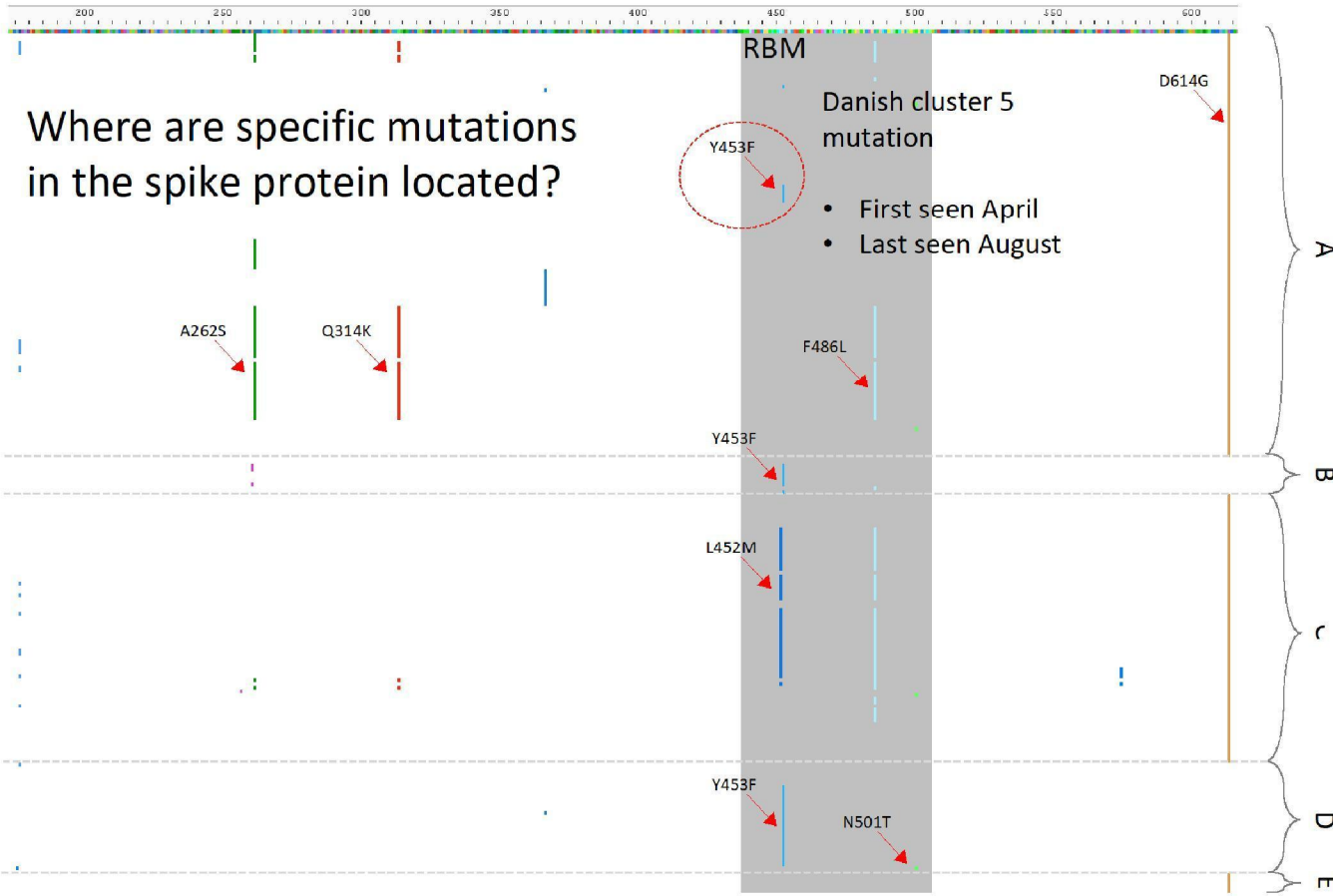


# Any relation with Danish mink mutation patterns?

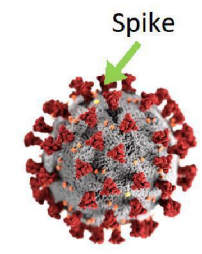
- Matching variants:
  - ORF3a
    - H182Y
  - Spike
    - Y453F



Where are specific mutations in the spike protein located?



