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On-demand Rioolwatersurveillance

Eindrapport

Samenwerkingspartners



GGD
Rotterdam-
Rijnmond



Noord- en Oost-Gelderland

KWR

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onderzoek & advies



Colofon

On-demand Rioolwatersurveillance

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Samenvatting

Rioolwatermetingen op landelijk niveau hebben hun meerwaarde bewezen voor de bestrijding van het Coronavirus. Voor een handelingsperspectief van de GGD ligt de meerwaarde van deze methode juist bij de inzet op lokale schaal en 'on-demand' bij hot-spots of kwetsbare locaties. Maar de installatie en bedrijfsvoering van meetkasten op veel punten in het rioolnetwerk die daarvoor nodig zou zijn, is complex, weinig flexibel en duur. Daarom is er behoefte aan een eenvoudige, snelle en 'on-demand' technologie.

1. Technologie

In dit project is een systeem ontwikkeld voor 'on-demand' lokale rioolwatermetingen. Het systeem is eenvoudig, goedkoop, snel en flexibel inzetbaar. Op een fijne ruimtelijke schaal kunnen er trends van COVID-19, maar ook van andere infectieziekten, mee worden gemeten.

2. Uitdaging

We hebben een systeem voor 'on-demand' rioolwatermetingen met passieve samplers voor SARS-CoV-2 ontwikkeld en gevalideerd. Samen met de gezondheids- en waterpartners is geëvalueerd welk handelingsperspectief dit systeem oplevert. Onderzoek is uitgevoerd naar materialen voor een hoge opbrengst (rendement virusvangst uit rioolwater), gevoeligheid, alternatieven voor debietmeting en het bepalen van nieuwe Variants-of-Concern, zoals de Omikron-variant. Nu COVID-19 is overgegaan van een pandemie naar een endemische infectie, is het van belang om te onderzoeken voor welke andere doelen de rioolwatermetingen de volksgezondheid van dienst kan zijn. Daarom zijn ook andere infectieziekten binnen dit project onderzocht.

3. Oplossing

Voor de watersector levert dit project een maatschappelijke verbreding op van de functie van rioolwatermetingen naar informatievoorziening over de publieke gezondheid. Door te helpen met het lokaliseren van infectie-hotspots, komt hier een aanpak uit voort die aansluit bij de huidige nationale rioolwatermetingen en die meer handelingsperspectief oplevert voor de GGD. Internationaal bestaat veel aandacht voor rioolwatermetingen en er is behoefte aan een dergelijke aanpak. De Nederlandse watertechnologie heeft hierin een voorlopersrol.

4. Resultaten

Het project heeft een unieke samenwerking tot stand gebracht tussen de gezondheids- en waterwereld. Gaandeweg zijn meer GGD's bij het onderzoek betrokken geraakt en hebben zij 'on-demand' casussen aangeleverd. Het onderzoek heeft zich daarmee gericht op actuele vragen van de GGD's over het gebruik van rioolwatermetingen als informatiesysteem om hun werk beter te kunnen doen. Het ging bijvoorbeeld over de vraag of contact tracing of quarantaine nog effectief zijn, of dat de variant of infectieziekte al breder is verspreid. Of: hoe verhoudt zich de informatie vanuit het riool (ondergronds) tot de patiëntgegevens (bovengronds)? En ook: zijn eventueel genomen maatregelen effectief, of gaat het virus nog ongezien rond en kunnen nieuwe gevallen worden verwacht? Naast nieuwe varianten van SARS-CoV-2 (omicron subtypes) hebben we in dit project laten zien dat de methode ook werkt voor andere infectieziekten zoals apenpokken (monkeypox), hepatitis A en de bof. Rioolwatermetingen werken zowel op het niveau van een stad en wijk, maar wanneer dat nodig is, is ook nog meer specifiek. Daarvoor is goede en snel inzetbare kennis over het rioolstelsel essentieel en ook over hoe dit op een representatieve wijze met passieve samplers is te bemonsteren. Verschillende materialen voor het bemonsteren van virussen in passieve samplers zijn vergeleken om de meest efficiënte combinatie te kunnen selecteren. Meerdere qPCR-analyses voor de detectie van infectieziekten zijn met succes ontwikkeld en getest binnen het project, waarmee ook is aangetoond dat snel op nieuwe infectieziekten kan worden ingespeeld.

5. Synthese en perspectief

Nieuwe mogelijkheden, met meerwaarde voor de GGD

Metingen van ziekteverwekkers in rioolwater (of wasmachinewater) kunnen voor veel meer ziektes informatie leveren. In dit onderzoek zijn toepassingen met waarde voor de GGD gedemonstreerd voor COVID-19, griep en bof (met als veroorzaker een respiratoir overgedragen virus (allen met envelop)), voor besmettelijke geelzucht (een fecaal-oraal overgedragen virus (zonder envelop)), voor mpox (een via huidcontact overgedragen virus) en schurft (een via contact overgedragen mijt). In de recente literatuur zijn nog veel meer voorbeelden te vinden, van:

- ziektes door respiratoire virussen en bacteriën, zoals respiratoir syncytieel virus (rs-virus), mazelen, tuberculose;
- ziektes door enterale virussen en bacteriën, zoals poliovirus, norovirus, hepatitis E virus, *Campylobacter*, cholera;
- ziektes overgedragen door muggen, zoals zika en dengue
- antibioticaresistentie
- drugs en geneesmiddelen, gezondheidsindicatoren, blootstelling aan giftige stoffen.

Metingen in rioolwater kunnen dus informatie verstrekken over een breed scala aan gezondheidsaspecten in de bevolking. Uit dit onderzoek is ook duidelijk dat als rioolmetingen meerwaarde voor de GGD willen hebben, er naast de nationale rioolsurveillance op de rioolwaterzuiveringen ook een flexibel systeem moet zijn dat vraaggericht snel lokaal kan worden ingezet. Dit onderzoek heeft laten zien dat zo'n systeem kan werken en meerwaarde voor de GGD kan opleveren. Het is vooral een *proof-of-concept*: voor een aantal ziektes (bof, schurft) is dit de eerste studie wereldwijd die laat zien wat er mogelijk is met rioolmetingen met passieve samplers. Het zou goed zijn als deze bevindingen in andere/vervolgstudies worden bevestigd.

Meten in het riool om een beeld te krijgen van ziekte in de bevolking

De metingen van het erfelijk materiaal van de ziekteverwekkers in rioolwater met passieve samplers leveren in ieder geval kwalitatieve informatie over de aan- of afwezigheid van de ziekteverwekker in het rioolwater. In de demonstratiestudies in dit onderzoek bleek dat gevoelig genoeg voor SARS-CoV-2, ook om de varianten daarvan te onderscheiden, voor Hepatitis A virus die beide in hoge concentraties via de faeces door besmette personen worden uitgescheiden. Maar ook voor bofvirus, uitgescheiden via urine en voor monkeypox virus, waarschijnlijk vooral van huidlaesies uitgespoeld bij wassen/douchen/baden is dat in rioolwater vermengd met dat van niet-besmette mensen te meten. In het geval van bofvirus lukte dat op het niveau van dorpen met ca 3000-5000 inwoners waaronder een aantal (6 bekend, waarschijnlijk meer) besmette inwoners aanwezig waren, maar niet meer als het rioolwater van deze dorpen werd bijgemengd met rioolwater van ruim 20000 mensen. Dat is een indicatie dat voor het meten van informatie over lokale uitbraken van bof ook echt op lokale schaal gemeten moet worden. Voor scabiës worden waarschijnlijk mijt(resten/DNA) gemeten die worden uitgespoeld uit kleding en bedlinnen van besmette personen. Daar was het DNA wel aantoonbaar in wasmachinewater, maar niet in het ontvangende riool in de straat. Daarvoor was de hoeveelheid scabiës-materiaal dat beschikbaar is waarschijnlijk te laag. Voor scabiës moet dus echt in de wasmachine gemeten worden.

Als de metingen van de ziekteverwekkers worden gecombineerd met een index voor de hoeveelheid humaan materiaal dat is opgevangen (in deze studie was dat de concentratie CrAssfaag voor de hoeveelheid humaan-fecale virussen) kan semi-kwantitatieve informatie over de concentratie ziekteverwekker in het riool worden verkregen. Dat is waardevol omdat niet alleen iets zegt over de aan- of afwezigheid, maar ook over de hoeveelheid. Voor SARS-CoV-2 is daar al veel over bekend, voor de andere ziekteverwekkers is dat nog niet goed bekend en zijn er andere indexparameters (bv Polyomavirus voor urine) nodig. Ook is betere informatie nodig over de mate waarin de ziekteverwekkers worden uit- of afgescheiden, de variatie daarin over de loop van de infectie en tussen personen, welke factoren die mate van uitscheiding bepalen en hoe representatief de rioolmetingen met de passieve sampler zijn voor de verschillende ziekteverwekkers.

Ethiek

Het is duidelijk dat er veel gemeten kan worden in rioolwater en dat daar waarde in kan zitten voor de infectieziektenbestrijding. Het verzamelen van informatie over de circulatie van (infectie)ziekten onder de bevolking via rioolwater is een terrein waar nog moet worden nagedacht over de ethische aspecten en de

randvoorwaarden voor de inzet en gebruik van rioolmeetgegevens voor de infectieziektenbestrijding. Dit terrein wordt juridisch niet gedekt door de bestaande ethische richtlijnen, dus wordt aanbevolen om gerichte richtlijnen op te stellen voor ethische inzet en gebruik van rioolmetingen.

Inhoud

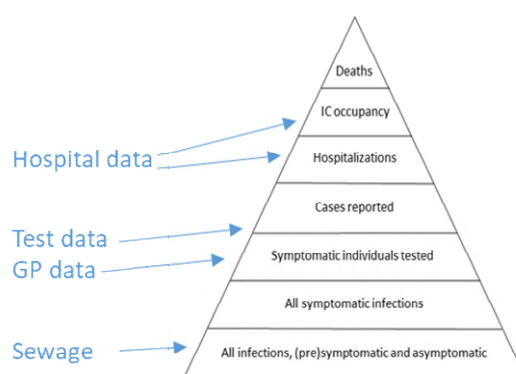
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1. Aanleiding en doel project

Rioolwatersurveillance is van grote aanvullende waarde op de nationale riol-surveillance en levert een aantal voordelen op:

1. het geeft een snelle indicatie van de trend in viruscirculatie in de populatie (early warning);
2. het is objectief, onafhankelijk van het testgedrag en ook asymptomatische virusdragerschap wordt meegenomen ('iedereen gaat naar het toilet, niet iedereen gaat naar de teststraat')
3. het is efficiënt: met één monster is een populatie van ca 100.000+ te volgen
4. binnenkomst van nieuwe Variants-of-Concern kan worden gevolgd.

Een belangrijke beperking van het huidige landelijke riolwatermeetprogramma is dat de schaal van de metingen in de grote steden onvoldoende is om handelingsperspectieven uit af te leiden. De riolwaterzuiveringsinstallaties bedienen in grotere steden meerdere 100,000-en mensen. Als het meetprogramma op die riolwaterzuivering aangeeft dat de infectiedruk in de stad omhoog gaat, wil de GGD dat signaal graag om kunnen zetten in actie, maar daarvoor wil ze weten waar in de stad de viruscirculatie toeneemt. Voor handelingsperspectief is een hogere resolutie van de informatie nodig. Maar installatie en bedrijf van monsterkasten op veel punten in het riolnetwerk is complex, weinig flexibel en duur. Er is behoefte aan een eenvoudig, snel en 'on-demand' in te zetten technologie.



Het doel van dit TKI-project was om een systeem voor 'on-demand' lokale riolwatermetingen te ontwikkelen, dat heel snel en flexibel inzetbaar is en waarmee op een fijnere ruimtelijke schaal (100-1.000-en inwoners) kan worden gemeten wat de trend is in de infectiedruk van COVID-19 of andere relevante infectieziekten en of/in welke mate de SARS-CoV-2 Variant-of-Concern (of relevante varianten van andere infectieziekteverwekkers) aanwezig is. Het onderzoek was gericht op het kunnen duiden van het riol-signaal van 'on-demand' kleinschalige metingen en de vertaling naar handelingsperspectieven voor GGD's. Dit werd nog niet toegepast in Nederland of daarbuiten.

2. Publiekssamenvatting “TKI-project riol-surveillance op aanvraag”

Manon Laterveer, op basis van interviews met samenwerkingspartners.

Tijdens de coronapandemie heeft de nationale riolwatersurveillance zijn kracht als monitoringtool bewezen. Deze monitoring werkt op het niveau van riolwaterzuiveringsinstallaties (rwzi's), waar het riolwater van bijvoorbeeld een hele stad samenkomt. Voor GGD's is het vooral ook belangrijk om snel en op kleinere schaal de uitbraak van een infectieziekte te volgen, zoals in de wijk of op een school. Een recent TKI-project stelde de mogelijkheid van zo'n vraaggestuurde aanpak op de proef. “On-demand riolwatersurveillance zou goed kunnen helpen om ondergronds aanwijzingen te krijgen voor wat je bovengronds moet doen”, denkt Ewout Fanoy van GGD Amsterdam.

Het gebruik van afvalwater om trends te signaleren in de verspreiding van besmettelijke ziekten, nam tijdens de uitbraak van het coronavirus een vlucht. KWR-onderzoeker Gertjan Medema memoreert hoe destijds vanuit de nationale riolwatersurveillance de behoefte ontstond om lokaal de opkomst van een nieuwe variant van dit virus op te merken. “In een voorgaand TKI-project werkten we met GGD Rotterdam-Rijnmond op wijkniveau aan de riolwatersurveillance. De bedoeling was om hiermee dieper in de stad te kunnen kijken, voor een beter handelingsperspectief. Toen daar de eerste Omikron-variant van het coronavirus werd gerapporteerd, wilde de GGD weten of het al breder onder de bevolking was verspreid. Met passieve samplers bleken we in staat om Omikron eruit te pikken. We zagen dat het al verder rondwaarde. Het ging dus niet om een lokale besmetting en de GGD wist dat isoleren van de bronbesmetting niet zinvol was.”

Stille transmissie

Het voorbeeld van Medema laat zien dat ook op fijnmazige schaal het riool inzetbaar is als ‘spiegel van de samenleving’. Volgens de onderzoeker opent de huidige postpandemische periode de weg voor een hele nieuwe set aan toepassingen voor snelle en flexibele riolwatersurveillance. “Daarmee krijgen GGD's de tools om te zien of er sprake is van ‘stille transmissie’. Dat is de overdracht van een infectieziekte die je niet ziet met de traditionele surveillance, maar wel in het riool. Zoiets was eerder niet mogelijk.” In het onlangs afgeronde TKI-project ‘Riolwatermetingen op aanvraag voor trendmetingen bij lokale uitbraken van infectieziekten’, is met pilots in Amsterdam en Rotterdam ingespeeld op de ontwikkeling die Medema schetst. Op grond van on-demand vragen vanuit de GGD's – bijvoorbeeld een uitbraak van hepatitis A, het apenpokkenvirus (monkeypox virus), of de bof – ging men lokaal speuren in het riool. “Dit vraaggestuurde werk betekent dat je heel snel je monitoringsnetwerk moet inrichten”, weet Jeroen Langeveld van Partners4UrbanWater. “Anders is de uitbraak alweer weg. Met onze kennis over de ondergrondse infrastructuur van het riool in verhouding tot die van de bovengrond, wisten we precies waar en wanneer we moesten meten. Je moet een sprintje trekken totdat je het antwoord hebt en elk geval is anders. Dat maakt het enorm interessant en uitdagend.”

Wattenstaafjes

De passieve sampler die op een specifieke plek in het riolstelsel wordt geplaatst, is een soort kleine torpedo met gaatjes erin, legt Medema uit. “Daarbinnen zit het monsternormaal, dat bestaat uit wattenstaafjes en membraanfilters die het langsstromende materiaal uit het riool absorberen. Eventuele virusdeeltjes plakken hieraan vast. We laten de passieve sampler 24 tot 48 uur in de stroming hangen. Dan hebben we een mooi representatief mengmonster. In het lab voeren we meerdere processtappen uit, waarna met een PCR-test wordt gekeken met welk virus we te maken hebben. Dankzij samenwerking met Erasmus Universiteit konden we vanuit de wereld van de klinische virologie de benodigde protocollen in huis halen voor de virussen waar we naar op zoek waren in het

rioolwater. We hebben gemerkt dat het on-demand werk heel dynamisch is. Je moet kunnen anticiperen op iets nieuws, waarbij je tijdens het onderzoek een proof-of-concept moet inbakken. Worden we ergens op afgestuurd waar een hoge virusuitscheiding wordt verwacht, en onze passieve sampler pikt dit op, dan weten we dat het werkt. Na de eerdere primeur van Omikron-variant van het SARS-CoV-2 virus hebben we in dit project ook Monkeypoxvirus en het bofvirus kunnen aantonen.”

Alert blijven

Behalve de successen die Medema noemt, ontsnapt ook hepatitis A niet aan de passieve sampler. Dit bleek bij een uitbraak hiervan op een school in Amsterdam, vertelt Fanoy. “We hadden daar een melding van hepatitis A gehad, maar in welke mate het virus was verspreid, wisten we niet. Bij kinderen kan het hepatitis A-virus rondsluimeren zonder dat je het merkt. Maar vooral de ouders kunnen er flink ziek van worden. Toen besloten we de passieve sampler in het rioolputje van de school en omliggende wijk te hangen. We konden het verloop daarna heel mooi volgen. Nadat bovengronds geen meldingen meer kwamen, bleven we toch ondergronds meten. We zagen eerst nog een lichte uitscheiding die daarna uitdoofde. Dat gaf de GGD bevestiging dat de uitbraak over was.”

Wasmachine

Voor Fanoy was een belangrijke reden om bij het project aan te haken dat het laat zien hoe GGD's hun surveillancemethodieken kunnen moderniseren. “Sinds corona is dit een enorm hot topic”, licht hij toe. “Natuurlijk is de nationale rioolwatersurveillance van het RIVM voor ons ook interessant. Maar je wilt ook zelf kunnen sturen. Zo hebben we geëxperimenteerd met schurft, dat niet via de ontlasting wordt uitgescheiden maar veroorzaakt wordt door een mijt die in de huid leeft. Bij een studentenhuus hebben we een passieve sampler in de wasmachine gedaan. En ja hoor, dat bleek te werken! Een interessant resultaat, dat bijvoorbeeld ook de deur opent naar het monitoren van een uitbraak in verpleeghuizen via wasmachines.”

Ethisch debat

Toch zijn er ook kritische kanttekeningen bij deze gerichte inzet van rioolwatersurveillance te plaatsen, haast Fanoy zich te zeggen. En dat is op ethisch gebied. “Als je bijvoorbeeld in een straat naar een SOA wilt gaan zoeken, hoe zit het dan met de privacy? Tijdens de coronapandemie was de urgentie voor iedereen duidelijk. Maar nu moeten we kijken naar de vraag: wat is nou echt nodig voor uitbraakbestrijding?” Bij het presenteren van de projectresultaten aan andere GGD's, merkt Fanoy dat sommigen vanwege deze kwestie afwachtend zijn. Dat is heel logisch vindt hij. “Daarom willen we een ethisch debat voeren, samen met het RIVM, ethici, juristen en andere stakeholders. Het is belangrijk om niet over grenzen heen te gaan en hier dus het gesprek goed over te voeren. Persoonlijk denk ik dat het een middel is dat ons bij uitbraken van infectieziekten heel effectief kan helpen. Als je ziet dat het verdwijnt uit het riool, geeft dat rust omdat je zekerder weet dat het ook echt weg is.”

