WHAT CAN ANALYSIS FOR VIRUSES IN WASTEWATER CONTRIBUTE WITH IN INFECTION CONTROL?

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Gothenburg is located on the west coast of Sweden and has about 770 000 inhabitans

We investigate the presence and divergence of viruses in wastewater in Gothenburg.

We have performed this investigation in 2013, 2017 and from 2020-and ongoing during the COVID-19 pandemic

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What does wastewater consist of?

Gryaab

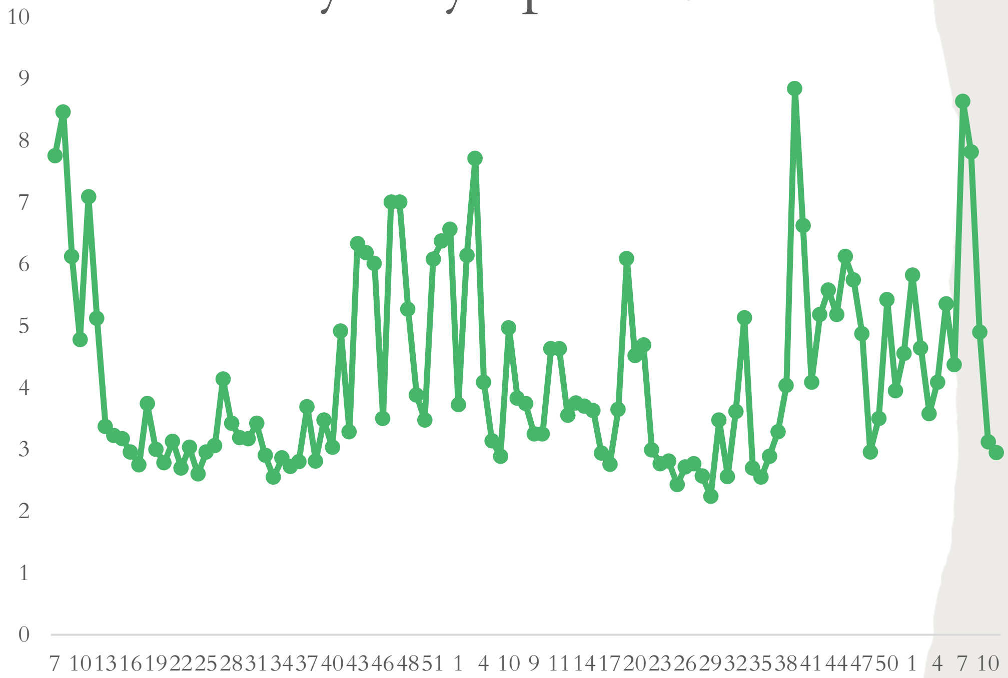
All wastewater from Gothenburg end up at the treatment plant GRYAAB

Rainfall and snow melting is the most variable parameter

We use flow weighted wastewater sampled every day to ensure that approximately the same amount from each of the 770 000 households in Gothenburg is analysed every week

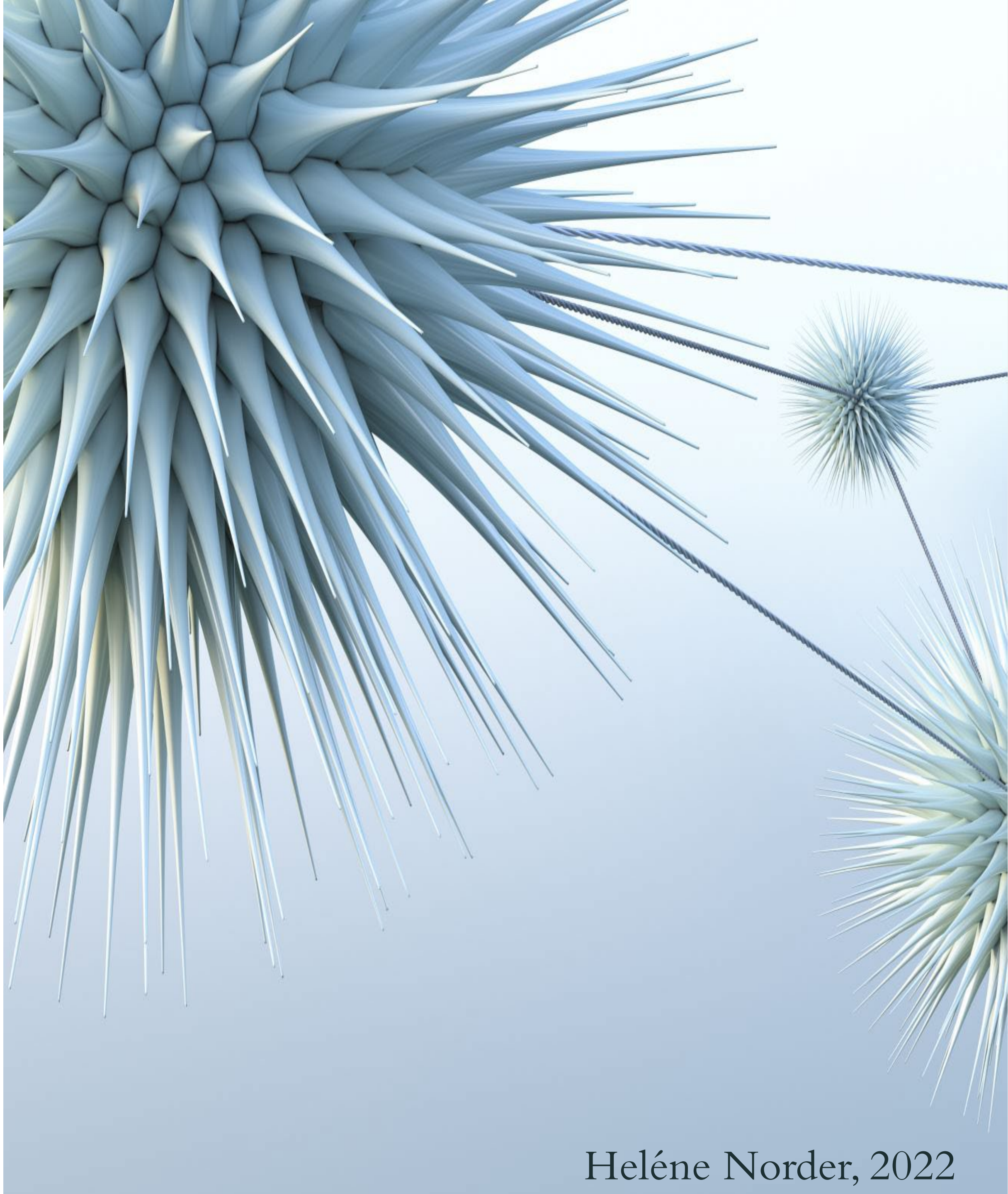
- For every 10,000 L wastewater entering the plant we sample an aliquot 24/7
- These aliquots are pooled to a weekly sample of 1 to 12 L
- Proteins, including viruses, in the sample are concentrated into a final volume of 2 mL
- Nucleic acids are extracted from the concentrate and used for the analysis
- We have performed these studies in 2013, 2017 and from the start of the pandemic in 2020 until now

The weekly wastewater flow may vary up to 10 times



Viruses are ancient, predating the divergence of life more than 3.5 billion years ago. They have co-evolved with their hosts and infect organisms from each of the three domains of life.

All viruses have genes with no cellular homologs which makes them quite easy to identify by molecular methods.



