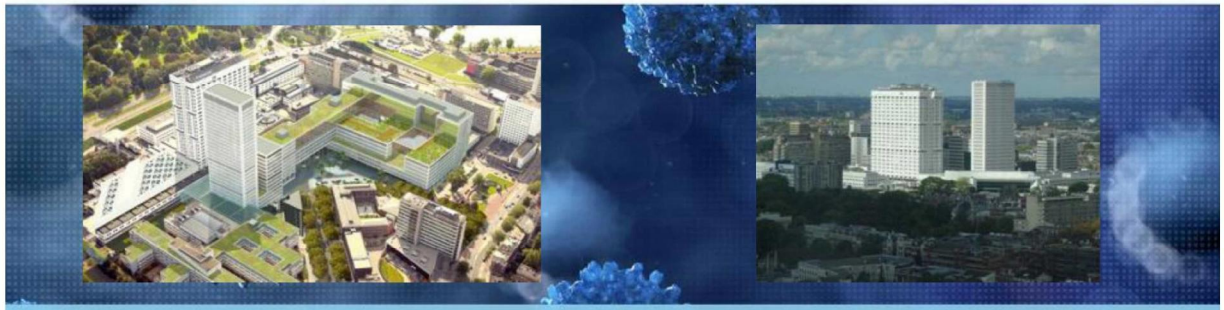
Erasmus MC

Viroscience lab

WHERE SKILLS MEET TO STUDY & PROTECT



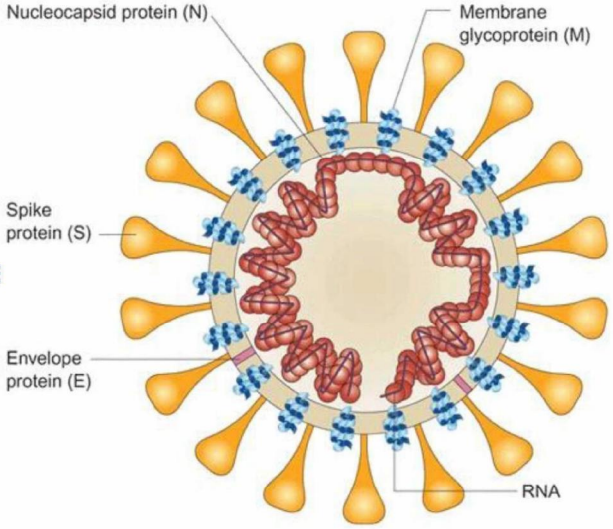
Rapid SARS-CoV-2 Whole Genome Sequencing

(10)(2e)

(10)(2e) @erasmusmc.nl

WGS for outbreak investigations

- Where did it come from?
- How different is it from what we have seen before?
- Is it part of an outbreak?



Peiris et al, Nature Medicine, 2004,

Nanopore MinION sequencing

- Promoted for outbreak based sequencing
- Examples during Ebola, Zika, Yellow fever

- Possible advantages:
  - **Real time base calling**
  - **Fast (library preparation)**
  - **Cheap**
  - Easy to implement

- Possible disadvantages:
  - **High error rates (~90% accuracy)**
  - Size of datasets / storage

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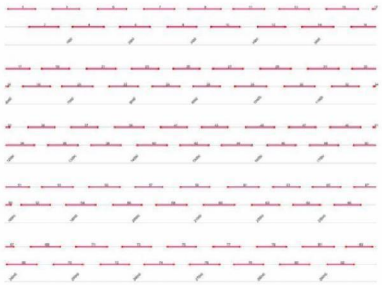
Amplicon based sequencing

PROTOCOL

Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples

Joshua Quick[1], Nathan D Grubaugh[2], Steven T Pullan[3], Ingra M Claro[4], Andrew D Smith[1], Karthik Gangavarapu[2], Glenn Oliveira[5], Refugio Robles-Sikisaka[2], Thomas F Rogers[2,6], Nathan A Beutler[2], R Burton[2], Lia Laura Lewis-Ximenez[7], Jaqueline Goes de Jesus[8], Marta Giovanetti[8,9], Sarah C Hill[10], Allison Black[11,12], Trevor Bedford[11], Miles W Carroll[3,13], Marcio Nunes[14], Luiz Carlos Alcantara Jr.[8], Ester C Sabino[4], Sally A Baylis[15], Nuno R Faria[10], Matthew Loose[16], Jared T Simpson[17], Oliver G Pybus[10], Kristian G Andersen[2,5] & Nicholas J Loman[1]

[1]Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Birmingham, UK. [2]The Scripps Research Institute, La Jolla, California, USA. [3]Public Health England, National Infection Service, Porton Down, Salisbury, UK. [4]Department of Infectious Disease and Institute of Tropical Medicine, University of Saõ Paulo, Saõ Paulo, Brazil. [5]Scripps Translational Science Institute, La Jolla, California, USA. [6]Massachusetts General Hospital, Boston, Massachusetts, USA. [7]Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil. [8]Fundação Oswaldo Cruz (FIOCRUZ), Salvador, Brazil. [9]University of Rome, Tor Vergata, Italy. [10]Department of Zoology, University of Oxford, Oxford, UK. [11]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA. [12]Department of Epidemiology, University of Washington, Seattle, Washington, USA. [13]University of Southampton, South General Hospital, Southampton, UK. [14]Instituto Evandro Chagas, Belem, Brazil. [15]Paul-Ehrlich-Institut, Langen, Germany. [16]DeepSeq, School of Life Sciences, University of Nottingham, Nottingham, UK. [17]OICR, Toronto, Canada. Correspondence should be addressed to N.J.L. (n.j.loman@bham.ac.uk).