Spin-off

Voor praktijkman Langeveld heeft het project aangetoond dat on-demand rioolwatersurveillance werkt. Hij beschouwt het als een extra tool voor de GGD's. “We zijn nu bezig om ervoor te zorgen dat zij het zien als een echt instrument om te kunnen gebruiken. Ik verwacht zeker een spin-off, waarbij Partners4UrbanWater een aanbieder kan zijn.” Als uitvoerende partij voor analyses in het lab, ziet Medema niet direct een rol weggelegd voor KWR. “Uiteraard willen we helpen als een GGD met een specifieke vraag voor een nieuw virus naar ons toekomt. Maar we zijn geen surveillance instituut, andere laboratoria kunnen deze service prima verlenen. Het is goed als de resultaten vanuit het TKI-project nu naar de praktijk toegaan.” Eindgebruiker Fanoy ziet dit wel gebeuren, nadat het ethische debat is gevoerd. Wel geeft hij aan behoefte te hebben aan een vertaling van de projectresultaten in een soort handboek voor de GGD's. “Het is geen complexe techniek, maar wel nieuw voor ons. Naast de gebruikelijke menselijke diagnostiek, vraagt het om ook naar het rioolwater te kijken. Dat maakt het enorm interessant.”

QR code link naar Engelstalige podcast over dit onderzoek die door kunstmatige intelligentie (Google LM Notebook) is gegenereerd.



3. Kennisontwikkeling en disseminatie

3.1 Leeswijzer

De verslaglegging van het project TKI On-demand rioolsurveillance is opgebouwd uit verschillende elementen die zijn samengebracht in dit eindrapport (Tabel 1). Hoofdstuk 1 geeft de aanleiding voor dit onderzoek. Om de resultaten breder toegankelijk te maken is een publieksvriendelijke samenvatting opgenomen in Hoofdstuk 2. In hoofdstuk 3 is een overzicht gegeven van de belangrijkste communicatie uitingen, waaronder presentaties op congressen, wetenschappelijk artikelen en diverse nieuwsitems en artikelen in vaktijdschriften. Hoofdstuk 4 bespreekt vijf case-studies naar de toegevoegde waarde van on-demand rioolsurveillance voor verschillende GGD/publieke gezondheidsdoelen ten tijde van de COVID-19 pandemie en ook mpox in de regio Rotterdam, in een wetenschappelijk artikel dat is gepubliceerd in Eurosurveillance. Hoofdstuk 5 presenteert een case-studie van on-demand rioolsurveillance in de regio Amsterdam bij een infectieuze geelzucht (hepatitis A) uitbraak. Hoofdstuk 6 bespreekt de toegevoegde waarde van afvalwater/wasmachinewater metingen voor een huis met een uitbraak van schurft (scabiës). Hoofdstuk 6 beschrijft de inbreng van rioolmetingen bij een griepvirus (influenza) uitbraak in een Rotterdams verpleeghuis in de zomer. Hoofdstuk 8 presenteert een case-studie in Noord-Oost Gelderland naar een uitbraak van de bof in die regio. Tenslotte volgen in Hoofdstuk 9 drie relevante presentaties die verschillende onderdelen van het project belichten. De eerste is de winnende poster presentatie van GGD Amsterdam op het Pandemic and Disaster Preparedness Centre congres in Utrecht in november 2023; de tweede een presentatie gehouden op WaterwaterBased Surveillance Summit, Canada, in september 2024, door Gertjan Medema van KWR.

Tabel 1 Leeswijzer en inhoudelijke verantwoording

Hfst	Auteurs	Soort output	Titel
1	Gertjan Medema	Introductie	Aanleiding en doel project
2	KWR, met podcast door Google Notebook LM	Publieksvriendelijke samenvatting	TKI-project Rioolsurveillance op aanvraag. De waarde voor de publieke gezondheid.
3	Diverse	Overzicht	Kennisontwikkeling en disseminatie
4	GGD Rotterdam Rijnmond en KWR, ism de overige partners	Wetenschappelijk artikel	Agile, on-demand wastewater surveillance of virus infections to support pandemic and outbreak response in Rotterdam-Rijnmond, the Netherlands
5	GGD Amsterdam ism de overige partners	Wetenschappelijk en vakblad artikel	Use of passive samplers as sewage surveillance tool to monitor a hepatitis A outbreak at a school in Amsterdam, the Netherlands, Oct 2022 – March 2023.
6	GGD Rotterdam ism de overige partners	Abstract voor presentatie op wetenschapscongres	Scabiës in studentenhuizen en instellingen
7	GGD Rotterdam ism de overige partners	Vakblad artikel (concept)	Uitbraak griep in verpleeghuis
8	GGD NOG ism de overige partners	Wetenschappelijk artikel (concept)	Uitbraak bof in Gelderland
9	Maarten de Jong, Gertjan Medema	Presentaties op wetenschapscongressen	

3.2 Uitingen via online en geschreven media, radio

Hieronder volgt een selectie van de belangrijkste media-uitingen.

1. TKI project website: Rioolwatermetingen op aanvraag voor trendmetingen bij lokale uitbraken van infectieziektes

3.3 Presentaties op conferenties

1. SIWW 6/2024. Wastewater surveillance Lee Kuan Yew Water Prize Award Lecture. 18 June 2024
2. Water Congress China 5/2024. Safe water supply. 24 May 2024.
3. GloWaCon 11/2023. IWA Cluster on WBES.
4. GloWaCon 11/2023. Hepatitis A virus outbreak in Amsterdam.
5. Pandemic and Disaster Preparedness Congress 10/2023. Hepatitis A virus outbreak in Amsterdam.
6. WEFTEC Chicago, 10/2023. Wastewater surveillance.
7. WHO workshop. Wastewater surveillance in Ukraine, 20 July 2023.
8. 7th Ministerial Conference on Water and Health. Wastewater surveillance use cases. 13 July 2023
9. Cannes Water & Health Seminar. Wastewater surveillance. 26 June 2023
10. Canadian Water Network. Wastewater surveillance on a global stage. 31 May 2023
11. Michigan Go with the Flow. Pandemic signals from the sewer. May 18 2023
12. UN Water Conference New York. Wastewater surveillance use cases. March 24 2023
13. GWRC meeting. Wastewater surveillance. March 17 2023
14. National water congress: Spiegeltje, spiegeltje. January 10, 2023
15. WHO Technical Advisory Group – Virus Evolution. Environmental surveillance for SARS-CoV-2 to complement public health surveillance. 12/2022
16. IWA WWCE Copenhagen. Keynote Wastewater going viral. 16 September 2022.
17. Nederlandse Vereniging voor Medische Microbiologie: Mirror, mirror in the sewers: added value of COVID-19 surveillance via wastewater. April 5, 2022

3.4 Bijzonderheden/prijzen

Rioolsurveillance in de prijzen:

- Maarten de Jong is winnaar van de posterprijs op het Pandemic and Disaster Preparedness Center Congress 2023
- Gertjan Medema is Lee Kuan Yew water Prize 2024 winnaar in Singapore.

3.5 Kennisvalorisatie en -disseminatie

De resultaten worden als ze beschikbaar komen direct gedeeld met alle partners en de GGD, ErasmusMC. Voor dit onderzoek is een begeleidingscommissie actief die bestaat uit een deelnemer van elk van de partners. De resultaten worden gedeeld –in overleg- in wetenschappelijke en sociale media. Het informeren van de populaties die worden bemeaten ligt bij de GGD. Metingen samen met RIVM worden met RIVM samen gerapporteerd. STOWA faciliteert de disseminatie naar de Waterschappen.



Figuur 3-1 Maarten de Jong bij de presentatie van de posterprijs van de PDPC jury



Figuur 3-2 Prijsuitreiking Lee Kuan Yew Water Prize 2024. Van links naar rechts: Senior Minister Teo Chee Hean, Prize Council Chairman; Professor Gertjan Medema, Lee Kuan Yew Water Prize 2024 Laureate; Mr Tharman Shanmugaratnam, President of the Republic of Singapore; Professor Leo Tan, Board Member of Temasek Foundation. (Foto: SIWW2024)

4. On-demand rioolsurveillance in Rotterdam Rijnmond: Covid-19 en mpox

Nederlandse samenvatting

Tussen 2020 en 2022 werd in de regio Rotterdam-Rijnmond een reeks pilotstudies uitgevoerd om de haalbaarheid en effectiviteit van rioolwaterbemonstering te onderzoeken als hulpmiddel tijdens infectieziekte-uitbraken om infectieziektebestrijding door de GGD te ondersteunen. Het onderzoek maakte gebruik van passieve bemonstering, een methode die eenvoudig inzetbaar is en een hoge spatiotemporale resolutie biedt. Het richtte zich op SARS-CoV-2 en later het apenpokkenvirus (MPXV) in verschillende gemeenschappen, waaronder studentenhuizen, buurten met uiteenlopende vaccinatiegraden en kwetsbare populaties.

Detectie van virusuitbraken:

Passieve bemonstering kon lokale uitbraken van SARS-CoV-2 snel detecteren. Zo werd in een buurt een piek in het RNA van SARS-CoV-2 in afvalwater opgemerkt voordat traditionele surveillance-methoden dit vaststelden. Dit stelde de GGD in staat om sneller te reageren met gerichte interventies, zoals mobiele testunits en informatiecampagnes. Toen de eerste persoon met infectie door de Omikron-variant in het gebied van de GGD Rotterdam-Rijnmond werd gemeld, werd rioolonderzoek gebruikt om te zien of isolatie en bron- en contactonderzoek nog effectief konden zijn. De riooldata wezen op bredere gemeenschapstransmissie, zodat isolatiemaatregelen rondom dit gemelde geval niet opportuun was.

Toepassing bij MPXV:

MPXV-DNA werd opgespoord in afvalwater nabij een bevestigde casus, maar niet in omliggende gebieden. Dit indiceerde dat er geen verdere lokale transmissie plaatsvond.

Invloed van vaccinatiëgraad:

Een vergelijking tussen buurten met hoge en lage vaccinatiëgraad toonde vergelijkbare niveaus van SARS-CoV-2-concentraties in het afvalwater. Dit suggereert dat de vaccinatiëgraad op zichzelf niet altijd een directe correlatie heeft de viruscirculatie in de populatie, zoals gemeten via het afvalwater.

Effectiviteit en betrouwbaarheid:

De resultaten van passieve bemonstering kwamen overeen met die van standaard waterbemonsteringsmethoden, wat de betrouwbaarheid van deze nieuwe techniek bevestigde. Het normaliseren van resultaten op basis van CrAssphage hielp bij het verbeteren van de nauwkeurigheid, met name in kleine populaties.

Ethiek en operationele uitdagingen:

Ethische overwegingen, zoals mogelijke stigmatisering van kwetsbare groepen, leidden tot de annulering van één casestudy. Operationele problemen, zoals verlies of beschadiging van monsters, werden opgelost door verbeteringen in de bemonsteringsprocedures.

Conclusie:

Passieve bemonstering van rioolwater is een waardevol instrument gebleken voor snelle, op maat gemaakte infectieziekte-surveillance op gemeenschapsniveau. Het biedt GGDs een eenvoudige en flexibele manier om uitbraken vroegtijdig te signaleren en gericht in te grijpen. Voor een bredere toepassing is het essentieel dat ethische richtlijnen worden ontwikkeld. Deze technologie biedt mogelijkheden om infectieziektebestrijding verder te verbeteren en kan bijdragen aan betere paraatheid bij toekomstige uitbraken.

Toekomstige uitbreidingen:

De methode is flexibel en uit te breiden naar andere virussen, zoals influenza, RSV en enterovirussen, en mogelijk zelfs op bacteriën en antimicrobiële resistentie.

Agile, on-demand wastewater surveillance of virus infections to support pandemic and outbreak response in Rotterdam-Rijnmond, the Netherlands, 2020 to 2022

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Background: Wastewater surveillance may support early and comprehensive detection of infectious diseases' community transmission, particularly in settings where other health surveillance systems provide biased or limited information. Amid the SARS-CoV-2 pandemic, deploying passive samplers to monitor targeted populations gained importance. Evaluation of the added public health value of this approach in the field can support its broader adoption. **Aim:** We aimed to assess the feasibility and utility of on-demand wastewater surveillance, employing passive samplers, for SARS-CoV-2 and monkeypox virus (MPXV) in small/targeted populations, also considering ethical aspects. **Methods:** Pilot case studies in the Rotterdam-Rijnmond region were used for a systematic assessment of the feasibility and utility of wastewater monitoring of SARS-CoV-2 (variants) and MPXV using passive sampling. Each case study was instigated by actual questions from the Public Health Service about disease transmission. **Results:** Case study results demonstrated the feasibility and utility of on-demand wastewater surveillance with successful identification of a local peak in SARS-CoV-2 transmission, early detection of wider Omicron variant transmission after the first case was reported, as well as indication of no emerging local MPXV transmission. Ethical considerations led to the abandonment of one case study involving a displaced population. **Conclusions:** The study confirms the feasibility and utility of passive sampling for real-time infectious disease surveillance, at desired spatiotemporal resolution. Ethical concerns and operational challenges were identified, highlighting the need for early stakeholder engagement and

ethical guideline adherence. The method could be used to study under-surveyed populations and be extended beyond SARS-CoV-2 and MPXV to other pathogens.

Introduction

Wastewater surveillance is the process of monitoring wastewater for pathogens in human communities. Pathogens that are excreted in bodily fluids and excrements may be detectable in domestic wastewater, sometimes before infected individuals are symptomatic [1,2]. During the COVID-19 pandemic, an objective of wastewater surveillance was to identify COVID-19 outbreaks early. Another aim was to obtain more insight into the circulation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus causing COVID-19. This included studying trends of SARS-CoV-2 RNA concentrations in wastewater over time, investigating the occurrence and spread of virus variants of concern (VOC) or variants of interest (VOI) [2], as well as surveying changes in the geographical and spatial distributions of SARS-CoV-2 infections coinciding with different socio-demographic contexts.

Detection of genetic material in wastewater using reverse-transcription quantitative PCR (RT-qPCR), allows for surveillance of circulating viruses in the population and a more complete representation of the bottom of the surveillance pyramid, including asymptomatic and mildly symptomatic infections that are not detected in conventional syndromic or clinical surveillance. While the added value of wastewater surveillance targeting a pathogen depends on the clinical profile of the infection with this pathogen

KEY PUBLIC HEALTH MESSAGE

What did you want to address in this study and why?

In a Dutch urban region, case studies in 2020–2022 tested if local community-level wastewater surveillance could provide comprehensive, real-time infectious disease transmission data upon acute demand from a public health (PH) service. Passive samplers for SARS-CoV-2 and monkeypox virus (MPXV) were deployed in small wastewater catchment areas to assess this method's feasibility, utility, adaptability, strengths and limitations in informing PH actions.

What have we learnt from this study?

A large increase in SARS-CoV-2 RNA concentration in wastewater was timely identified in a locality, triggering the PH service to further inform people about the virus there and offer testing for it. Omicron detection in wastewater where the first Omicron case was reported confirmed this variant's wider circulation in the community. For MPXV, wastewater data suggested no further local transmission. One case study highlighted ethical constraints.

What are the implications of your findings for public health?

The case studies suggested that on-demand wastewater surveillance using passive samplers can be a valuable and agile tool for tracking the spread of SARS-CoV-2 and MPXV at community level. The method's ability to detect emerging SARS-CoV-2 variants and early transmission patterns allowed to improve the situational awareness of the PH service and guided targeted PH interventions. Ethics and stakeholder engagement are important for the application.

(such as incubation time, severe vs mild vs no symptoms while infected, virus shedding etc.), it has been clearly demonstrated for trend-monitoring of SARS-CoV-2 [3] and as an early warning tool for poliovirus [4]. The potential added value of wastewater surveillance has also been discussed for other infections, including among others, those with monkeypox virus (MPXV), influenza virus and respiratory syncytial virus [5].

Wastewater sampling has typically been conducted at intakes of large wastewater treatment plants, using (costly) auto-sampling equipment. However, this approach is not easily applicable to small catchment areas, such as sewer manholes. Yet, such small-scale sampling could offer more rapid and agile deployment and high spatial resolution measurements, facilitating public health action aimed at specific community or facility levels, like long-term care facilities or university campuses [6]. Recently, small, cheap, easily deployable passive sampler units have been developed and validated for SARS-CoV-2 detection, making rapid 'on-demand' surveillance possible at such localised scales [6,7]. Their application in a quality-assured, standardised manner for such surveillance has nevertheless not been widely evaluated, nor has the method's feasibility, timeliness, and reliability to yield results that are interpretable and actionable for public health. This lack of evaluation potentially limits incentives for public health authorities to use or rely on this 'on-demand' surveillance.

A regional Public Health Service (PHS) in the Netherlands commenced with on-demand wastewater

surveillance to evaluate its feasibility for SARS-CoV-2 and later for MPXV in public health practice and its added value compared with traditional surveillance to inform targeted control measures to limit the spread of infectious disease. In this article, we consider the feasibility and utility, as well as the strengths and limitations of the technique through a series of pilot studies conducted in the Public Health Service Rotterdam-Rijnmond (PHS-RR) region. We also discuss the direction of future development of the tools for public health action.

Methods

Study setting

Rotterdam-Rijnmond is a densely populated urban region hosting 1.3 million people of considerable ethnic diversity. The Port of Rotterdam, the largest port in Europe, adds to the flow of diverse people to the city, and there is wide variation in socioeconomic, educational and health status in the region [8].

Pilot studies' objectives

Six pilot studies were conducted, selected based on public health needs as identified by the PHS. The objectives of the case studies were as follows: to determine the feasibility and reliability of wastewater surveillance using passive sampling for the detection of SARS-CoV-2 in wastewater (case study1, October 2021); to evaluate community transmission around the first reported case of the Omicron variant in the service area of the Rotterdam Rijnmond public health agency (case study2, December 2021); to detect and monitor

viral spread among groups of interest based on their risk profile for SARS-CoV-2 infection (case studies 3–5), with case study 3 (May 2022) focusing on displaced populations, case study 4 (September–November 2022) on university students and case study 5 (September–November 2022) on people with different vaccination rates; and to identify if there was evidence of community MPXV spread after the diagnosis of the first case of mpox (case study 6, June–July 2022).

Wastewater passive sampling methodology

A passive sampler is a plastic porous container with absorption materials inside, such as the cotton tip used for this study. Two types of passive samplers shown in Supplementary Figure S.1 in the Supplementary Information were used in these pilot studies: a torpedo (larger, for application in pumping stations, and pensive (smaller, for application in sewer lines) sampler, both obtained from D. McCarthy, Monash University, Australia [7].