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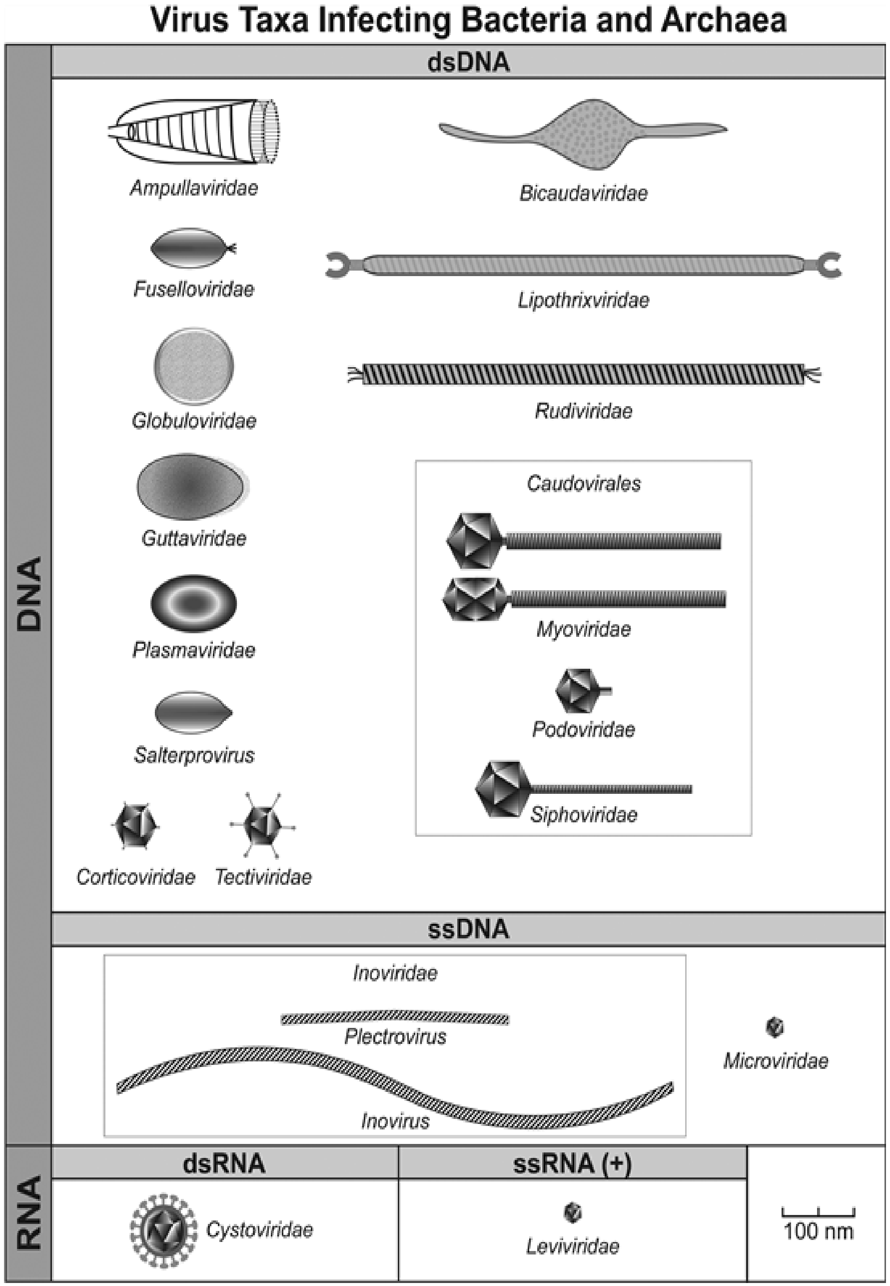
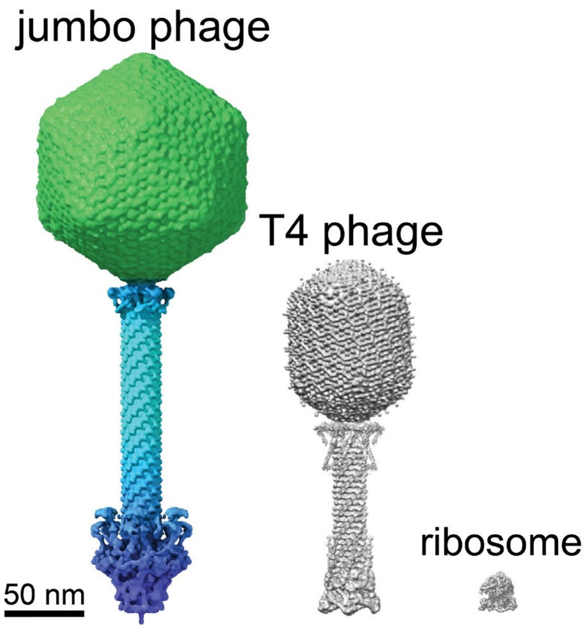


ICTV



- Jumbo phages have large genomes of over 200,000 – 500,000 bp ds DNA.
- They grow a shield around its genetic material, as a nucleus, inside the infected bacterium.
- They possess special genes that reduce their dependence on their hosts.
- They have a wide host range

