Wastewater gone viral
Pandemic signals from the sewer
Viruses monitoring of sewage

1940 Charleston, Detroit, Buffalo
Poliovirus epidemic

Rationale:
transmission via water?

Method:
inoculation of Rhesus monkeys

Outcome:
Infectious poliovirus in wastewater during outbreak

II. POLIOMYELITIC VIRUS IN URBAN SEWAGE*

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(Received for publication, March 21, 1940)

In the preceding paper (1) attention has been called to the fact that poliomyelitic virus can be readily isolated from the stools of some patients with this disease. Our own experiments, and those of many others, now testify to the ease with which this can be accomplished; not only with paralytic cases but also with the more common abortive types; and not only during active stages of the disease but also during convalescence. Obviously, therefore, when an epidemic of poliomyelitis occurs within a city there must be ample opportunity for the virus to enter the local sewage system. And, considering the frequency of mild and unrecognized forms of poliomyelitis, and the length of time which such cases may be potentially infectious, it seems possible that the concentration of virus in urban sewage may become appreciable. Prior to 1939, however, poliomyelitic virus had never been

Journal of Experimental Medicine
Sewage surveillance of polio

1992 Netherlands Poliovirus epidemic

Rationale:
Surveillance of poliovirus circulation in at-risk communities

Method:
Virus isolation and cell culture

Outcome:
Early circulation of outbreak virus (retrospectively)
Sewage surveillance of polio: added value
Wilkinson et al, 2022

Early warning before cases of paralysis occur
9 countries

Monitor trend
Increase in 1, decline in 2 countries

Confirm absence

‘Variants of concern’
27 detected in 32 countries
Water virology pandemic response 1

Can SARS-CoV-2 be transmitted via water?

Are workers, recreational waters, drinking water safe?

Amoy Gardens, Hong Kong, 2003
Health risk to workers?

- No epidemiological signals
- No case reports
- In stool:
  - high RNA concentrations
  - but few reports of infectious virus
  - rapid inactivation in intestine
- In wastewater:
  - no reports of infectious virus
  - virus is not robust in wastewater
- Standard personal protection (to protect against other viruses)
Availability of sufficient clean water is a societal issue all over the world.

How can we say that?

Because we know about other viruses that survive better in sewage and water, are more resistant to disinfection, and have confirmed that our drinking water supply systems can adequately remove/inactivate these.
WHO notified of pneumonia cases Wuhan

Identified as new coronavirus

First protocols for clinical samples

First cases in Italy (travel from China)

First wastewater analysed with qPCR

Dec 31, 2019

January 11

January 23

January 31

February 5

Virus test: science at warp speed
Sewage surveillance at WWTP in the Netherlands

- Clear increase in reported cases coincides with increase in concentration in wastewater
- Two other WWTP: virus detected in wastewater 6 days before first reported case
- National surveillance (RIVM)

Medema et al, 2021, EST Letters
Objective surveillance of SARS-CoV-2

Objective indicator of SARS-CoV-2 circulation, independent of human testing

everybody is going to the toilet, not everybody is going to get tested

- test availability
- testing strategy
- testing willingness
- asymptomatic ‘cases’
National dashboard RIVM

The situation in the Netherlands

- Coronavirus thermometer

The position
Expectation for 9 May until 16 June 2023

Low

The influence on society and healthcare due to the disease burden of the virus is low.

The position 3 is decided on 9 May 2023. The position remained the same.

Source: RIVM

Development of the virus

Tests
0.3%

The number of individuals who tested positive for COVID-19 as a percentage of the total number of tests.

Value from 8 - 14 May 2023 - Source: Infektionsschutz

Wastewater
-5%

The national average number of virus particles decreased slightly (5%) in week 19 (1 - 7 May).

The number of virus particles decreased (7%) in the first half of week 19 (8 - 10 May).

Value from 1 - 10 May 2023 - Source: RIVM

Virus variants
XBB

XBB (including XBB.1.5 and XBB.1.6) is responsible for most infections.

These subvariants do not appear to be more pathogenic than the earlier Omicron variants. This also applies to XBB.1.6.

