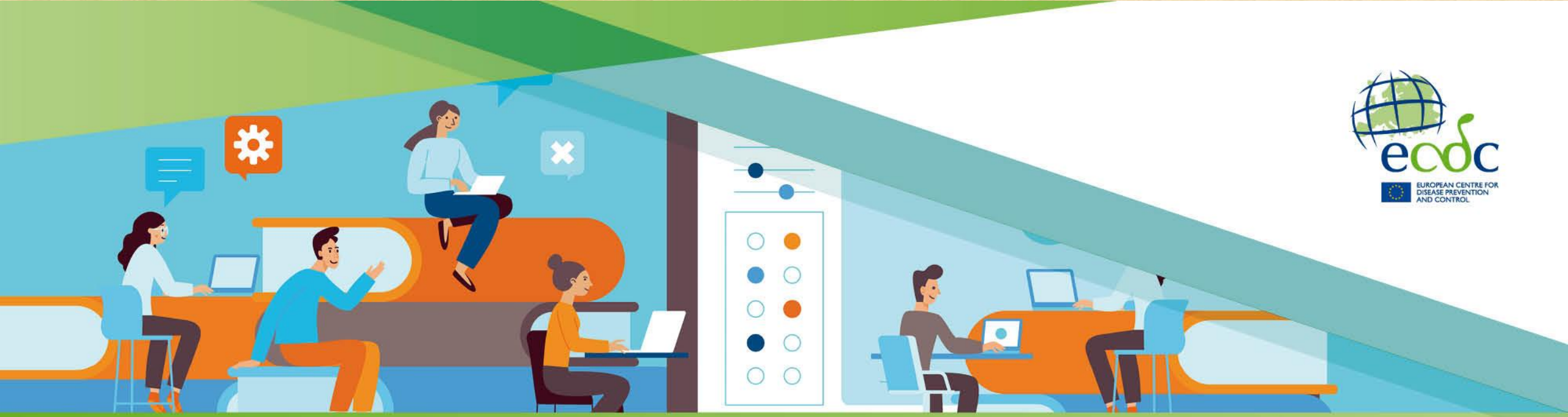


GenEpi-BioTrain Virtual Training 29 - Genomic epidemiology of mpox

Whole genome sequencing for outbreak response

05-10-2026

Bas Oude Munnink

Aim and learning objectives

Aim:

Learn how you can use WGS for outbreak investigations

Specific learning objectives

At the end of this session, you will be able to:

1. Understand how WGS was used during the beginning of the SARS-CoV-2 outbreak in the Netherlands
2. Understand how WGS was used during the outbreak of SARS-CoV-2 on mink farms
3. Understand how WGS was used during the Andes Hantavirus outbreak on a cruise ship

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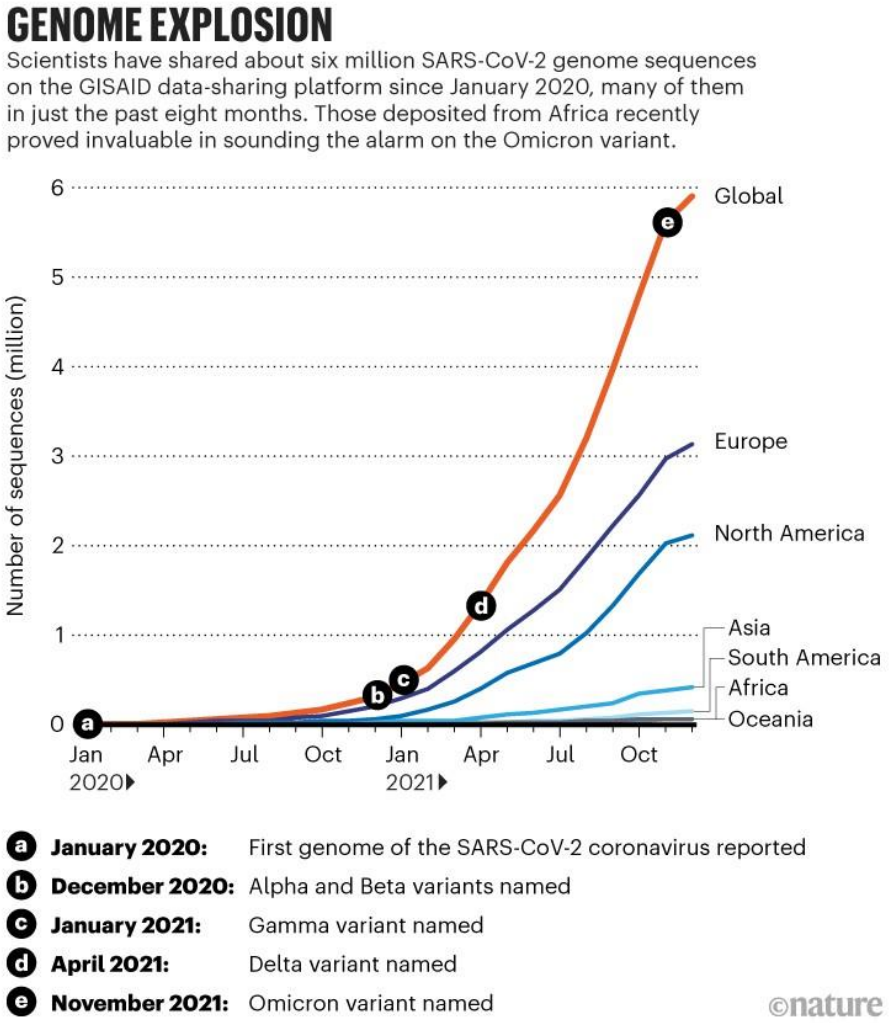
What do you think WGS can be used for during an outbreak response?

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WGS for outbreak investigations

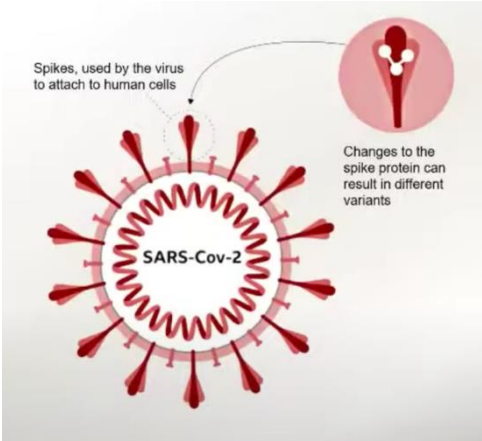
- Where did it come from?
- How different is it from what we have seen before?
- Is it part of a transmission cluster?
- Is it part of an outbreak?
- Is there ongoing circulation or a new introduction?



https://www.nature.com/articles/d41586-021-03698-7

SARS-CoV-2 -- All viruses change over time

- Emergence of new variants is expected, although:
  - Most mutations have no impact on function or structure of virus
  - Some can be harmful for virus survival
  - Some can result in advantage for virus:
    - Increased transmissibility
    - Increased disease severity, virulence
    - Influence efficacy of vaccines/therapeutics



- Mutation rate of SARS-CoV-2 is low relative to other RNA viruses
- **Chance on mutations increases with frequency of human and animal infections**

Amplicon-based Nanopore 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], Dennis 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 São Paulo, São 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).