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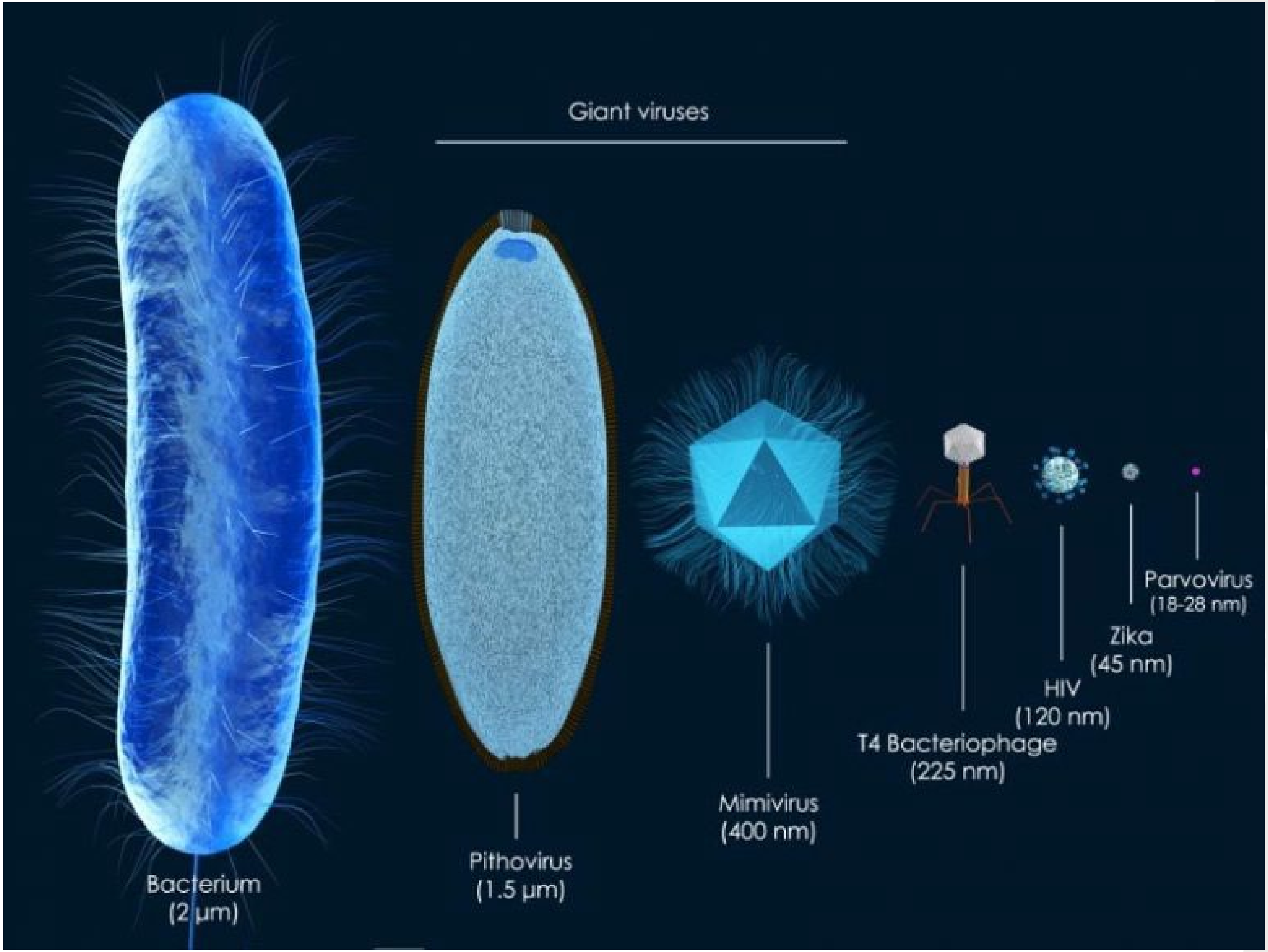
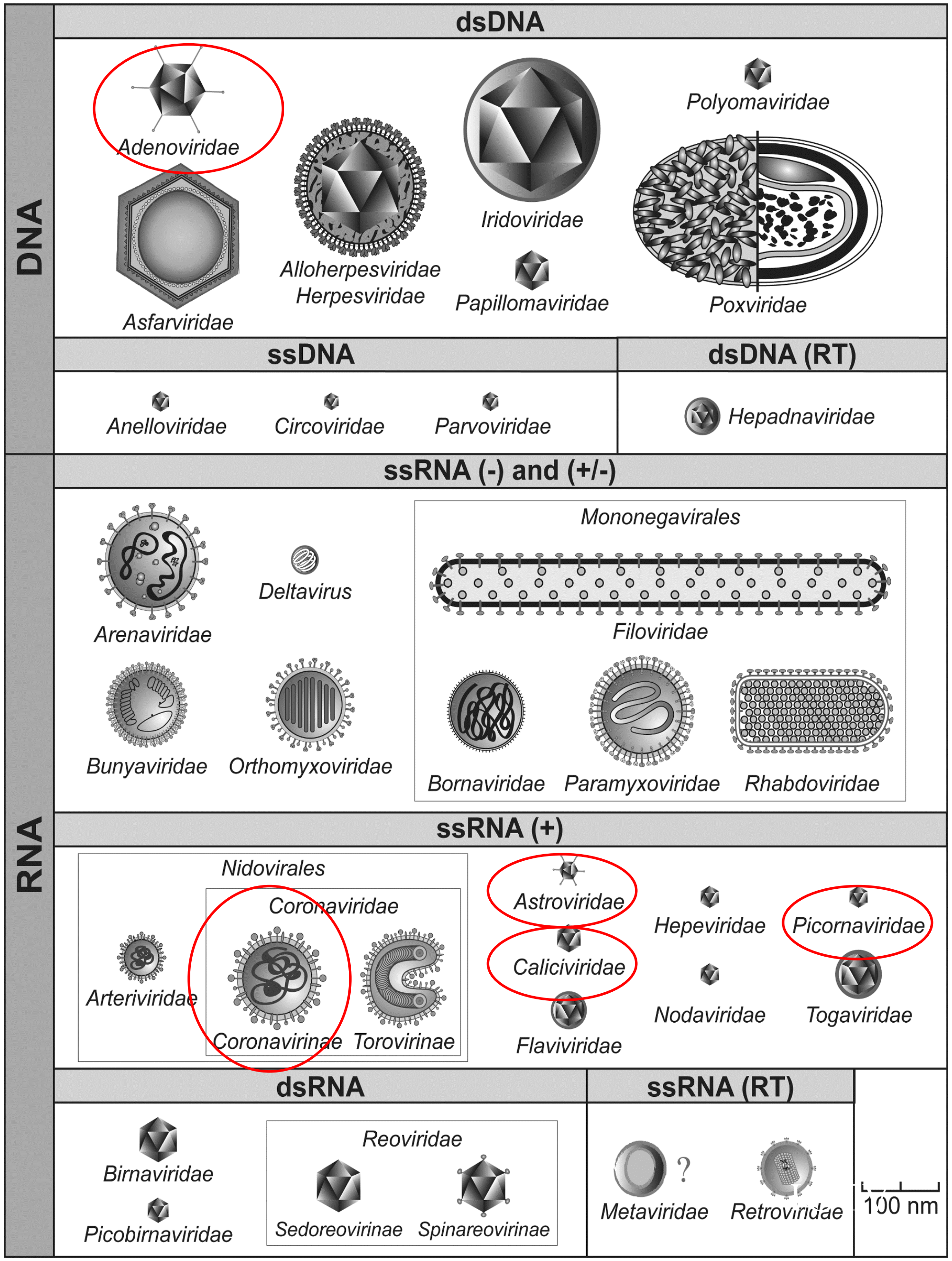

Giant viruses
Bacterium
(2 µm)
Pithovirus
(1.5 µm)
Mimivirus
(400 nm)
T4 Bacteriophage
(225 nm)
HIV
(120 nm)
Zika
(45 nm)
Parvovirus
(18-28 nm)

Virus Taxa Infecting Vertebrates



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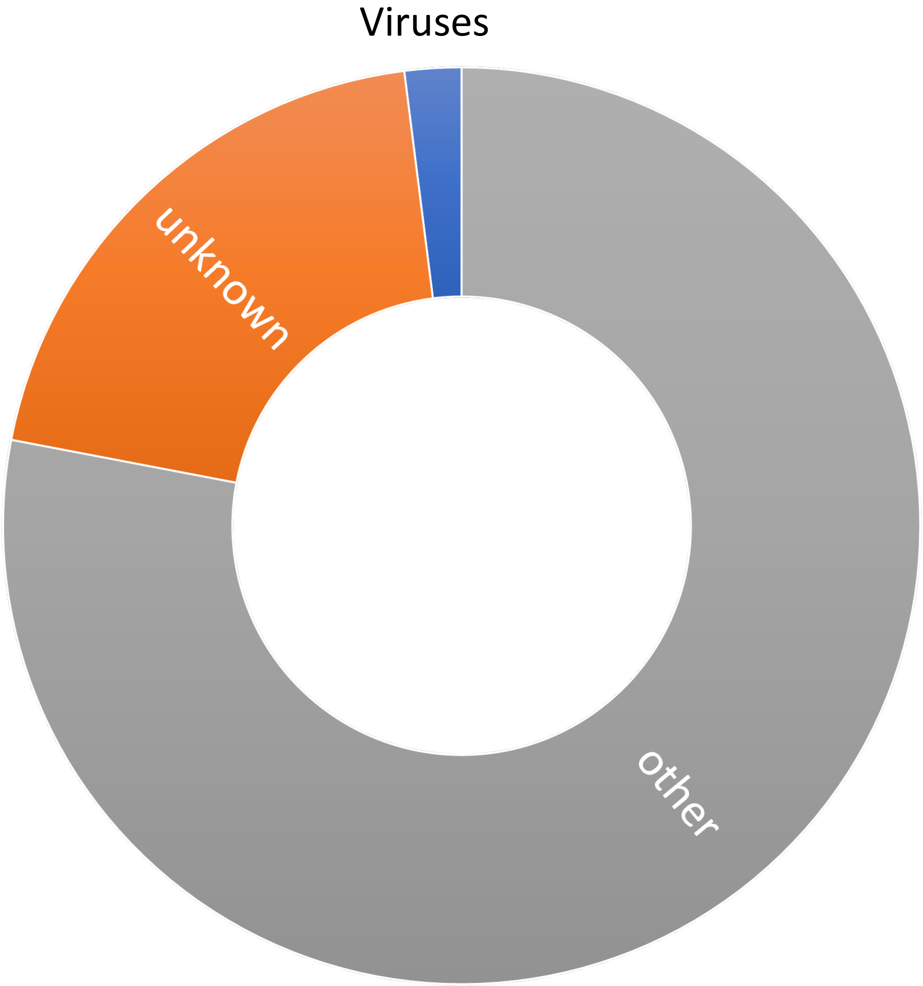
- NGS on the genomic material in wastewater showed that most genomes were derived from bacteria, archea and eukaryotes

- about 33% could not be determined to a known sequenced organism

- only 3-5% of the genomes derived from viruses.