The samplers were installed at the desired locations (e.g. sewage treatment plant inlet, sewer pipe or pumping station) using ropes. After being in place for 24 to 72 hours, the passive samplers were collected and taken to the laboratory. The plastic casing was removed, and the cotton swab that was inside the casing was immersed in nucleic acid extraction buffer (Nuclisens, Biomerieux, Amersfoort, the Netherlands). Nucleic acids (RNA and DNA) were extracted from the material in the cotton swabs in combination with the semi-automated KingFisher mL (Thermo Scientific, Bleiswijk, the Netherlands) purification system and tested for the presence of SARS-CoV-2 RNA (N2 gene) and for CrAssphage DNA using RT-qPCR and qPCR respectively, as prior described [3,9]. Viral concentration was quantified in genome copies per mL (GC/mL). Next generation sequencing (NGS) and data analysis were conducted as described previously [10]. Presence of signature mutations of specific VOC's was determined using droplet digital (dd) RT-PCR conducted on 5 µL of nucleic acid extract using G339D and N856K assays [9]. ddPCR was also used for MPXV DNA [11], using the dEXD51818561 research assay of BioRad, with positive controls from the gBlock described by BioRad, as well as MPXV DNA provided by Erasmus University Medical Center. In addition, qPCRs were conducted for both orthopoxvirus [12] and MPXV [13] on an aliquot of the nucleic acid extracts of the passive samplers.

Sampling locations were selected based on the specific objectives of the pilot while also taking account of the safety and accessibility of the test locations. The information on the local sewer network (map, location and diameter of sewer pipes, manholes, pumping stations, wastewater treatment systems, combined or separate sewer) was obtained from the municipality and site visits provided information on accessibility, (road) safety, fouling and ability to deploy passive samplers, sewage flow, water depth and flow direction at the sampling sites. Sewer networks in the Netherlands are typically

mazed due to the low gradient and, consequently, flow directions are not always clear from sewer maps and need to be confirmed by site visits.

Test validity and quality control

To distinguish between actual variations in SARS-CoV-2 levels and apparent variations caused by differences in the dilution of the sewage due to precipitation, extraneous waters or industrial wastewater or different amounts of material captured by the passive sampler, the results from the samplers were normalised based on levels of CrAssphage, by dividing the number of gene copies of SARS-CoV-2 by those of CrAssphage for each sample [14]. CrAssphage is a bacteriophage uniquely present in high concentrations in human faeces. The results of the separate measurements of SARS-CoV-2 RNA (N2 gene) by RT-qPCR or MPXV DNA by ddPCR and the CrAssphage DNA by qPCR can be combined to allow for a quantitative comparison of the results between different measuring points. Laboratory analyses on each sampler material were conducted in duplicate, also including positive and negative controls. Mouse hepatitis virus (MHV), an animal coronavirus, was added to monitor inhibition of the extraction and RT-qPCR.

Further quality control was based on photos and videos of the passive samplers before and after installation. Additionally, measurements were also taken at two reference locations to investigate the comparability between the 'normal' 24-hour volume-proportional sampling by automatic sampling cabinets and passive samplers. Results were evaluated at the end of each measurement week and at the conclusion of the pilot.

Evaluation of on-demand wastewater passive sampling surveillance

For each pilot study, specific attributes of a quality public health surveillance system [15] were evaluated (Table 1). Attributes focused on quantitative aspects that reflected the overall reliability of the methods used, including how well the presence of a virus or its variants observed in the targeted population was reflected in the wastewater monitoring data (sensitivity), to what extent differential viral distribution between communities or viral distribution by place and time was mirrored by the wastewater surveillance (representativeness), how adequately the wastewater sampling covered the targeted community (completeness), the percentage of passive samplers recovered and yielding adequate results and how the molecular findings compared with defined controls (validity) and the timeliness in days to get results from the wastewater monitoring. Other attributes were more qualitative in nature, evaluating the acceptability within communities, the feasibility and ease of implementation (simplicity), the flexibility based on changing demands of the public health agency, the consistency in different populations and their sewer catchments and the usefulness in informing public health action and policy.

TABLE 1

Quality attributes of wastewater surveillance that were evaluated during pilot case studies conducted in Rotterdam-Rijnmond, the Netherlands, 2020–2022 (n = 5 case studies)^a

Attribute	Outcome	Endpoint
Sensitivity	Detection of SARS-CoV-2 (including VOC) or MPXV.	A positive signal for SARS-CoV-2 and VOC or MPXV in the target area with known case(s) can be obtained by the on-demand wastewater surveillance; positivity of passive samples for SARS-CoV-2 can be confirmed as similar to the positivity of 24-hour volume proportional samples, which are used as a reference.
Representativeness	Occurrence of a reported infectious pathogen and its distribution in the population by time and place is reflected in the wastewater of that same population.	Differential viral circulation at community level observed in reported cases is mirrored by the signals in wastewater in carefully matched wastewater samples in the same geographic area, neighbourhood, or institution or over time.
Completeness	Ability to monitor the complete target community during the monitoring period.	Targeted neighbourhoods or communities can be adequately covered with passive samplers deployed at the sewer network servicing this population.
Validity	Results are trustworthy.	In the laboratory, positive and extraction controls yield a positive result, PCR blanks and negative controls yield a negative result. Percentage of placed passive samplers that are recovered and yield adequate results.
Timeliness	Rapid availability of results for implementation of timely virus spread control measures.	Three time-intervals in days are considered: (i) between agreeing on the pilot objectives among stakeholders and placement of passive samplers; (ii) between retrieval of passive samplers and receipt of laboratory test results; (iii) between an increase in the proportion of positive reported test from public health PCR testing and increase in viral circulation in wastewater.
Acceptability	Willingness of organisations or people to cooperate.	Communities affected agree or not to participate.
Simplicity	Feasibility and ease of implementation and operation.	Pilot studies can be conducted in practice within available resources (personnel, time, cost).
Flexibility	Quick implementation at various locations and potential to adapt when information needs change.	Proportion of desired locations that are successfully sampled in the desired period.
Consistency	Virus circulation is captured systematically based on circulation in the population.	Similar circulation in different populations is mirrored by results of the passive water surveillance of their respective sewer catchments.
Usefulness	Results can inform decision-making and public health action.	Was public health action implemented based on the results? (yes/no)

MPXV: mpox virus; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; VOC: variant of concern.

^a A total of six studies were initially envisaged but one study involving a displaced population had to be cancelled based on ethical considerations.

Each attribute was operationalised into study outcomes and endpoints as in Table 1.

Results

Details of the characteristics of each pilot, including specific objectives, hypotheses and some assessed attributes of the wastewater surveillance (i.e. sensitivity and usefulness) are summarised in Table 2. An itemised evaluation of all the attributes for each case study is presented in Supplementary Table S.1.

Case study 1 from 6 to 29 October 2021 ran for 4 weeks in three different areas of the city, where wastewater was sampled with passive samplers twice a week. This case study showed that deployment of passive samplers in the sewer via manholes and at pumping stations was feasible. The monitoring sites served population sizes ranging from ca 7,000 to 21,000

inhabitants. The CrAssphage concentrations that were recovered showed that 72 of 76 passive samplers consistently picked up human faecal material. The four passive samplers that picked up very low amounts of human faecal material were either blocked by deposition of wipes and other materials on the outside of the sampler or were moved outside of the wastewater flow (Figure S.1). As indicated in Figure 1, the correlation between the concentrations of the virus captured from wastewater by passive samplers that were deployed for 24 hours at the pumping stations and those of the composite water samples collected over the same 24 hours at the same pumping stations (n=24) was good (Pearson's $R^2=0.62$). Normalisation of the passive sampler results with CrAssphage reduced the correlation (Figure S.2). Comparing the patterns in the SARS-CoV-2 concentration observed in wastewater from different communities in the different city areas, with

the patterns in reported cases in these areas, found peaks in reported cases reflecting peaks in wastewater concentration of SARS-CoV-2. Supplementary Figure S.3 shows concurrent peaks in reported cases and wastewater in city area 3 in the week of 11–17 October and in city area 2 in the week of 25–31 October. In contrast, a high incidence of reported cases in city area 3 in the week of 18–25 October was not reflected by a high concentration in wastewater. Smaller trends in virus circulation in the community were more difficult to observe due to the variability in both wastewater and case data. An estimation of the timeliness was made based on the experience in this case study. Provided that the monitoring objectives are clear and the information on the sewer network is already available, the minimum time between start and first result was 3 days. This would be in a situation where all information and stakeholders are available, and the objectives are (pre)planned.

Case study 2 from 7 to 30 December 2021 investigated community transmission after the first case of the Omicron variant was identified in the region. Samples ($n=26$) were taken at a sewer pumping station serving the hamlet where the Omicron index case resided (ca 1,000 inhabitants), as well as a sewer pumping station in a town 6 km downstream (ca 5,000 inhabitants) and a wastewater treatment plant 5 km downstream of the town (serving ca 15,000 inhabitants). Like in the first case study, the passive samplers and the 24-hour volume-proportional samplers showed comparable results, as illustrated in Supplementary Figure S.4. All samples yielded valid results. Elevated levels of SARS-CoV-2, but not Omicron, were initially detected in the pumping station of the hamlet of the index case. Subsequently, the Omicron variant was successfully detected in wastewater, not only at the hamlet, but also downstream at the pumping station and the wastewater treatment plant of the wider community. The proportion of Omicron was generally low (average 8.0%; standard deviation 11.7%; $n=23$) but was not lower in the samples from the larger population. This indicated that, despite the isolation measures rapidly instituted for the case, wider transmission in the local area was already ongoing and further quarantine and contact tracing measures would thus no longer be successful to contain onward spread. In this case study, we aimed to be as timely as possible, achieving a time of 6 days between the decision to start sampling at a specific location and first result of Omicron assays.

Case study 3 study was abandoned due to ethical considerations and therefore no results are reported. The population was regarded as both traumatised and sensitive to stigmatisation. In addition, the housing situation involved additional parties that were reluctant to participate in this case study. It was therefore decided to not pursue this case study.

Case study 4 from 7 September to 14 November 2022, tested the hypothesis that students might be

associated with the introduction of SARS-CoV-2 to their local communities, picking up the infection during summer holidays abroad, as was suggested after the summer holiday of 2020 [16]. A sampling campaign of student houses was preplanned during the summer (2 months), including selection of student houses and corresponding sampling sites. Sampling was started in the first week of the academic year. Five student-houses, inhabited by ca 220–350 students each, were sampled 2–3 times (48 or 72 hours) per week with passive samplers ($n=20-30$ per student house). In total 140 samples were taken and 134 yielded a valid result. The samples of a week were analysed as one batch, making the time-to-result 1–5 days. Normalised SARS-CoV-2 concentrations in wastewater from the student houses were highly variable (Figure 2). Some houses, such as house 3, were consistently positive with relatively high (normalised) concentrations, while other houses, like house 4, fluctuated from negative to positive over time, with concentrations lower than the SARS-CoV-2 levels in wastewater from the larger catchment areas servicing Rotterdam. Overall, there was no consistent evidence of earlier or higher transmission in the student houses compared with the general population and the hypothesis was rejected (Figure 2).

Case study 5, from 7 September to 14 November 2022, sought to explore the relationship between vaccination coverage and SARS-CoV-2 circulation by analysing wastewater concentrations in two neighbourhoods with higher vaccination coverage ($>60\%$ 1st vaccine, $>45\%$ 3rd vaccine [8]) than three other neighbourhoods ($<45\%$ 1st vaccine, $<30\%$ 3rd vaccine [8]). The vaccination coverage was assessed in the month before the start of the sampling campaign. Neighbourhood sizes ranged from 1,300 to 29,100 inhabitants and these were monitored, depending on the sewage network at one to three sampling sites ($n=8$ sampling sites in total). Each sampling site was investigated with 2–3 passive samples per week (i.e. 29–30 samples per sampling site over the study period). Of 238 samples in total, 229 gave a valid result. The samples of a week were analysed as one batch, making the time-to-result 1–5 days. Initially, our hypothesis was that a low vaccination rate would be associated with higher transmission since immunity reduces transmission of the virus [17]. However, our findings showed similar normalised SARS-CoV-2 concentrations in wastewater of the different areas, as illustrated in Supplementary Figure S.5, and one area with high vaccination coverage even had a relatively high amount of SARS-CoV-2 in wastewater in October 2022. The similar normalised SARS-CoV-2 concentrations in the area with high versus low vaccination rate indicated that viral shedding in these areas was similar, regardless of the differences in vaccination coverage in the examined areas. Sequencing data of the wastewater samples showed that the dominant VOC at that time was BQ1, as shown in Supplementary Figure S.6, which aligned with the prevalent VOC seen in clinical samples in the Netherlands in the autumn of 2022 [18].

TABLE 2

Details of pilot case studies using passive sampling for wastewater surveillance in Rotterdam-Rijnmond, the Netherlands, 2020–2022 (n=6 studies)

Cases study (period)	Objective/hypothesis to test	Sensitivity	Usefulness
1 (6–29 Oct 2021)	Testing, if detecting SARS-CoV-2 in wastewater in different community contexts, with passive samplers is feasible and reliable.	In a locality of Rotterdam-Rijnmond large coinciding peaks were observed both in COVID-19 case numbers detected through COVID-19 testing centres in the last week of October and in SARS-CoV-2 concentration in wastewater tested on 27 October 2021. Similar positivity rate and concentrations of SARS-CoV-2 RNA in 24-hour volume-proportional water samples (taken with an autosampler) and passive samples; good correlation between the virus concentrations observed, and similar concentration-patterns over time observed in both sampler types.	The result triggered the public health service to focus information campaigns and further testing in the locality.
2 (7–30 Dec 2021)	Evaluating community transmission after the first case of the Omicron variant was identified in the Rotterdam-Rijnmond region in late November 2021.	Higher concentrations of SARS-CoV-2 were detected in the first sampling week (7, 8 and 9 December) compared with the second sampling week (28, 29 and 30 December 2021). No Omicron was identified in the first sampling week (ddPCR and sequencing) but its presence was confirmed in passive samples of 28, 29 and 30 December 2021 taken in the hamlet where the first case of Omicron had been detected by surveillance in people, as well as in a sewer pumping station and a wastewater treatment plant located downstream ^a . Numerical values of the Omicron proportion relative to overall SARS-CoV-2 (mostly Delta) were variable per sampling day and location, probably due to relatively low proportions of Omicron (average: 8.0%; standard deviation: 11.7%). The passive samplers and the 24-hour volume-proportional samplers showed comparable results as illustrated in Supplementary Figure S.4.	The occurrence of Omicron VOC at each of the wastewater sampling sites, indicated that this VOC was broadly present within the community. Authorities thus improved real-time situational awareness in the community and informed the medical and PH community about local ongoing transmission, while also concluding that quarantining and contact tracing were no longer appropriate to restrict the spread of this VOC.
3 (7 Apr–15 May 2022)	Early warning of viral introduction in displaced populations.	Due to potential stigmatisation of this population and the complexity of obtaining consent from all involved the case study was cancelled.	
4 (7 Sep–14 Nov 2022)	Determining if student houses are hotspots for the introduction of SARS-CoV-2 in Rotterdam after the summer holidays.	SARS-CoV-2 was detected at each student home, but at different positivity rates and (normalised) concentrations. CrAssphage indicated that similar amounts of human faeces were sampled in each sample. It was not possible to take 24-hour volume proportional samples ^b .	Variability of the observed concentration in sewers from the student houses hindered firm conclusions about the students being 'introducers' of SARS-CoV-2 after the summer holidays. These findings did not call for specific public health action to further contain SARS-CoV-2 spread in/from student houses.
5 (7 Sep–14 Nov 2022)	Determining if neighbourhoods with lower vaccination coverage have more current SARS-CoV-2 infections than neighbourhoods with higher coverage.	Virus circulation was shown among all the populations measured through passive sampling of the community's wastewater. Distribution of viral circulation was equal in most neighbourhoods, except for one of the neighbourhoods with high vaccine coverage, which had a high amount of SARS-CoV-2 present in October 2022. There was no explanation for why this area had higher virus-circulation. It was not possible to take 24-hour volume proportional samples ^b .	Differences in vaccination coverage were not very large, data on acquired immunity due to prior infections were not complete and information on frequency and diversity of contacts of the communities was not available. Due to limitations, the study findings as such could not support additional public health measures to stop the spread of SARS-CoV-2 at local level.
6 (27 Jun–1 Jul 2022)	Detecting local transmission of MPXV.	In the street section of a person with MPXV, the virus DNA was detected in the passive samples of the wastewater, in 20–80 copies per passive sample, both through the Orthopox qPCR and MPXV qPCR and ddPCR assay. It was not possible to take 24-hour volume proportional samples ^b .	The presence in the wastewater in the street of the case, but not in the surrounding area which was also studied by on-demand passive surveillance, showed proof-of-concept of MPXV detection in wastewater and indicated that there was no further local spread of MPXV. No further/additional public health action was needed.

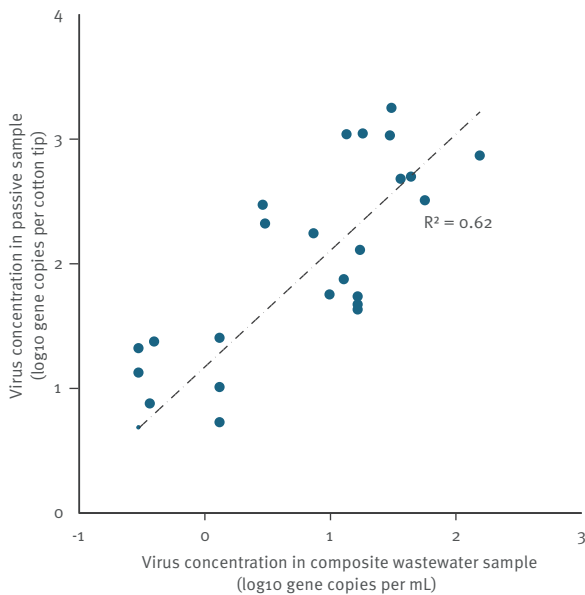
ddPCR: droplet digital PCR; MPXV: mpox virus; PH: public health; qPCR: quantitative PCR; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; VOC: variant of concern.

^a Samples processed from passive samplers could be sequenced just as well as from sewage water samples. Omicron was identified through ddPCR and sequencing.

^b Since case studies 1 and 3 showed comparable results between the two, we considered that the sensitivity of the passive samplers was demonstrated convincingly in the earlier case studies where sampling with both methods concurrently was a possibility.

FIGURE 1

Correlation between SARS-CoV-2 concentrations from 24-hour passive samplers (y-axis) and the 24-hour volume-proportional wastewater samples for virus capture from wastewater (x-axis)



In the case study 6 from 27 June to 1 July 2022, when the introduction of MPXV in the PHS-RR region by a traveller was reported, the question was whether there was wider transmission than the reported cases. Dedicated passive samplers were deployed in the sewer at the end of the street section where a documented person with MPXV resided and in two neighbouring areas. The first series of passive samplers were installed within 2 days of receipt of the request. Over the course of 1 week, three successive passive samplers collected the wastewater from a few hundred residents, each for 48 or 72 hours. All samples were valid. The samples of this week were analysed as one batch, making the time-to-result 1–5 days. MPXV presence was detected in the passive samples through a combination of qPCR assays for orthopoxvirus and MPXV assays and was further confirmed by additional ddPCR assay for West African MPXV. This demonstrated that MPXV was detectable in wastewater using passive samplers downstream of a reported case. The same methodology was repeated for a second person with MPXV, in another street in a different city area, yielding similar results (data not shown).