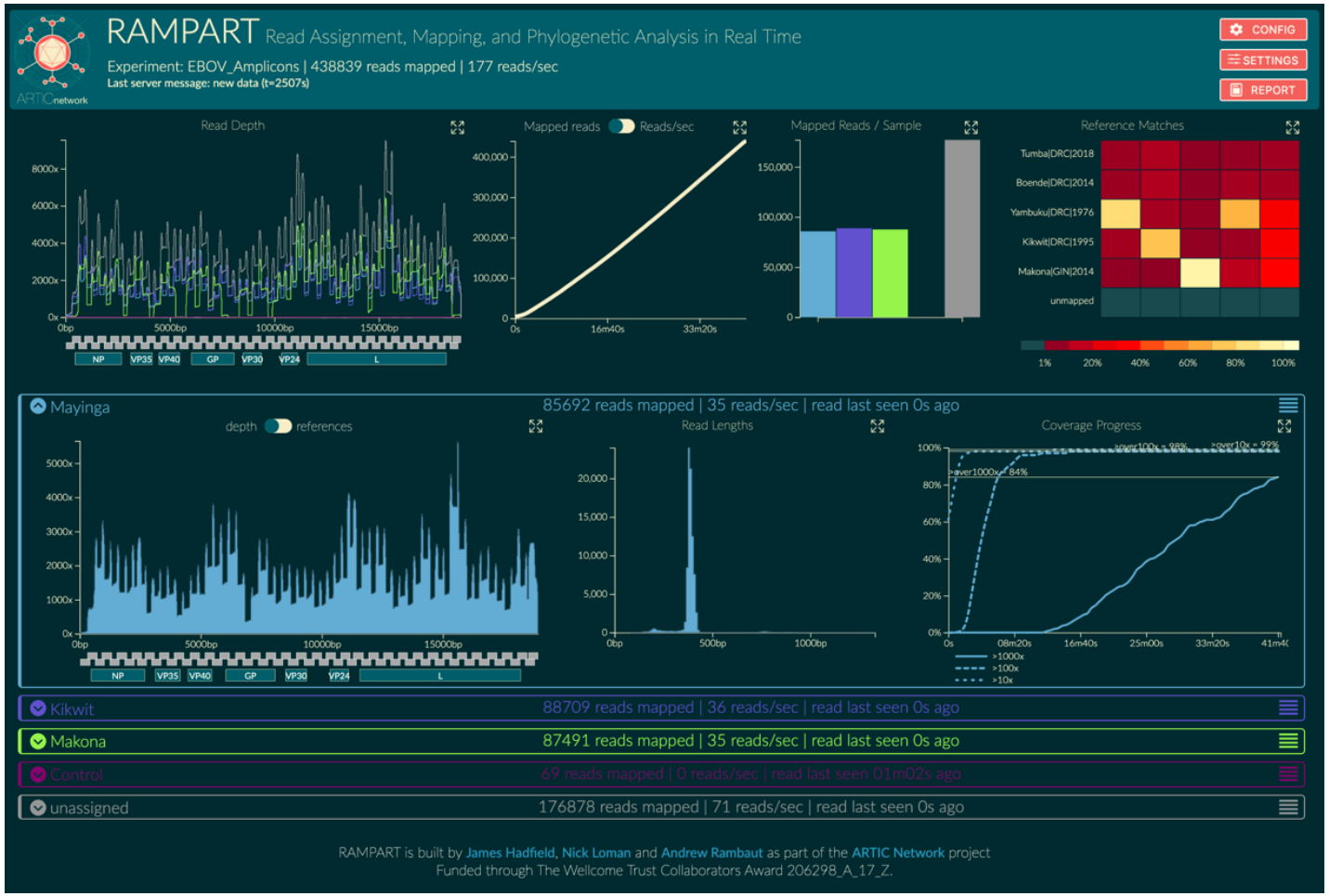






89 overlapping amplicons 500bp
75 bp overlap

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)

https://github.com/artic-network/rampart

Beginning of the pandemic

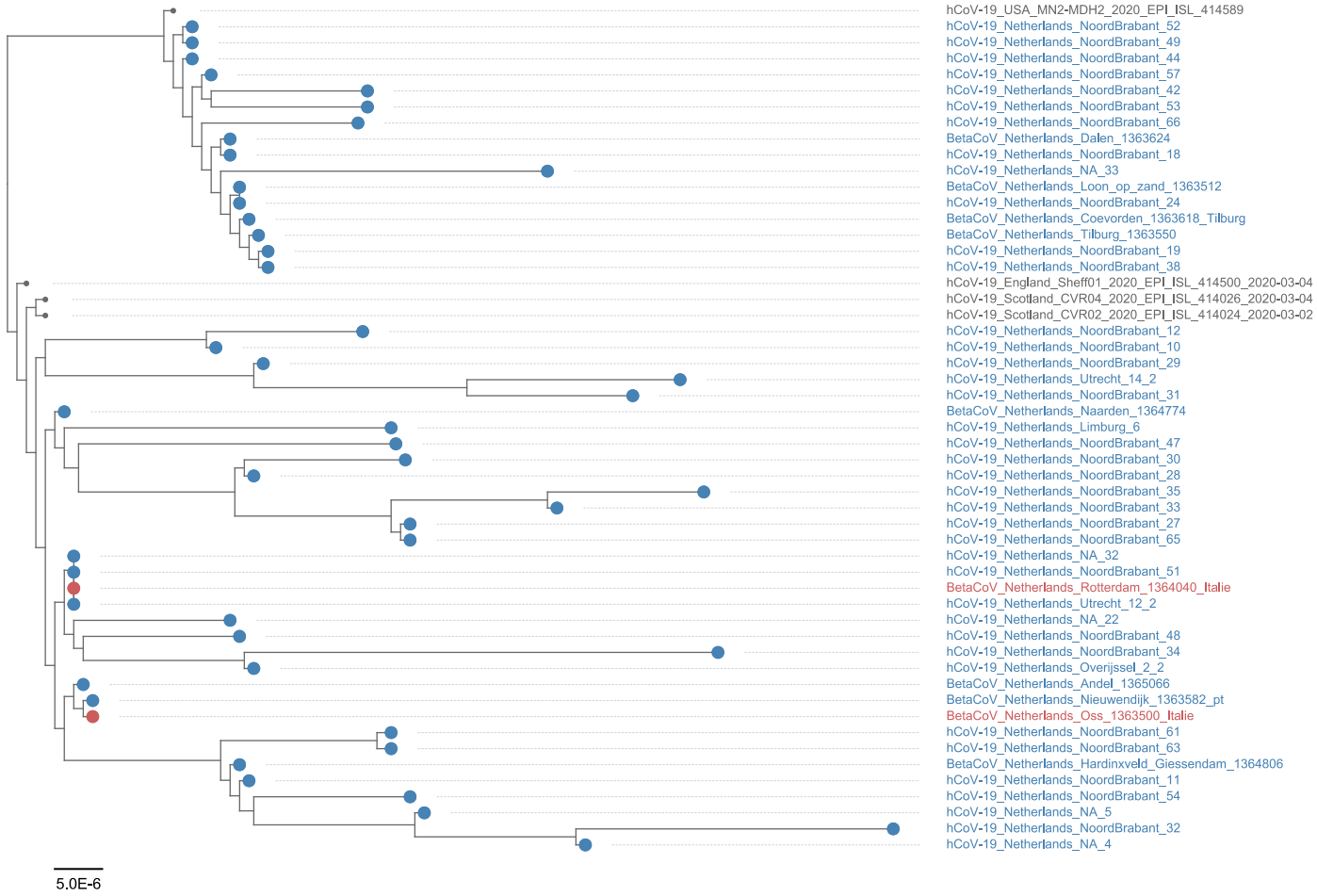
The first SARS-CoV-2 infection in the Netherlands was confirmed on February 27th and an additional case one day later. The genomes of these first two positive samples were generated and analyzed by the February 29th.



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

Oude Munnink et al., Nature Medicine (2020)

Systematic sequencing during first phase of the pandemic



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.