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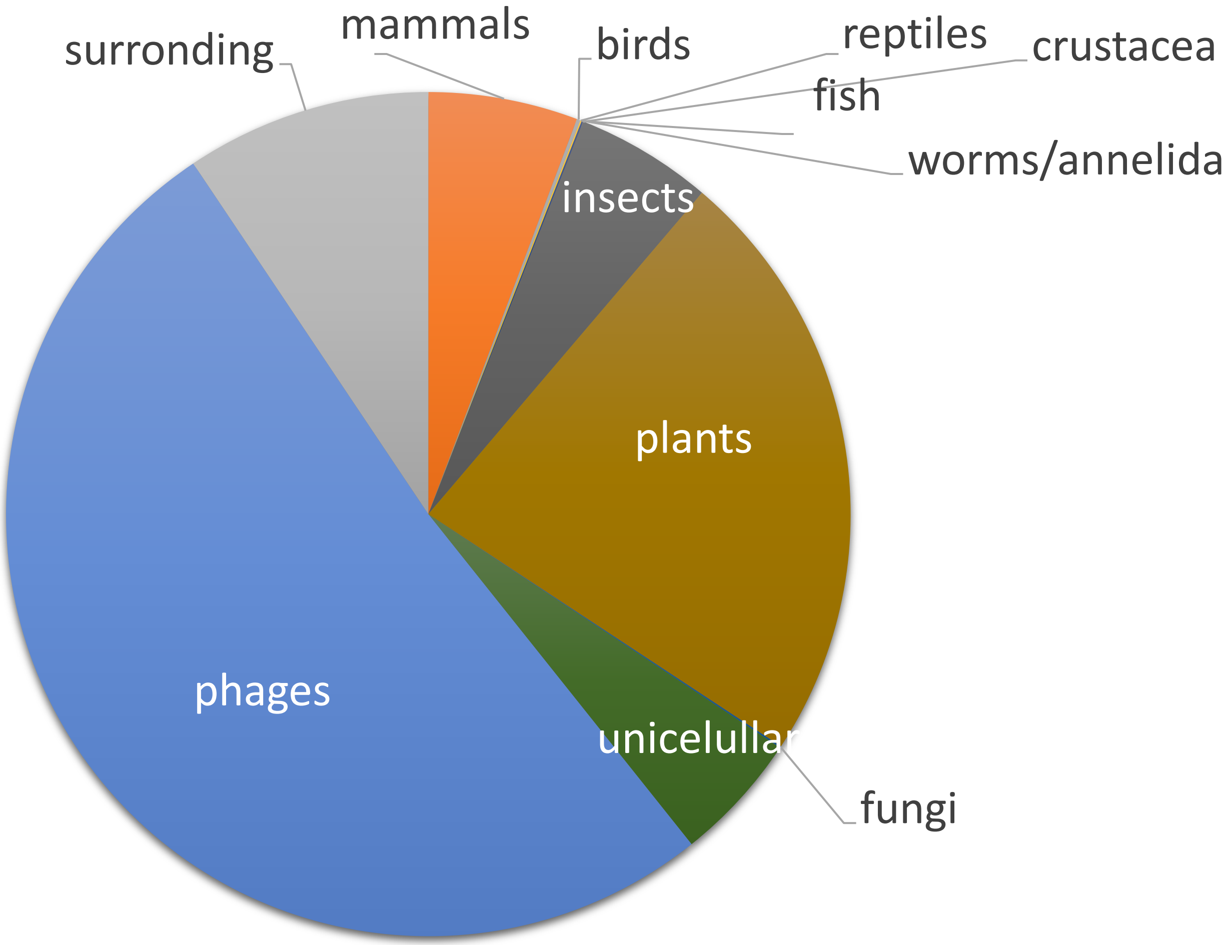
Viruses in wastewater



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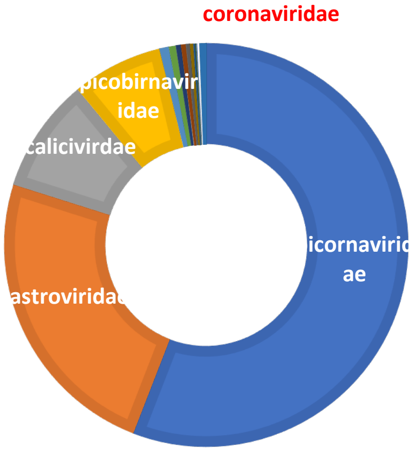
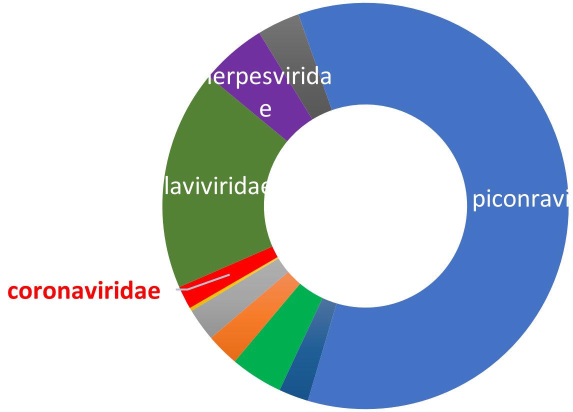
Viruses in wastewater infecting mammals determined by NGS