Report of 16 May 2023 - Source: RIVM
National data (dashboard RIVM)

Hospitalizations

SARS-CoV-2 load wastewater

Positive tests
Nationale data (dashboard RIVM)

Hospitalizations
SARS-CoV-2 load wastewater
Population study (self reported positive tests)
Infectieradar
Use case: understand COVID-19 dynamics: the infectious disease surveillance pyramid

High resolution surveillance:
- Time: 3/week
- Space: city districts
- Matched population

![Diagram of surveillance pyramid]

- Deaths
- ICU occupancy
- Hospitalizations
- Cases reported
- Symptomatic individuals tested
- All symptomatic infections
- All infections, (pre)symptomatic and asymptomatic

- Hospital data
- Test data
- GP data
- Sewage
Rain dilutes SARS-Cov-2 signal

Wet weather Rotterdam district 3
Lower SARS-CoV-2 concentration
Up to factor ≈3 to 4

Normalisation of SARS-CoV-2 concentration with domestic wastewater flow (inhabitants * 120 l pppd), checked with conductivity and CrAssphage
Comparing sewage and clinical surveillance
Second wave (sep – dec 2020)

Trends in clinical (red) and sewer (blue) data

Autocorrelation between clinical and sewer data

Highest correlation with 0 / -2 (= sewers are 6 days ahead)
Delay in clinical testing (disease onset vs nose swab)
Simple linear regression positive tests onset day vs sewage
Model started to diverge in Dec 2020
Dynamics in clinical testing (Rotterdam data)
Model improvement: include testing rate as index of testing behaviour
Relation between concentration in wastewater and newly reported cases

Normalisation of wastewater concentration with flow (with EC)
Langeveld et al, 2022, STOTEN

Normalisation of newly reported cases
- For test delay (to symptom onset day)
- For test behaviour (#tests per 100,000)
De Graaf et al, 2023 STOTEN

Wastewater = newly reported cases
- Also with Alpha and Delta
- Also with vaccination
- Except for July 2021 (open nightlife if tested)
Data analysis Rotterdam data

Sewage as objective indicator of virus circulation

Undertesting of humans in certain city areas?

Sewage data used to mobilize testing facilities to city areas with low case number/sewer signal ratio

Linking sewage data to human testing data: correct for human testing behaviour via total number of tests

KWR, GGD Rotterdam, Erasmus MC, Partners4UrbanWater, RHDHV, RIVM, Water authorities: Hollandse Delta, Delfland, Schieland & Krimpenerwaard
dd-PCR to detect signature mutations of VoC

1. Divide wastewater extract in ca 20,000 droplets (each droplet with very few virus-RNA strands)
2. Run PCR in each droplet, using two probes with different colours: one for the wild type and one for the mutant (differ only a SNP)
3. Absolute quantification: count droplets in which the wild type vs in which the mutant (or both)
4. Determine relative abundance of wild type and signature mutation in sample
Use case: Variant of Concern Alpha introduction N501Y mutation vs ‘wild type’ by ddPCR (2020/2021)

Heijnen et al, 2021, STOTEN
Wastewater surveillance N501Y (alpha) vs Kiemsurveillance (alpha)
Kiemsurveillance national data RIVM; ddPCR data Rotterdam; new variant primers/probes by BioRad
SARS-CoV-2 VoC mutations over time in Rotterdam sewage (NGS)

Izquierdo et al, submitted
“We’ve done a decade’s worth of science in the first year”

Doug Manuel (epidemiologist at the University of Ottawa)
The WSPHERE global data center and public health use cases
View trends and data (for quantitative datasets)
Open access data center

**CA - Ottawa - ROPEC**

*Ottawa Public Health*

Wastewater samples are from the Robert O. Pickard Environmental Centre (ROPEC) which collects and treats wastewater from ~91.6% of Ottawa's population, 2,846 km of sanitary sewers, 108 km of combined sewers, 71 wastewater pumping stations,...

2x csv  1x geojson

**LU - country data - LIST**

*Environmental Microbiology and Biotechnology research group*

This dataset presents the results of national-wide wastewater monitoring efforts in Luxembourg through the sampling of 13 different WWTP across the country from March 2020.

2x csv  1x geojson

**SI - country data - NIB**

*NIB - National institute of biology*

Wastewater influent samples from slovenian WWTPs.
Public health use cases

Use cases
Have your wastewater data been used to underpin public health actions?

Our goal is to help health authorities understand the added value of wastewater surveillance, by showcasing good examples of how wastewater data have been used to support public health actions in different settings (national, regional, local, building-level surveillance; high and low resource settings: sewered and non-sewered settings).