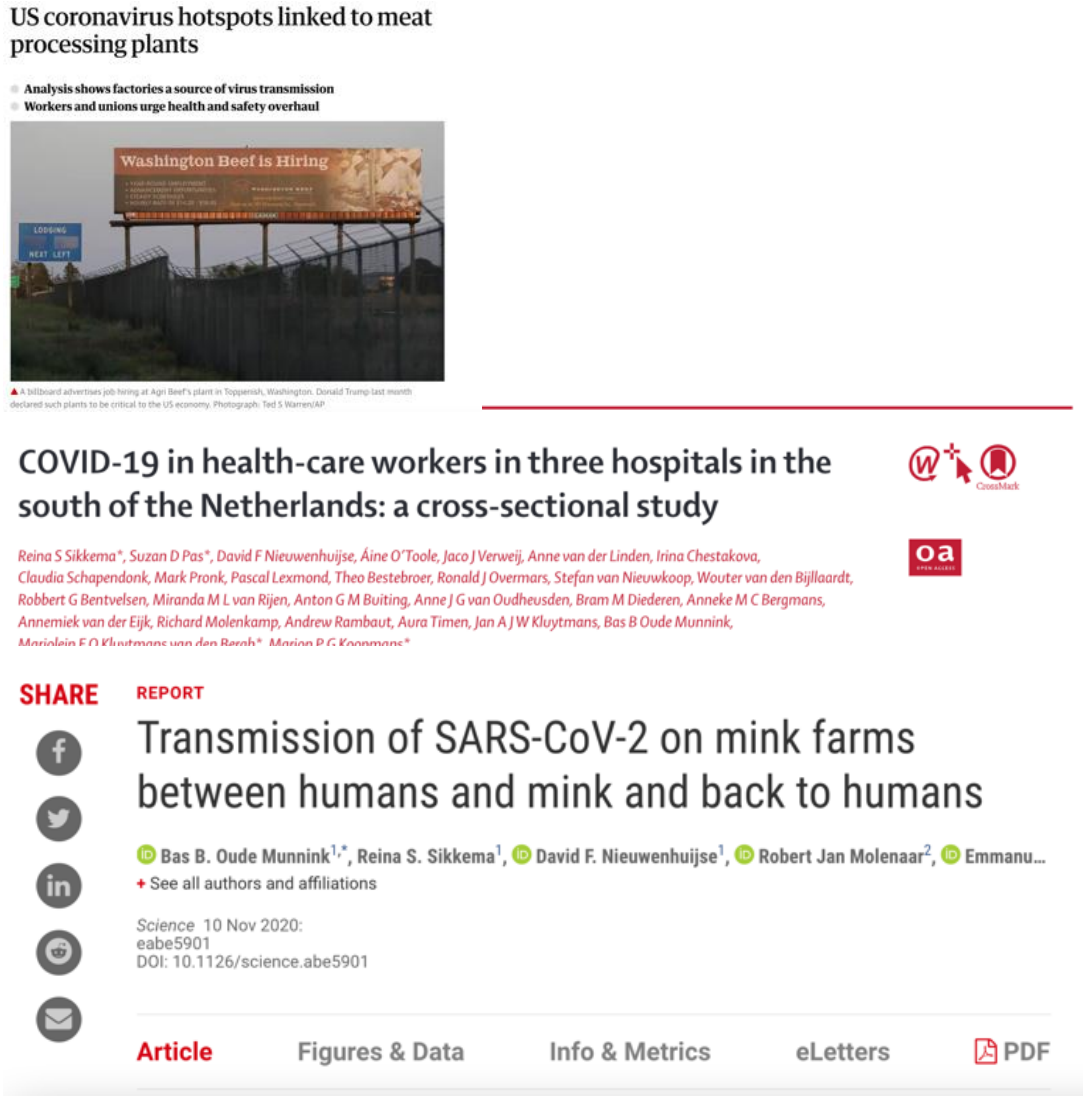
WGS sequencing during the rest of the pandemic

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: Ted S Warren/AP

COVID-19 in health-care workers in three hospitals in the south of the Netherlands: a cross-sectional study

Reina S Sikkema*, Suzan D Pas*, David F Nieuwenhuijse, Áine O'Toole, Jaco J Verweij, Anne van der Linden, Irina Chestakova, Claudia Schapendonk, Mark Pronk, Pascal Lexmond, Theo Bestebroer, Ronald J Overmars, Stefan van Nieuwkoop, Wouter van den Bijllaardt, Robbert G Bentvelsen, Miranda M L van Rijen, Anton G M Buiting, Anne J G van Oudheusden, Bram M Diederen, Anneke M C Bergmans, Annemiek van der Eijk, Richard Molenkamp, Andrew Rambaut, Aura Timen, Jan A J W Kluytmans, Bas B Oude Munnink, Marjolein F Q Kluytmans-van den Bergh*, Marion P G Koopmans*

SHARE

REPORT

Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans

Bas B. Oude Munnink[1,*], Reina S. Sikkema[1], David F. Nieuwenhuijse[1], Robert Jan Molenaar[2], Emmanu…

+ See all authors and affiliations

Science 10 Nov 2020:
eabe5901
DOI: 10.1126/science.abe5901

Article | Figures & Data | Info & Metrics | eLetters | PDF

SARS-CoV-2 in mink farms

Increased mortality was reported at mink farms on the 19th and 20th of April 2020

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

Farm 2: 7,500 animals of which 90 (1,2%) died

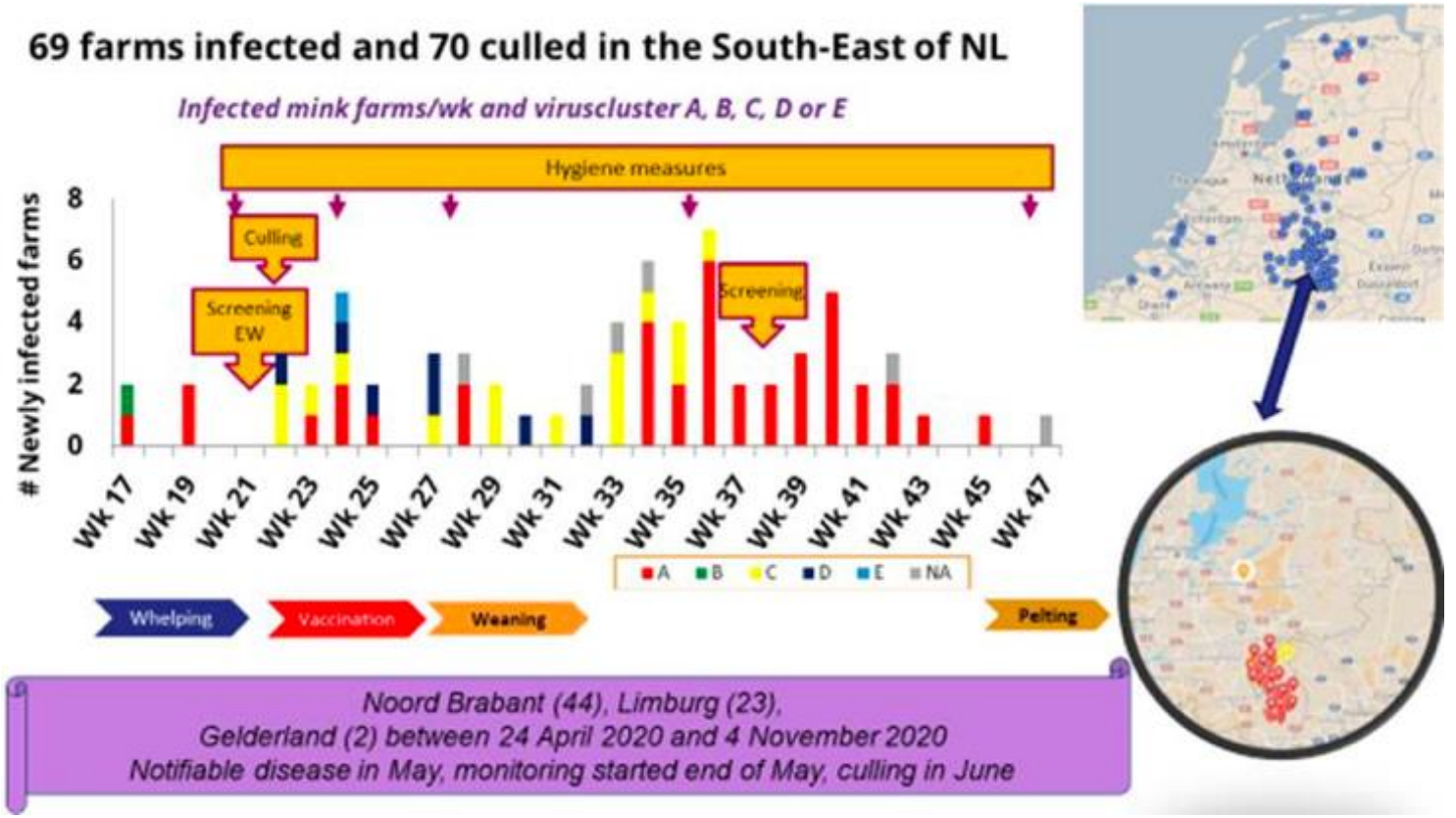
SARS-CoV-2 was identified as causative agent and later more and more mink farms were infected



https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(20)30912-9/fulltext#f10