week 19 April/May 2020



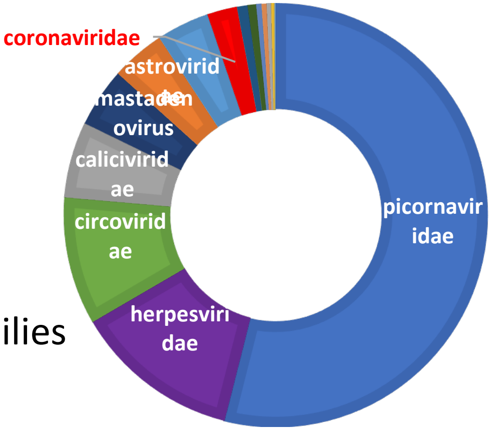
18 virus families

week 26 June 2020



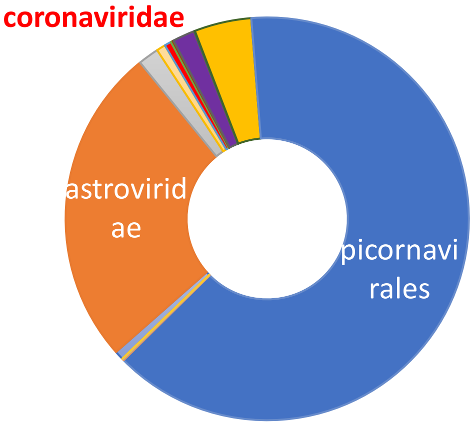
10 virus families

week 44 October 2020



17 virus families

week 50 December 2020



16 virus families

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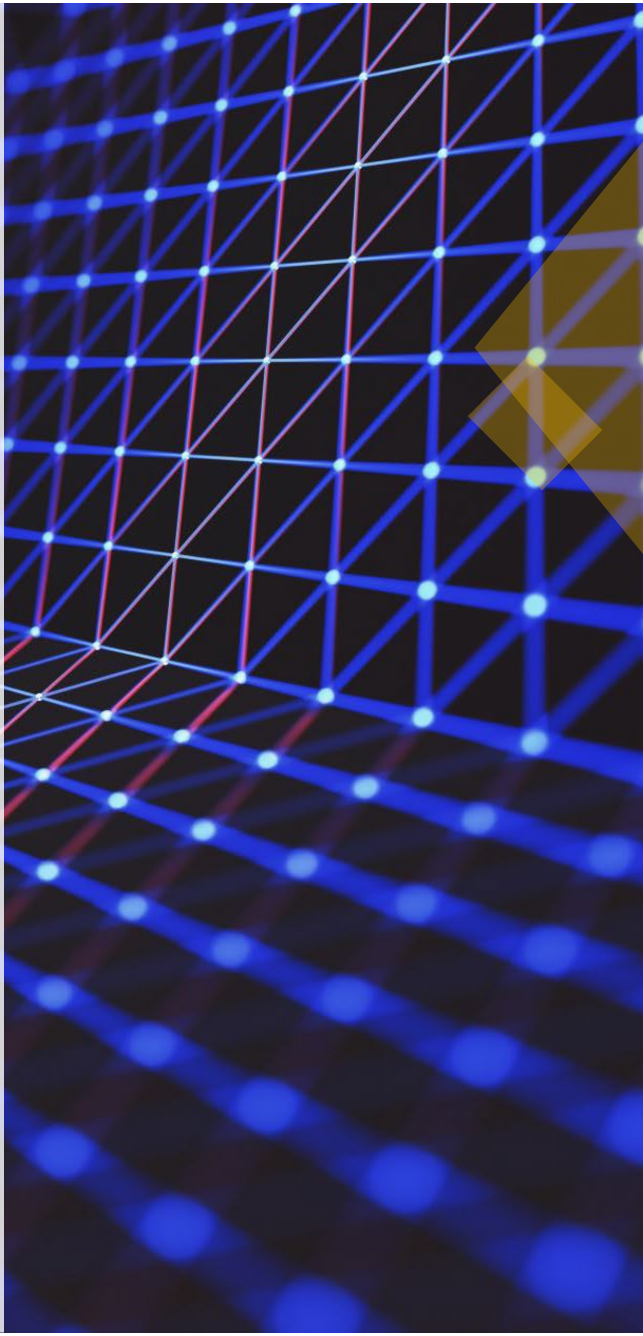
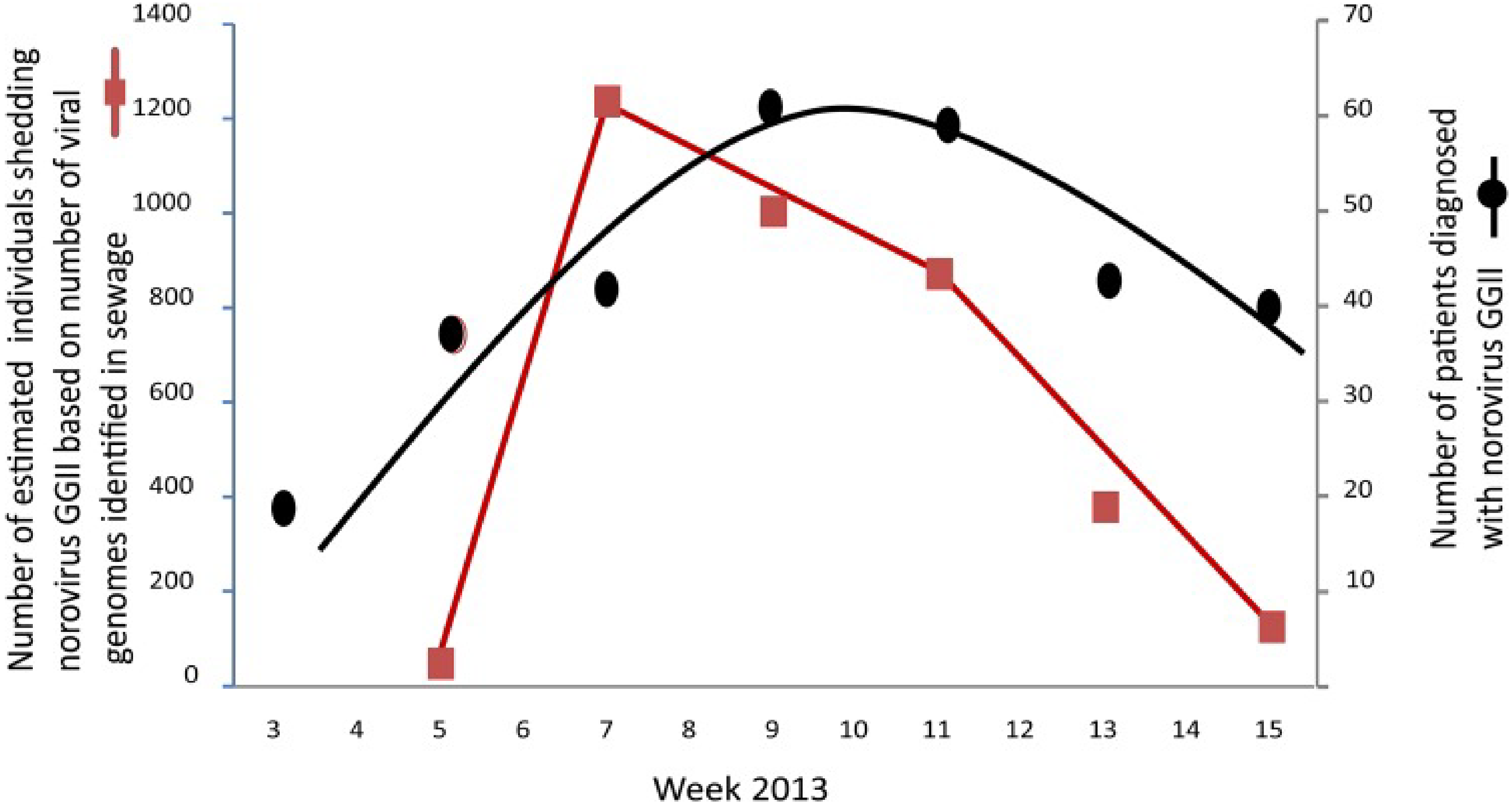
Mammalian viruses we have identified during the pandemic

- 20 genera of picornaviruses infecting many mammals - humans, porcine, bovine caprine, ovine, feline, rodent,
- Calicivirus - human, feline, bovine, porcine
- Coronavirus - human, feline, canine
- Hepatitis E virus- human and rodent
- Bastrovirus- human, porcine, rodent
- Herpesviruses- human, equine, feline
- Boccavirus- human, bovine
- Border disease virus (Flavivirus)- ovine, caprine
- Equine arteritis virus - equine
- Papillomavirus - human- bovine
- Parvovirus - human, feline, canine
- and more…



