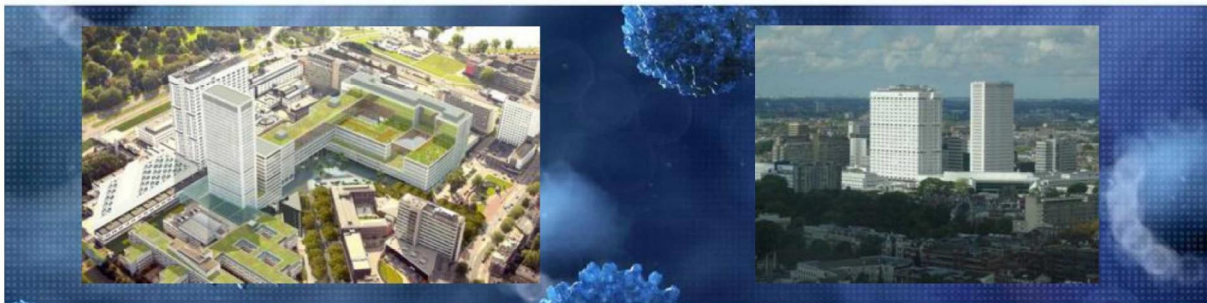




Viroscience lab

WHERE SKILLS MEET TO STUDY & PROTECT



Rapid SARS-CoV-2 Whole Genome Sequencing

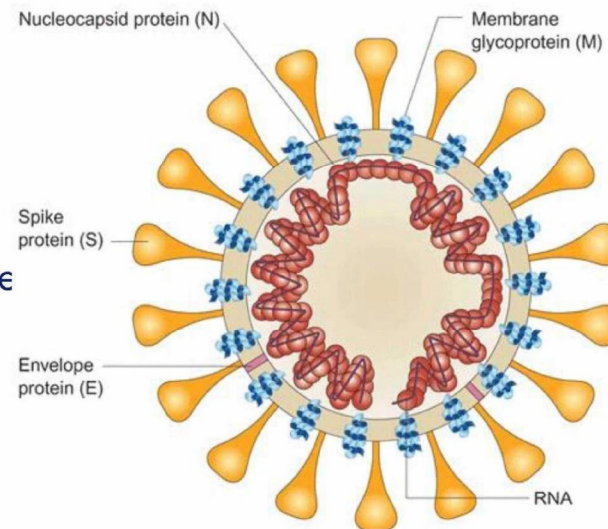
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[@erasmusmc.nl](https://www.erasmusmc.nl)

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, A DEPARTMENT OF **ErasmusMC**



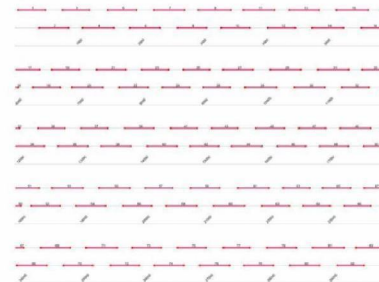
Amplicon based sequencing

PROTOCOL

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

Joshua Quick¹, Nathan D Grubaugh^{2,3}, Steven T Pullan⁴, Ingra M Claro⁴, Andrew D Smith¹, Karthik Gangavarapu¹, Glenn Oliveira⁵, Refugio Robles-Sikisaka², Thomas F Rogers^{2,6}, Nathan A Beutler^{2,7,8,9,10}, R Burton², Lia Laura Lewis-Ximenez⁷, Jaqueline Goes de Jesus⁸, Marta Giovanetti^{8,9}, Sarah C Hill¹⁰, Allison Black^{11,12}, Trevor Bedford¹¹, Miles W Carroll^{13,14}, Marcio Nunes¹⁴, Luiz Carlos Alcantara Jr.⁸, Ester C Sabino⁴, Sally A Baylis¹⁵, Nuno R Faria¹⁰, Matthew Loose¹⁶, Jared T Simpson¹⁷, Oliver G Pybus¹⁰, Kristian G Andersen^{2,5} & Nicholas J Loman¹