Discussion

We evaluated the attributes of a quality surveillance system in six pilot studies that were conducted during real-time, community-level infectious disease transmission events of SARS-CoV-2 and MPXV in the Rotterdam-Rijnmond region of the Netherlands. While one study had to be cancelled, for the remainder, once the aim and objectives of sampling were agreed between the PHS and key stakeholders, the underground sewage catchment areas were rapidly aligned with the above-ground location, and deployment of passive samplers was feasible at the various locations and in

the contexts selected, including street-level communities, neighbourhoods and student residences. Passive samplers were generally installed within a day after the request was made by the public health service for acute case studies (1, 2 and 6) and on defined days for pre-planned case studies (4 and 5) and although some passive samplers were initially lost or yielded inadequate results due to clogging or movement outside the wastewater flow, adjustments to the attachment process proved successful (data not shown). Sampler losses were minimal and the feasibility of the method at a practical level was confirmed.

In terms of quality assurance, sample results were only regarded valid when positive, negative and inhibition controls showed valid results, technical duplicate analyses showed comparable results and CrAssphage indicated that human faecal material was captured by the passive sampler. A key element of passive sampling is the need to relate the concentration of the target virus to the amount of human faecal wastewater monitored. CrAssphage normalisation was conducted for SARS-CoV-2 and passive samples yielded comparable results to 24-hour volume-proportional composite wastewater samples from pumping stations, further confirming the sensitivity of the monitoring and normalisation process, as also shown by Schang et al. [6], who used pepper mild mottle virus as faecal normaliser. Differences in CrAssphage shedding rates may lead to higher uncertainty in the normalisation as the population that is captured by the passive sampler becomes smaller [14].

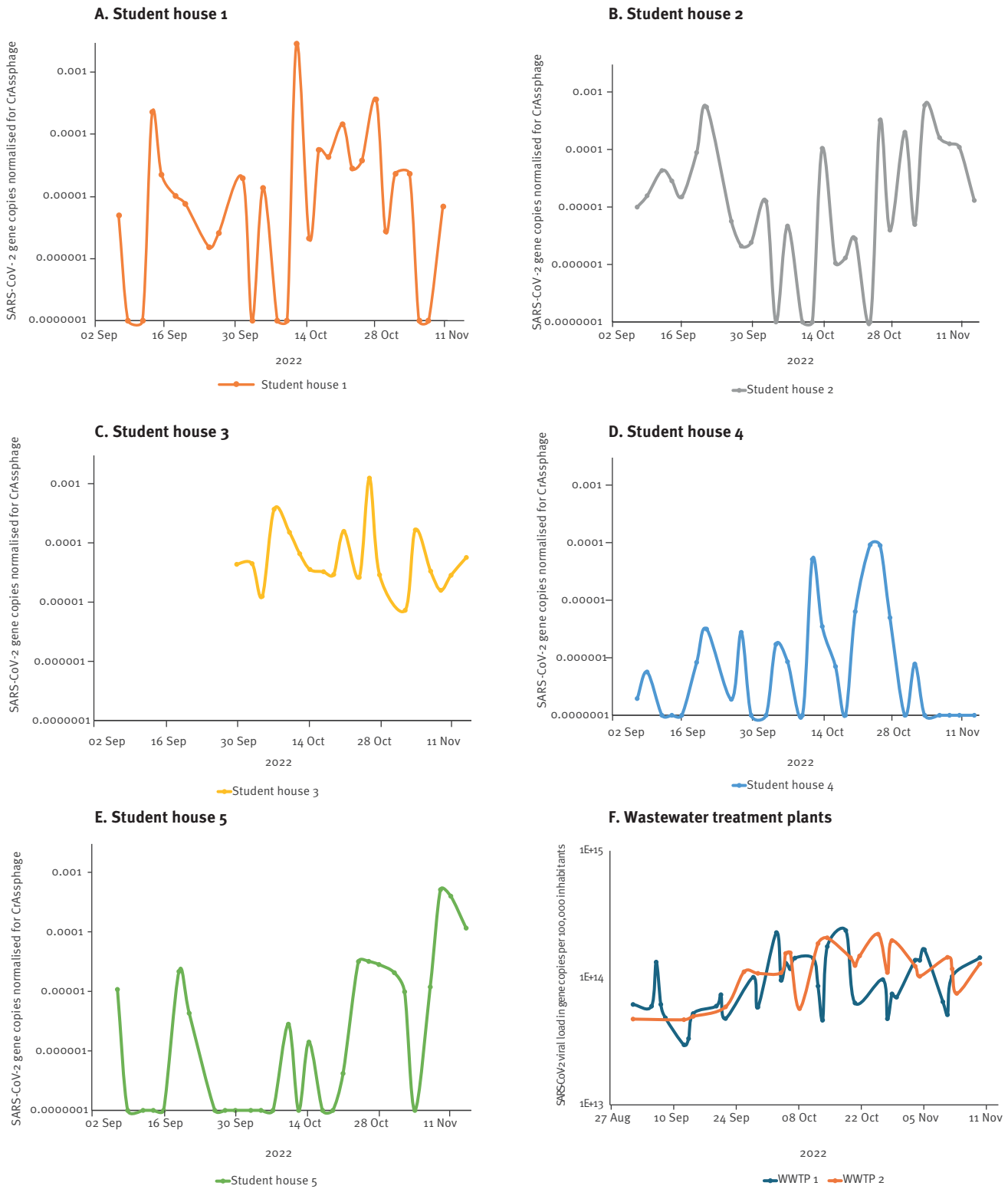
The results from wastewater testing were considered timely, as the first results (i.e. detection of the virus by PCR) were available within 6 days of deciding to commence sampling at a specific location. Sequencing results followed within 14 days. Consequently, once the PHS identifies a need for targeted surveillance in a specific area, actionable data can be obtained within a week. This timeframe is considered sufficiently fast to initiate an effective response in standard practice.

In each case study, results from wastewater monitoring were interpreted in the context of wider public health surveillance systems. When comparing wastewater markers and reported cases in the same neighbourhood, both datasets exhibited concurrent patterns, as illustrated in Supplementary Figure S.3, indicating a good relationship between the two measures, as reported by Pico-Tomas [19].

A large increase in SARS-CoV-2 RNA concentration in one of the neighbourhoods was detected in a timely fashion (Figure S.3, city area 2), consistent with a large increase in reported cases in the same neighbourhood. This signal triggered the PHS to deploy additional mobile testing units to the area, and targeted information campaigns to intensify awareness on testing and tracing, the need for personal protective measures, the importance of vaccination, and hygiene and isolation

FIGURE 2

Normalised SARS-CoV-2 concentration in passive samplers at student residences (case study 4) and monitoring data from the Rotterdam wastewater treatment plant obtained from the national sewage surveillance at RIVM, Rotterdam-Rijnmond, the Netherlands, September 2022–November 2022



RIVM: Rijksinstituut voor Volksgezondheid en Milieu (National Institute for Public Health and the Environment); WWTP: wastewater treatment plant.

and quarantine measures. Other successes included the early detection of the Omicron variant in the community sewers adjacent to the location where the first imported case was diagnosed in Rotterdam-Rijnmond. Evidence that community circulation was already ongoing allowed authorities to improve real-time situational awareness in the community and to inform stakeholders about local ongoing transmission, while also concluding that a ramping up of control measures would no longer be timely and effective.

In contrast, while MPXV was detected in the wastewater downstream of the home of a reported case, no detection was observed in surrounding areas, indicating no further local transmission. In this research effort, the selection of the sewer in the street of a known case was done to determine if MPXV was detectable in passive wastewater samples, rather than suggesting this as a common site for MPXV monitoring.

Sampling at student houses did not show high or early circulation of SARS-CoV-2 after the 2022 summer holidays. In the autumn of 2022 SARS-CoV-2 was detected in similar concentrations in wastewater of city areas with either higher or lower vaccination coverage. This observation is interesting against the background that immunity reduces transmission of the virus. Other observations on potential differences in these communities that may have affected SARS-CoV-2 transmission, such as the frequency and diversity of contacts [17], or acquired immunity due to previous infections with SARS-CoV-2, were difficult to make. This was because it was not possible to get an exact match between the population served by the sampled sewer network and the population for which vaccination or demographic data were reported in the available reporting systems [8]. Generally, the resolution of the vaccination rate or demographic data was lower than that of the sewer monitoring data. This makes conclusions from this case study difficult to draw.

A clear advantage of passive sampling over other forms of wastewater sampling is that it provides a high-level spatial and temporal resolution, allowing to study small or localised populations and, for example, locations that may be underserved by healthcare services or where residents do not otherwise access services. This study showed wastewater sampling can be done at the level of a student house with 220–350 residents. On the other hand, zooming in on small populations may pose certain ethical and operational challenges [20], which was reflected in two of our case studies. In case study 3, cooperation was sought to survey wastewater at locations where a displaced population was housed but local stakeholders did not agree, arguing that detection of SARS-CoV-2 in wastewater from the residential sites and related control measures such as lockdowns, isolation and/or quarantine, could lead to further stigma and trauma for residents. This pilot study was subsequently abandoned. In case study 5, it appeared that the rope anchoring the sampler inside

the manhole had been cut. The context in which this occurred was unknown, but it may have indicated resistance among the local population to wastewater surveillance.

Sewage surveillance in the Netherlands complies with the legal frameworks for privacy, and independent assessment at national level has confirmed that medical ethical approval is not required. We ensured that in our case studies, each sewer receives contributions from a minimum of 20 households/houses and findings could not be traced back to individuals, as suggested by Hruđey et al. [21]. Discussion about the ethical and juridical implications of sewage sampling are still ongoing in the Netherlands [22]. To address ethical concerns and facilitate early targeted deployment, engagement with local populations and close collaboration between public health, environmental and clinical stakeholders in advance or as early as possible in the process is recommended. Outcomes can be optimised: protocols should specify the necessity for sampling (such as outbreak response and health benefits); include strict criteria for data sharing (e.g. selective sharing of clinical data while ensuring patient anonymity); the timing and placement of passive samplers; sampling method to be applied and how results would be used.

A further limitation at the small area level is the daily fluctuations in people's behaviour and virus shedding, water flow and viral concentration, which may lead to substantial variability (Figure 2). To control for this, technical duplicates and a relatively high measurement frequency (such as three 24-hour periods per week) is recommended for good coverage of short-term trends and early warning (and some robustness for missing data points). Where data needs were more semiquantitative, such as for the local transmission of Omicron or MPXV, the variability is less of a concern. In addition, boundaries of the wastewater catchment area were not always distinct and mixing from other sewage systems is possible. Detailed knowledge of the sewage system and catchment area is always key. Similarly, people travel for work or school from their residential area, so are not always represented via their residential sewers.

Conclusion

Our study confirmed the feasibility and effectiveness of passive wastewater sampling for tracking the spread of SARS-CoV-2 and MPXV at the community level. We detected increased circulation of these viruses through surges in their nucleic acid concentrations in wastewater. This method provides a valuable tool for tracing outbreaks in a way that is simple and accessible. Passive samplers can be deployed rapidly and at many different places, yielding an agile, on-demand infectious disease surveillance system that can monitor at the desired resolution. Where the public health rationale for its application is clearly specified, agreed, and meets ethical guidelines, passive sampler technology has wide application in similar contexts across Europe

where most of the population is linked to a sewage network. Despite our efforts to carefully match the area/population of the data obtained ‘above-ground’ (positive tests, vaccination rates, demographics) and ‘below-ground’ (wastewater monitoring), it is important to recognise that differences in resolution and areas may hamper a direct comparison. As the technology continues to evolve, it could be extended to other viruses, including respiratory viruses (e.g. influenza, respiratory syncytial virus) and enteric viruses (e.g. polio, entero, noro, hepatitis A and E virus), as well as bacteria (*Campylobacter*, Enterohaemorrhagic *Escherichia coli*) and antimicrobial resistance. Optimal stakeholder engagement and consideration of ethical and legal issues, especially on local sewage sampling, will be essential to ensure political and social acceptance.

Ethical statement

This was an ecological study of pathogens in wastewater using data collected in Rotterdam. The analysis was based on population wastewater samples that were not traceable to individuals and no investigations of individual human subjects were conducted. Ethical approval was therefore not required for the study.

Funding statement

This study was funded by Public Health Service Rotterdam-Rijnmond, Rotterdam; Public Health Service Amsterdam, Amsterdam; Partners4UrbanWater, Nijmegen; IMD, Apeldoorn; STOWA, Amersfoort and the Water Technology Topconsortium for Knowledge and Innovation, all in the Netherlands.

Use of artificial intelligence tools

None declared.

Data availability

Requests to access or use anonymised case data will be addressed on a case-by-case basis. Wastewater virus data are available upon request.

Conflict of interest

None declared.

Authors' contributions

- Emma Besijn: study design, interpretation of the results, writing the manuscript, reviewing the manuscript.
- Jane Whelan: study design, interpretation of results, writing the manuscript, reviewing the manuscript.
- Paul Bijkerk: study conception, study design, interpretation of the results, writing the manuscript, reviewing the manuscript.
- Gregorius J. Sips: interpretation of the results, writing the manuscript, reviewing the manuscript.

- Jeroen Langeveld: study conception, study design, data collection, data extraction, production of figures, interpretation of the results, reviewing the manuscript.
- Ray W. Izquierdo-Lara: data analysis, interpretation of the results, reviewing the manuscript.
- Elvira van Baarle: study design, interpretation of results, reviewing the manuscript.
- Remy Schilperoort: study design, data collection and curation, data extraction, production of the figures, interpretation of results, reviewing the manuscript.
- Marion Koopmans: study conception, interpretation of the results, reviewing the manuscript.
- Miranda de Graaf: study conception, study design, data analysis, interpretation of the results, reviewing the manuscript.
- Gertjan Medema: study conception, study design, data analysis, production of figures, interpretation of the results, writing the manuscript, reviewing the manuscript.
- Ewout Fanoy: study conception, study design, interpretation of the results, writing the manuscript, reviewing the manuscript.

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5. Uitbraak Hepatitis A virus in Amsterdam

Nederlandse samenvatting

Het onderzoek van de Jong et al. (2024) onderzocht de toepasbaarheid van rioolwatermetingen met passieve samplers om een uitbraak van hepatitis A-virus (HAV) op een school in Amsterdam te volgen. In oktober 2022 werden vijf HAV-gevallen geïdentificeerd op een school, en rioolwatermonitoring in de wijk rond de school werd toegepast om te volgen of er verdere overdracht van HAV was of dat de maatregelen die de GGD nam om de uitbraak te bestrijden effectief waren. Dat was vooral relevant omdat jonge kinderen symptoomloos drager kunnen zijn, maar het virus wel over kunnen dragen. Passieve bemonsteringsapparaten verzamelden rioolwatermonsters die werden getest op HAV-RNA om te zien of het virus nog rondging. Positieve rioolwatermonsters werden ook gesequenced om overeenkomsten met de uitbraakstam vast te stellen.

Belangrijke bevindingen:

Detectie-efficiëntie: In 25% van de 88 rioolwatermonsters werd HAV-RNA gedetecteerd, waarmee zowel bekende als stille (niet-gerapporteerde) circulatie van HAV effectief werd gedetecteerd.

Virusidentificatie: Sequencing bevestigde de aanwezigheid van de uitbraakstam (subgenotype IB) in het rioolwater tot februari 2023, waarna een andere stam (subgenotype IA) opdook, wat wijst op voortgezette, maar niet-gerelateerde HAV-circulatie.

Implicaties voor de ziektebestrijding: Rioolwatermonitoring op basis van passieve bemonstering bleek een waardevol hulpmiddel bij uitbraakbestrijding, waarmee vroege detectie en tracking van virusuitscheiding mogelijk was, zelfs bij asymptomatische gevallen.

De studie concludeert dat deze methode een veelbelovende, kosteneffectieve en niet-invasieve surveillancetool is voor het opsporen en beheersen van HAV- en mogelijk andere infectieziekte-uitbraken.

RESEARCH

Open Access



Use of passive samplers as sewage surveillance tool to monitor a hepatitis A outbreak at a school in Amsterdam, the Netherlands, Oct 2022 – March 2023

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Abstract

Background Enteric hepatitis A virus (HAV) infections during childhood are often asymptomatic but may cause severe illness in adults. To improve public health surveillance we assessed the applicability of sewage monitoring during an HAV outbreak at a primary school.

Methods Between October 19 and December 27, 2022, five symptomatic HAV cases were notified to the Public Health Service Amsterdam; all attended the same primary school. Passive samplers, small absorbent tools, were deployed in sewage near the school from November 14, 2022, to March 22, 2023. The absorbents were subjected to RNA extraction, HAV PCR testing, and, if positive, sequencing. PCR and sequencing were also performed on plasma and feces samples of HAV cases.

Results In 22 out of 88 (25%) of sewage samples, HAV RNA was detected. All HAV-RNA-positive sewage samples until 8 February 2023 were subgenotype IB, matching the strain detected in all cases. Another strain of HAV (subgenotype IA) was detected in sewage from 15 February 2023 onwards, without associated cases.

Conclusions Passive sampler-based sewage monitoring is an effective method to rapidly detect HAV shedding linked to diagnosed cases. It detects unnoticed viral infections and allows monitoring of outbreaks. This suggests that passive sampler-based monitoring is a promising tool supporting the public health response during HAV and other outbreaks.

Keywords Sewage sampling, Passive samplers, Hepatitis A virus, Outbreak detection, Sewage monitoring

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Background

Hepatitis A virus (HAV) is the cause of acute hepatitis A infection. During early childhood the symptoms can vary from asymptomatic to mild disease, but in adults it may cause severe illness with jaundice and liver failure that, in rare cases, can be fatal [1]. In 2021, 3,864 HAV cases were reported by 30 countries in the European Economic Area; people 45 years of age or older accounted for almost a third of the cases [2]. Transmission mainly takes place via the faecal-oral route via person-to-person contact and through consumption of contaminated food or water [3, 4]. The incubation period of hepatitis A ranges from 15 to 50 days with a mean of 30 days [5]. HAV infection is a notifiable disease in the Netherlands; its incidence is low, with 85 cases reported in 2022 [6] and a considerable proportion occurring in men who have sex with men [7]. It can be contracted by unvaccinated residents travelling to high or intermediate endemic countries. Upon return to the Netherlands HAV infection can result in silent transmission among children, due to a predominantly immune-naïve population regarding hepatitis A virus, a relatively long incubation period and an often asymptomatic course of infection. This may result in HAV-clusters that pose health threats for specific risk groups such as people at occupational risk of exposure [8]. These include unprotected staff in schools or child day care centres who have not been vaccinated or not previously been exposed to the virus. The risk of infection is increased in children who travel to endemic HAV countries, often for visits to family in their parents' countries of birth [9, 10].