89 overlapping amplicons 500bp
75 bp overlap

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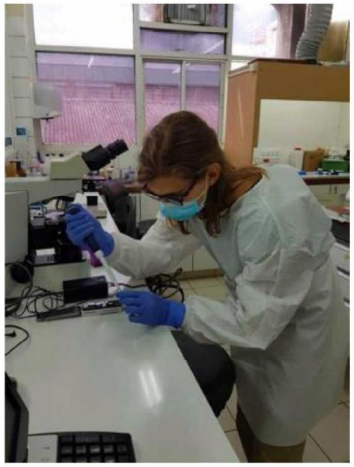
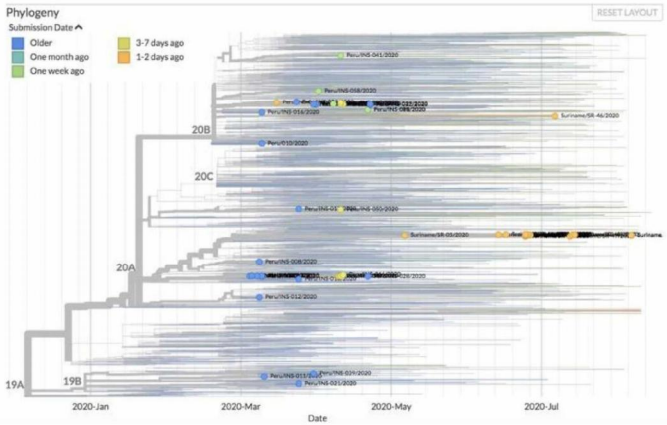
Surinam



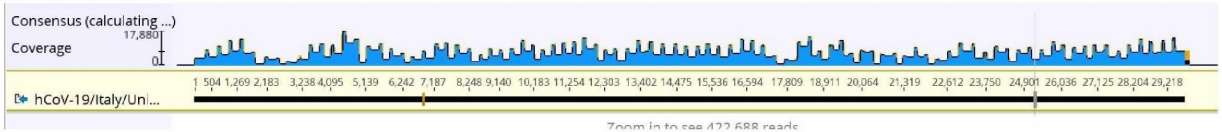
Built with nextstrain/ncov. Maintained by the Nextstrain team.

Showing 67 of 4802 genomes sampled between Mar 2020 and Aug 2020. Filtered to × Peru (40) × Suriname (27).



Reconstruct the genome

- Around 12,000,000 sequences are generated per sequence
- Around 10GB of data per sequence run
- Now almost 160 sequence runs performed



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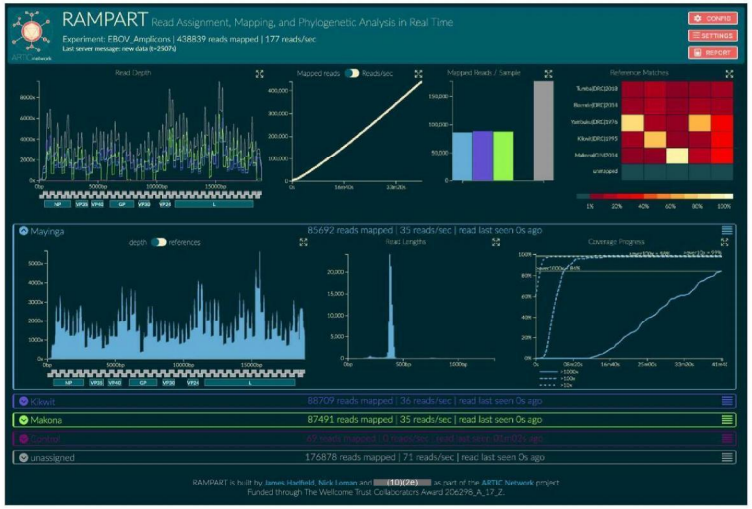
Data analysis



Custom script to automatically collect data from the machine and transfer to the server

Real-time automated data-analysis:

- Demultiplex
- Trim primers
- Align to reference
- Generate consensus sequence (10x, 30 and 100x coverage)

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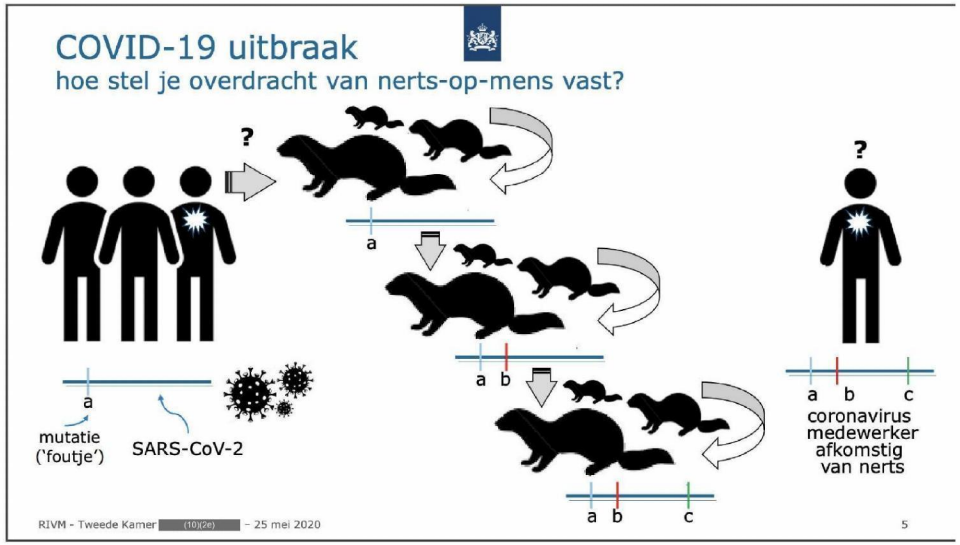
Mutations in the viral genome



(10)(2e), RIVM

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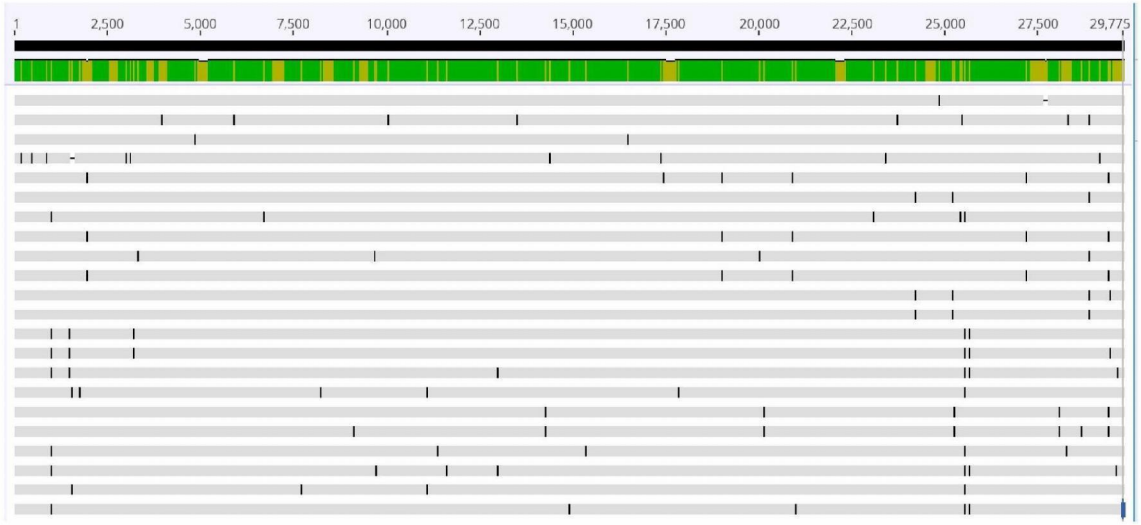
Multiple sequence alignment