Manuscript in preparation

Norovirus GII in wastewater and diagnosed patients in 2013

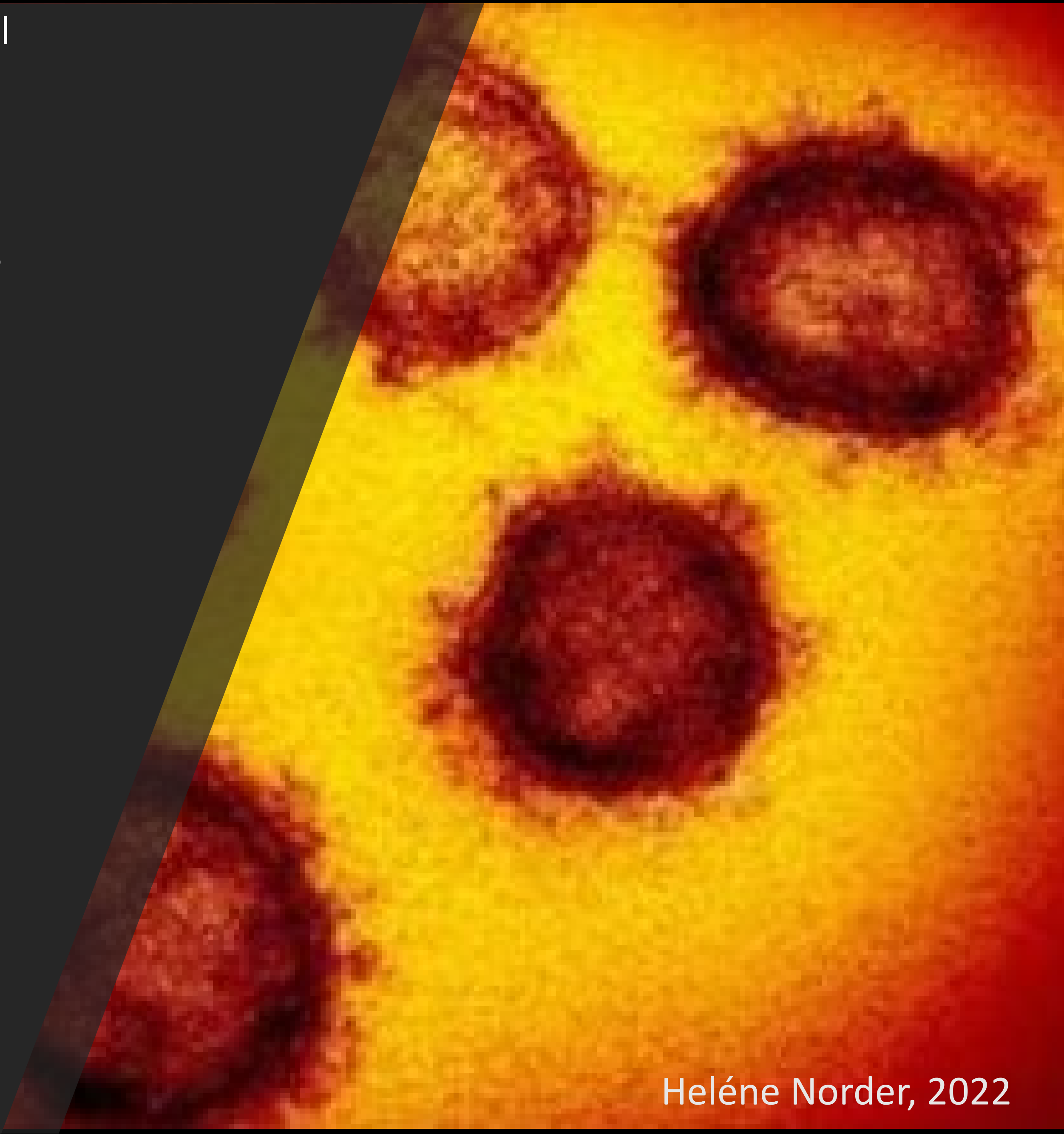


Hellmér M, Paxéus N, Magnius L, Enache L, Arnholm B, Johansson A, Bergström T, Norder H. Detection of pathogenic viruses in sewage provided early warnings of hepatitis A virus and norovirus outbreaks. Appl Environ Microbiol. 2014 80:6771-81.

- Most viruses with fecal/oral transmission are non-enveloped since the hostile environment in the gut and the bile would destroy the outer membrane of the virus.

- The coronavirus SARS-CoV-2 causing the COVID-19 pandemic is enveloped.
- Early 2020 Chinese scientists published that they had detected SARS-CoV-2 in feces from infected patients. Therefore, we wanted to investigate if we could find this virus in wastewater in Sweden.

- We started this investigation before the first case in Gothenburg was diagnosed



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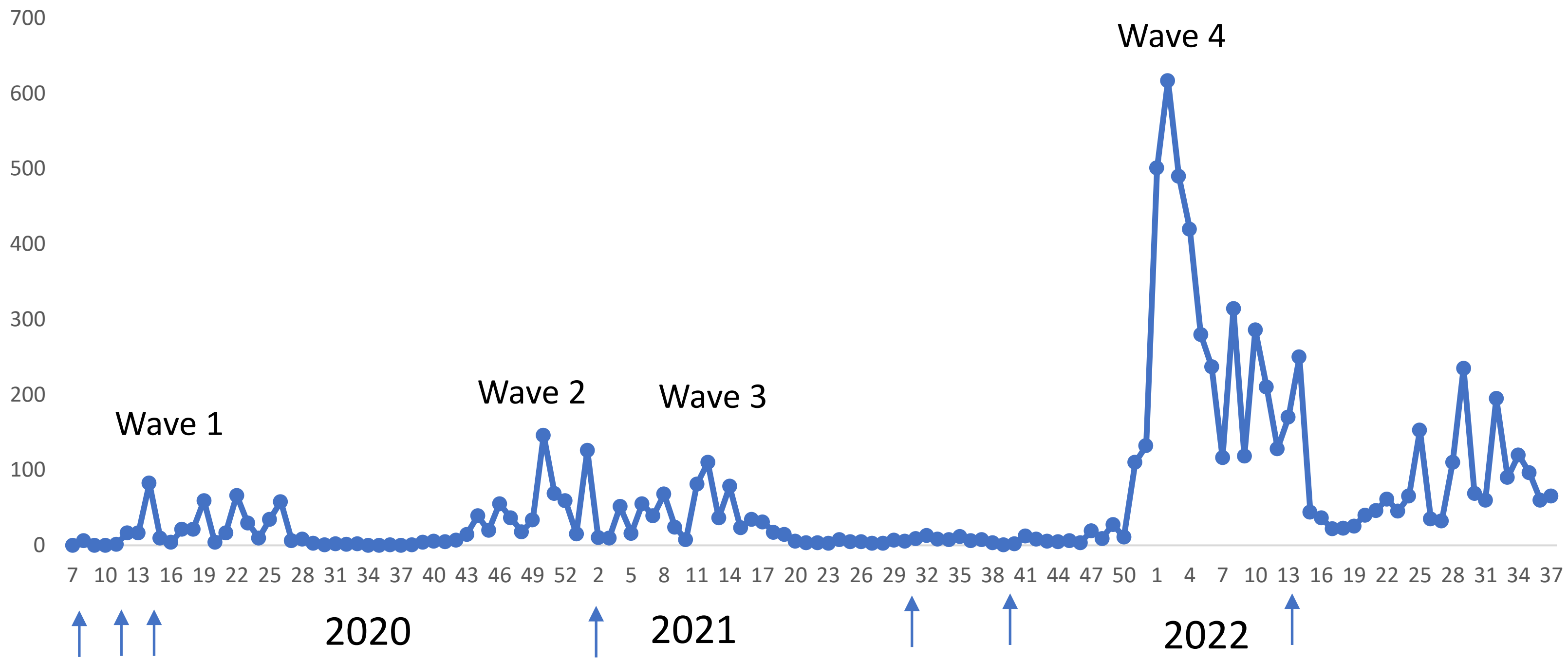
Weekly relative amount of SARS-COV-2 in wastewater from week 6 2020 until week 37 2022



w8w11w14

w8 first case in Gothenburg
w11 Covid-19 is classified as a pandemic
w14 the first restrictions are introduced in Sweden

w1
Vaccination starts in VGR

w32
50% of the population in VGR has received two doses of the vaccin

w39
Most restrictions are removed

w13
COVID-19 is no longer a notifiable disease in Sweden

Saguti F, Magnil E, Enache L, Churqui MP, Johansson A, Lumley D, Davidsson F, Dotevall L, Mattsson A, Trybala E, Lagging M, Lindh M, Gisslén M, Brezicka T, Nyström K, Norder H. Surveillance of wastewater revealed peaks of SARS-CoV-2 preceding those of hospitalized patients with COVID-19. Water Res. 2021 89:116620.