Sewage monitoring using passive samplers is a method for tracking and analysing the presence of various substances such as genomic material of pathogens in sewage. The first passive sampler described was a folded gauze attached to a string which was submerged in a sewer to detect *Salmonella typhi* [11]. In addition to gauze, electronegative membranes and cotton swabs are considered appropriate absorbing material to be placed within passive samplers [12]. Passive samplers absorb faecal matter, including viruses, from the sewage and they are strategically placed within sewer systems to collect sewage samples over time as introduced during the coronavirus disease (COVID-19) pandemic [13]. This monitoring approach might also detect silent transmission of other infections, such as HAV.

In October 2022 three Dutch children attending the same school in Amsterdam were diagnosed with HAV; they had recently returned from a visit to an HAV endemic country. To monitor potential ongoing transmission, sewage monitoring around the school was initiated. This study focuses on the application of this targeted sewage monitoring, using passive samplers, to detect ongoing circulation of HAV.

Methods

Setting of the study

On 18 October 2022 a case of HAV was notified by an Amsterdam hospital to the Public Health Service (GGD) Amsterdam. After source and contact tracing, a second and third case were notified on 20 October 2022 and a fourth case on 3 November 2022 by the Regional Laboratory of the GGD Amsterdam. A fifth case was notified by the same hospital on 27 December 2022. All five cases were attending the same primary school. No faeces of other children at the school was collected to monitor transmission, as this was not a standard procedure in the outbreak protocol.

Case definitions

A suspected case was defined as a person attending or working at the primary school with at least one of the following symptoms after 8 October 2022: jaundice, discoloured stool, dark urine, fever, loss of appetite, nausea, diarrhoea, fatigue and abdominal pain. A confirmed case was defined as a person with immunoglobulin M (IgM) anti-HAV detected in the serum, or a positive polymerase chain reaction (PCR) of faeces or plasma, and with reported fever or jaundice.

Specimen collection

EDTA-plasma of two suspected cases was obtained on 17 October 2022 (case #1) and on 22 December 2022 (case #5) by the local hospital and faeces specimens of three suspected cases were collected on 20 October 2022 (cases #2 and #3) and 3 November 2022 (case #4) by the GGD for routine HAV PCR diagnostic testing. All PCR positive specimens were sent to the National Institute for Public Health and the Environment (RIVM) for sequencing analysis.

Outbreak investigation

Confirmed cases and their parents or guardians were interviewed by telephone by trained nurses using a standardized questionnaire on the day their positive test result became available, or the next day. The questions included a list of symptoms experienced, HAV vaccination status, visited locations, history of recent travel abroad, mapping of the family situation and attendance to the school main building or annex building. The GGD regularly contacted the school during the outbreak to enquire if other children had developed symptoms that could be related to HAV infection.

HAV sewage monitoring methods

On 9 November 2022 it was decided to employ sewage monitoring. After city sewage employees shared the map of the city sewage flows, the sewage sampling locations

were identified that same day. HAV sewage monitoring using 3-D printed torpedo passive samplers with cotton tips (Fig. 1) was implemented on 10 November 2022, 23 days after the notification of the first case, until 21 December 2022 in district X and Y (Fig. 2) in two sewer manholes that serviced the two buildings of the school and two sewer pumping stations that serviced the city area ($\pm 20,000$ residents) around the school of the cases [14]. When a new HAV case (case #5) was notified to the GGD Amsterdam on 27 December 2022, the sewage

monitoring was expanded to district Z from 18 January 2023 until 22 March 2023 with one sewer pumping station near the home address of case #5 and one wastewater treatment plant that collected sewage of both the school and the residence of case #5. In the first week one passive sampler was deployed per manhole and sewer pumping station for 96 h; after the first week passive samplers were deployed for 48 h. When collected, samples including torpedo and the passive sampler material (inside a Zip bag) were transported to the laboratory of KWR Water

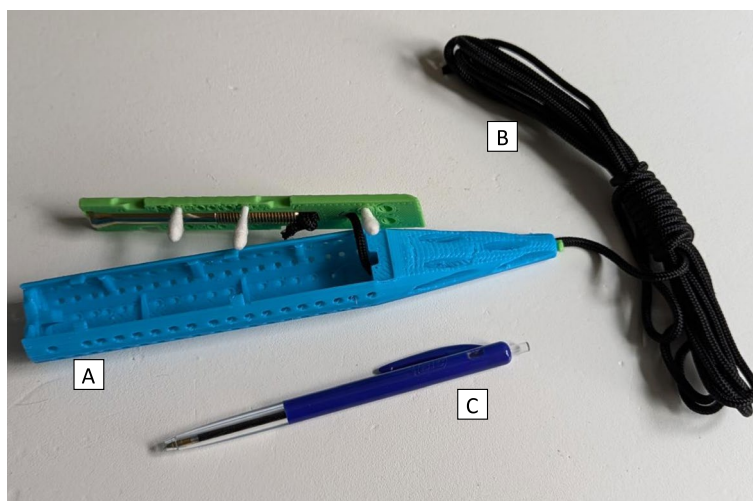


Fig. 1 Passive samplers used for sewage sampling around Amsterdam school, the Netherlands, November 2022 – March 2023. **A** 3-D printed torpedo passive sampler with cotton tips **B** Suspension cord **C**: Pen for reference

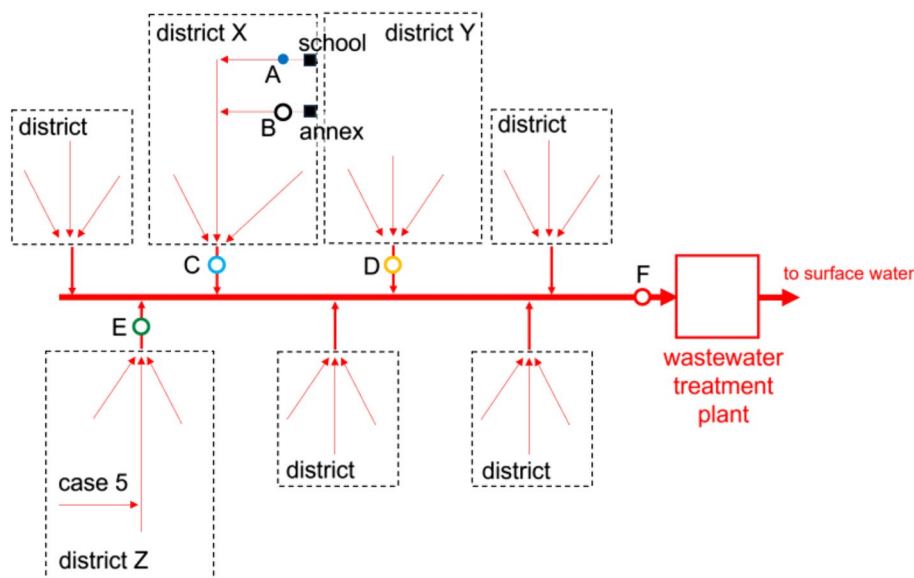


Fig. 2 Schematics of sewage sampling locations around Amsterdam school, the Netherlands, November 2022 – March 2023. **A** Sewer manhole directly downstream of the school main building. **B** Sewer manhole directly downstream of the annex building. **C** Sewer pumping station district X. **D** Sewer pumping station district Y. **E** Sewer pumping station district Z. **F** Wastewater treatment plant (influent)

Research Institute (Nieuwegein, Netherlands) on ice and processed the same day. All HAV PCR positive sewage extracts were sent to the RIVM for sequencing analysis.

Laboratory analysis

Diagnosics human specimens

Routine HAV PCR testing was performed on blood (two) and faeces (three) specimens of suspected cases using specimen type specific protocols. In short, for faeces specimens ribonucleic acid (RNA) was extracted from 250 µl faeces suspension using the Nordiag Arrow (Isogen Life Science, De Meern, the Netherlands) according to manufacturer's instructions. For EDTA-plasma 200 µl of plasma was used for RNA extraction using TriPure Isolation Reagent (Roche Diagnostics, Germany) according to manufacturer's instructions, spiked with 5 µl of Phocine herpes virus (PhHV) as an internal control. The residual pellet was dissolved in 500 µl 0.2 u/ml T10-RNasin (Sigma Aldrich, USA). From extracted RNA via both methods, 5 µl was used in a hepatitis A real-time PCR on a Rotor Gene Q Real Time PCR system (Qiagen), using the Quantinova Pathogen + IC kit (Qiagen, cat.no. 208652) using primers HAV F-San6 (5'-GCTCTCCCC TTGCCCTAG-3'), HAV R-San4 (5'-TCCCCAATTTAG ACTCCTACAGC-3') and HAV probe San12 ([FAM]-CGGGGTCAACTCCATGATTAGCATGG- [15]) according to manufacturer's protocol. Resulting S-curves were visually checked by a trained medical molecular microbiologist and the final diagnostic result clinically authorized by the medical microbiologist.

Analysis sewage samples

The cotton swab (still containing a small volume of wastewater) from the passive samplers was placed in 1.5 ml lysis buffer from the Biomerieux Nuclisens kit (Biomerieux, Amersfoort, the Netherlands) and vortexed four times for four seconds and the lysis buffer was transferred to a 2 ml centrifuge tube. To the tube with the cotton swab 1.5 ml fresh lysis buffer was added and vortexed again four times for four seconds and the lysis buffer was transferred to a new 2 ml centrifuge tube. Total contact time in the Nuclisens buffer was 10 min. The 2 ml centrifuge tubes were centrifuged for 1 min at 13.000xg and the supernatant (2.8–2.9 mL) was pooled and processed using the semi-automated KingFisher mL Purification System (Thermo Scientific, Bleiswijk, The Netherlands) [16]. Extracted nucleic acid was eluted in a volume of 100 µl. The digital droplet reverse transcription PCR (RT-PCR) method of Persson et al. was used to quantify HAV in the nucleic acid extracts [17]. CrAssphage was used as index of the human faecal load captured by the passive sampler and quantified from the same extracts as previously described [18]. Since the amount of human faecal

matter captured can vary from (passive) sample to sample, the amount of CrAssphage captured by each passive sampler was used as index for the amount of human faecal matter captured. The observed concentration of HAV RNA in the extract of the passive sampler was normalized by dividing this through the observed CrAssphage concentration in the same extract. RT-ddPCR reactions performed on PCR grade and RNase free distilled water and RNA extracted from PCR grade and RNase free distilled water were used as negative controls. RT-ddPCR performed on both approximately 500 and 50 genome copies of HAV were used as positive controls. Assays were performed in technical duplicates, each in 20 µl reaction volumes containing the reagents from the One-Step Advance RT-ddPCR for probes: 5 µl RTddPCR One-Step Advanced Supermix, 2 µl Reverse Transcriptase, 1 µl DTT (300 mM) supplemented with 3.7 µl PCR grade and RNase free water (Applied Biosystems, Fisher Scientific, Landsmeer, the Netherlands) and 5 µl sample-RNA. Primers and probe were the same as Persson et al., 2021, and were added as 1 µl HepA-F (500 nM), 1.8 µl HepA-R (900 nM), probe 0.5 µl FAM-labeled HepA-P (250 nM) with minor groove binder-eclipse quencher. The BioRad QX200 droplet generator partitioned sample-RNA and reagents in (at least) 77,000 droplets. The temperature profile used for RTddPCR was as follows: 60 min. 50 °C, 10 min 95 °C, 50 cycles with 30 s. 95 °C and 1 min. 60 °C followed by 10 min. 98 °C, 30 min. 4 °C and hold at 12 °C. Samples were scanned using the QX200 system (BioRad) and analyzed using the QuantaSoft-Analysis software (BioRad). For each sample, the number of negative and HAV ddPCR positive droplets were recorded and used to determine the HAV concentration. HAV RNA (kindly provided by ViroScience at Erasmus Medical Center, Rotterdam, the Netherlands) was used as positive control with each batch of samples, in two concentrations, yielding at least 500 and 50 positive droplets. PCR blanks (with 5 µl PCR grade water as sample) were run with each sample batch. When positive for RNA of HAV (when at least 1.2 genome copies per reaction), 25 µl of nucleic acid extract of the sewage samples were shipped to RIVM for sequencing.

Sequencing human specimens and sewage samples

Sequencing analysis was performed on nucleic acid extracts originating from HAV PCR positive blood and faeces specimens and sewage samples (22). For sequence based typing we routinely use the HAVNet protocol [19]. Briefly, cDNA synthesis and nested-PCR were used to obtain a 460bp fragment of the VP1-P2A junction, which was sequenced by Sanger sequence analysis. For serum samples and faeces, the protocol was used exactly as described. For sewage samples the yield of fragment

Table 1 Characteristics of hepatitis A virus cases attending Amsterdam school, the Netherlands, October—December 2022

Case	Age (years)	Sex	Region of origin ^a	Recent ^b travel history	Symptomatology	HAV vaccination status	Date start symptoms	Diagnostics		HAV typing
								Specimen taken	Specimen result	
1 (index)	5–8	F	Eastern Mediter-ranean	Somalia	Jaundice, dark urine, discolored stool, fever, loss of appetite, nausea, fatigue	Not vaccinated	15 Oct 2022	17 Oct 2022	18 Oct 2022	Type 1B
2	0–4	F	Eastern Mediter-ranean	Morocco	Jaundice, abdominal pain, discolored stool	Not vaccinated	Unknown	20 Oct 2022	20 Oct 2022	Type 1B
3	5–8	M	Eastern Mediter-ranean	Morocco	Fever, diarrhea	Not vaccinated	26 Sep 2022	20 Oct 2022	20 Oct 2022	Type 1B
4	9–12	F	Eastern Mediter-ranean	Somalia	Fever	Not vaccinated	15 Oct 2022	3 Nov 2022	3 Nov 2022	Type 1B
5	5–8	M	Eastern Mediter-ranean	None reported	Jaundice, dark urine, discolored stool, loss of appetite, nausea	Not vaccinated	19 Dec 2022	22 Dec 2022	27 Dec 2022	Type 1B

^a Continent of birth of at least one parent^b In last three months

was not enough for Sanger sequencing. These samples were analysed on the Oxford Nanopore platform, with sequences assembled using Canu, and trimmed to the 460bp fragment between the primers used for amplification. For Oxford Nanopore based sequence analysis we used the ligation sequencing kit (SQK-LSK109) with the Native Barcoding Expansion 96 kit (EXP-NBD196), and we used flowcell FLO-MIN106D R9.4.1. Resulting sequences were compared by alignment and phylogenetic analysis with sequences from reference strains from known geographic origin.

Results

Description of cases

From 18 October 2022 to 27 December 2022, five hepatitis A cases were associated with the outbreak. All five cases were confirmed by HAV PCR testing and they were all infected with the same strain of subgenotype IB (Table 1). The age range of the cases was 0 to 12 years. The five confirmed cases all attended different school classes; three of the five cases also attended the annex of the school. Case #1 and #4 were siblings, and cases #2 and #3 were also siblings. None of the five confirmed cases had been vaccinated against HAV and four confirmed cases reported recent travel to Somalia or Morocco, both known to be HAV endemic [20]. For cases #1–3, their travel occurred before the incubation period, making it unlikely to be the source of infection. Case #4 was identified through source and contact tracing related to case #1. It was discovered that case #4 had experienced a prior fever, which had not been addressed as it resolved on its own. This case was recognized as the missing link to Somalia, likely serving as the initial source of the outbreak.

Sewage monitoring

HAV sewage monitoring around the school (Fig. 2) from 10 November 2022 until 22 March 2023 resulted in the collection and analysis of 88 sewage samples. Of these, 22 (25%) were positive for RNA of HAV. In the first weeks of the sewage monitoring HAV RNA was only detected in low concentrations in sewage of the sewer manhole of the annex building, later also in the sewage of the sewer manhole of the school main building and sewer pumping stations in districts X, Y and Z (Fig. 3). Eight out of 17 sewage samples taken from the sewer manhole of the annex building and one out of 17 sewage samples taken from the sewer manhole of the school main building were positive for HAV RNA. Of the sewage samples taken from sewer pumping stations in districts X and Y, two and seven out of 17 were positive for HAV RNA, respectively. Three and two out of 10 sewage samples taken from sewer pumping station in district Z and the

wastewater treatment plant were positive for HAV RNA, respectively.

Genotyping showed that the HAV RNA positive sewage samples all contained the same subgenotype IB up to 8 February 2023 and this subgenotype was indistinguishable from the outbreak strain. From 15 February 2023 onwards, the subgenotype IB was no longer detected in sewage samples, but another subgenotype (IA) was detected in 10 positive sewage samples. During the subgenotype IB outbreak, RNA of HAV was detected most frequently in the sewer manhole directly downstream of the school annex building and the sewer pumping station in district Y (both seven times), and once from the sewer manhole of the school main building. The average detected concentration of HAV RNA genome copies/CrAssphage DNA genome copies was 2.24×10^5 , with a minimum of 0.12×10^5 and a maximum of 13.06×10^5 . In the sewer pumping station in district Y the highest concentration of subgenotype IB HAV RNA was observed, from 9 until 21 December 2022. One week later, case #5 was diagnosed. Based on time, place and subgenotype we considered this case part of the outbreak. From 15 February 2023 onwards, HAV subgenotype IA was detected mostly in different sewage sampling sites: district X and Z and the wastewater treatment plant. Following the first detection of subgenotype IA strain on 15 February 2023, an increased concentration of HAV RNA was detected in five out of six sewage monitoring locations until 22 March 2023, whereafter it was decided to end sewage monitoring since no new hepatitis A cases had been notified to the GGD Amsterdam since 27 December 2022.

Microbiological investigations

Sequencing analysis of all blood and faeces specimens that were positive for HAV RNA identified HAV subgenotype IB strain which matched the strain of the sewage samples until 8 February 2023 (Table 1 & Fig. 3). The HAV subgenotype IB strain was genetically closely related to strains previously isolated from travellers returning from Somalia. Case #4 had returned from Somalia a couple of weeks prior to onset of symptoms, suggesting Somalia as the most likely country of acquisition. HAV subgenotype IA strain was identified in sewage samples from 15 February until 22 March 2023, but no HAV suspected cases or diagnoses with an epidemiological or geographical link to the school were notified to the GGD during this period or in the following months.