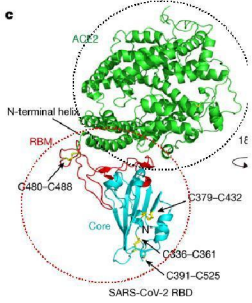
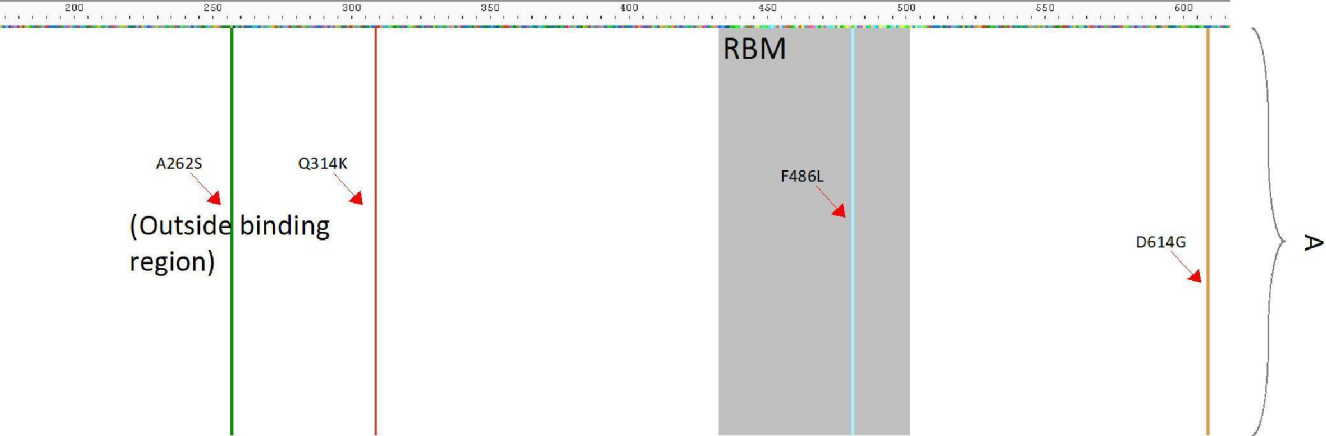
Lan, J., Ge, J., Yu, J. *et al.* Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* 581, 215–220 (2020).  
<https://doi.org/10.1038/s41586-020-2180-5>