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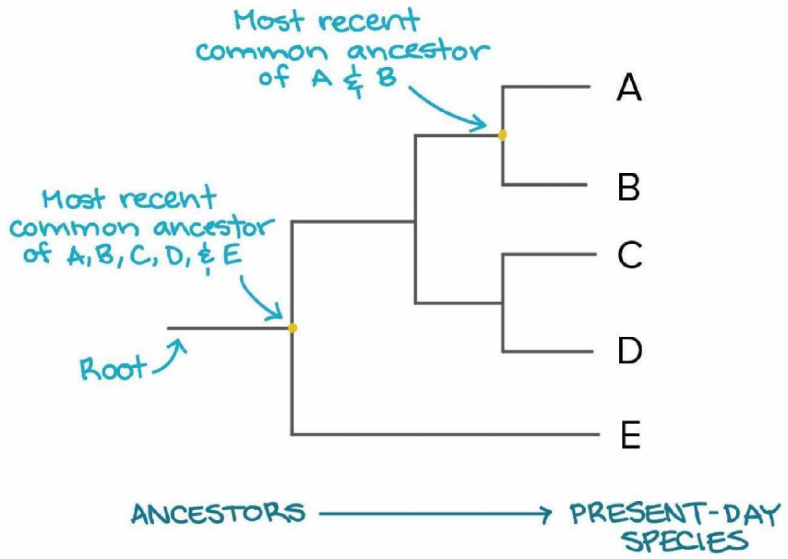
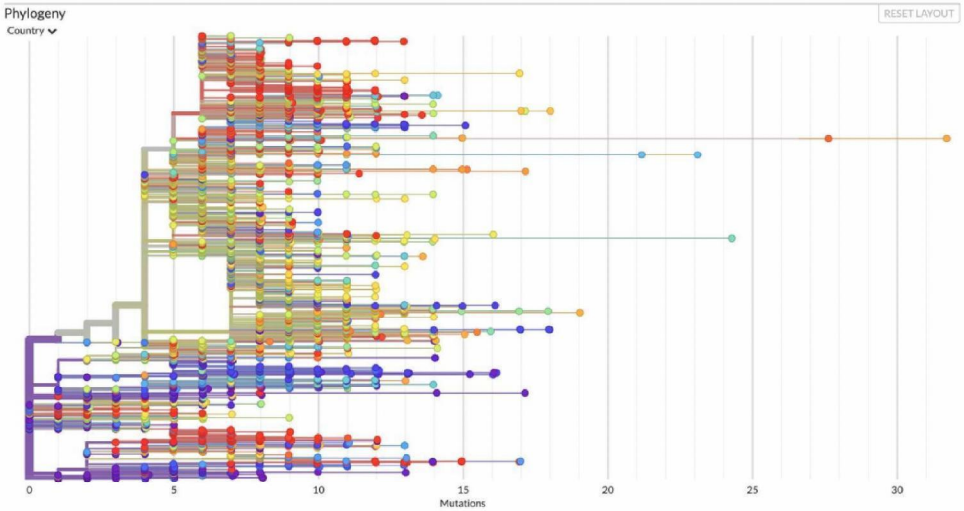
Phylogeny



Nextstrain

Showing 4307 of 4307 genomes sampled between Dec 2019 and May 2020.



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Rapid SARS-CoV-2 whole genome sequencing

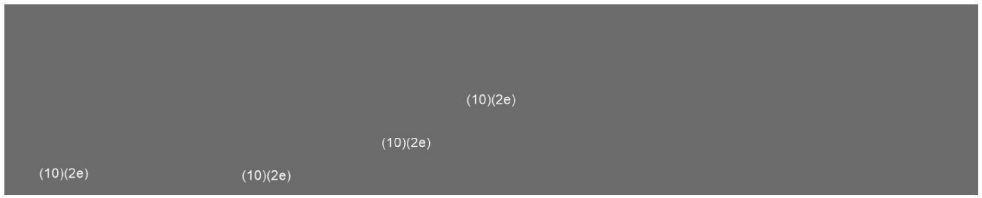
nature medicine

LETTERS

https://doi.org/10.1038/s41591-020-0997-y

Check for updates

Rapid SARS-CoV-2 whole-genome sequencing and analysis for informed public health decision-making in the Netherlands



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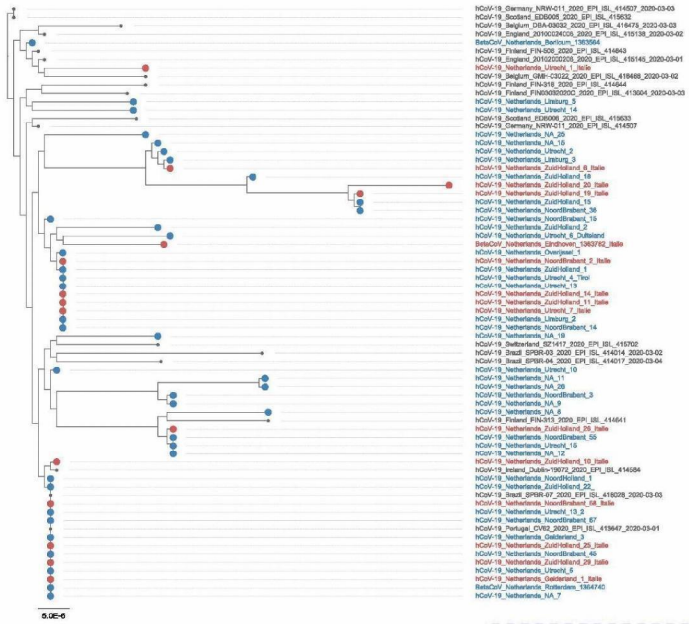
Initial testing of travelers according to the WHO and ECDC case definitions

The first SARS-CoV-2 infection in the Netherlands was confirmed on February 27th and an additional case one day later. The genomes of these first two positive samples were generated and analyzed by the February 29th.