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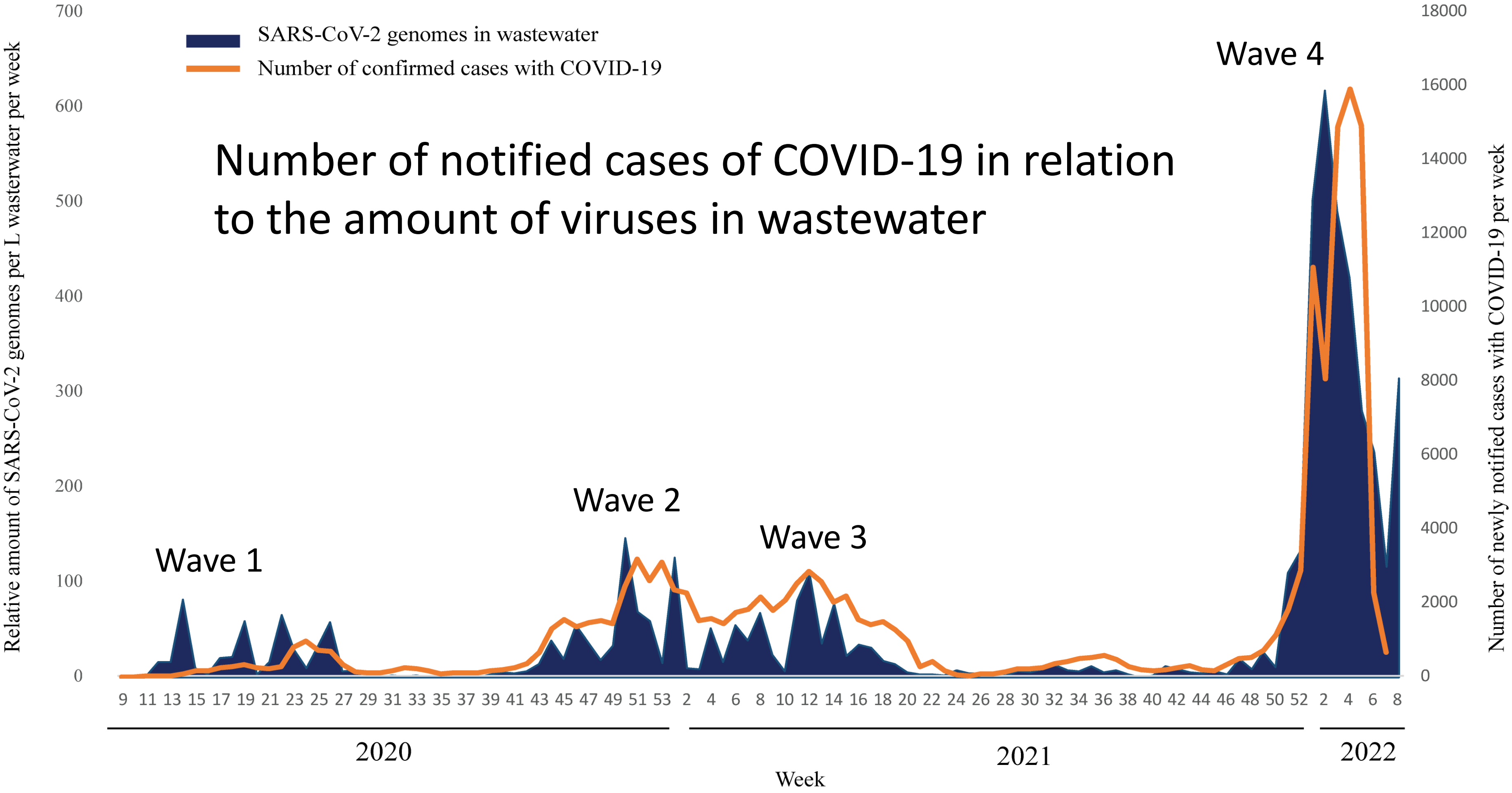
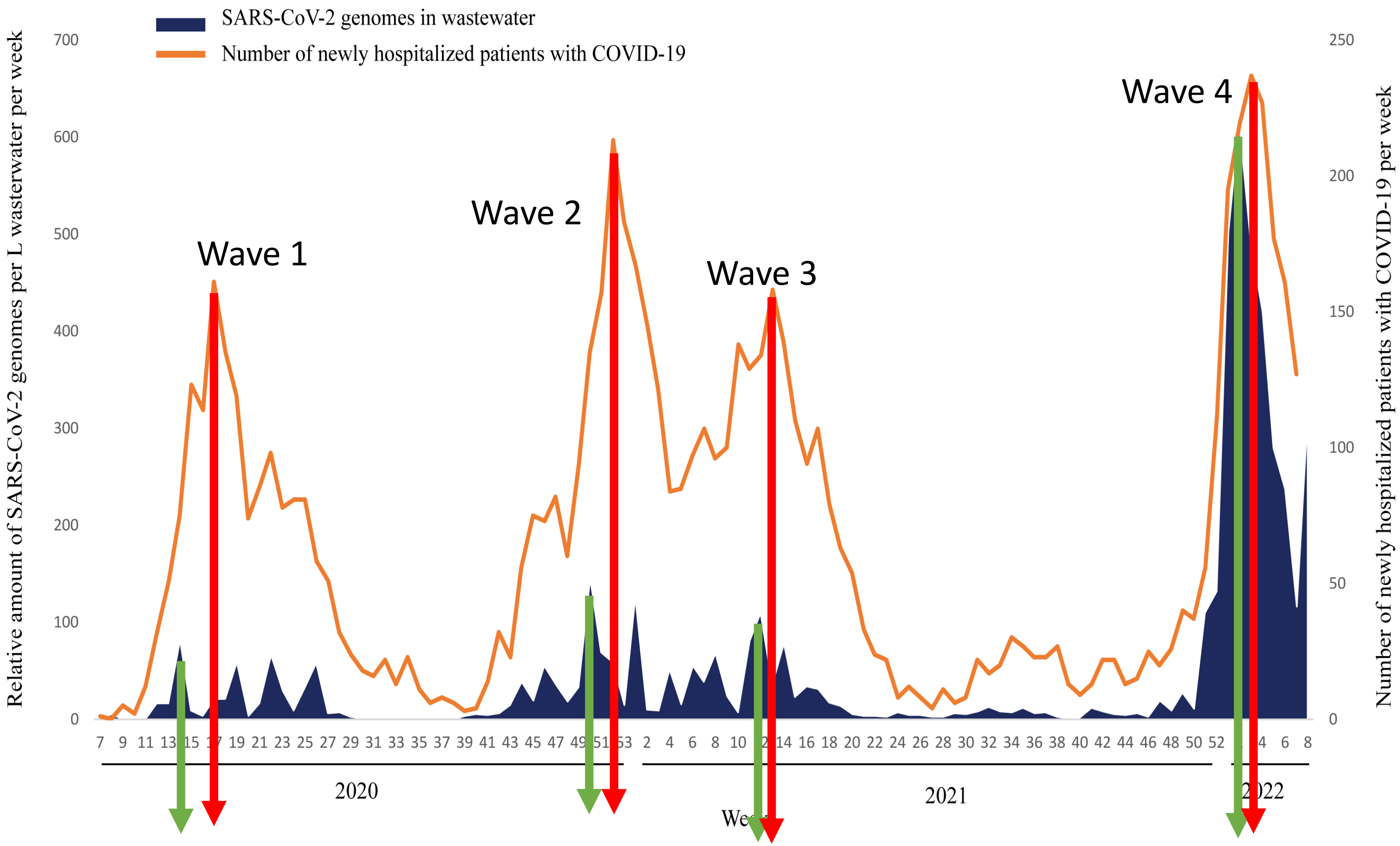
Number of notified cases of COVID-19 in relation to the amount of viruses in wastewater



During the pandemic the recommendation for testing changed

Wang H, Churqui MP, Tunovic T, Enache L, Johansson A, Kärmander A, Nilsson S, Lagging M, Andersson M, Dotevall L, Brezicka T, Nyström K, Norder H. The amount of SARS-CoV-2 RNA in wastewater relates to the development of the pandemic and its burden on the health system. iScience. 2022 25:105000.