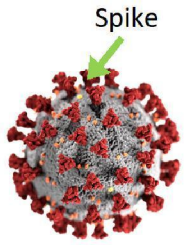
A  
B  
C  
D  
E

# Where are specific mutations in the spike protein located?

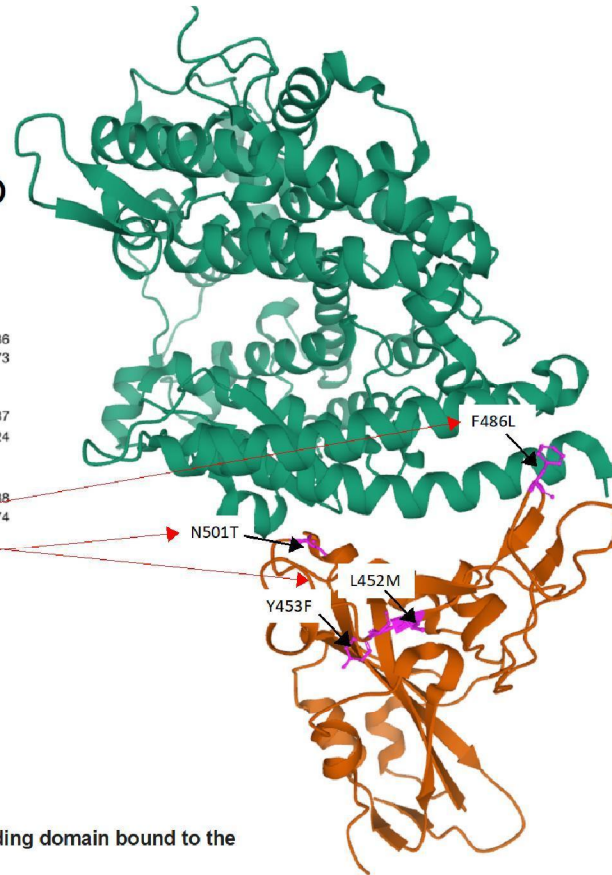
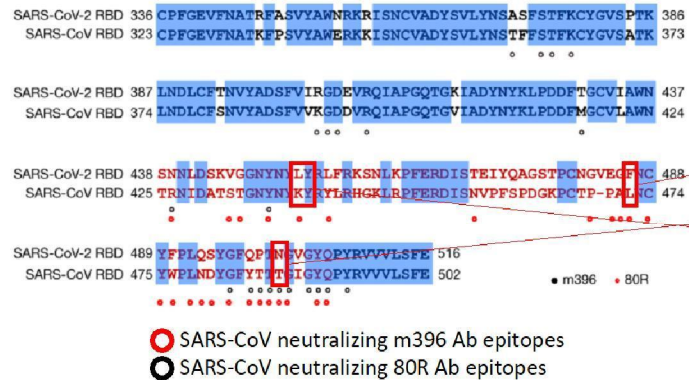
Latest cases in Cluster A:  
(6<sup>th</sup> Nov)



Lan, J., Ge, J., Yu, J. *et al.* Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* 581, 215–220 (2020). <https://doi.org/10.1038/s41586-020-2180-5>

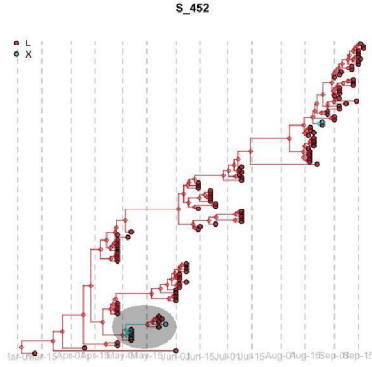
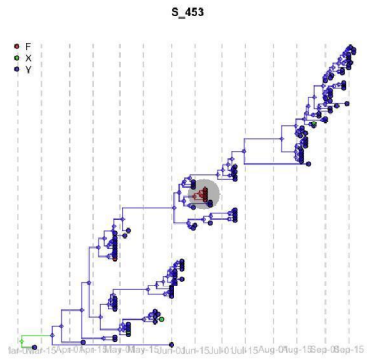
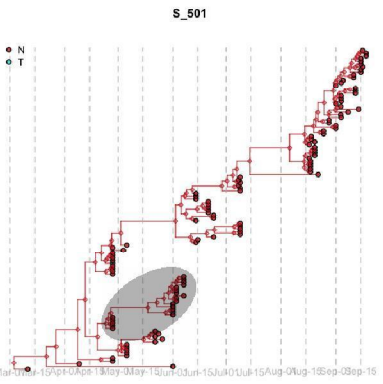
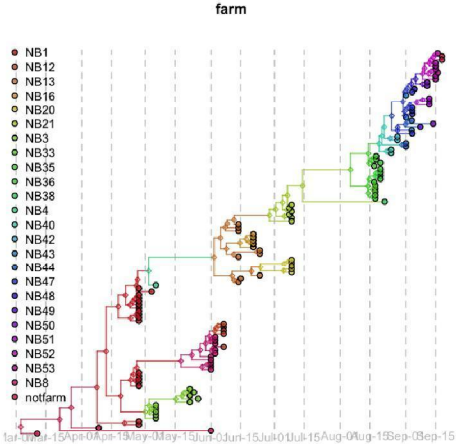