Not from one recent source, e.g. unlikely connected

Systematic sequencing during exponential growth phase



In the third phase sequencing of new cases with emphasis on HCW and severe hospitalized cases was continued.

The diversity was also observed in cases with similar travel histories

At that moment this represented 27,1% of the total number of full genome sequences produced worldwide.

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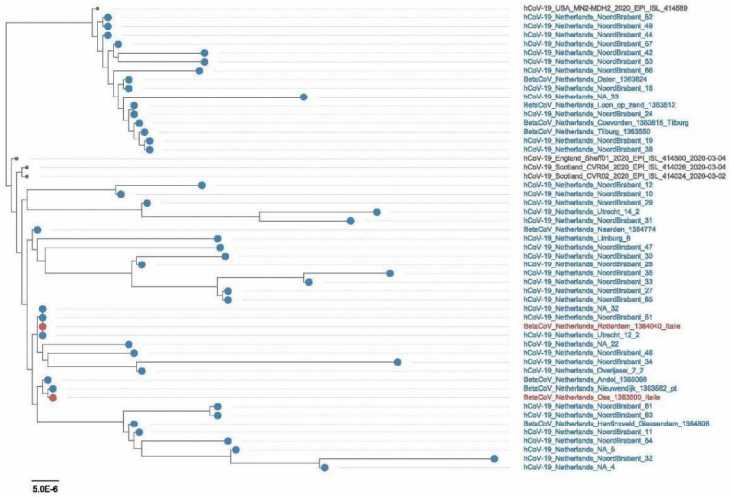
Systematic sequencing during exponential growth phase



The sequences detected in the Netherlands were diverse and revealed the presence of multiple co-circulating sequence types, found in several different clusters in the phylogenetic tree.

The increase in COVID-19 patients as well as increasing affected geographic areas and occurrence of local clusters provided further support for the increased movement restrictions.

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Discussion

- We show that whole genome sequencing in combination with epidemiological data strengthened the evidence base for public health decision making in the Netherlands.
- Although implementation of WGS in the Dutch disease prevention and control has shown its added value, there is still only a limited amount of genomic information available from certain parts of the world.
- The combination of real-time WGS with the data from the National Public Health response team has provided information that helped decide on next steps in the decision making.
- In order to fully capitalize on the potential added value of WGS for public health decision making, systems for combined analysis of data are needed that are in agreement with general data protection rules.

Ongoing research

- Determine the geographical signature (if any)
- Monitor significant changes in the genome
- Outbreak investigations
  - Hospitals
  - Nursing homes
  - Slaughterhouses
  - Gyms
  - Schools
  - Fruit industry
  - **Mink farms**

US coronavirus hotspots linked to meat processing plants

Analysis shows factories a source of virus transmission

Workers and unions urge health and safety overhaul

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SARS-CoV-2 in animals

- First detected on a sea food and live animal market
- Although the virus could be identified in environmental samples, no animal tested positive
- Cats, ferrets, hamsters, rhesus macaques, cynomolgus macaques and fruit bats are susceptible
- Dogs, cats, lions, tigers and minks can be infected

SARS-CoV-2 in mink farms

- Increased mortality was reported at mink farms on the 19th and 20th of April

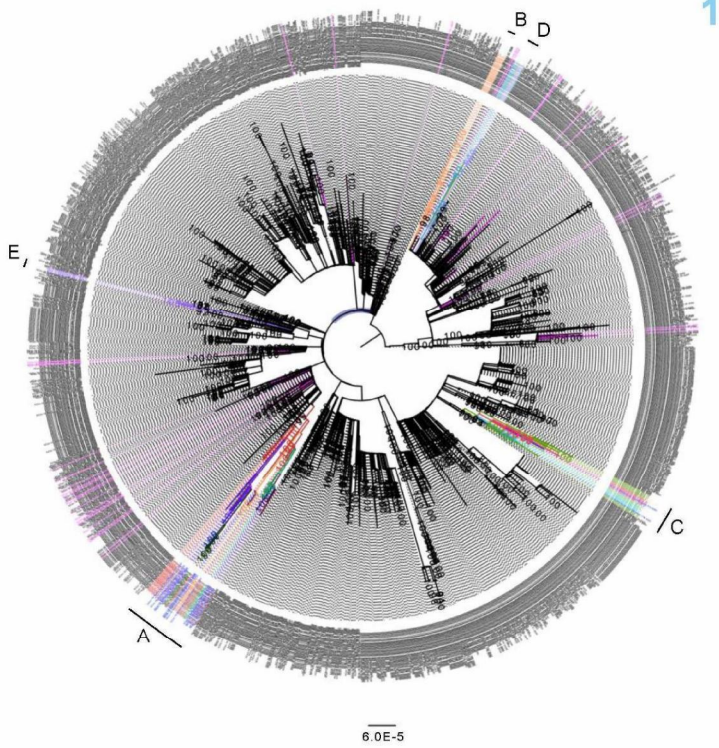
- Farm 1: 12,000 animals of which 285 (2,4%) died
- Farm 2: 7,500 animals of which 90 (1,2%) died

- Current states: 40 mink farms in the Netherlands and 2 mink farms in Denmark with infections

- Situation report of the first 16 SARS-CoV-2 infected mink farms

Human cases

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%)



16 mink farms

- Mink farm related sequences compared to all Dutch sequences
- A lot of divergence compare to the human population
- 3 main clusters
- 5 different introductions