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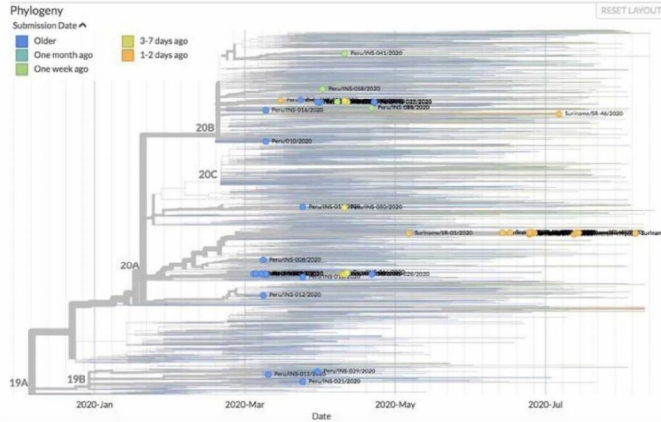
Erasmus

Surinam



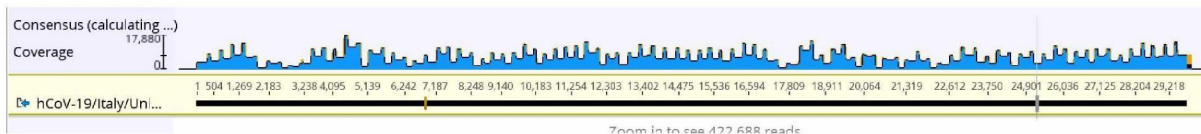
Built with nextstrain/ncov. Maintained by the Nextstrain team.

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



Reconstruct the genome

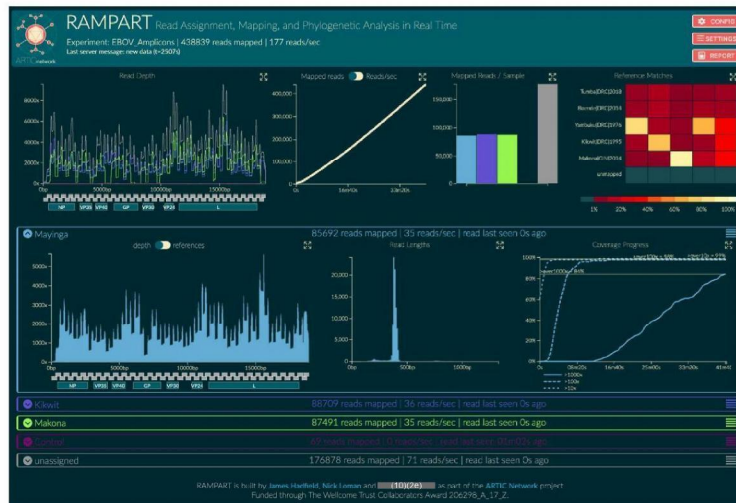
- Around 12,000,000 sequences are generated per sequence
- Around 10GB of data per sequence run
- Now almost 160 sequence runs performed



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Data analysis

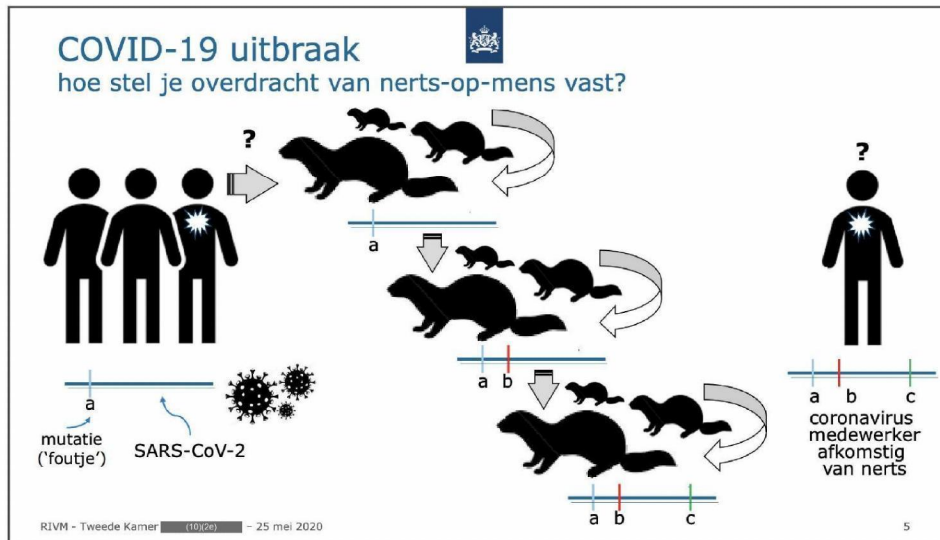


Custom script to automatically collect data from the machine and transfer to the server