Outbreak control measures

GGD Amsterdam issued a letter to the school management on 24 October 2022 informing them, that a pupil had been notified with HAV. The school forwarded this letter to all parents or guardians of the pupils. Parents,

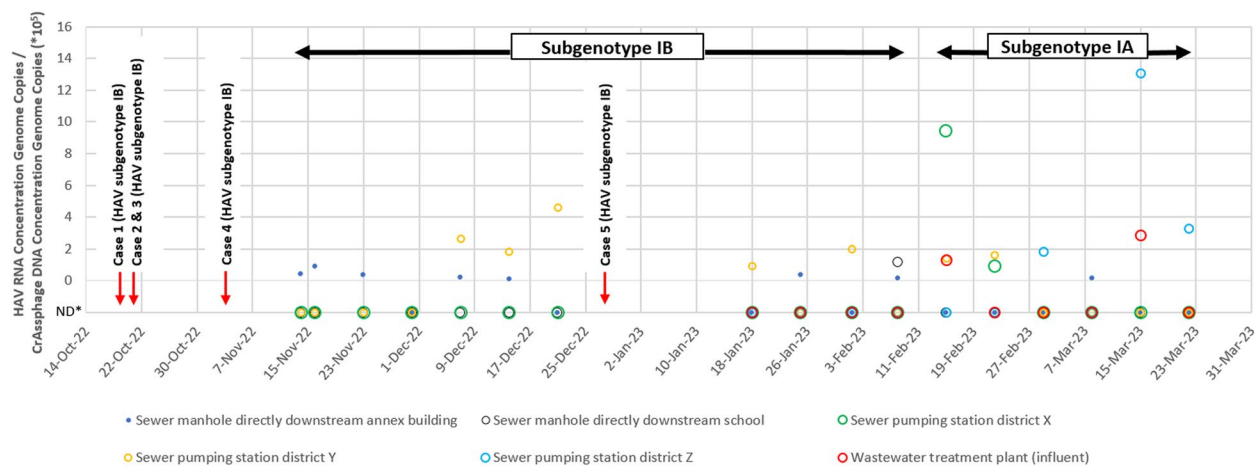


Fig. 3 HAV concentrations and strain typing in sewage samples around Amsterdam school, November 2022 – March 2023. *Non-Detect

guardians and school staff were advised to be vigilant for HAV related symptoms in their children and pupils and to contact the general practitioner should children display any symptoms. Parents or guardians were also informed that the majority of HAV infections is asymptomatic and measures of transmission prevention were provided. Subsequently, GGD Amsterdam organized an HAV vaccination campaign at the school on 26 October 2022. Out of 234 pupils 33 (14%) were vaccinated; none of the school staff wanted to be vaccinated. An information session at the school was organized on 6 March 2023 about vaccines, their benefits, and to dispel any misconceptions that might exist. This did not lead to additional vaccination uptake among pupils and school staff.

Discussion

This study demonstrates to our knowledge for the first time the application of passive samplers to detect HAV RNA in sewage while monitoring an outbreak. The described sewage monitoring approach enabled public health authorities to detect and sequence HAV RNA in sewage around the school of the first case and to match the identified HAV strains in sewage samples with the human specimens of all identified cases. Prior studies in endemic regions conducted sewage monitoring as a surveillance tool to track HAV circulation and other studies detected HAV in sewage using grab sampling: a method that involves sewage collection at one point in time [21–32]. Here, we introduced and evaluated another approach: passive sampler-based sewage monitoring that can detect HAV for a defined period of time. The sewage data showed that silent transmission of HAV was ongoing after the identified outbreak, and indicated that the outbreak was over by showing that the outbreak HAV strain disappeared from the sewage around the school

and the city district. Interestingly, the sewage monitoring detected circulation of another HAV strain (subgenotype IA) indicating silent transmission of another HAV strain in that city area. Identifying the source of this strain was outside the scope of this outbreak investigation, but the positive signal from the sewage could have been combined with other epidemiological approaches to benefit infectious disease control.

The described sewage monitoring technique shows the potential of using passive samplers in sewage to become an added value for outbreak investigation and management of hepatitis A or potentially other infectious diseases by offering a relatively new, non-invasive and near-real-time approach to monitor (re)emerging infectious diseases. Compared to autosamplers that collect wastewater, passive samplers are simple in design and operation, are easy to deploy and require minimal maintenance. In line with our findings, studies have shown that passive samplers are a powerful tool for viral detection and can provide robust information for infectious disease control [33, 34]. Passive sampler-based sewage monitoring is a promising tool for future outbreak responses because public health authorities can take prompt action to control the spread of the disease, such as implementing increased testing, contact tracing, isolation measures and vaccination campaigns when the pathogens are detected in sewage. It can also potentially lead to improved targeting of public health interventions: sewage monitoring using passive samplers can provide information on the location of outbreaks, help to identify areas with more cases (hot-spots) and monitor trends over time [35, 36].

The organized vaccination campaign targeting the pupils and staff of the school resulted in a low vaccination uptake of 14%. The pre-outbreak vaccination coverage

was estimated to be very low by the youth health care physician affiliated with the school. Next to hesitancy of the school to participate in the promotion of vaccination, the relatively short window for campaign announcement, coinciding with the autumn holidays from 15 till 23 October 2022 could have attributed to the low uptake. Possibly the education efforts of the GGD were not appropriate or suitable, or not timely, for the parents or guardians of the pupils of this school. We think the effect of vaccination on the course of this outbreak at the school was probably minimal. Low impact of vaccination was also observed in an HAV outbreak report at a British school in 2019 [37]; it suggests that outbreaks in these settings may be effectively managed without resorting to vaccination campaigns.

No faeces of pupils without symptoms was collected to monitor silent transmission as this supplementary screening tool was not a standard procedure of the local outbreak protocol. Requesting faeces of pupils for source and contact tracing may be a more direct method to detect silent transmission [38]. This could also include children who do not defecate at school. However, obtaining faeces of asymptomatic children may be a challenge, is fully dependent on informed consent of parents or guardians, and may be hard to repeat on a weekly basis for a long period. If during a potential outbreak of HAV or another (a)symptomatic infectious agent it is not possible to collect these faeces samples, sewage monitoring using passive samplers would be recommended for detecting (silent) transmission. Future studies around hepatitis A outbreaks should try to collect faeces of asymptomatic persons as well as deploy passive samplers, so that the findings of both approaches can be compared, and relative advantages of each evaluated. If the use of passive samplers is validated, and perhaps shown to be simpler and more feasible for ongoing monitoring, their use may be incorporated in outbreak protocols. Future studies could also focus on the concentrations of DNA or RNA of HAV or other infectious agents in sewage by passive samplers: how does it relate to concentrations identified in grab sampling and might this be an indication for the number of infected individuals, as is done in SARS-CoV-2 sewage monitoring [39].

Conclusions

Passive sampler-based sewage monitoring offers an effective approach for swift identification of HAV shedding, which can be directly associated with diagnosed cases. This method is adept at uncovering viral shedding from asymptomatic cases, enabling the near-real-time tracking of outbreaks and monitoring the effectiveness of interventions. Consequently, it emerges as a highly promising tool for public health authorities, empowering them

to tailor their response strategies during HAV and other infectious disease outbreaks. This is the case in not only high-income countries like the Netherlands, but also in middle- and low-income countries [32]. We recommend piloting this sewage monitoring method during future outbreaks of infectious diseases to assess the potential of passive sampler-based surveillance systems in local (resource limited) settings and contribute to more effective infectious disease monitoring and control strategies.

Abbreviations

HAV	Hepatitis A Virus
COVID-19	Coronavirus Disease
GGD	Public Health Service
IgM	Immunoglobulin M
PCR	Polymerase Chain Reaction
RIVM	National Institute for Public Health and the Environment
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RNA	Ribonucleic Acid
WPG	Wet Publieke Gezondheid

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Authors' contributions

MJ, CW, EF and GM coordinated the investigations of the outbreak. MJ, RS, JL, MS, EF and GM contributed to data collection, case information and data analysis. HV, MW and GM were involved in the laboratory investigations. MJ and MS drafted the manuscript and all authors were involved in revising the manuscript. All authors reviewed and approved the final version.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Permission of the (guardians of the) cases for diagnostics and sequencing of the human specimens was not required because the GGD has legal permission, provided by Dutch national public health law (Wet Publieke Gezondheid (WPG))[40], to process patient information for surveillance and outbreak analysis of notifiable diseases and can request microbiological laboratories to

perform additional testing (including whole genome sequencing) on available clinical materials (article 25 section 5 WPG). Therefore, medical ethical clearance for this study was not required.

Consent for publication

Not applicable, see section 'Ethics approval and consent to participate'.

Competing interests

The authors declare no competing interests.

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Een passive sampler die zojuist in het riool is geplaatst

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RIOOLWATERMETINGEN ONDERSTEUNEN GGD BIJ EEN UITBRAAK VAN GEELZUCHT OP EEN BASISCHOOL

Nu de Corona-teststraten verdwenen zijn is de Nationale Rioolwater Surveillance van het RIVM hét instrument om de viruscirculatie te blijven volgen. Maar rioolwatermetingen kunnen ook bij andere virusziekten nuttig zijn, zo bleek bij een uitbraak van hepatitis A op een school in Amsterdam.

In de Coronajaren kende het instrument van de rioolwatermetingen een snelle ontwikkeling, en het leverde een duidelijke meerwaarde voor het volgen van de virusverspreiding en de volksgezondheid. Riolsurveillance bleek ook op wijkniveau betrouwbare en nuttige informatie op te leveren over viruscirculatie [3]. Dat was aanleiding voor GGD Rotterdam-Rijnmond om nader onderzoek te doen naar de bruikbaarheid van rioolwatermetingen. Het is uitgevoerd samen met GGD Amsterdam, KWR, Partners4UrbanWater, IMD en Erasmus MC en mede gefinancierd door STOWA en TKI Watertechnologie.

Geelzucht in Amsterdam

Toen zich in 2022 een uitbraak van een hepatitis A-virus (HAV) op een school in Amsterdam voordeed, vroeg de GGD zich af of rioolmetingen hen konden helpen om zicht te krijgen op de effectiviteit van hun maatregelen. Hepatitis A (besmettelijke geelzucht) is een virale ontsteking van de lever. De ziekte verloopt bij jonge kinderen vaak ongemerkt, maar ze kunnen het virus aan volwassenen overdragen die er vaak wel ziek van worden.

De infectie komt vooral voor in (sub)tropische landen. In Nederland zijn er tussen de 100 en 200 infecties per jaar, waarvan 40 procent importinfecties: mensen raken besmet tijdens een bezoek aan landen waar hepatitis A veel voorkomt [5]. Omdat kinderen vaak asymptomatisch zijn en omdat de incubatietijd lang is (4 weken), kan het virus al een tijd ongemerkt rondwaren voor het opgemerkt wordt (stille circulatie).

Hepatitis A-virus wordt via ontlasting en opname via de mond overgebracht. Hepatitis A is een meldingsplichtige infectieziekte, artsen en laboratoria moeten een geval doorgeven aan de GGD. De GGD kan bron- en contactonderzoek doen en bestrijdingsmaatregelen instellen. Net als bij Corona kunnen rioolmetingen HAV-infecties in de school en in wijken eromheen zichtbaar maken. Bij een effectieve bestrijding zou HAV uit het rioolwater moeten verdwijnen.

De uitbraak

In oktober 2022 meldde een Amsterdams ziekenhuis een kind met geelzucht bij de GGD Amsterdam. Na bron- en contactonderzoek zijn eind oktober en begin november 2022 nog drie besmette kinderen geïdentificeerd en eind december 2022 was er een vijfde geval. Alle vijf kinderen gingen naar dezelfde basisschool, naar verschillende klassen en zowel in het hoofdgebouw als een dependance. Na genetische typering bleken alle vijf besmet te zijn met dezelfde virusstam. De zieke kinderen zijn thuisgehouden om uit te zieken en verspreiding te voorkomen. Ondertussen ging GGD Amsterdam aan de slag. Eind oktober 2022 ging er een brief naar de schoolleiding met het verzoek om ouders en verzorgers te informeren en hen te vragen contact op te nemen met de huisarts als hun kinderen symptomen van HAV zouden vertonen.



Afbeelding 1. Een passieve sampler wordt na 48 uur monsternamming uit het riool gehaald

De school stuurde deze brief door naar alle ouders en verzorgers. Vervolgens organiseerde GGD Amsterdam snel een HAV-vaccinatiecampagne op de school, waaraan slechts weinig ouders gehoor gaven (c.q. 14 procent van de kinderen). Vanwege de lage opkomst is vervolgens nog een informatiesessie voor school en ouders georganiseerd. Medio januari werd nog een zesde HAV-besmetting in dezelfde wijk gemeld. Deze persoon had geen link met de andere gevallen of de school.

Opzet rioolmetingen

In het rioolnetwerk rondom het school- en dependancegebouw zijn twee rioolputten en twee rioolgemaalenselecteerd om representatieve rioolwatermonsters te



Afbeelding 2. Digital droplet RT-PCR voor het aantonen van HAV in rioolwater

nemen. De twee rioolputten ontvingen rioolwater van de beide schoolgebouwen, de gemalen van de omliggende wijken (20.000-30.000 personen). Voor het aantonen van HAV zijn 'passive samplers' gebruikt, die meerdere dagen op een locatie hingen om ontlasting met daarin HAV uit het langsstromende rioolwater te vangen. Het betrof 3D-geprinte torpedovormige samplers met wattenstijps die in het rioolwater werden geplaatst en gefixeerd. De monsternamen liep van half november 2022 tot in maart 2023. Omdat er eind december 2022 een vijfde besmetting op de school bekend werd en dit kind in een andere wijk woonde, werden de rioolmetingen uitgebreid naar het rioolgemaal van deze wijk en naar de betreffende rwzi.

In de eerste meetweek in november 2022 is per rioolput en rioolgemaal in 96 uur één passieve sampler ingezet; in de weken daarna zijn ze 48 uur ingezet. De samplers werden op ijs naar het laboratorium vervoerd en dezelfde dag onderzocht. Het wattenstaafje uit de passieve samplers werd op dezelfde manier opgewerkt als eerder voor SARS-CoV-2 [2]. De extracten zijn met een speciale PCR-methode ingezet, de digital droplet RT-PCR (ontleend aan [4]), met positieve controles.

Als controles zijn 5 passieve samples uit november 2022 uit het rioolnetwerk van Rotterdam genomen (dus van

buiten het uitbraakgebied). In deze monsters werd zoals verwacht geen HAV aangetroffen. Extracten waarin RNA van HAV is aangetroffen werden voor sequentie-analyse naar het RIVM gestuurd.

Uit de monsters werd naast HAV ook crassfaag gemeten. Crassfaag komt vrijwel alleen in menselijke darmen voor en is hier gebruikt als maat voor de hoeveelheid ontlasting die door de passieve samplers uit het rioolwater is gevangen [3].

Resultaten

In alle passieve samples werd Crassfaag aangetroffen in redelijk vergelijkbare hoeveelheden ($5,2 \pm 1,1$ miljoen genkopieën per sampler), wat aangaf dat alle samplers vergelijkbare hoeveelheden menselijke ontlasting hadden gevangen.

In de eerste weken werd HAV alleen in het rioolwater van de dependance aangetroffen, later ook in het water van de rioolgemalen en het hoofdgebouw. 8 van de 17 samples die van november 2022 tot en met maart 2023 van het bijgebouw waren genomen waren positief voor HAV, telkens in lage hoeveelheden. Van het hoofdgebouw was 1 van de 17 samples positief (in februari 2023). Van de rioolgemalen waren 2 en 7 van de 17 samples positief voor HAV, ook telkens in lage hoeveelheden. Ook in het

rioolgemaal en de rwzi die in januari 2023 werden toegevoegd naar aanleiding van het vijfde ziektegeval werden in respectievelijk 3 en 2 van de 10 samples HAV aangetroffen, weer in lage hoeveelheden.

De genetische typering van HAV in het rioolwater bleek waardevol: van november 2022 tot begin februari 2023 lukte het om in 7 samples HAV genetisch te typeren en bleek het gedetecteerde HAV genetisch identiek aan de stam die bij de zieke kinderen was aangetroffen. Na eind december werden er geen nieuwe gevallen meer gemeld die gekoppeld waren aan deze uitbraak. Na begin februari 2023 werd deze stam niet meer in rioolwater gevonden. Dit was voor de GGD de bevestiging dat er geen stille circulatie van de uitbraakstam meer plaatsvond op en rond de school. Intrigerend was wel dat er vanaf begin februari 2023 een andere HAV-stam in het riool werd aangetroffen. In deze periode lukte het om in 4 samples HAV te typeren, telkens van dit andere type. Er kwamen geen meldingen bij de GGD binnen van mensen met deze HAV-stam. Meting en typering van HAV in rioolwater gaf dus aan dat er stille transmissie van deze andere HAV-stam heeft plaatsgevonden in hetzelfde gebied in Amsterdam.

Conclusies

De nauwe samenwerking met de GGD'en heeft laten zien dat rioolwatermetingen ook bij een uitbraak van een ander virusziekte dan COVID-19, namelijk hepatitis A, waardevolle informatie opleveren. Bij beide infectieziekten verloopt een (groot) deel van de infecties mild of symptomeloos, zeker bij jonge kinderen, waardoor ze in de reguliere surveillance makkelijk gemist kunnen worden. Een andere overeenkomst is dat een deel van de infecties ernstig kan verlopen. Daarom is het belangrijk

om de overdracht zo veel mogelijk in te perken.

Bij de uitbraak op de school in Amsterdam bleek rioolwatersurveillance waardevol om eventuele stille transmissie te kunnen volgen, en bevestigden de rioolmetingen dat de uitbraak over was. Bovendien bleek dat vroege, wekelijkse metingen met passieve samplers op vier (later zes) locaties, een efficiënte methode kan zijn om de HAV-circulatie te volgen zonder veel moeite en belasting voor de school en de betreffende woonwijken.

Genetische typering bleek in dit onderzoek noodzakelijk, zowel om te bevestigen dat met de PCR-methode daadwerkelijk HAV was aangetoond, als om te bevestigen dat de uitbraakstam in het riool was aangetroffen. Bij toeval kwam via de rioolmetingen de import en mogelijke transmissie van een andere HAV-stam aan het licht, vlak na en vlakbij de eerste uitbraak.

Voor de GGD kan rioolwateronderzoek bij een Hepatitis A-uitbraak (en mogelijk ook uitbraken van andere virale darm- en luchtweginfecties) zowel ondersteunen bij bron- en contactonderzoek als bij de bestrijding, om zichtbaar te maken of en waar er viruscirculatie 'onder de radar' plaatsvindt.

Met dank aan George Sips en Paul Bijkerk (GGD Rotterdam Rijnmond), Goffe Elsinga en Leo Heijnen (KWR), Jeroen Langeveld (Partners4UrbanWater), Ewout Fanoy (GGD Amsterdam), Bert Pasma (STOWA), J. Guldemeester (Erasmus MC) en Dave McCarthy (Queensland University of Technology, Australië).

Gertjan Medema (KWR), Remy Schilperoort (Partners4UrbanWater), Miranda de Graaf (Erasmus MC), Harry Vennema (RIVM), Maarten de Jong (GGD Amsterdam)

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SAMENVATTING

Bij een uitbraak van hepatitis A (HAV) op een school in Amsterdam zijn metingen aan rioolwater ingezet om de virusverspreiding te volgen. Via monsternamen in rioolputten en -gemalen werden twee schoolgebouwen en de wijken eromheen gemonitord. In vier maanden waren er vijf ziektegevallen. In alle gevallen betrof het dezelfde virusstam. Dezelfde HAV stam kwam geregeld in lage concentraties voor in het rioolwater, en werd na begin februari niet meer aangetroffen. Dat bevestigde dat de uitbraak van die HAV-stam over was. Wel werd er later via de rioolmetingen 'stille verspreiding' van een andere HAV-stam in dezelfde wijk aangetroffen.

6. Scabiës in studentenhuizen en instellingen

Nederlandse samenvatting

De toenemende incidentie van schurft in West-Europese landen gaat gepaard met meer uitbraken in huishoudens en zorginstellingen. Deze proof-of-conceptstudie onderzocht de effectiviteit van PCR-diagnostiek op afvalwater van wasmachines voor het snel detecteren van schurft-DNA in diverse settings.