Outbreak investigation

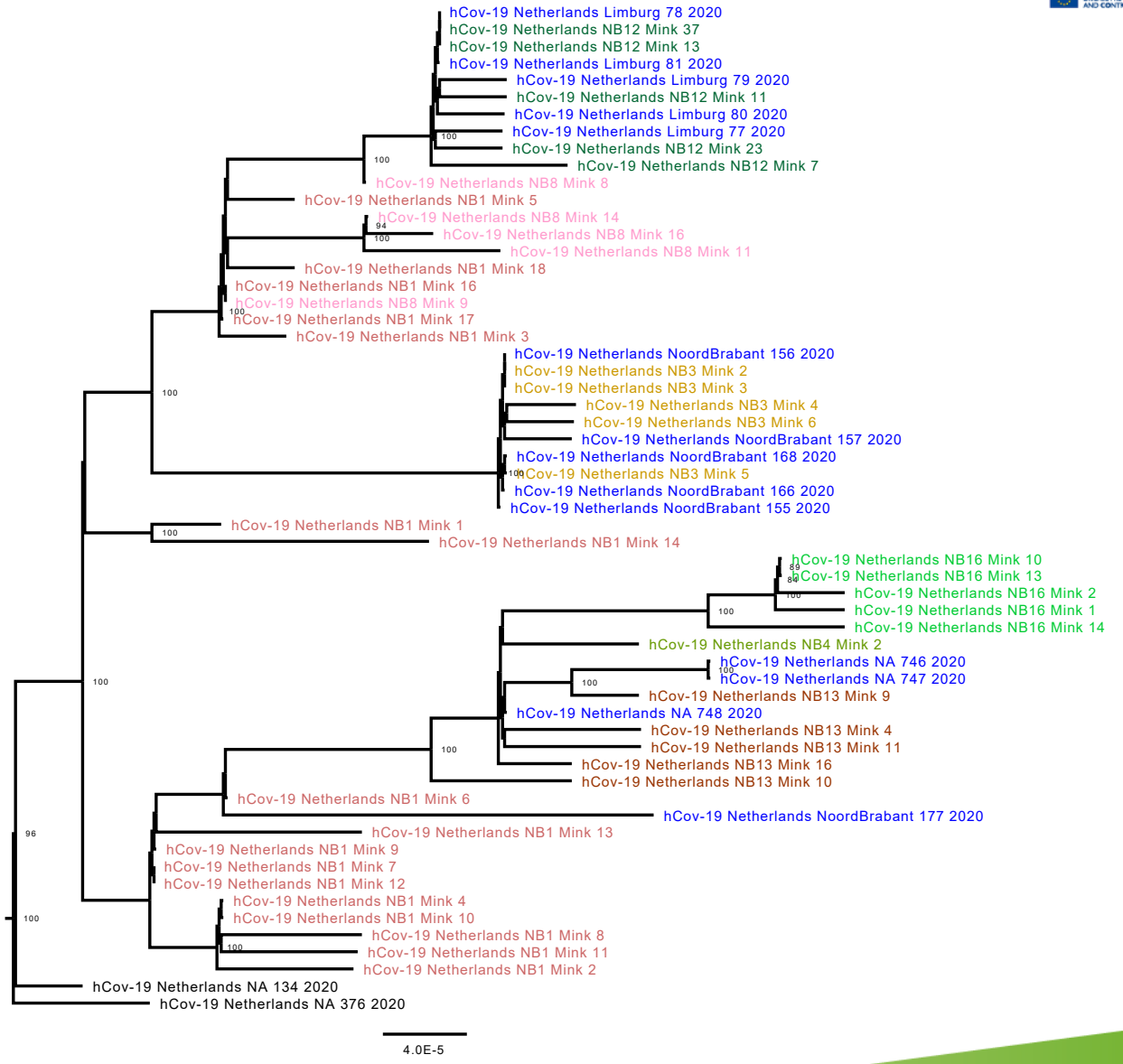
- 69/128 farms were infected in the Netherlands with one of the five "mink" virus clusters
- 57/99 humans on an infected mink farm were infected
- EWS in place to rapidly detect SARS-CoV-2 in mink and if found positive the mink farms will be culled



- **How is the virus spreading, mutating and what is the risk for humans?**

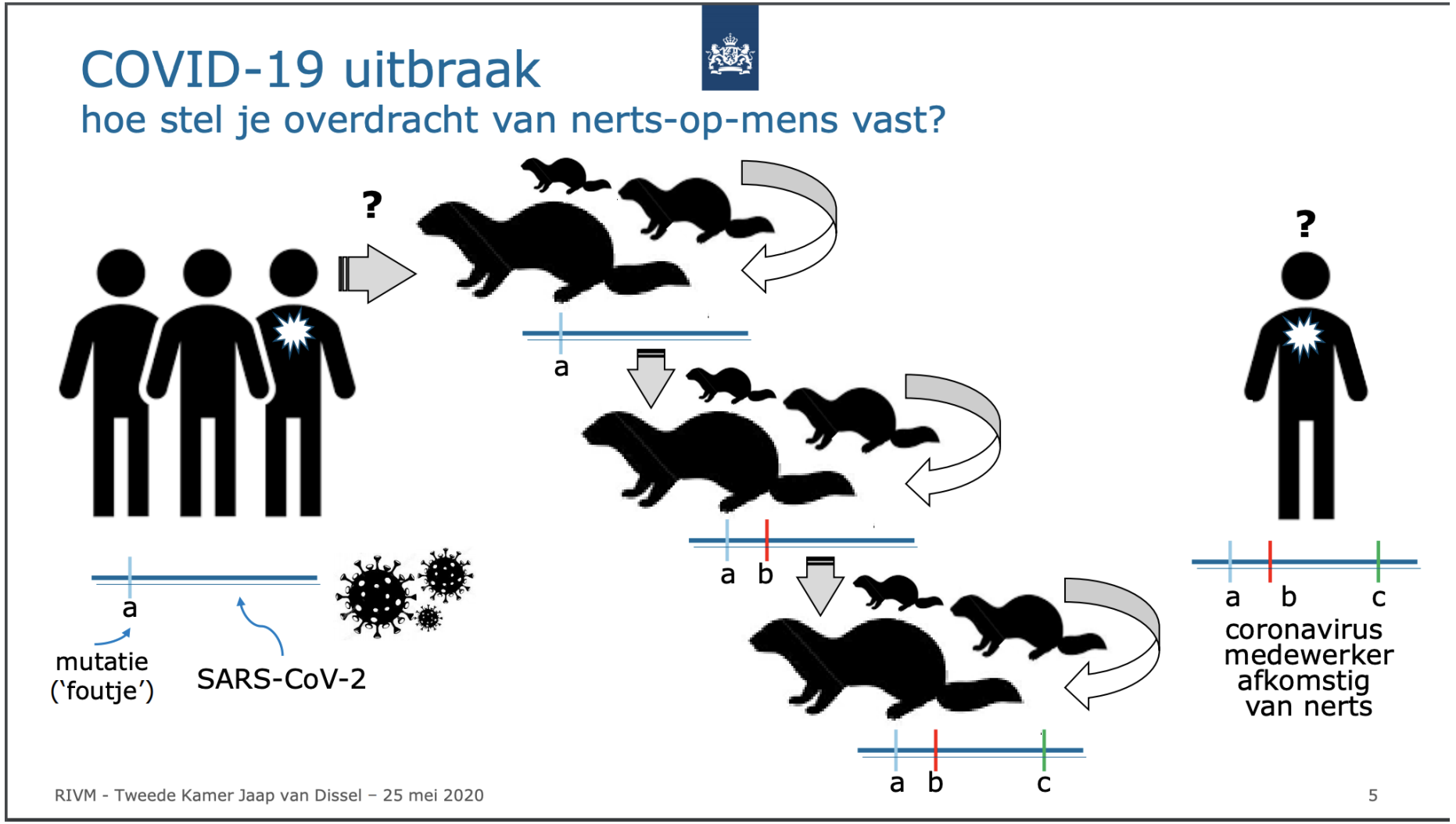
Proof of mink to human infection

- Employees got infected after the detection of SARS-CoV-2 in minks
- Clustering in the phylogenetic tree and timing of the samples
- Probably not all infected at the farms but also family clusters



Oude Munnink et al., Science (2020)