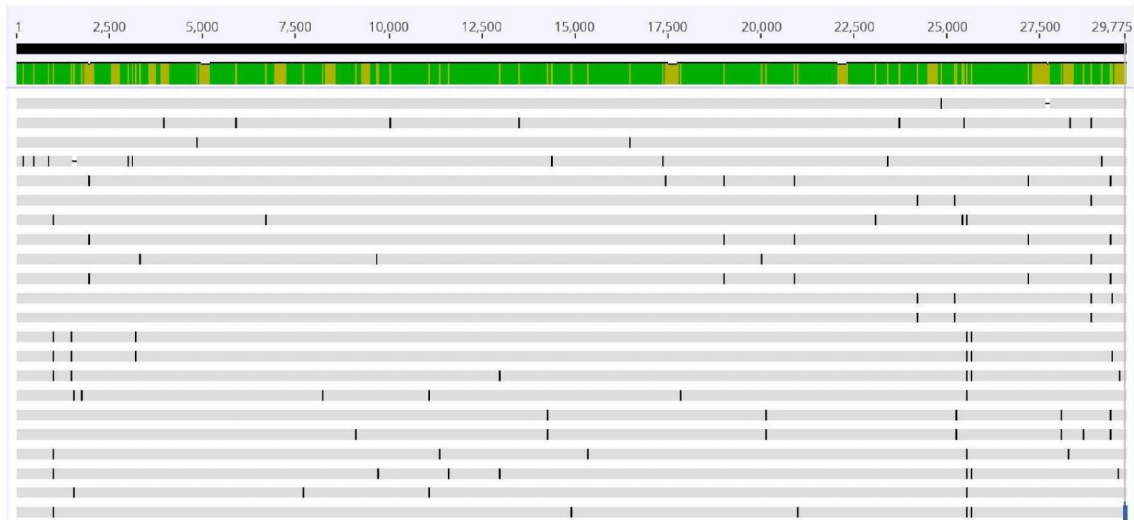
Real-time automated data-analysis:

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

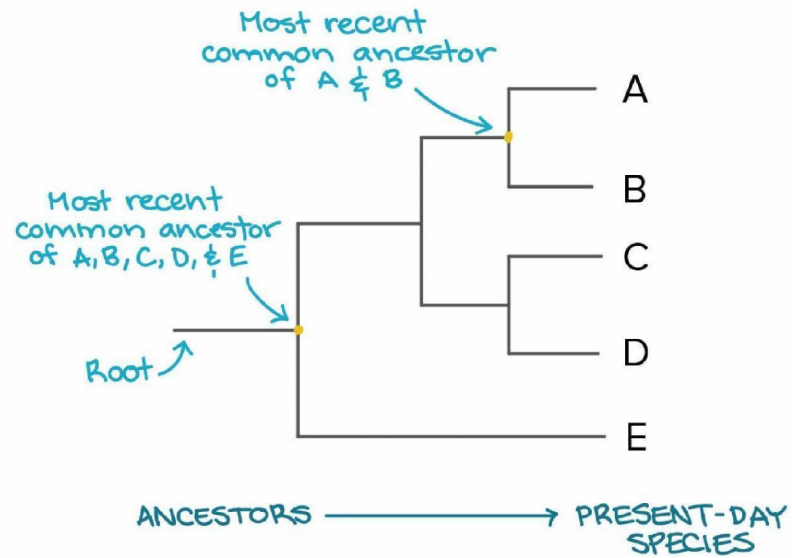
Mutations in the viral genome



Multiple sequence alignment



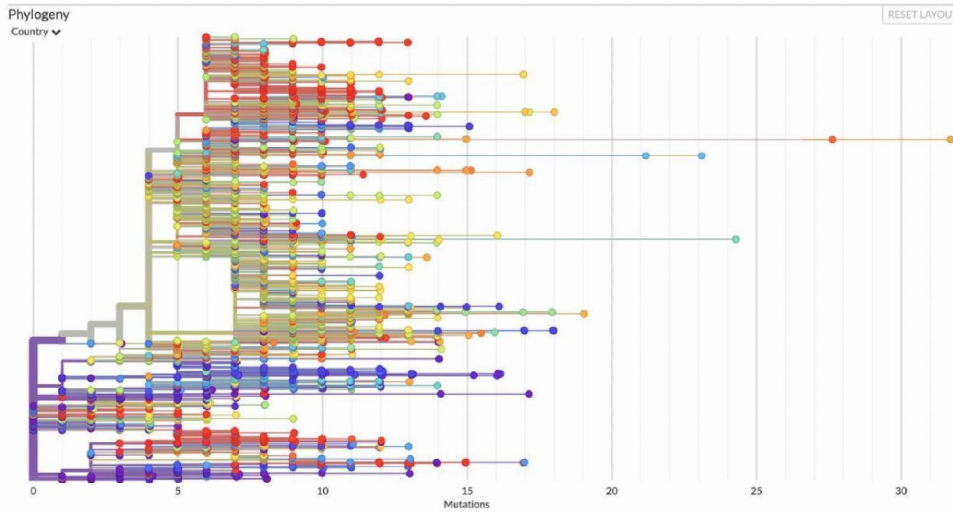
Phylogeny



Nextstrain



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




Rapid SARS-CoV-2 whole genome sequencing



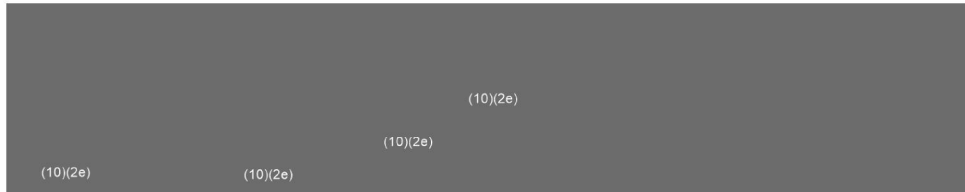
nature
medicine

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.

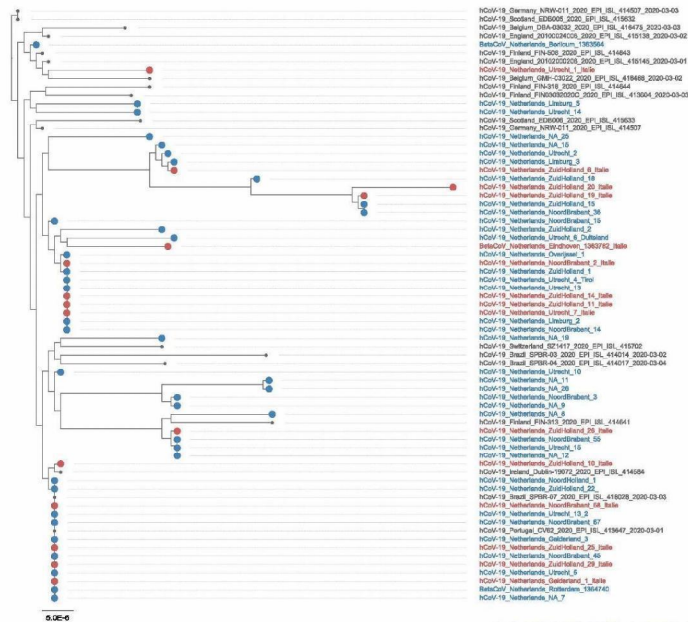


Not from one recent source, e.g. unlikely connected

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Systematic sequencing during exponential growth phase