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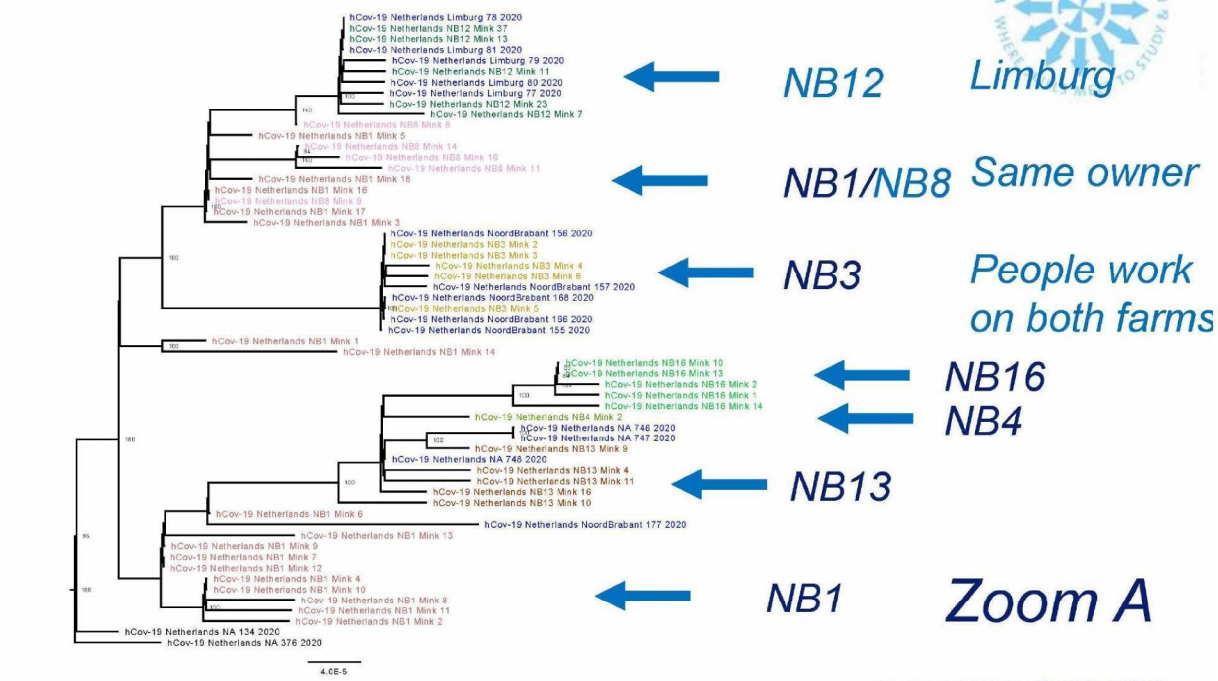
hCov-19 Netherlands Limburg 78 2020
hCov-19 Netherlands NB12 Mink 37
hCov-19 Netherlands NB12 Mink 13
hCov-19 Netherlands Limburg 81 2020
hCov-19 Netherlands Limburg 79 2020
hCov-19 Netherlands NB12 Mink 11
hCov-19 Netherlands Limburg 80 2020
hCov-19 Netherlands Limburg 77 2020
hCov-19 Netherlands NB12 Mink 23
hCov-19 Netherlands NB12 Mink 7
hCov-19 Netherlands NB8 Mink 8
hCov-19 Netherlands NB1 Mink 5
hCov-19 Netherlands NB8 Mink 14
hCov-19 Netherlands NB8 Mink 16
hCov-19 Netherlands NB8 Mink 11
hCov-19 Netherlands NB1 Mink 18
hCov-19 Netherlands NB1 Mink 16
hCov-19 Netherlands NB8 Mink 9
hCov-19 Netherlands NB1 Mink 17
hCov-19 Netherlands NB1 Mink 3
hCov-19 Netherlands NoordBrabant 156 2020
hCov-19 Netherlands NB3 Mink 2
hCov-19 Netherlands NB3 Mink 3
hCov-19 Netherlands NB3 Mink 4
hCov-19 Netherlands NB3 Mink 6
hCov-19 Netherlands NoordBrabant 157 2020
hCov-19 Netherlands NoordBrabant 168 2020
hCov-19 Netherlands NB3 Mink 5
hCov-19 Netherlands NoordBrabant 196 2020
hCov-19 Netherlands NoordBrabant 155 2020
hCov-19 Netherlands NB1 Mink 1
hCov-19 Netherlands NB1 Mink 14
hCov-19 Netherlands NB16 Mink 10
hCov-19 Netherlands NB16 Mink 13
hCov-19 Netherlands NB16 Mink 2
hCov-19 Netherlands NB16 Mink 1
hCov-19 Netherlands NB16 Mink 14
hCov-19 Netherlands NB4 Mink 2
hCov-19 Netherlands NA 746 2020
hCov-19 Netherlands NA 747 2020
hCov-19 Netherlands NB13 Mink 9
hCov-19 Netherlands NA 748 2020
hCov-19 Netherlands NB13 Mink 4
hCov-19 Netherlands NB13 Mink 11
hCov-19 Netherlands NB13 Mink 16
hCov-19 Netherlands NB13 Mink 10
hCov-19 Netherlands NB1 Mink 6
hCov-19 Netherlands NoordBrabant 177 2020
hCov-19 Netherlands NB1 Mink 13
hCov-19 Netherlands NB1 Mink 9
hCov-19 Netherlands NB1 Mink 7
hCov-19 Netherlands NB1 Mink 12
hCov-19 Netherlands NB1 Mink 4
hCov-19 Netherlands NB1 Mink 10
hCov-19 Netherlands NB1 Mink 8