Mutations in the viral genome

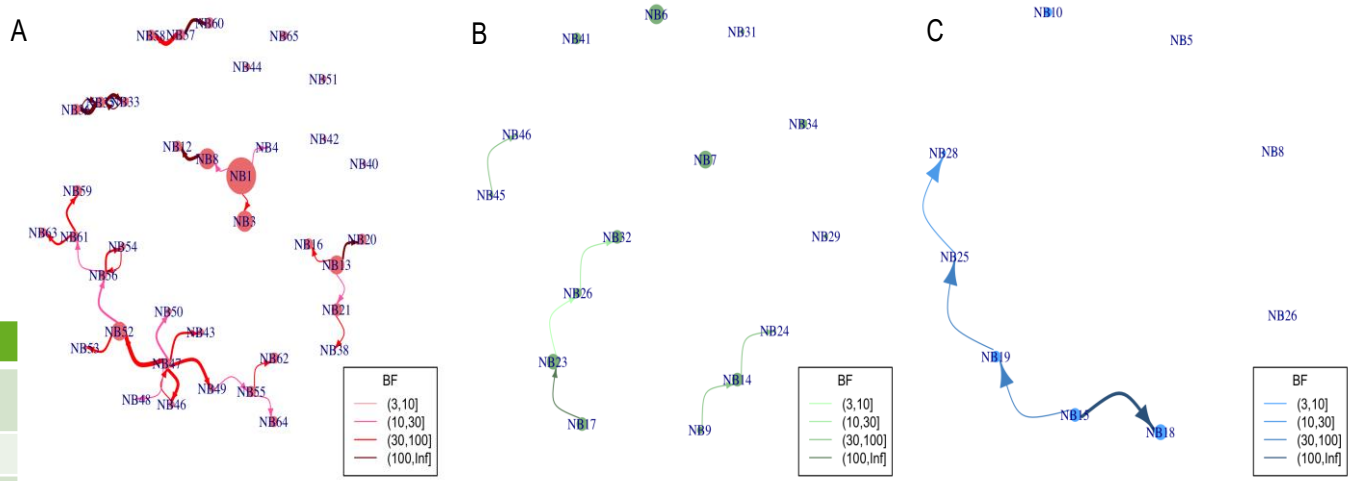


Van Dissel et al, 2de kamer briefing

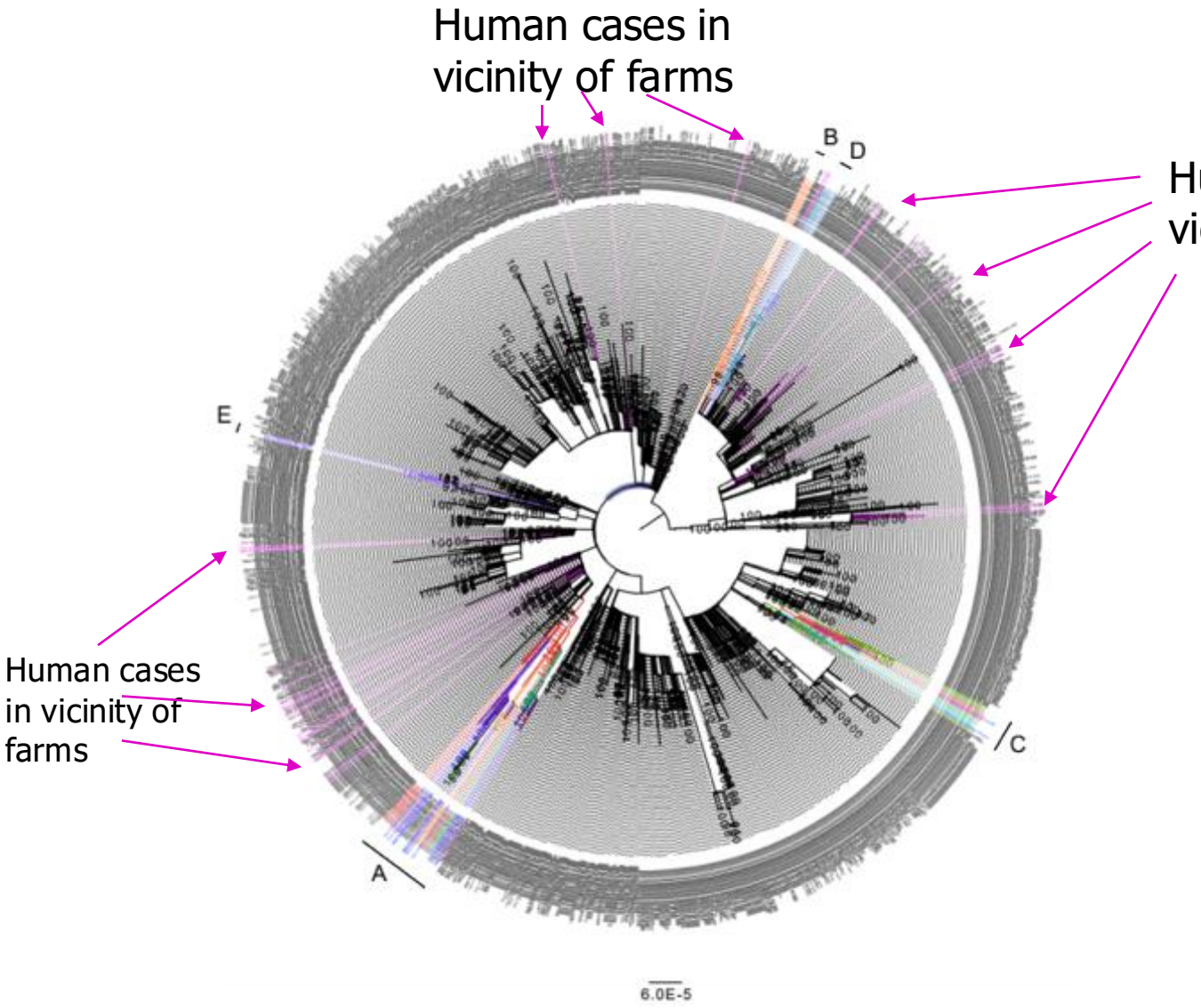
Potential links between the mink farms

- Distance
- Same owners
- Shared personal
- Feed supplier
- Veterinarian

Predictor	Coefficient	95% HPD interval	Inclusion Prob
distance between farms*	-0.67	[-0.90, -0.41]	0.99
if share owners *	1.74	[0.64, 2.81]	0.97
mink density origin	0	[-3.95, 3.72]	0.01
mink density destination	-0.01	[-3.86, 3.85]	0
human density origin	-0.12	[-3.71, 3.91]	0.14
human density destination	0.04	[-3.82, 3.87]	0.01
sample size of the origin	-0.03	[-4.10, 3.78]	0
sample size of the destination	0.02	[-3.96, 3.73]	0
days to cull origin	-0.01	[-3.85, 3.86]	0.01
days to cull destination	0.04	[-4.05, 3.76]	0



No mink related strains in surrounding Dutch population



Limited spill over to the human population observed based on the sequencing samples (n=34) with the same 4-digit postal code and ”kiemsurveillance”

Only once detected in the kiemsurveillance

Hantaviruses

Hantaviruses are primarily zoonotic viruses hosted by rodents without causing illness

60 different orthohantaviruses

38 different reservoir species

30 pathogenic to human

2 main severe clinical syndromes in humans

- HFRS: Hemorrhagic Fever with Renal Syndrome
  - North- and South-America
- HCPS: Hantavirus Cardiopulmonary Syndrome
  - Europe and Asia