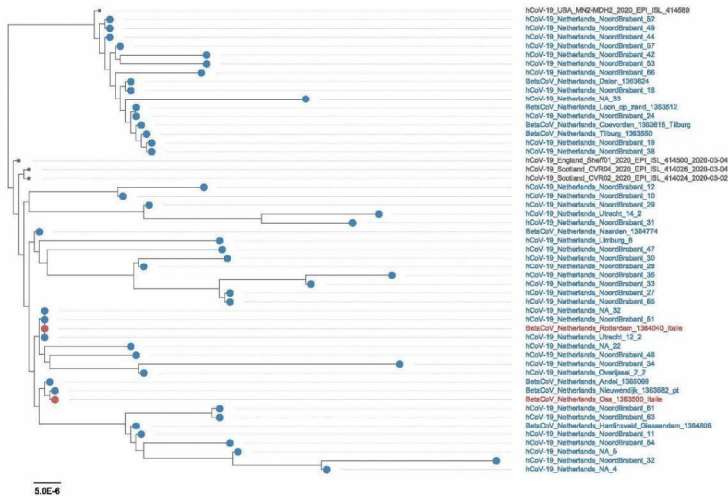
In the third phase sequencing of new cases with emphasis on HCW and severe hospitalized cases was continued.

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.



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.

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.

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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



▲ A billboard advertises job hiring at Agri Beef's plant in Toppenish, Washington. Donald Trump last month declared such plants to be critical to the US economy. Photograph: Paul A. Hester/AP



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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