Published use cases

South Africa sewage surveillance use case
ZA - Water Research Commission

Ghana sewage surveillance use case
GH - Greater Accra

Canadian sewage surveillance use case
CA - Windsor

Switzerland sewage surveillance use case
CH - Eeweg

Dutch sewage surveillance use case
NL - Rotterdam

Catalan Surveillance Network of SARS-CoV-2 in Sewage
ES - Catalonia

ICRA
Public health use case Victoria (Australia)

**Victoria Australia**

Early warning

Adjunct outbreak response

Wastewater surveillance of SARS-CoV-2 (2021)

**Aug 12: Positive sample in Shepparton, no reported cases**
State and local Health Dept notified: public notification and expanded wastewater surveillance

**Aug 19: 4 more positive wastewater samples**
Further press coverage, expanded clinical testing services

**Aug 20: first case reported**
Lockdown, expand wastewater testing to towns in region and localised surveillance at facilities with large vulnerable populations
Public health use case Rotterdam (NL)

Undertesting of humans in certain city areas

Sewage data used to mobilize testing facilities to city areas with low case number/sewer signal ratio
Undertesting of humans in certain city areas

Sewage data used to mobilize testing facilities to city areas with low case number/sewer signal ratio
Switzerland

Comprehensive and early detection of VoC at high resolution

ETH Zürich, EAWAG, EPFL
Public health use case South Africa

Establishing the framework for water quality based surveillance in non-sewered settlements
Identifying the appropriate sampling area

Through trial-and-error, it was decided that the **run-off and streams nearby settlements** were the most appropriate sampling points and that **passive sampling** overcame the challenges of routine sampling in non-sewered settlements.
**Passive sampling**

Passive sampling devices were placed for 24 h in the selected areas.

They were selected because of:

1. Low yield during high dilution periods
2. Easy and cheap sample transfer
3. Fast sample processing
Added value for pandemic response

- Early warning
- Objective, independent of human test behaviour
- Efficient: one sample for a population sample
- Low cost
- Versatile: city, city area, building, on-demand, non-sewered
- Feasible for emergence of Variants-of-Concern
- qPCR platform expendable

Mirror of society
International experience (EU and beyond)

Collecting data from wastewater to support public health response
- Atypical for health sector (why do we want sewage data?)
- Atypical for water sector (why do we collect health data?)
- Atypical for academia (surveillance is not research)

Successful implementation of (actionable) sewage surveillance
- Public health institutions that bridge health and environment (see Netherlands, Finland, Hungary) or are ‘early adopters’ (US-CDC, Victoria Dept of Health)
- Local level Public Health agency
- Public health is driver, water sector pro-active support
Supersites

Does Wastewater surveillance of Supersites reveal different story compared to the city?
Omicron via air travel

Schiphol & Frankfurt: 3 days before first positive sample

Figure 6. Early detection of Omicron in airport wastewater samples. Samples from the indicated airports were analyzed by RT-PCR with both the JRC-CoV-UCE and the Omicron-specific OmMet [22] assays. SARS-CoV-2 (most likely the Delta variant, which was highly predominant at that time) was identified in all of the samples while Omicron was only detected at lower concentrations (higher Ct numbers) in two samples from airports in Frankfurt and Amsterdam from the middle and end of November 2021. The presence of Omicron was confirmed by genome sequencing [44]. Neg: no detection of Omicron by using the OmMet assay.

Marchini et al 2023, Viruses
Sewage as an indicator of public health

Sewage research is now being used to detect the coronavirus (SARS-CoV-2). This type of monitoring has been used for some time to detect poliovirus and antibiotic-resistant bacteria. RIVM is investigating options for expanding sewage research to include other data that could provide information about public health.

- Drug use: Heroin, cocaine, new party drugs
- Forensic research: Drug production
- Use of muscle-building supplements: Anabolic steroids, sequestering agents*, weight-loss supplements*
- Smoking and drinking: Nicotine, alcohol consumption
- Dangerous substances: PFAS, substances of very high concern (SVHC/ZZS), pesticides, flame retardants*, new substances*
- Pathogens: SARS-CoV-2, poliovirus, norovirus, antibiotic-resistant bacteria
- Personal hygiene and care products: Microplastics, parabens*, UV filters*
- Obesity: Biomarkers correlated to BMI
- Nutrition: Mycotoxins, vitamins*, isoflavones*
- Medication use: Regimen compliance*, insight into over-the-counter medicines*
- Non-communicable diseases: Diabetes*, cancer*, allergies*
- A healthy lifestyle: Oxidative stress*

* indicates specific substances or categories.
Poliovirus circulation in London and New York detected via sewage

Extra polio vaccine dose for children in London

News – 9 September 2022

The NHS is inviting children aged 1 to 9 in London to receive a dose of vaccine against. For some children this may be an extra dose on top of their routine vaccinations. In other children it may bring them up to date with their routine vaccinations.

Protect your child from polio.

Children aged 1 to 9 years old in London are being offered a dose of polio vaccine.