# Can RBM mutations influence ACE2 binding?



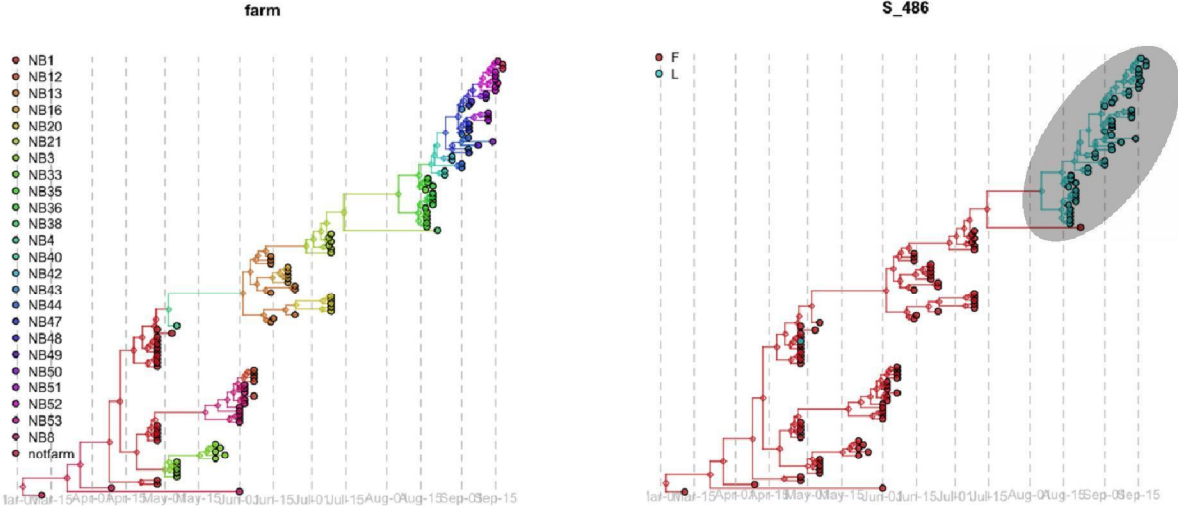
Lan, J., Ge, J., Yu, J. *et al.* Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* 581, 215–220 (2020). <https://doi.org/10.1038/s41586-020-2180-5>

# Spike 501, 453 and 452 mutation present in early clusters



Lu Lu et al.

# Spike 486 mutation is dominant in latest farms



Lu Lu et al.

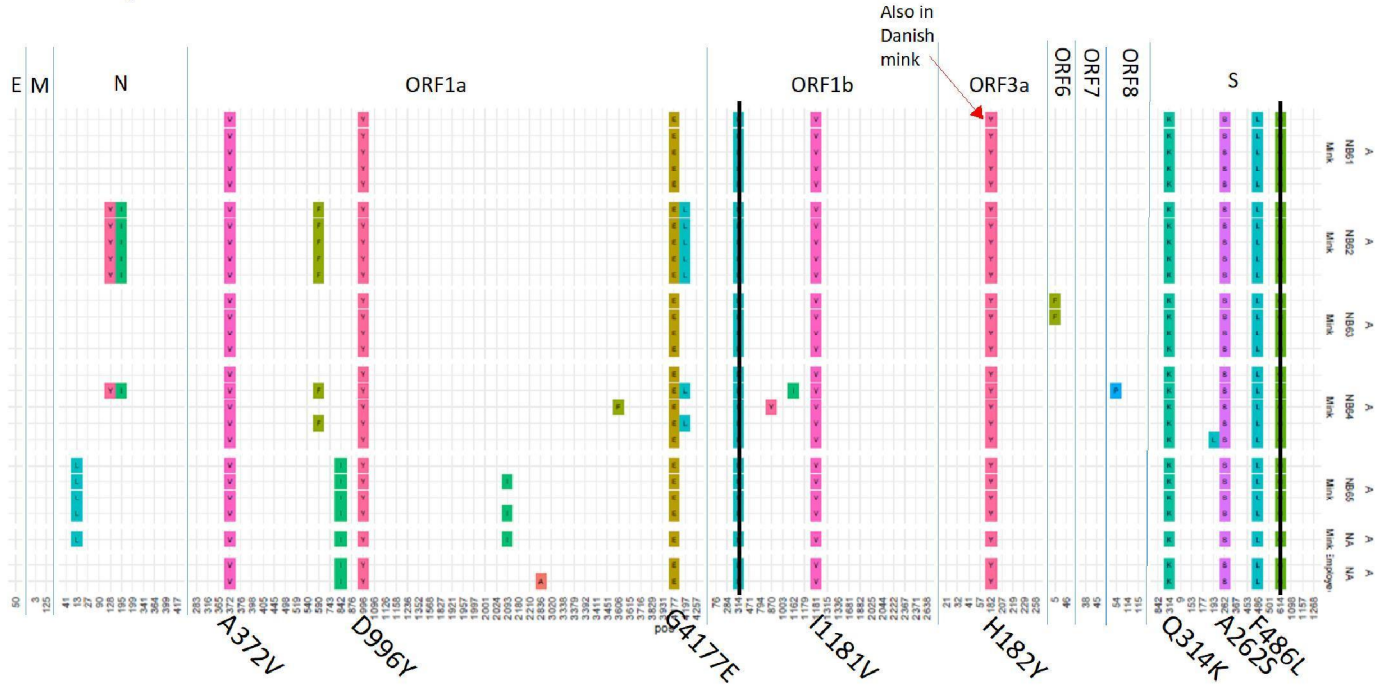
# F486L in only 9 other sequences worldwide