hCov-19 Netherlands NB1 Mink 11
hCov-19 Netherlands NB1 Mink 2
hCov-19 Netherlands NA 134 2020
hCov-19 Netherlands NA 376 2020
4.0E-5
NB12
Limburg
NB1/NB8
Same owner
NB3
People work on both farms
NB16
NB4
NB13
NB1
Zoom A
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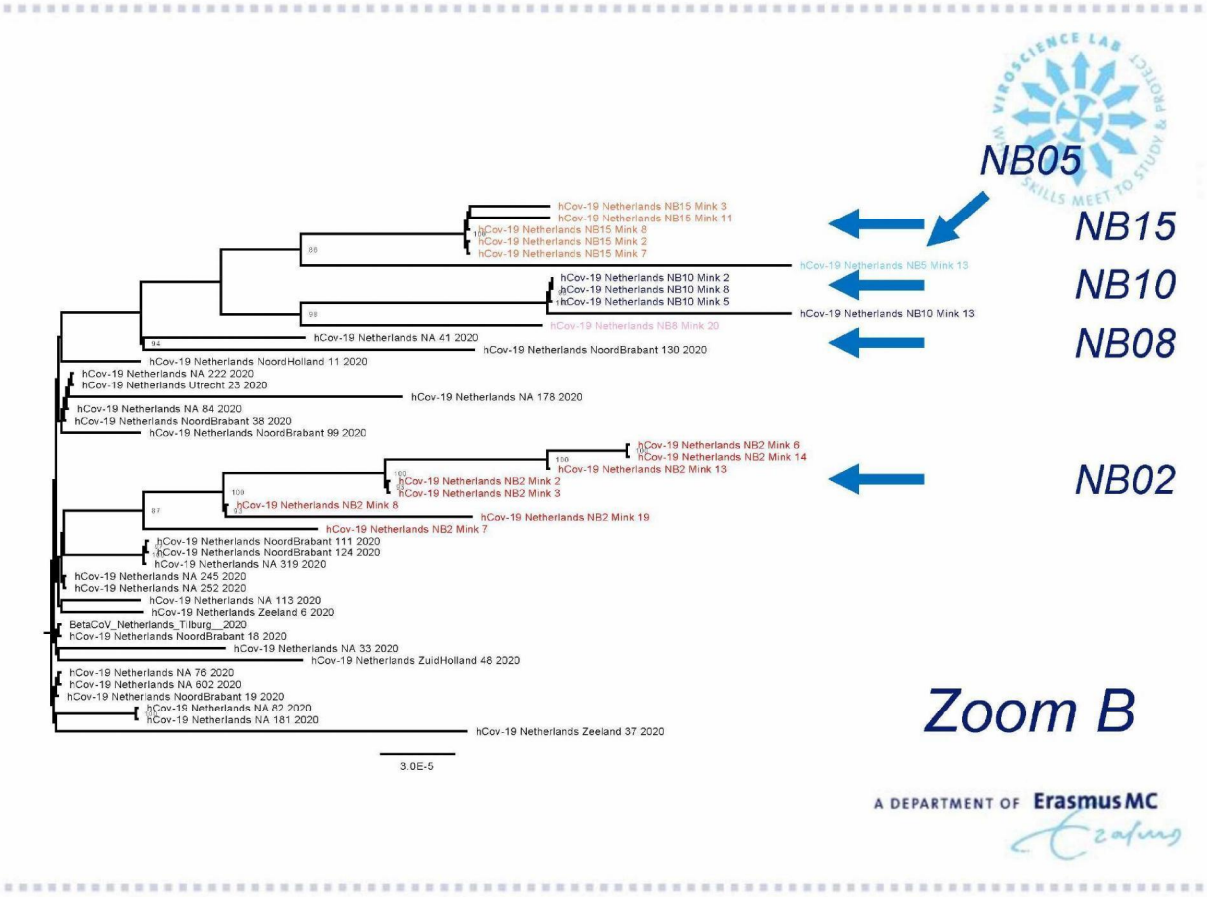
NB05
NB15
NB10
NB08
NB02
hCov-19 Netherlands NB15 Mink 3
hCov-19 Netherlands NB15 Mink 11
hCov-19 Netherlands NB15 Mink 8
hCov-19 Netherlands NB15 Mink 2
hCov-19 Netherlands NB15 Mink 7
hCov-19 Netherlands NB5 Mink 13
hCov-19 Netherlands NB10 Mink 2
hCov-19 Netherlands NB10 Mink 8
hCov-19 Netherlands NB10 Mink 5
hCov-19 Netherlands NB10 Mink 13
hCov-19 Netherlands NB8 Mink 20
hCov-19 Netherlands NA 41 2020
hCov-19 Netherlands NoordBrabant 130 2020
hCov-19 Netherlands NoordHolland 11 2020
hCov-19 Netherlands NA 222 2020
hCov-19 Netherlands Utrecht 25 2020
hCov-19 Netherlands NA 178 2020
hCov-19 Netherlands NA 84 2020
hCov-19 Netherlands NoordBrabant 38 2020
hCov-19 Netherlands NoordBrabant 99 2020
hCov-19 Netherlands NB2 Mink 6
hCov-19 Netherlands NB2 Mink 14
hCov-19 Netherlands NB2 Mink 13
hCov-19 Netherlands NB2 Mink 2
hCov-19 Netherlands NB2 Mink 3
hCov-19 Netherlands NB2 Mink 8
hCov-19 Netherlands NB2 Mink 19
hCov-19 Netherlands NB2 Mink 7
hCov-19 Netherlands NoordBrabant 111 2020
hCov-19 Netherlands NoordBrabant 124 2020
hCov-19 Netherlands NA 319 2020
hCov-19 Netherlands NA 245 2020
hCov-19 Netherlands NA 252 2020
hCov-19 Netherlands NA 113 2020
hCov-19 Netherlands Zeeland 6 2020
BetaCoV_Netherlands_Tilburg__2020
hCov-19 Netherlands NoordBrabant 18 2020
hCov-19 Netherlands NA 33 2020
hCov-19 Netherlands ZuidHolland 48 2020
hCov-19 Netherlands NA 76 2020
hCov-19 Netherlands NA 602 2020
hCov-19 Netherlands NoordBrabant 19 2020
hCov-19 Netherlands NA 181 2020
hCov-19 Netherlands Zeeland 37 2020
3.0E-5
Zoom B
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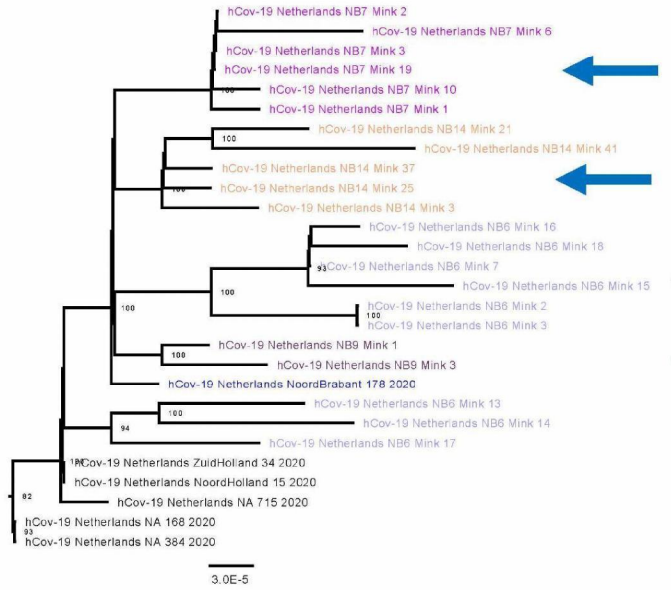