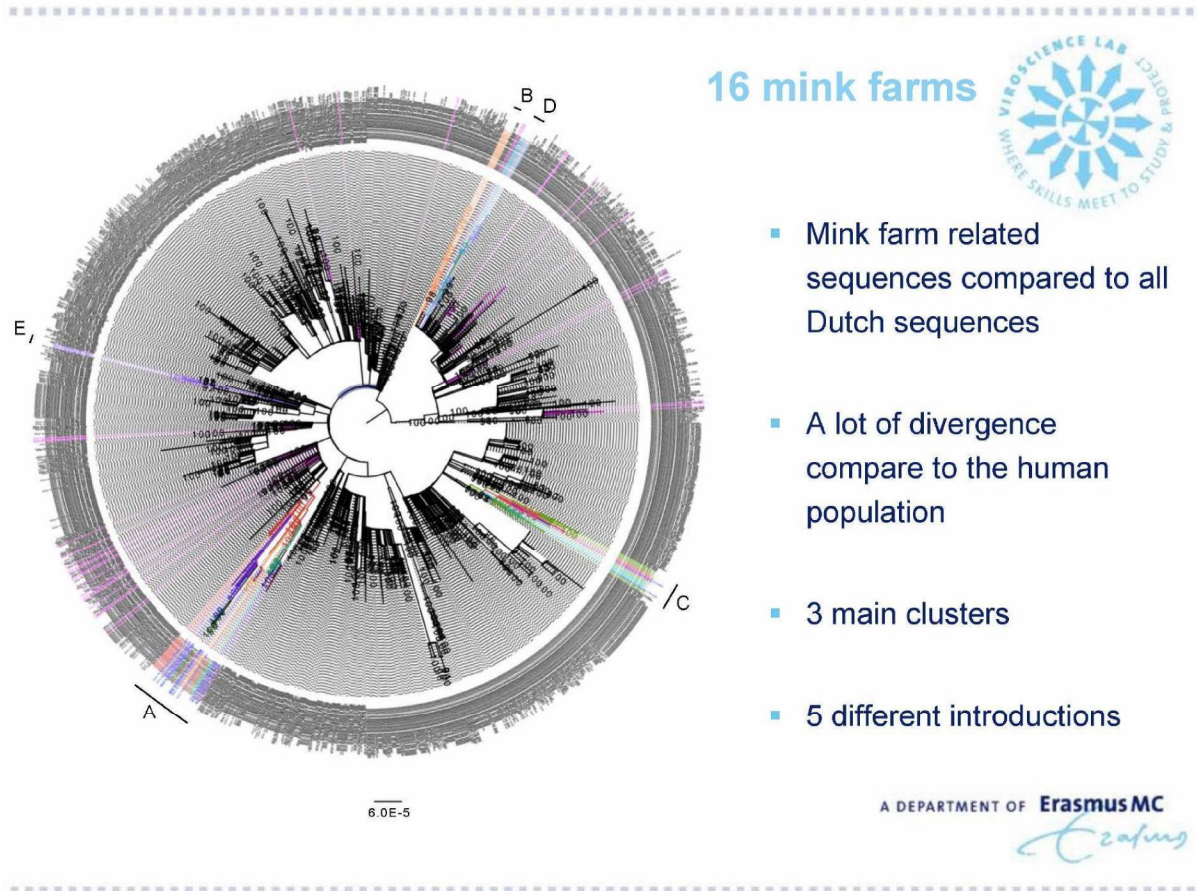
Human cases

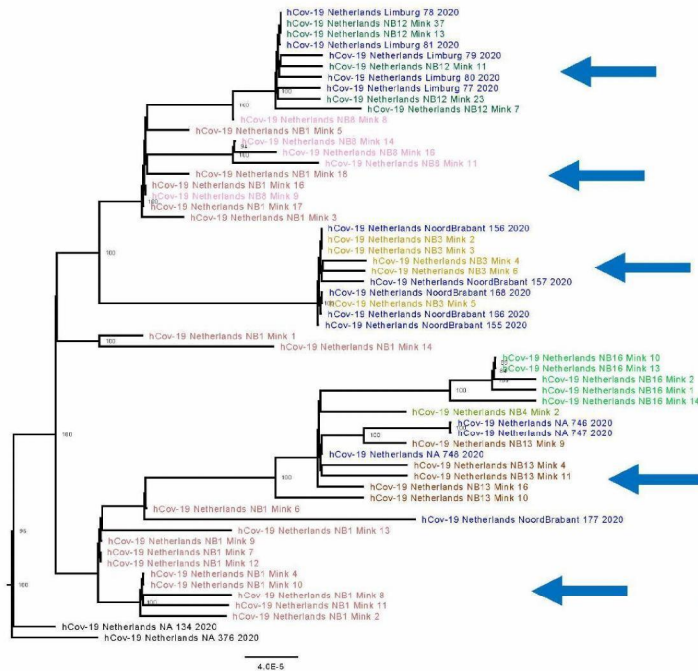


Farm:	First diagnosis in animals:	Date(s) of sampling employees and family members:	PCR positive (%)	Serology positive (%)	Employees and family members tested positive (PCR and/or serology)
NB1	24-04-2020	28-04-2020 – 11-05-2020	5/6 (83%)	5/5 (100%)	6/6 (100%)
NB2	25-04-2020	31-03-2020 – 30-04-2020	1/2 (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%)