Methode:

Afvalwater werd verzameld uit wasmachines in drie huishoudens met bevestigde schurftgevallen. Daarnaast werd afvalwater vóór en na behandeling van besmette personen met permetrine verzameld in een zorginstelling. Monsters werden handmatig en met passieve bemonstering uit de wasmachines gehaald en geanalyseerd met PCR.

Resultaten:

- Schurft-DNA werd gedetecteerd in alle afvalwatermonsters uit huishoudens.
- Zowel handmatig als passief verzamelde monsters uit de zorginstelling toonden aanwezigheid van schurft-DNA, ook na behandeling.
- Stijgende Ct-waarden na behandeling suggereerden een dalende schurft-DNA-belasting.
- Het 'meewassen' van een waszakje met passieve bemonsteringsmaterialen in de trommel van de wasmachine waarin beddengoed en kleding van geïnfecteerde bewoners wordt gewassen is een eenvoudige en niet-invasieve manier voor schurft-diagnostiek.
- Ondanks dat geen actieve mijtinfestatie werd waargenomen na behandeling, bleef DNA detecteerbaar in het wasmachinewater, wat er op wijst dat de behandeling onvoldoende effectief was of herinfectie is opgetreden.

Conclusie:

PCR-diagnostiek van waswater biedt een snelle, niet-invasieve methode om in instellingen schurft-DNA op te sporen. Deze aanpak is veelbelovend voor vroege detectie in huishoudelijke en institutionele settings en voor het monitoren van behandelingsucces. Dankzij de flexibiliteit kan deze methode een waardevol instrument zijn in volksgezondheidsinterventies bij schurftuitbraken.

Abstract submitted to ESCMID Global 2025, 11-15 April, Vienna

Abstract title: Using washing machine water to detect scabies (*Sarcoptes scabiei*)

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Presenter: G.J. Sips

Abstract

Background:

With an increasing incidence of scabies in Western European countries, the number of scabies outbreaks in household settings and residential care facilities also increases. We performed a proof-of-concept study to test the efficacy of PCR diagnostics on water from washing machines, i.e. laundry wastewater, to quickly detect scabies in various public health settings.

Methods:

We collected laundry wastewater taken from washing machines from three individual household settings with confirmed scabies to assess the feasibility of detecting scabies DNA. Additionally, we obtained pre- and post-treatment laundry wastewater during an outbreak in a residential facility, using both manual collection of water samples as well as collection via insertion of so-called passive samplers into the washing machine. PCR analysis was performed on these samples.

Results:

All collected laundry wastewater samples from household settings tested positive for scabies DNA. In the outbreak setting, both manually collected laundry wastewater samples as well as those collected via passive samplers tested positive. Notably, we observed increasing Ct-values indicative of a decreasing scabies DNA load following treatment of infected individuals.

Conclusions:

PCR diagnostics on laundry wastewater can be used to detect scabies. This opens up a variety of applications of this quick and non-invasive approach, such as early institutional and household detection of scabies and the monitoring of treatment effects via washing machine water.

7. Uitbraak griep in verpleeghuis

Nederlandse samenvatting

Op 7 september 2023 werd in een Rotterdams verpleeghuis een vroege influenza-A-uitbraak gemeld bij de GGD Rotterdam-Rijnmond. Deze uitbraak, ongebruikelijk voor de tijd van het jaar, trof 12 bewoners en 7 medewerkers. Whole genome sequencing (WGS) wees uit dat de uitbraak veroorzaakt werd door een enkele introductie van een H3-virus (clade 3C.2a1b.2a2a.3a.1), overeenkomend met het quadrivalente influenzavaccin voor 2023/2024. De seizoensvaccinatie was op dat moment echter nog niet beschikbaar.

Beheersmaatregelen, gebaseerd op Verenso-richtlijnen, werden snel geïmplementeerd, wat resulteerde in een relatief snelle indamming van de uitbraak. Onderzoek naar de bron bleef beperkt door gebrek aan deelname van medewerkers aan diagnostiek. Rioolwatersurveillance werd uitgevoerd, maar toonde geen detecteerbare virusniveaus, mogelijk door lage concentraties.

Deze casus benadrukt het belang van vroegsignalering, samenwerking en de mogelijke rol van rioolwatersurveillance bij het identificeren en beheren van vroege influenza-uitbraken, vooral vóór de beschikbaarheid van de seizoensvaccinaties.

8. Uitbraak bof in Gelderland

Nederlandse samenvatting

Het doel van deze studie was om de haalbaarheid te onderzoeken van rioolwatermonitoring voor het opsporen van bofuitbraken in gebieden met een lage vaccinatiegraad, met de focus op een religieuze subregio in Nederland tijdens een uitbraak in 2023. Onderzoekers gebruikten passieve samplers op strategische locaties, waaronder nabij de school en verschillende rioolgemalen die een bepaalde plaats afwaterden.

De belangrijkste bevindingen waren:

Detectie van Bofvirus-RNA: Bofvirus-RNA werd succesvol gedetecteerd in de lokale rioolgemalen, waarbij de RNA-niveaus toenamen naarmate meer klinische gevallen werden gerapporteerd. Positieve signalen werden ook waargenomen in een nabijgelegen stad zonder gemelde gevallen, wat wijst op mogelijke onderrapportage.

Correlatie met klinische gegevens: De bevindingen van rioolwatermonitoring kwamen goed overeen met de klinische gevallen die door lokale huisartsen werden gemeld. Ondanks onderrapportage door zorgverleners hielpen rioolwatermetingen bij het inschatten van de werkelijke verspreiding van de uitbraak en vulden zij zo de traditionele klinische rapportage aan.

Effectiviteit van passieve sampling: De studie bevestigde dat passieve samplers bof-RNA in rioolwater kunnen bemonsteren, zelfs in kleine, gelokaliseerde gebieden. Verdunning door regenval had een minimale invloed op de detectie, terwijl verdunning bij een stroomafwaarts gelegen rioolgemaal met een groter voorzieningsgebied (verdunning met het rioolwater van meer huishoudens) de signalen wel beïnvloedde.

Het onderzoek toonde succesvol aan dat rioolwatermonitoring met behulp van passieve samplers een haalbare methode kan zijn voor het detecteren en volgen van bofuitbraken, met name in ondergevaccineerde regio's. Deze aanpak kan de *situational awareness* tijdens uitbraken verbeteren door gevallen te identificeren die niet in de klinische surveillance worden gezien en biedt daarmee een veelbelovende aanvulling op de traditionele monitoring van infectieziekten.

9. Presentaties

1. Poster Maarten de Jong en co-auteurs op PDPC Congress 2023 – Moonshots of Pandemic & Disaster Preparedness Research.
2. Presentatie Gertjan Medema en co-auteurs op de Singapore International Water Week
3. Presentatie Gertjan Medema en co-auteurs op het World Water Congress in Toronto

Bijlage

Use of passive samplers as sewage surveillance tool to monitor a hepatitis A outbreak at a school in Amsterdam, the Netherlands

Maarten de Jong¹, Rémy Schilperoort², Harry Vennema³, Charlie van der Weijden¹, Jeroen Langeveld², Matthijs Welkers¹, Maarten Schim van der Loeff^{1,4}, Maria Prins^{1,4}, Ana Maria de Roda Husman³, Ewout Fanoy¹, Gertjan Medema⁵

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The authors thank the sewage consortium members including M. de Graaf, Erasmus Medical Center, Rotterdam, the Netherlands; P. Bijkerk, RIVM, Bilthoven, the Netherlands and G. Sips, GGD Rotterdam, Rotterdam, the Netherlands for their input during deployment and microbiological analysis of the passive samplers

Background

- Enteric hepatitis A virus (HAV) infections during childhood are often asymptomatic but could cause severe illness in unprotected adults
- Sewage monitoring using passive samplers could be useful for detecting ongoing circulation of HAV
- We report the application of sewage monitoring during an HAV outbreak at a primary school



Methods

- Between 19 October 2022 and 27 December 2022 five symptomatic HAV cases were notified and all attended the same primary school
- Passive samplers, small absorbent tools, were deployed in sewage near the school from 14 November 2022 until 22 March 2023
- Every week passive samplers were deployed for 48 hours and the absorbent sent for RNA extraction, HAV PCR testing, and, if positive, sequencing
- PCR and sequencing were also performed on blood and feces of the HAV cases

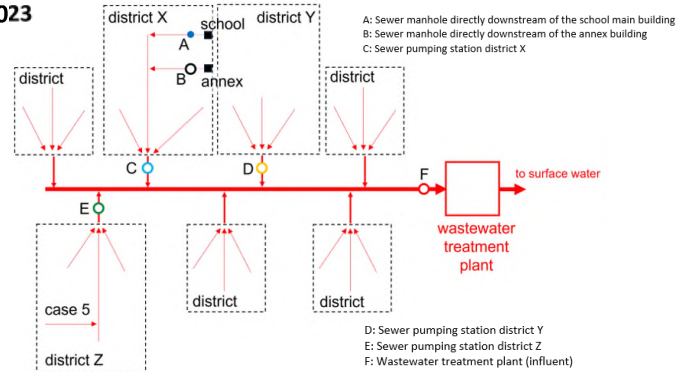
Conclusions

Identified HAV genosubtype IB in sewage samples matched with human specimens of all cases identified. Interestingly, sewage monitoring detected another HAV strain (genosubtype IA) in adjacent city districts indicating silent transmission of this strain.

Passive sampler-based sewage monitoring:

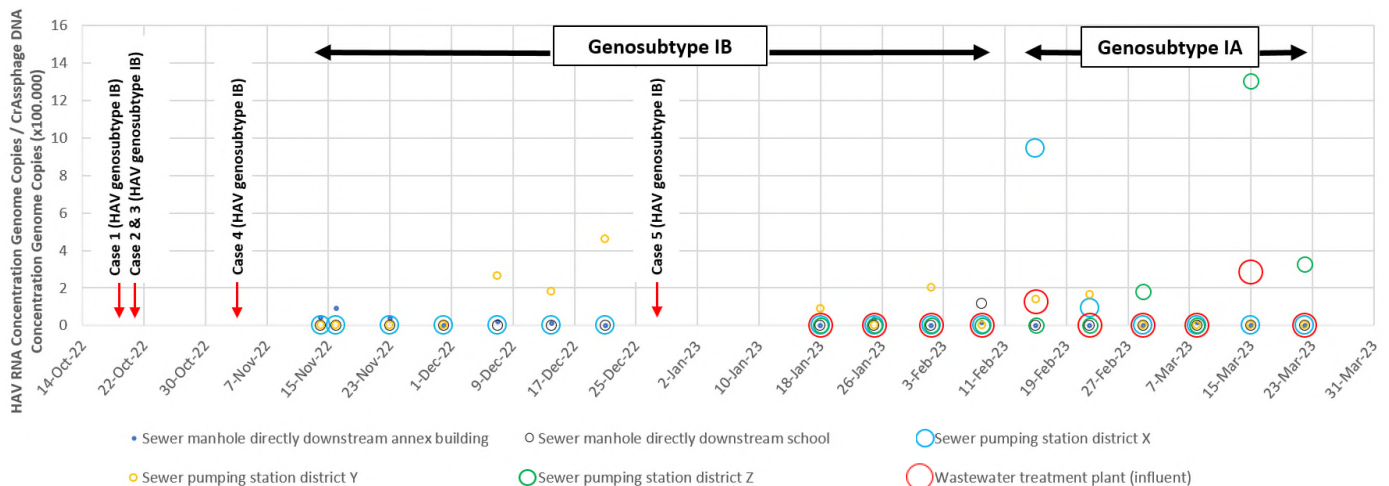
- can rapidly detect HAV shedding linked to diagnosed cases
- confirmed that outbreak control measures were effective by showing the outbreak HAV strain disappeared from sewage
- is a promising tool to guide public health response during HAV and other outbreaks

Figure 1: Schematics of sewage sampling locations around Amsterdam school, the Netherlands, 14 November 2022 – 22 March 2023



Results

Figure 2: HAV concentrations and strain typing in sewage samples around Amsterdam school, the Netherlands, 14 November 2022 – 22 March 2023

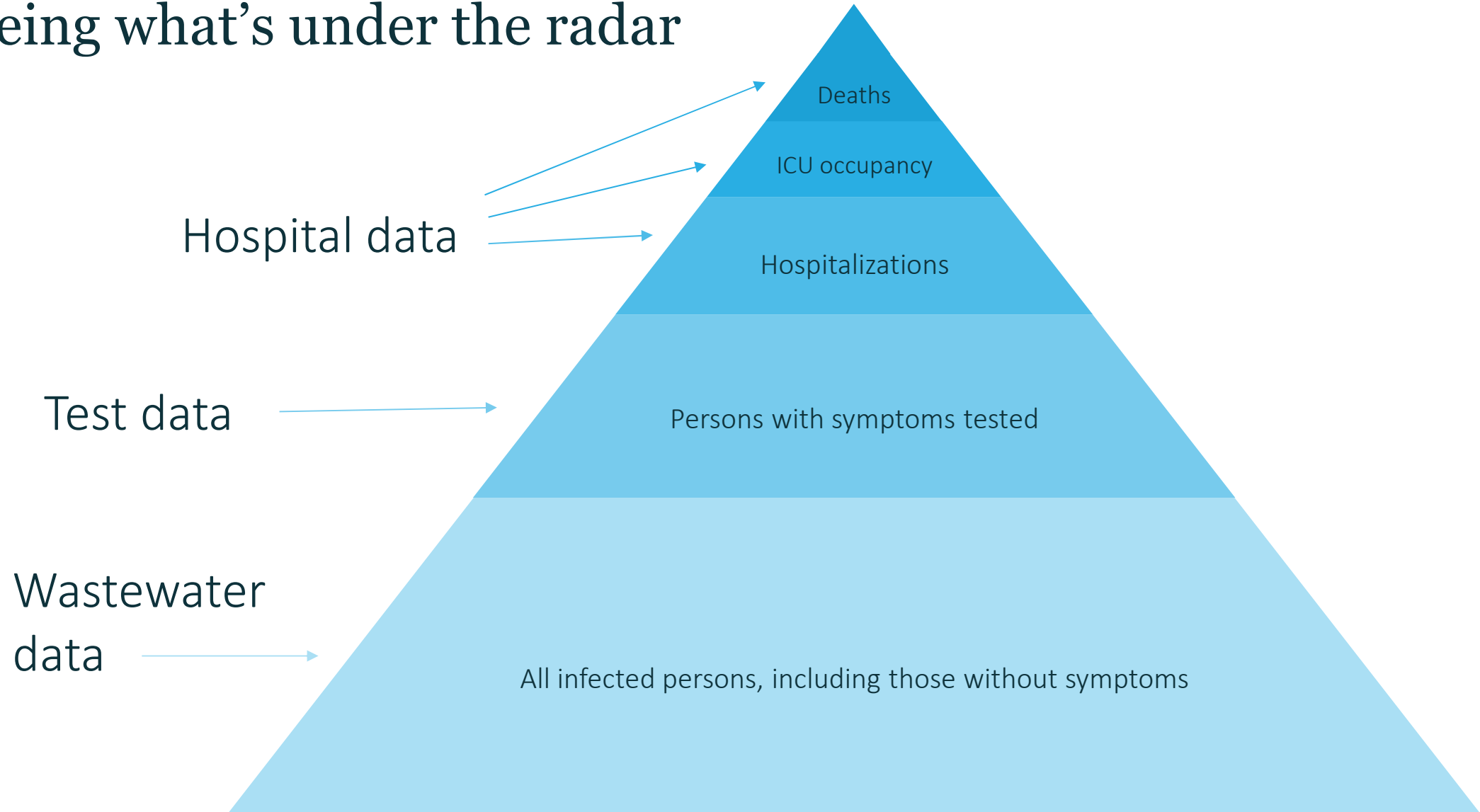




The future of wastewater-based epidemiological surveillance

G. Medema

Seeing what's under the radar



⚠️ Coronadashboard ends April 2nd 2024

The situation in the Netherlands

Week summary

- 👁️ **Development of the virus:** The spread of the virus is decreasing.
- 🏥 **Influence on healthcare sector:** The occupancy rate and patient influx are low, for both COVID ICU as well as for clinic beds.
- 📊 The [Corona thermometer](#) position is **1 Low**

View the most up-to-date figures of these subjects on the page [National](#).

👁️ Development of the virus

Infectieradar

0.2%

The percentage of Infectieradar participants with a positive corona test result slightly increased to **0.2%**.

Value of 26 February 2024 - 3 March 2024
Source: Infectieradar

[Read more about Infectieradar >](#)

Wastewater

-30%

The national average number of virus particles decreased (**-30%**) in week 9 (26 February - 3 March).

Value of 26 February - 3 March 2024
Source: RIVM

[Read more about wastewater >](#)

Virus variants

JN.1

Mainly BA.2.86 variants are found in sewage water and in germ surveillance and in particular subvariant JN.1, in addition to several other XBB variants.

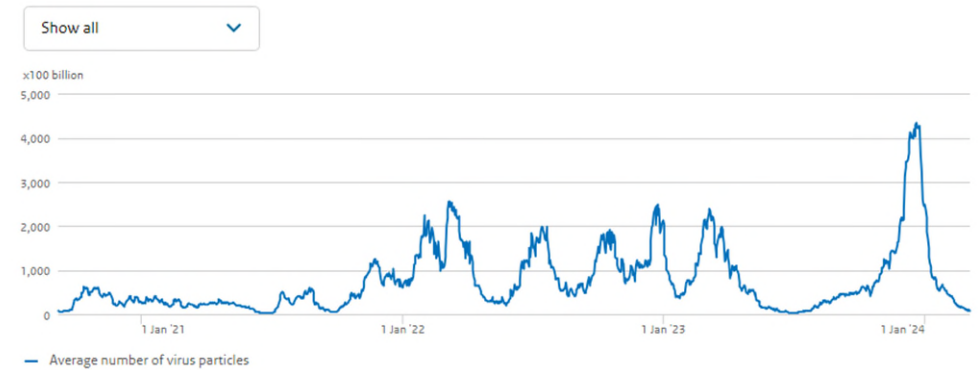
These subvariants do not appear to be more pathogenic than previous omikron variants.

Value of 20 February 2024
Source: RIVM

[Read more about virus variants >](#)

Virus particles over time

This graph shows the average number of virus particles per 100,000 inhabitants over time.



Source: RIVM

Virus particles in waste water

This chart shows the average number of virus particles per 100,000 inhabitants per municipality.