(checked on 12-11-2020, GISAID)



Seq ID	Sampling Date	GISAID id	Type sample
Scotland/CVR4501/2020	9/21/2020	EPI_ISL_585364	Original (Human)
USA/MI-MDHHS-SC22125/2020	10/6/2020	EPI_ISL_614176	Original (Human)
USA/MI-MDHHS-SC22140/2020	10/6/2020	EPI_ISL_614191	Original (Human)
bat/Yunnan/RaTG13/2013	7/24/2013	EPI_ISL_402131	Original
pangolin/Guangxi/P1E/2017	2017	EPI_ISL_410539	Original
pangolin/Guangxi/P2V/2017	2017	EPI_ISL_410542	Vero E6
pangolin/Guangxi/P4L/2017	2017	EPI_ISL_410538	Original
pangolin/Guangxi/P5E/2017	2017	EPI_ISL_410541	Original
pangolin/Guangxi/P5L/2017	2017	EPI_ISL_410540	Original

# Other persisting mutations in latest farms may be related to F486L



## Conclusions & ongoing research

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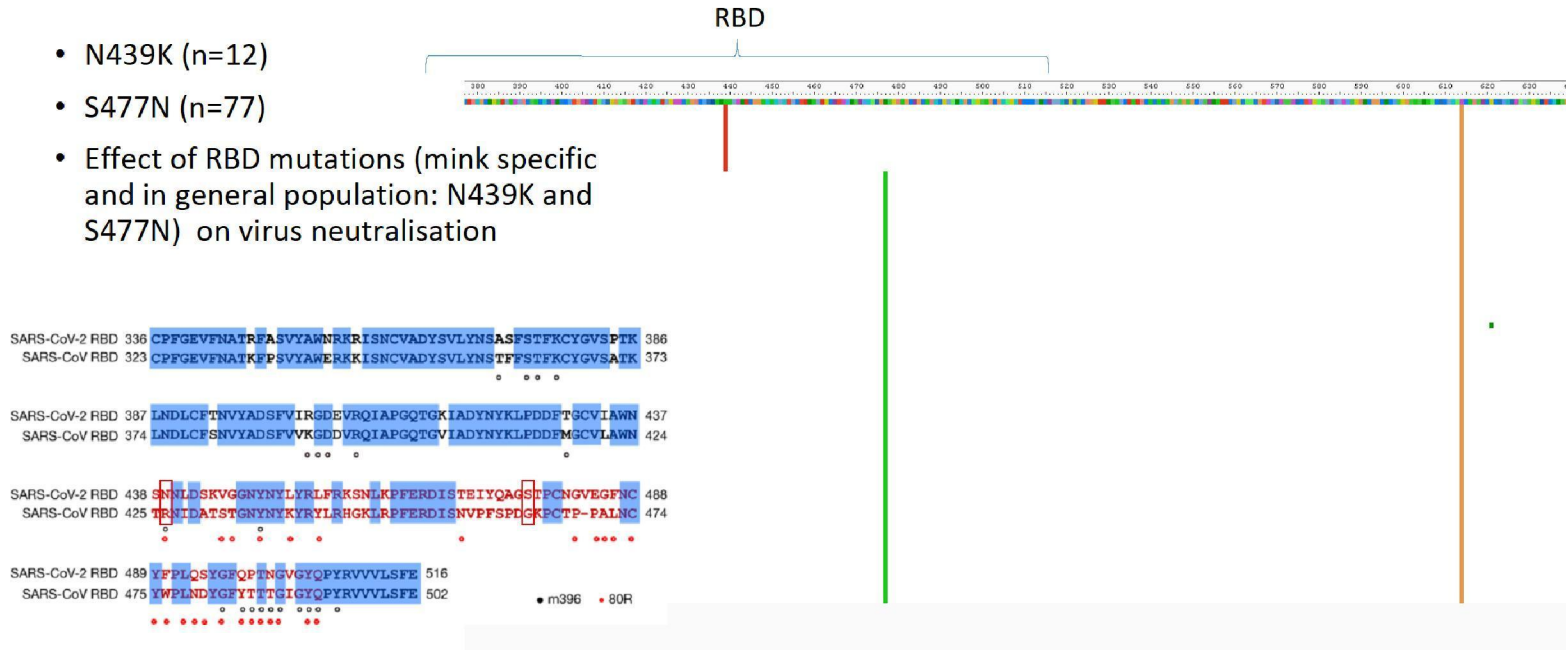
- Dutch mink viruses contain unique S protein (and other) mutations
- F486L mutation in mink predominant in infected farms
- No spread of F486L in Dutch population
  
- Ongoing research to determine phenotypic effects:
  - Organoids
  - Animal experiments



Photograph: Vasily Fedosenko/Reuters

# Ongoing: Investigation of other RBD mutations in general population

- N439K (n=12)
- S477N (n=77)
- Effect of RBD mutations (mink specific and in general population: N439K and S477N) on virus neutralisation



## Ongoing: assessment of the potential risk or role for Wildlife

Route	Risk to species likely to be susceptible for SARS-CoV2