NB7

NB14

NB6

NB9

Zoom C

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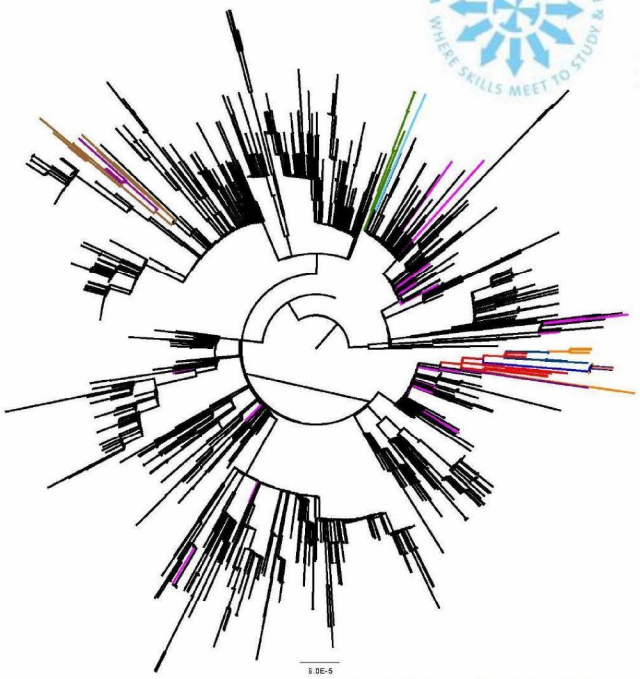
Reflection of local circulation

- 34 samples from SARS-CoV-2 positive individuals from the same 4-digit postal code area were tested

- Not related to the mink farms
  - No increased risk
  - Not a reflection of what is going on the area



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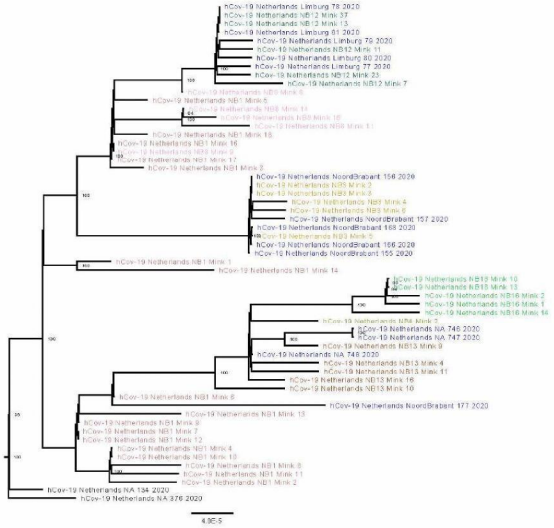
Proof of mink to human infection

- Employees got infected after the detection of SARS-CoV-2 in minks
- Clustering in the phylogenetic tree
- Detection of the virus in air samples (~CT 28)
- Probably not all infected at the farms
  but also family clusters



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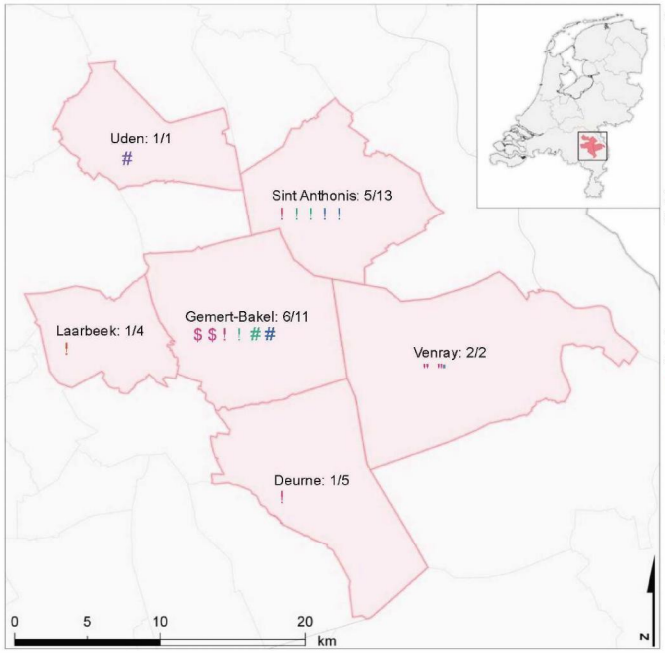
No clear link