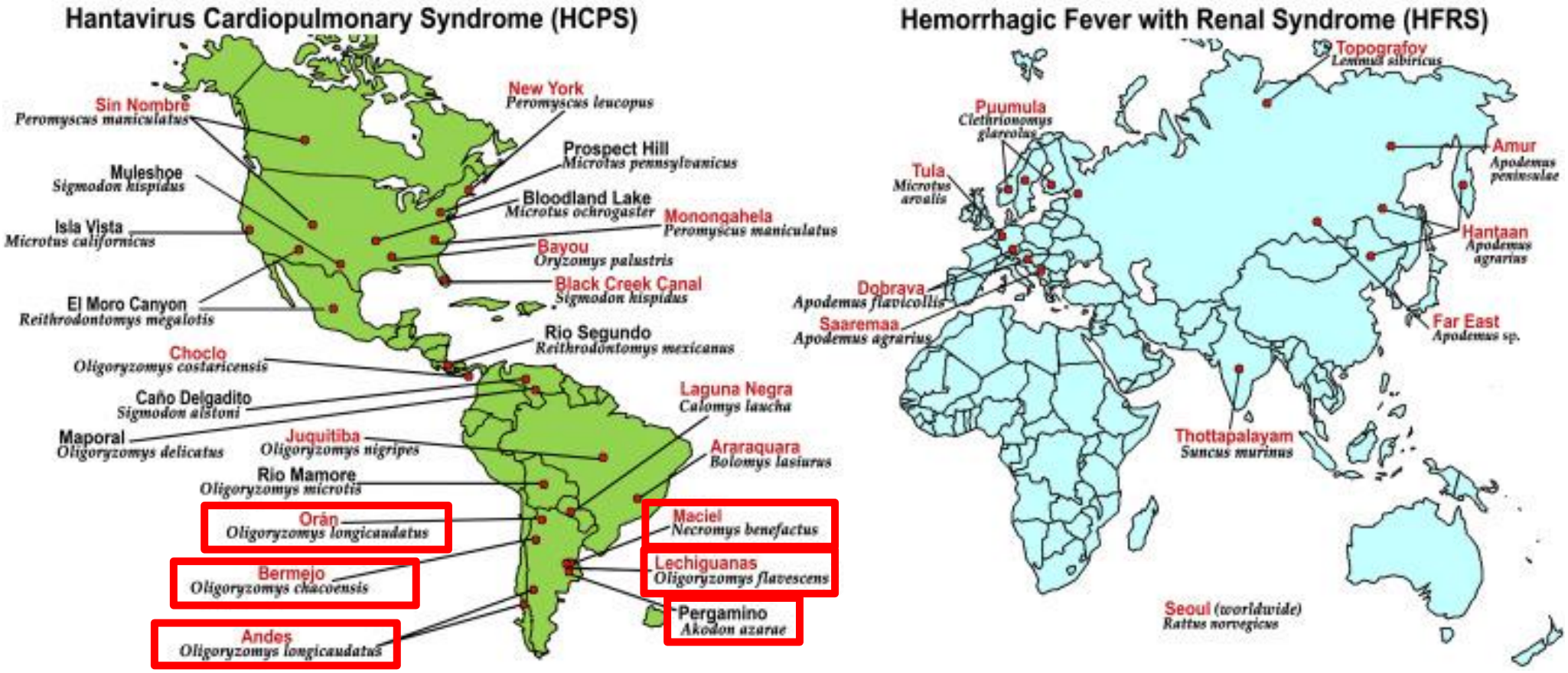
	Rodent host	Syndrome	Case fatality rate (%)	Country
The Americas				
Sin Nombre virus	*Peromyscus maniculatus*	HCPS	35%	USA, Canada
Andes virus	*Oligoryzomys longicaudatus*	HCPS	21·4–35·9%	Argentina, Chile
Araraquara virus	*Bolomys lasiurus*	HCPS	44·5%	Brazil
Choclo virus	*Oligoryzomys fulvescens*	Fever, HCPS	12–15%	Panama
Laguna Negra virus	*Calomys laucha*, *Calomys callosum*	Fever, HCPS	11·7%	Paraguay, Argentina, Bolivia, Peru
Juquitiba virus	*Oligoryzomys nigripes*	HCPS	32·5%	Brazil
Europe and Asia				
Hantaan virus	*Apodemus agrarius*	HFRS	1%	China
Puumala virus	*Myodes glareolus*	HFRS (NE)	0·1–0·4%	Finland, Sweden, Belgium, Germany, France, Russia, northeast Europe
Dobrava virus	*Apodemus flavicollis*, *Apodemus ponticus*	HFRS	9·8–12·0%	Balkans, southeast Europe
Seoul virus	*Rattus norvegicus*	HFRS	<1%	Worldwide
Tula virus	*Microtus arvalis*	HFRS	..	Russia, Europe

All information was obtained from several sources.[8,19–28] HCPS=hantavirus cardiopulmonary syndrome. HFRS=haemorrhagic fever with renal syndrome. NE=nephropathia epidemica.

Table: **Common or important hantaviruses causing disease in the Americas, and Europe and Asia**

Vial et al. Hantavirus in humans: a review of clinical aspects and management. Lancet ID 2023

Geographical distribution of hantaviruses



https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/hantavirus

Andes hantavirus – human-to-human transmission

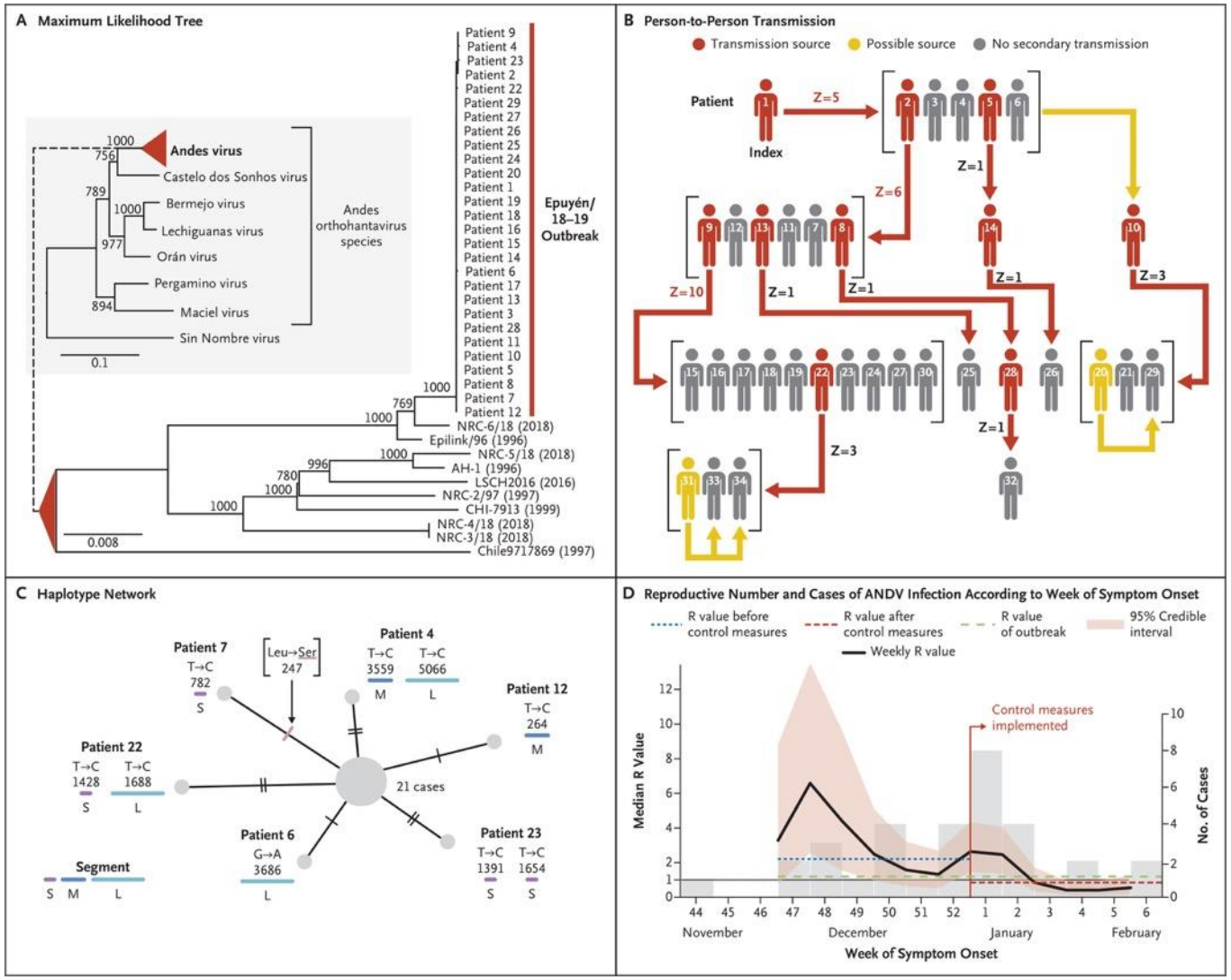
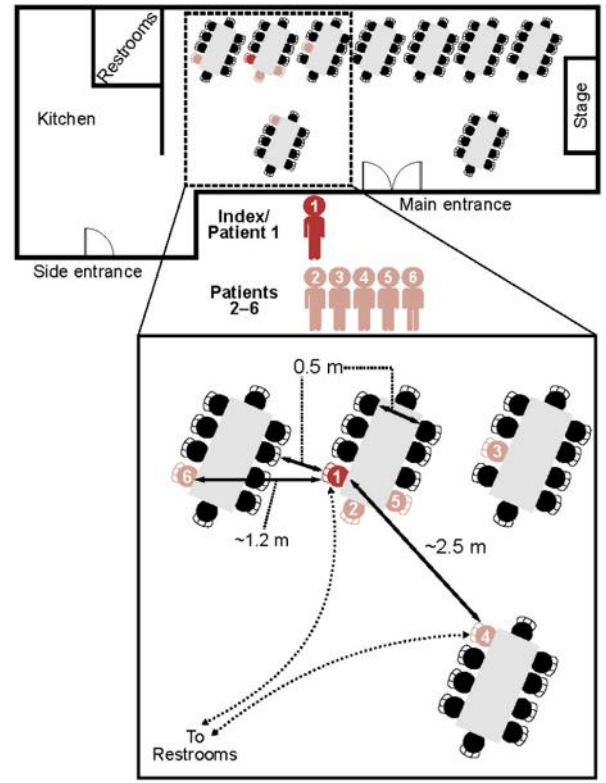