<b>Wild carnivores / escaped mink</b>	<p>Fenced farmyards and catching cages on premises</p> <p>In progress: Risk assessment of possible role of wild carnivores in the spread of SARS-CoV-2 between mink farms</p> <ul style="list-style-type: none"> <li>- Observations by zoologists on/around infected and non-infected farms for accessibility and presence of wild carnivores</li> </ul> <p>→ Results of first 10 visits: part of farms are accessible for martens, foxes and mink</p> <ul style="list-style-type: none"> <li>- Collection and testing of dead Mustelids (including minks) in risk areas + feces of martens, foxes and badgers</li> </ul> <p>→ 2 escaped minks PCR positive, 10 wild mustelids PCR negative</p>
<b>Wildlife as mechanical spreaders: birds, bugs and rodents</b>	<p>Rodents: only small numbers of mice and rats caught after culling:</p> <ul style="list-style-type: none"> <li>• 1 <i>Rattus rattus</i> tested 24-8 (PCR negative).</li> </ul> <p>In progress:</p> <ul style="list-style-type: none"> <li>- Risk assessment of possible role of birds by ornithologists (SOVON)</li> </ul>
<b>Bats</b>	<p>In progress: Risk assessment of possible role of wild carnivores in the spread of SARS-CoV-2 between mink farms</p> <ul style="list-style-type: none"> <li>- Observations by bat experts to assess suitability and likely presence of different bat species</li> <li>- Collection and testing of feces (validates for coronavirus surveillance in bats) and dead bats in risk area</li> <li>- Bat tracking data</li> </ul>