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← NB12

Limburg

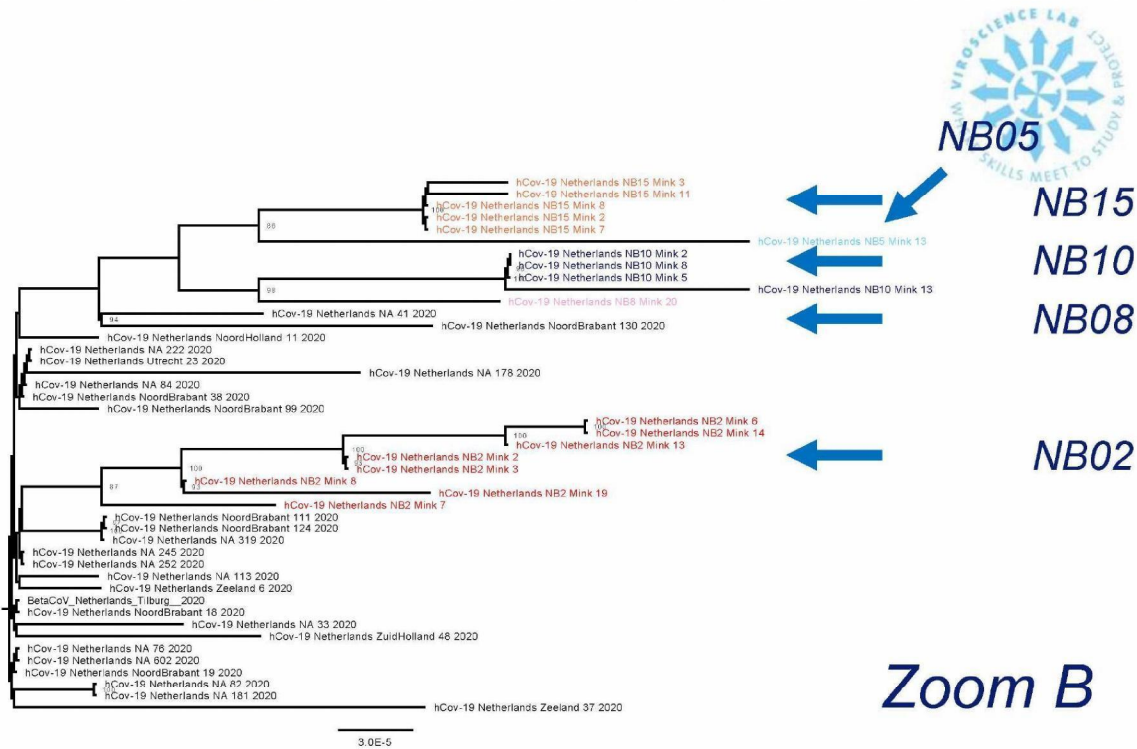
← NB1/NB8 Same owner

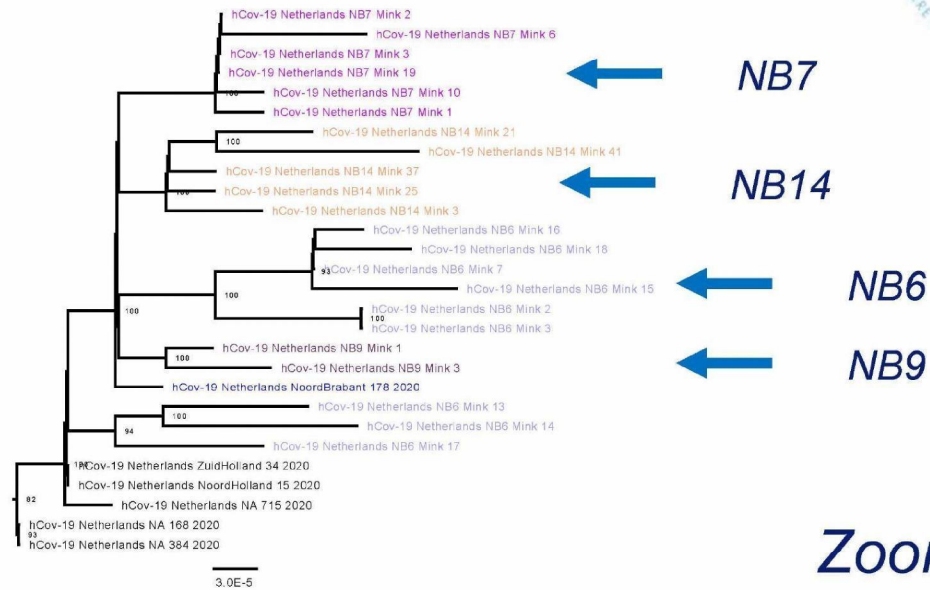
← NB3 People work on both farms

← NB16
← NB4

← NB13

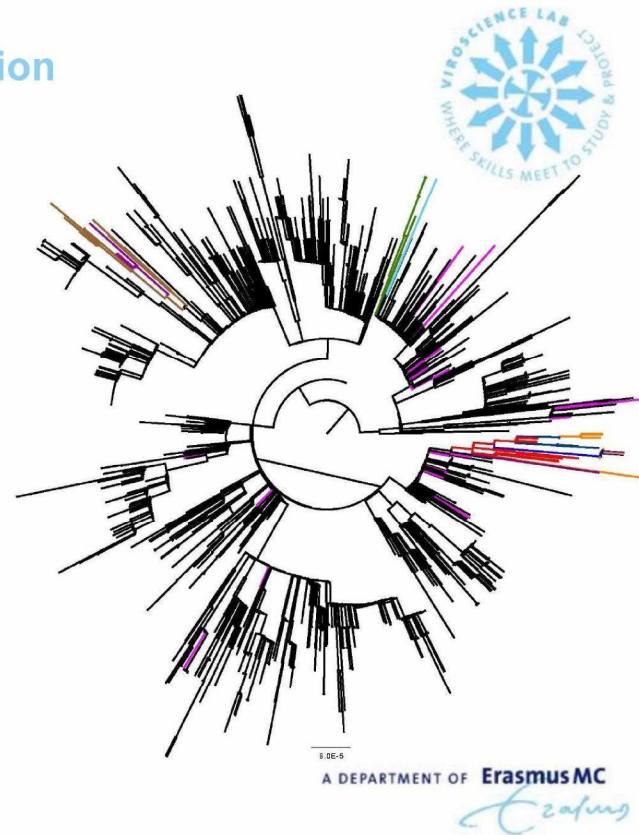
← NB1 Zoom A





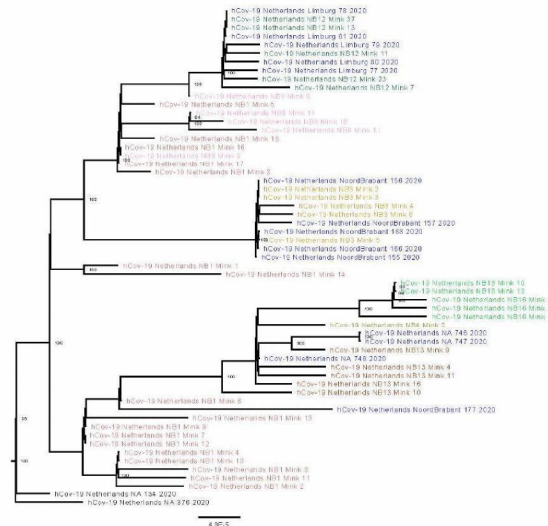
Reflection of local circulation

- 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



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

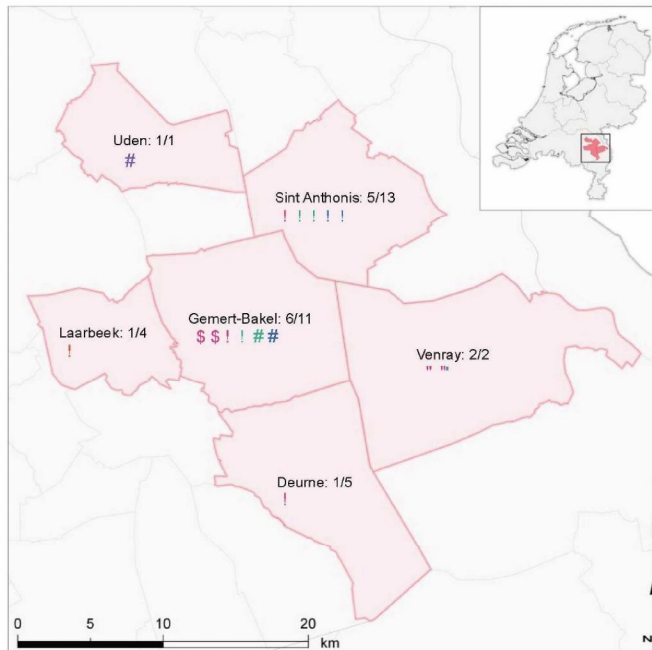


Farm:	Date of diagnosis :	Sequence cluster:	Same owner:	Feed supplier:	Vet**:	Number of sequences (human):	Sequence diversity (average):	Mink population size:	Detection***:
NB1	24-04-20	A	NB1, NB4	1	I	17 (1)	0-9 (3.9)	75,711	Notification
NB2	25-04-20	B		1	II	8	0-8 (3.6)	50,473	Notification
NB3	07-05-20	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

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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

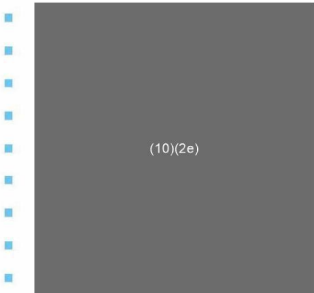


Discussion



- 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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And a lot of other people