Figure S3. Diagram of first superspreading event at a birthday party



https://www.nejm.org/doi/full/10.1056/NEJMoa2009040

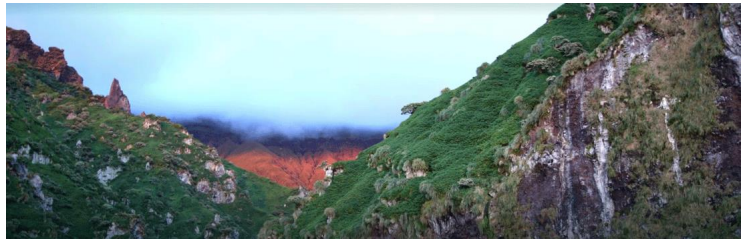
Andes hantavirus outbreak on a cruiseship



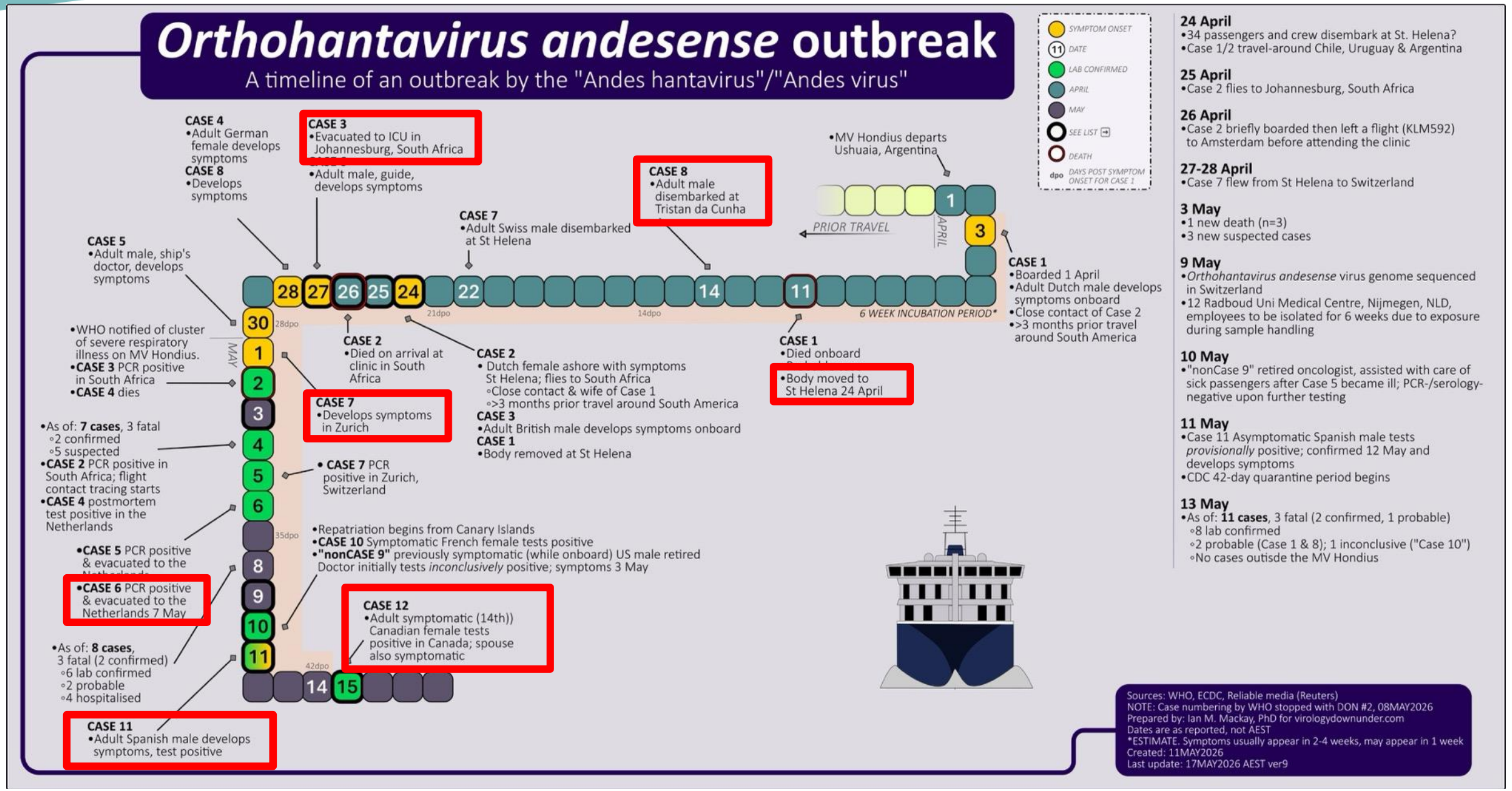






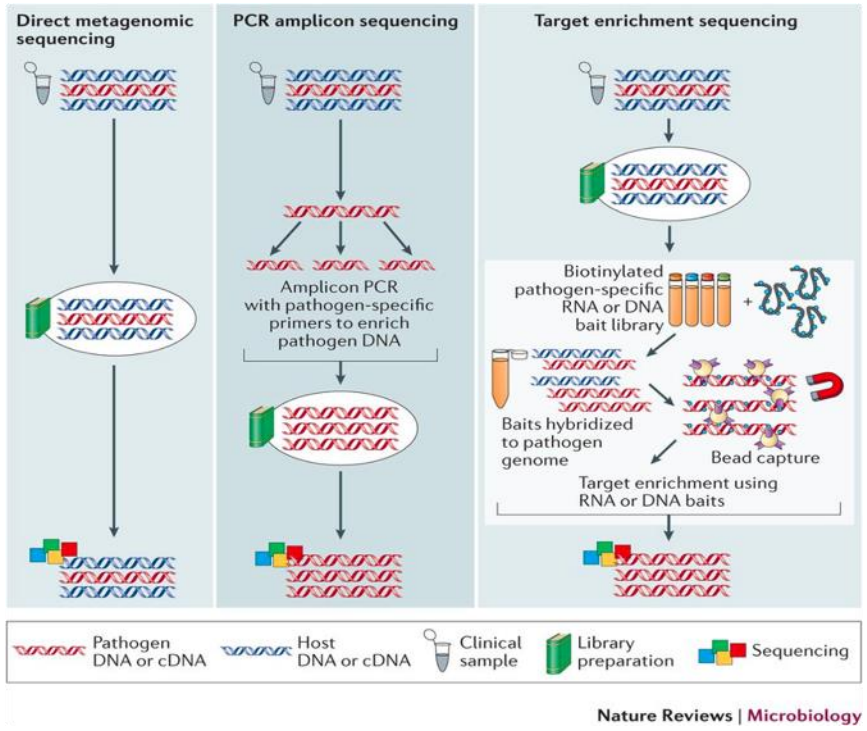


- Luxurious cruise ship to explore remote regions (90 passengers and 60 crew members)



https://x.com/MackayIM

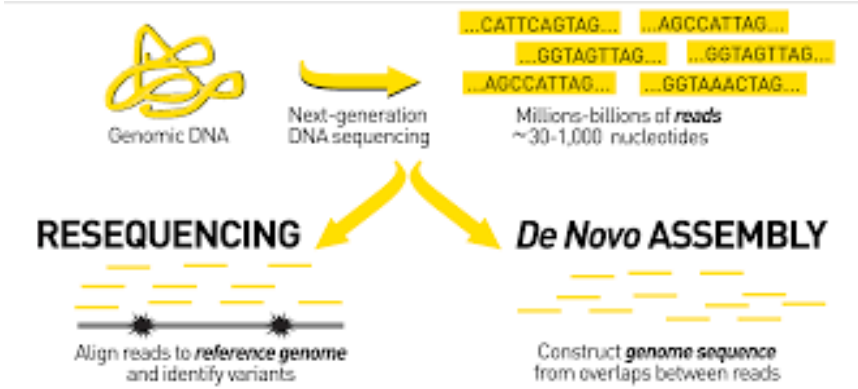
Different countries, different wetlab methods – different drylab methods