For every one case of paralytic polio observed, there may be hundreds of others infected. Coupled with the latest wastewater findings, it’s clear: polio is a threat to unvaccinated New Yorkers and children today. We must meet this moment by getting ourselves and our children by 2 months old immunized against polio as soon as possible – the protection against this debilitating virus we all need.

Dr. Mary T. Bassett
New York State Health Commissioner
Mpox testing of wastewater

Rotterdam Health Service

Could wastewater surveillance be used to
Hepatitis A virus outbreak in Amsterdam school

HAV imported disease in Amsterdam, by travellers to endemic countries

Asymptomatic carriers

Long incubation time

5 cases reported in primary school in Amsterdam

Question: can wastewater surveillance be used to detect if:
  • outbreak control measures are effective?
  • there is silent circulation of HAV?
Design

School 2 locations

Most cases at 1 location

Examine local sewer network lay-out

Evaluate sampling sites via manholes

4 sites:
1 sewer draining school building 1
1 sewer draining school building 2
2 in sewer draining city residential areas of school students

Passive samplers 48h-72h, 3/wk
Sampling human fecal material
CrAssphage as normalizer
Sampling for HAV
CrAssphage as normalizer
Sequencing HAV from wastewater and cases

Sanger and Nanopore

Identical strain
• in wastewater samples
• in cases
• IB 22-197
• Somalia

Wastewater surveillance
• confirmed outbreak
• confirmed silent circulation
• supported determination of end
• Found silent circulation of Morocco strain
Human circulation of avian influenza?

In case of transmission and infection in human(s)

Symptomatic carrier(s)

Asymptomatic/mildly symptomatic carriers?

Silent circulation

Wastewater surveillance in affected community

Now: establish proof of concept
Scabies in (student) homes

Waste/wash water monitoring as non-invasive surveillance tool

To monitor if control measures are effective

Proof of concept study: bed linen swaps PCR positive. Will be detectable in wastewater? First test wastewater from laundry

Current location: asylum home for minors

Too sensitive: minors, language barrier, asylum status still unclear
Illicit drugs in sewage

Trends in use (unbiased)
Illicit drugs in sewage

Trends in use (unbiased)
Illicit drugs: market size estimation from sewage Amsterdam

<table>
<thead>
<tr>
<th>Drug</th>
<th>Weight consumed (/year)</th>
<th>Market size (/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannabis</td>
<td>1370 kg</td>
<td>€ 84 mln</td>
</tr>
<tr>
<td>Cocaine</td>
<td>750 kg</td>
<td>€ 53 mln</td>
</tr>
<tr>
<td>MDMA</td>
<td>250 kg</td>
<td>€ 6.5 mln</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>170 kg</td>
<td>€ 2.4 mln</td>
</tr>
</tbody>
</table>
Opioids

Opioids

Opioid Use Monitoring via Compliance to NPDES Permit Program

**Sample Collection**
Effluent and influent composite samples are collected by wastewater operators at NPDES.

**Analysis**
Conduct chemical analysis (e.g., LC-MS/MS) on influent and effluent samples to determine opioid metabolites present in sample.
- Conduct internal analysis
- Provide contract environmental laboratories

**Outcomes**
- Local public health officials use collected data to:
  - Allocate more resources to prevent opioid use
  - Increase additional funding

**Distribution of Results**
Data on opioid metabolites and specific events identified within water systems is distributed to local public health officials:
- Submitters: local municipalities, providers, and hospitals
- Health educators
- Health officials
- Healthcare professionals

**Record in Public Databases**
- GAPDH Supplemental Efficacy Database (Efficacy 13)
- CDC National Narcotics Surveillance System
- CDC’s Vital Signs Online: Opioids for Pain/Recipes

**Public Policy Impact**
Potential pathway for new policy that motivates the implementation of the EPA and CDC to monitor opioid use through wastewater surveillance or the NPDES regulatory program.
Lead exposure in Flint

Roy, Tang & Edwards, 2019
PFAS exposure?

Gadden et al, 2023
All set!

Demonstrated added value for public health

Connections between water and health sector reinforced

Water sector: monitoring infrastructure for operations and water quality

Dissemination of PCR: multi-target platform, environmental testkits
Metagenomic “catch-all” techniques

Global resistome via wastewater

Munk et al, Nat Commun. 2022; 13: 7251

Global (vertebrate) virome via wastewater

Frontrunner INSPECT
International Network Surveillance for Pandemic EmergenCe via Transport hubs

Targeted (d/qPCR) and semi-targeted (Coronaviridae; genotype-to-phenotype methods; genotype + antibody capture)
Global disease surveillance and pandemic sentinel system