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

Geographical distribution



SARS-CoV-2 affected mink farms

Colored by cluster

! A
! B
! C
! D
! E

For farms with same owner, shaped by owner

⬟
■
▲

Municipalities with SARS-CoV-2 affected mink farms

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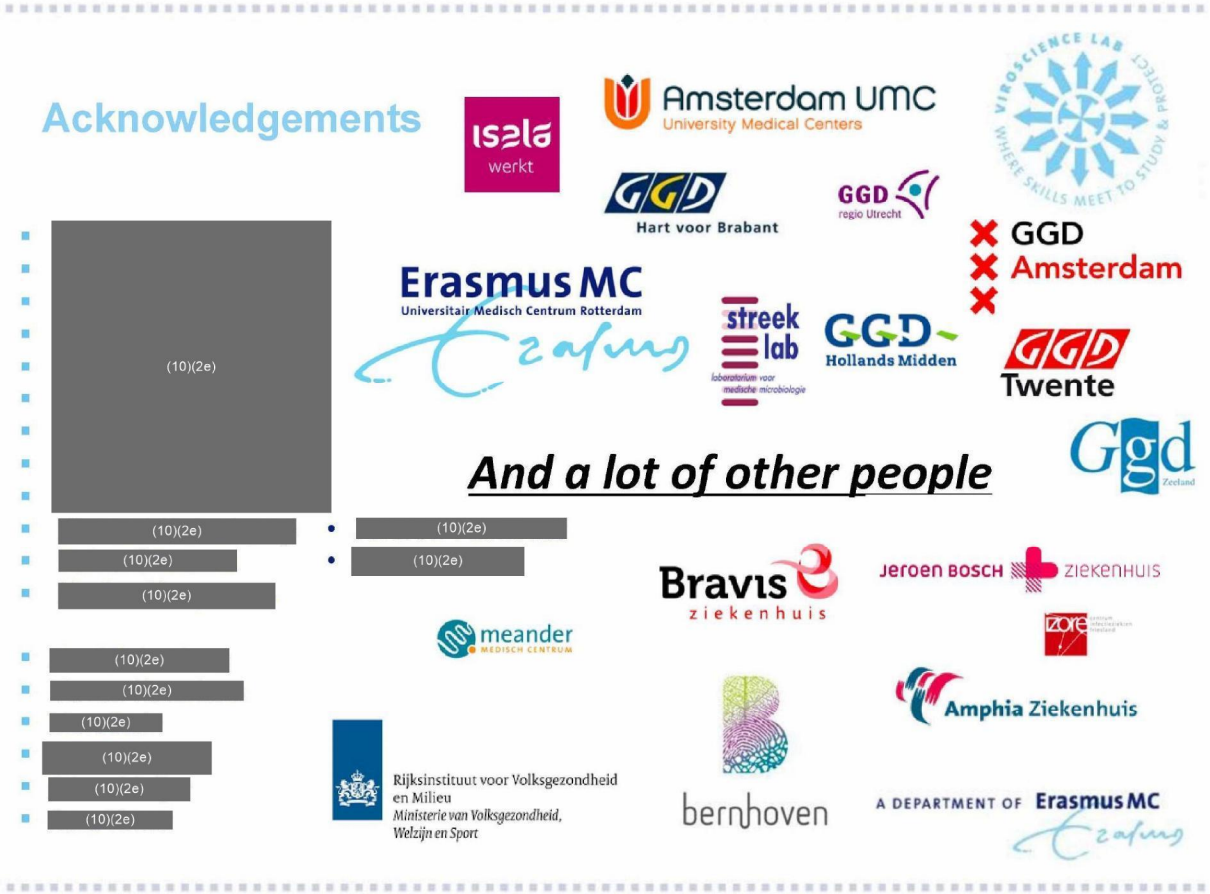
Discussion

- SARS-CoV-2 outbreaks have been detected in 16 mink farms in the Netherlands
- Phylogenetic signal indicates a wide/fast spread
- No specific adaptation mutation were observed in all mink samples
- Three main clusters have been identified without obvious link
  - Same food supplies?
  - People working at different farms?
  - Exchange of material?


Acknowledgements
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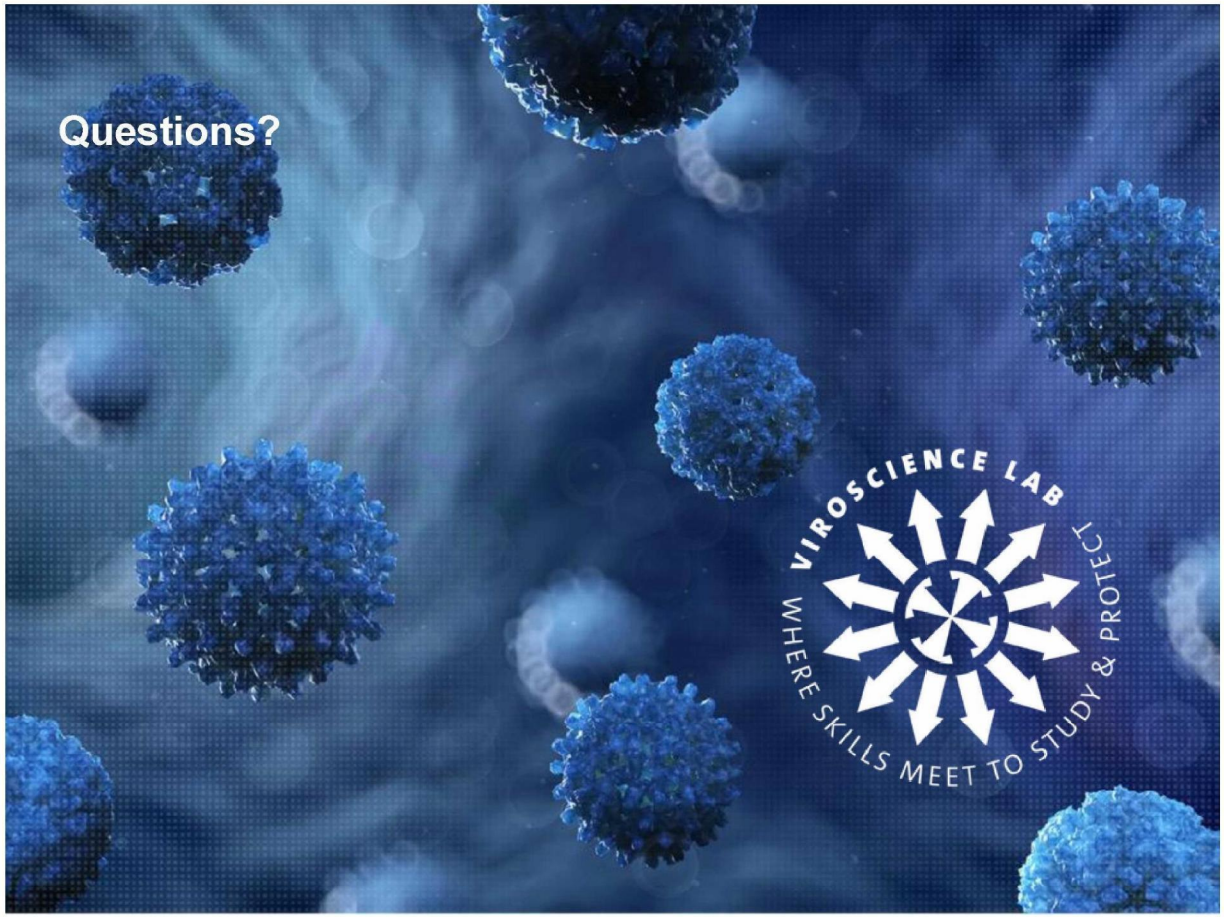
Rijksinstituut voor Volksgezondheid en Milieu
Ministerie van Volksgezondheid, Welzijn en Sport
And a lot of other people


Questions?
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