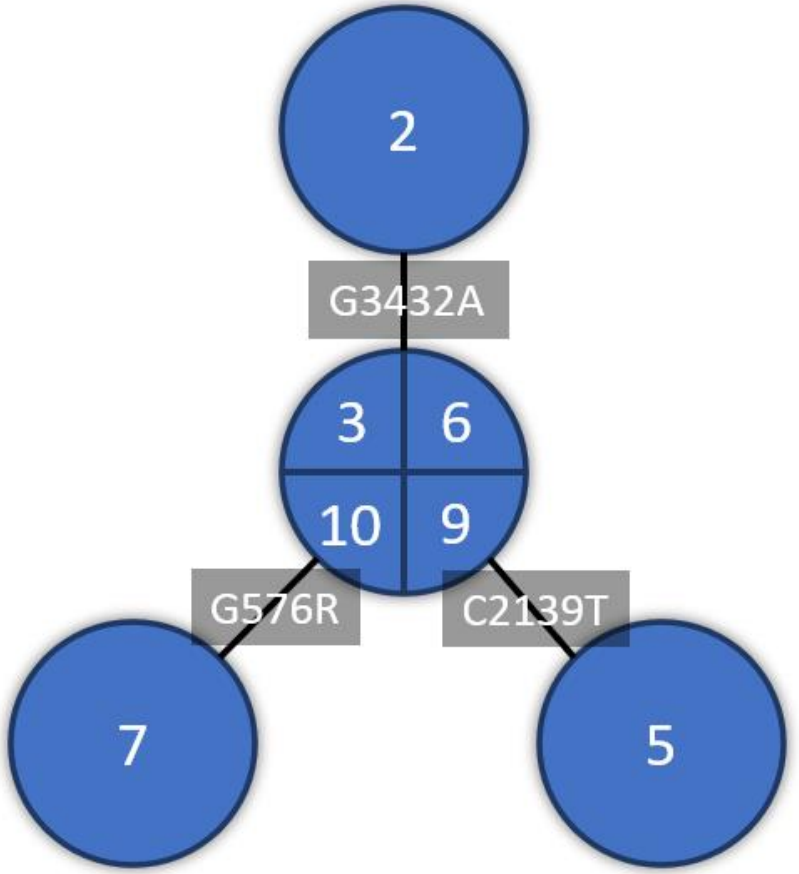
While using different references and read cut-offs for consensus calling

Initial observations

- Several SNPs can be seen already (>10 SNPs)
- Suggesting there might be different introductions on the cruise ship

-> Cross comparison of the data by sharing raw data files within the group of data generators

- Identical M and S segments, 3 unique SNPs in L segment in 3 cases




Preliminary analysis of Orthohantavirus andesense virus sequences from a cruise-ship related cluster, May 2026

Hantavirus

gustavo_palacios 1 5d

May 10

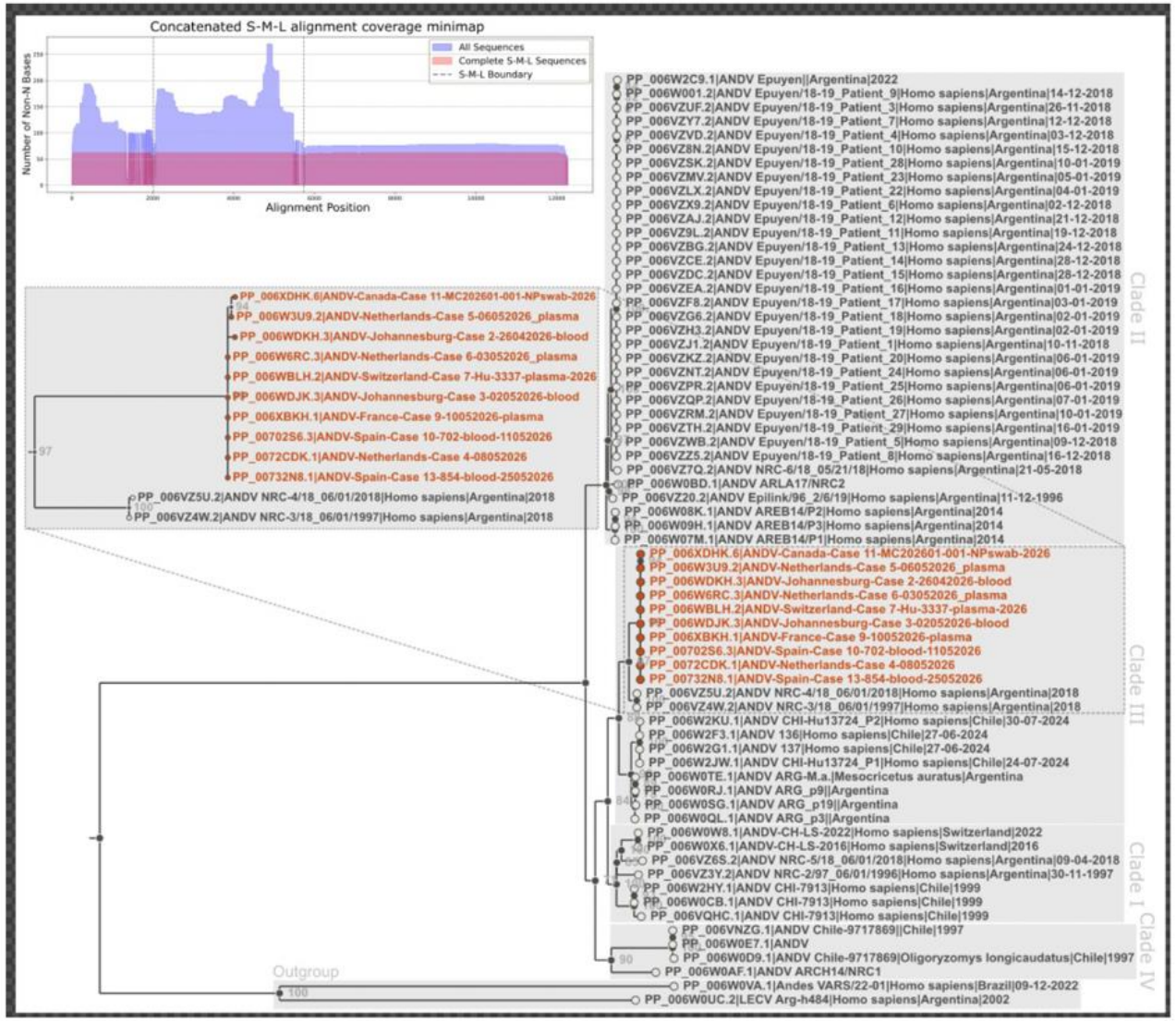
Preliminary analysis of *Orthohantavirus andesense* virus sequences from a cruise-ship related cluster, May 2026.

In May 2026, an Andes virus (ANDV) outbreak occurred aboard the Dutch-flagged cruise ship MV *Hondius*. The ship initially departed from Ushuaia, Argentina, on 1 April 2026 for a transatlantic voyage via remote South Atlantic islands. The index case (case 1) reportedly developed fever,

1 / 1

May 10

Phylogeny



- The overall high level of genetic similarity suggests that the outbreak originated from a single zoonotic spillover event (or a limited number of closely related spillover events.)
- Clusters with sequences for which person-to-person transmission has been described before

Oude Munnink et al, Science, 2026 (in press)

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What do you think WGS can be used for during an outbreak response (part 2)?

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In summary

Whole genome sequencing (in combination with the right metadata) can be used to:

- See how many times a virus got introduced in a certain settings or population
- Determine the direction of spread
- Determine the number of zoonotic transmission events (if the data is generated and analysed in a similar fashion)

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