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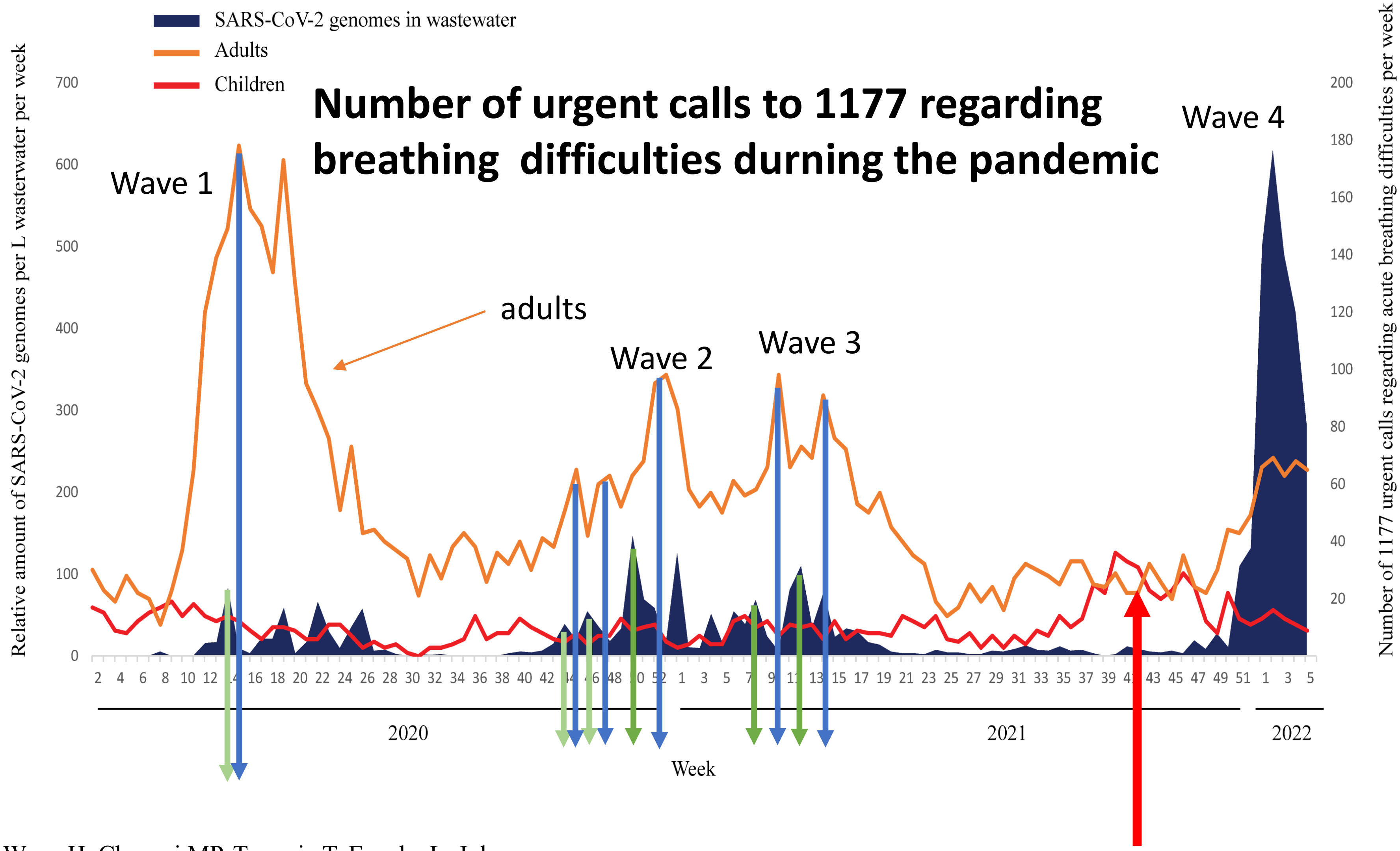
Number of urgent calls to 1177 regarding breathing difficulties durning the pandemic



Respiratory syncytial virus (RSV) outbreak

Wang H, Churqui MP, Tunovic T, Enache L, Johansson A, Kärmander A, Nilsson S, Lagging M, Andersson M, Dotevall L, Brezicka T, Nyström K, Norder H. The amount of SARS-CoV-2 RNA in wastewater relates to the development of the pandemic and its burden on the health system. iScience. 2022 25:105000.

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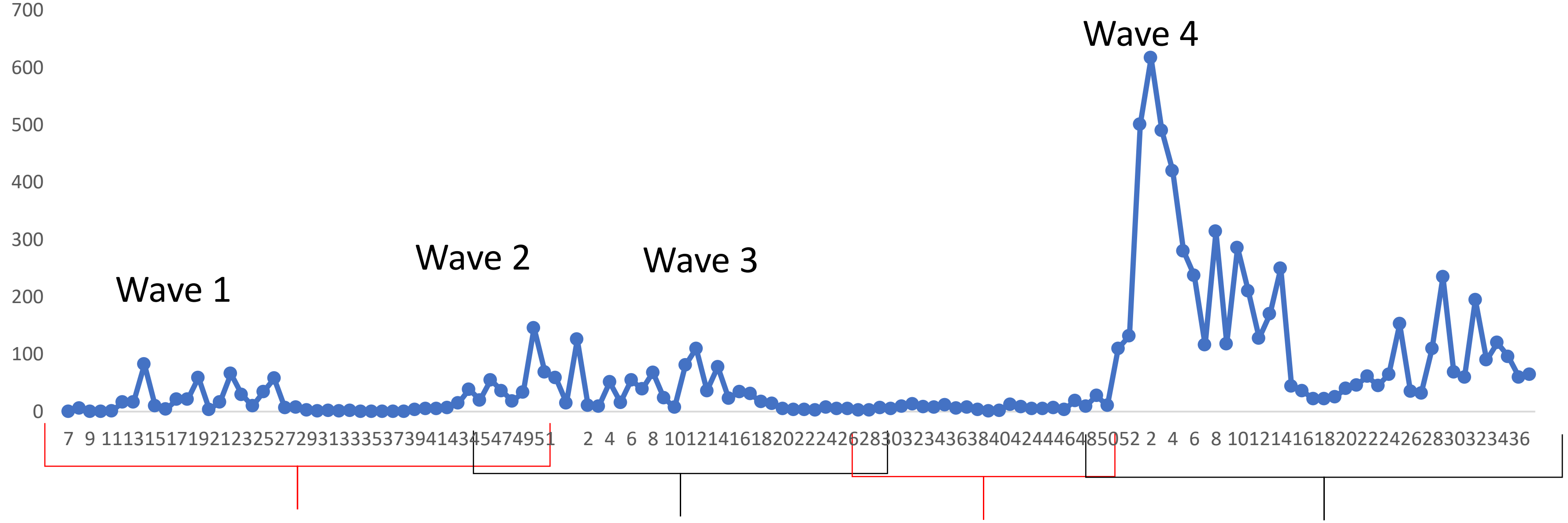
Each wave of SARS-CoV-2 was caused by a new variant which was found in wastewater three to four weeks before it caused a wave

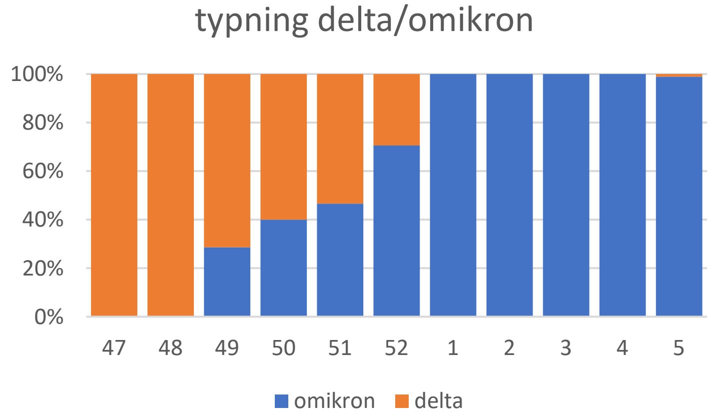


SARS-CoV-2 variants
B.1.1, B.1.177, B.1.160, P.1.16

SARS-CoV-2
alpha variant

SARS-CoV-2
delta variant

SARS-CoV-2
omikron variant



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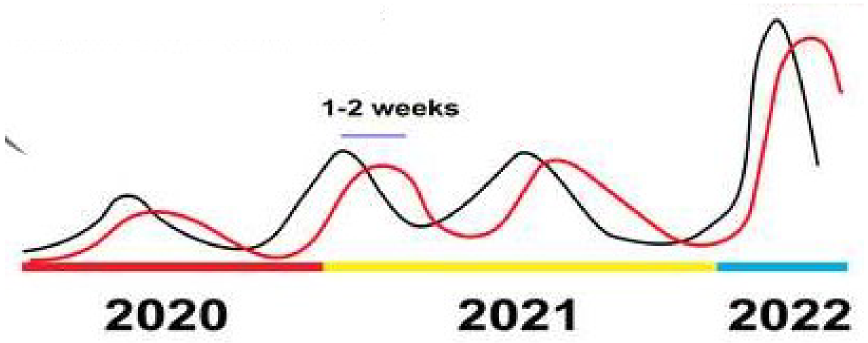
Conclusions

Monitoring of viruses in wastewater identifies

- which viruses are circulating in society, also those not identified by the health system such as HAV, HEV, Aichi, Bastrovirus, animal viruses, and many more
- temporal changes in virus compositions, both for specific viruses and for entire virus families
- changes in virus levels, which can be used to predict the future burden on the health system, since the increase in the amount of a specific virus in wastewater usually occurs one to two weeks before it causes illness in those who need help from the health system
- changing patterns of viral spread. Some viruses have now returned to pre-pandemic patterns while others have changed their patterns, which may represent the new normal or the spread pattern may return to the pre-pandemic seasonality in a few years

Virus in wastewater in relation to number of diagnosed patients



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