Average number per 100,000 inhabitants

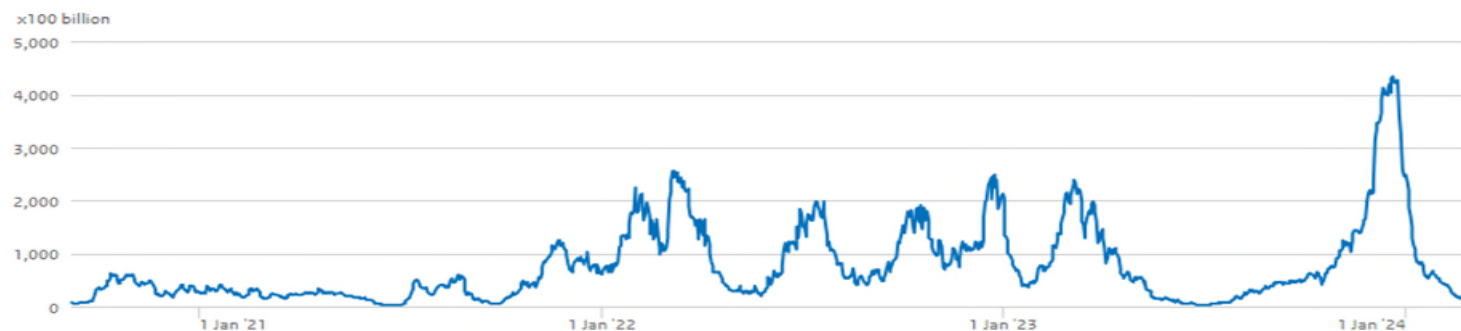
- 🟡 No recent measurement
 - 🟠 No virus particles measured
 - 🟢 More than 0 to 50
 - 🔵 50 to 250
 - 🟠 250 to 500
 - 🟡 500 to 750
 - 🟢 750 to 1,000
 - 🔵 1,000 to 1,500
 - 🟠 1,500 to 2,000
 - 🟡 2,000 and more
- x100 billion



National data (dashboard RIVM)



Hospitalizations



SARS-CoV-2 load wastewater

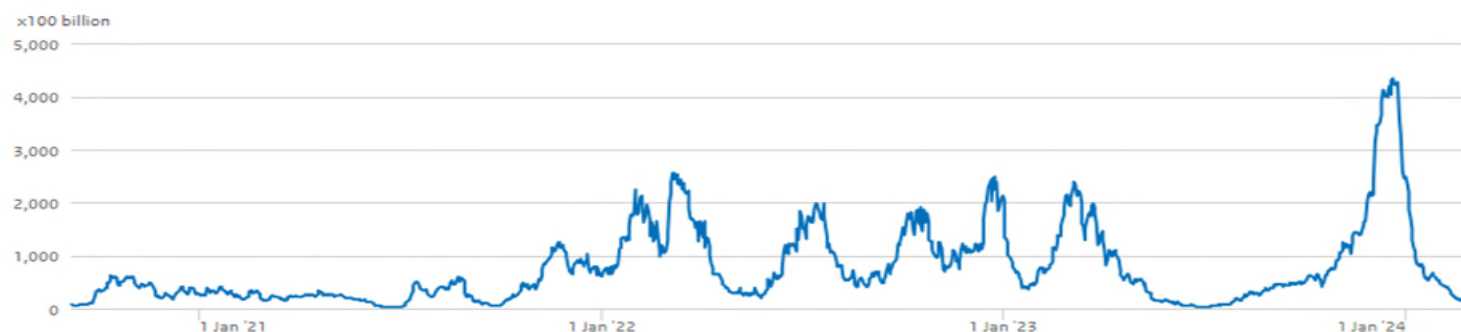


Positive tests

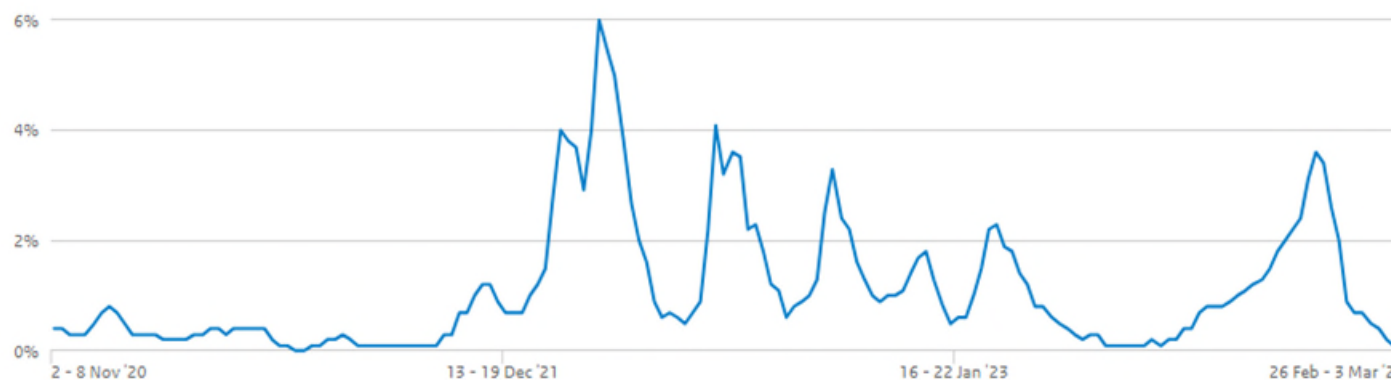
National data (dashboard RIVM)



Hospitalizations



SARS-CoV-2 load wastewater



Infection radar

Use case: understand COVID-19 dynamics: the infectious disease surveillance pyramid

City areas with low ratio of reported incidence vs normalised wastewater concentrations case

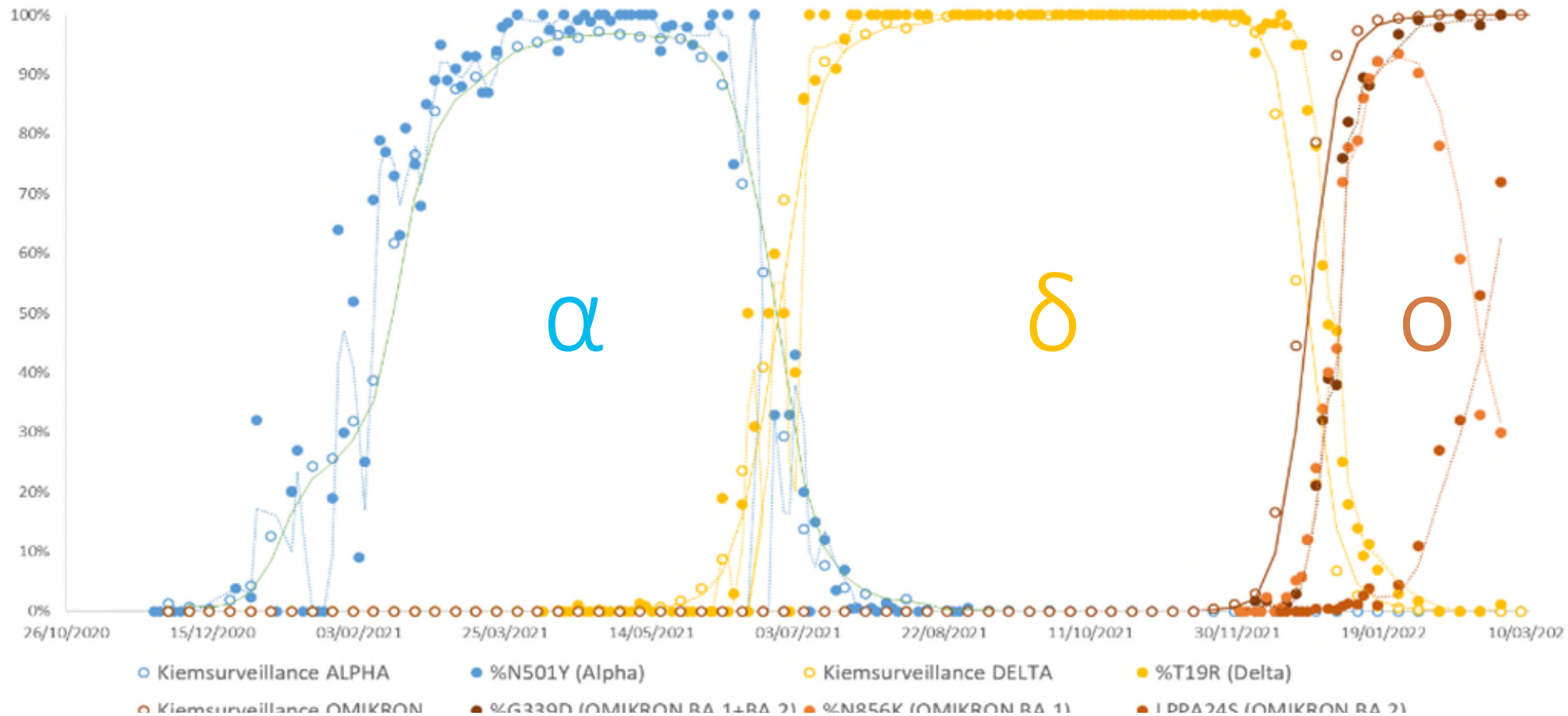
Suggested undertesting of humans in these city areas

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



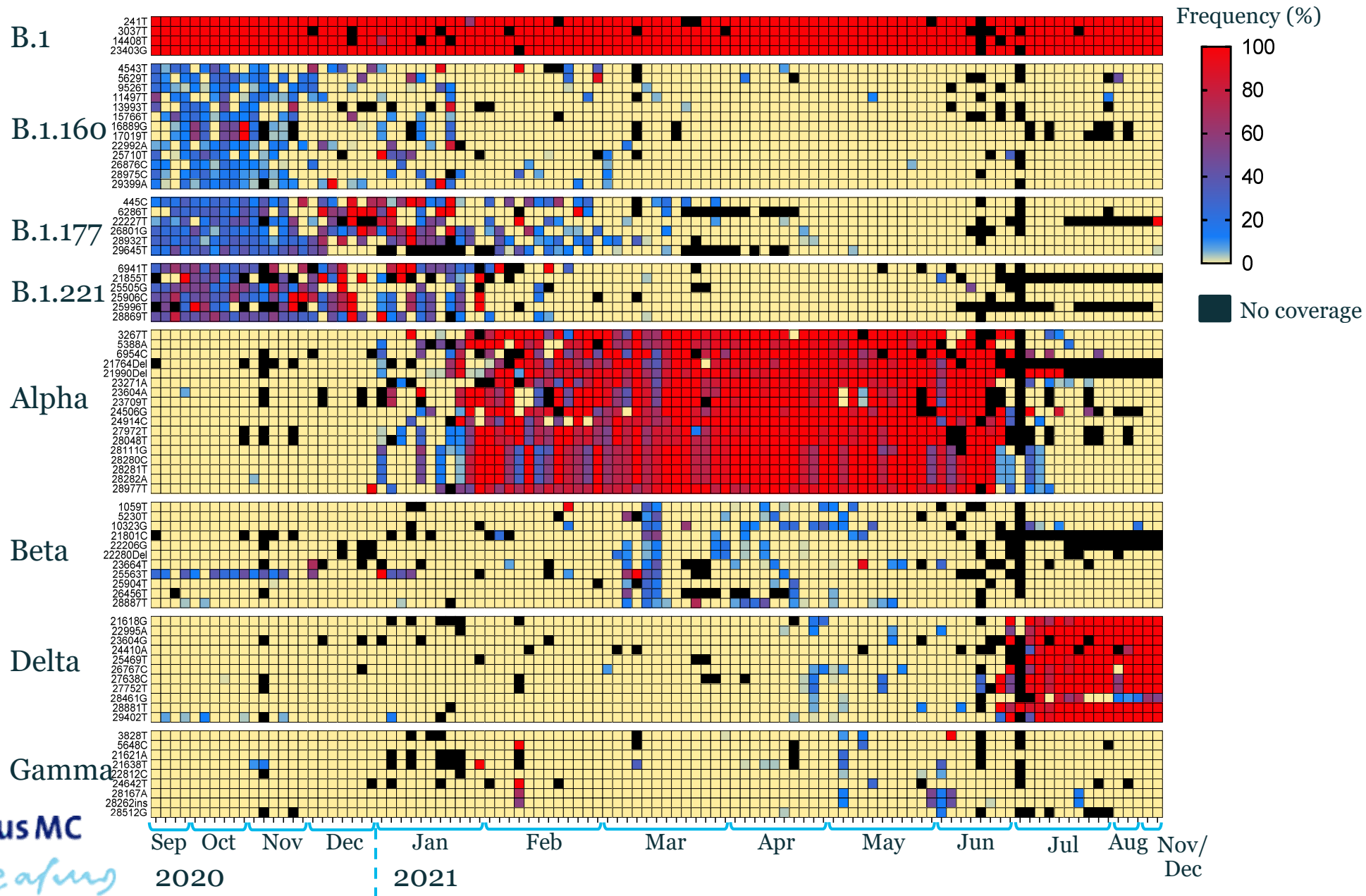
Photo: GGD

New variants in clinical vs sewage surveillance



SARS-CoV-2 VoC mutations over time in Rotterdam sewage (NGS)

Izquierdo et al, STOTEN, 2023



Erasmus MC



Why added value for public health?



POPULATION METRIC

Supports broad population-based surveillance, including people with no symptoms



UNBIASED

Everyone goes to the toilet, not everyone is tested



NON-INVASIVE

No effort required for citizens



RAPID

Short time-to-result, supporting early warning



LOW COST

One sample to test a large population



Rotterdam

Taking a composite sample of wastewater from 300.000 citizens

What can we do with wastewater monitoring?



KWR Water Research Institute

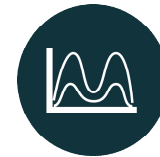
Producing public health information from wastewater samples

Video: NOS TV



SUPPORT CONTROL

Early warning about virus emergence in community



SEE TRENDS

Observe trends in virus transmission in communities,



SEE NEW VIRUS VARIANTS

Observe emergence of new virus variants in the community



BE AGILE

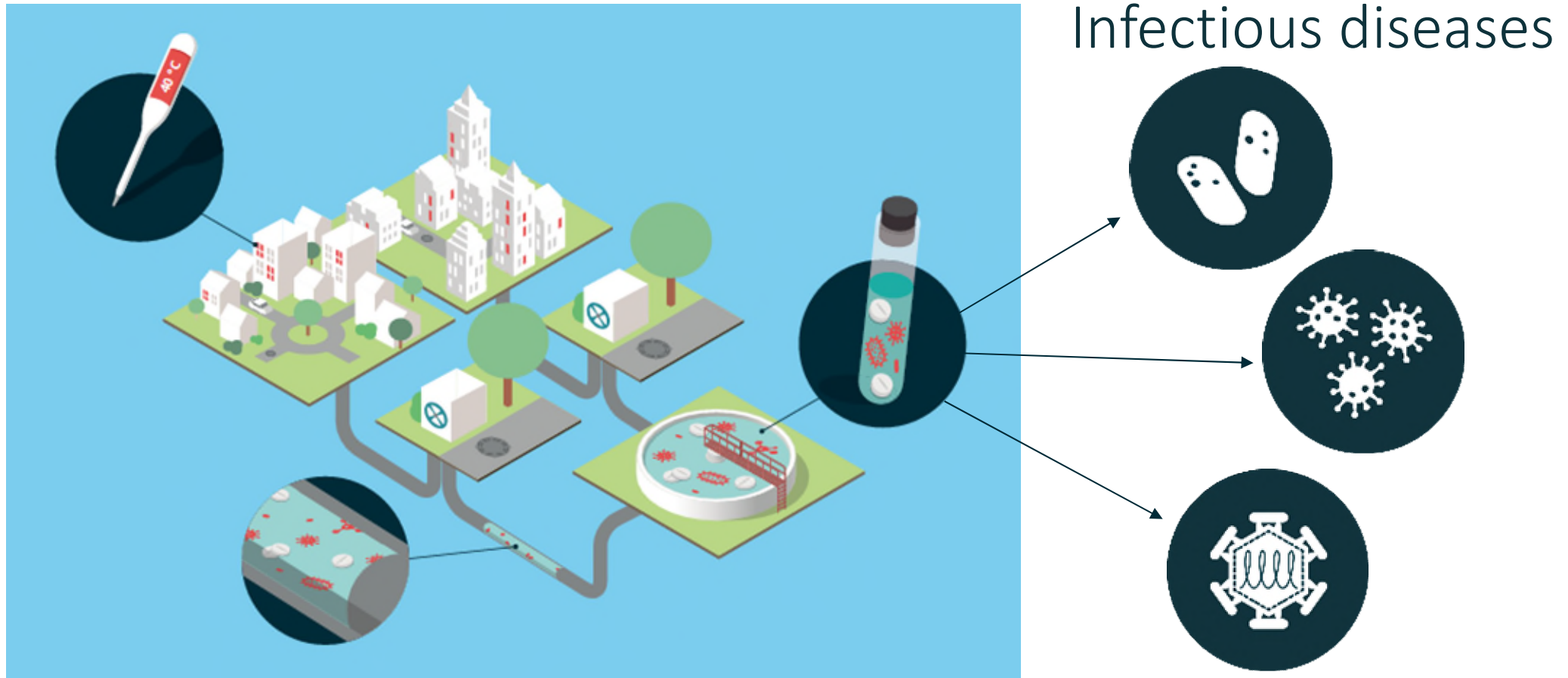
Enables focus on vulnerable populations



EXPAND

Many other viruses, bacteria, chemicals can be tested from the same sample

Wastewater as a mirror of society



Poliovirus (RIVM NL)

Objective

early warning, evaluate global eradication initiative

Design

Monthly grab samples in schools of communities with low vaccination rate

Governance

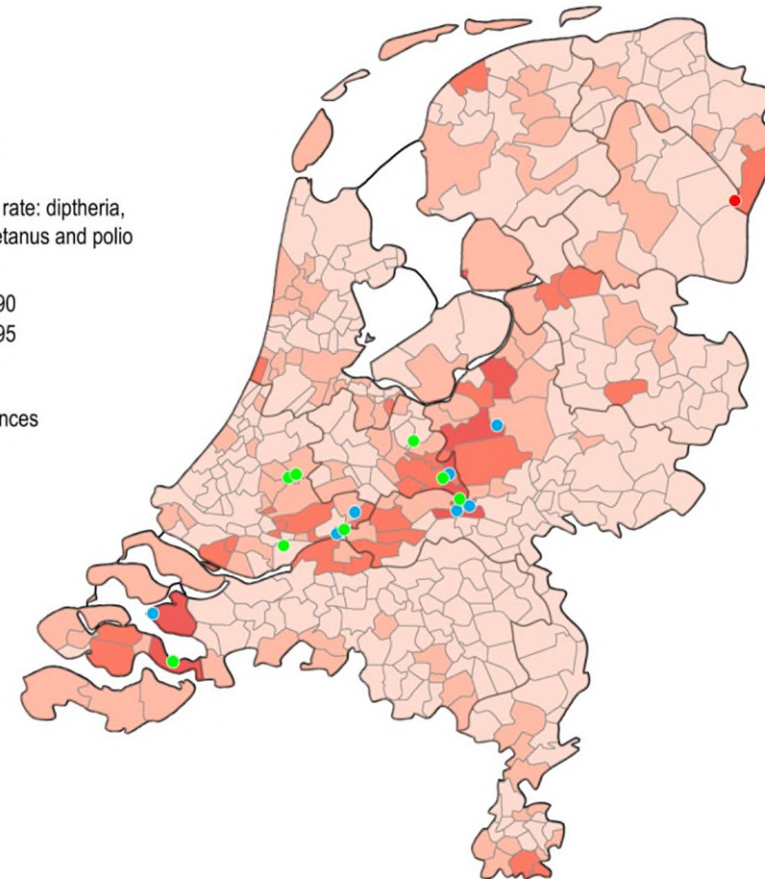
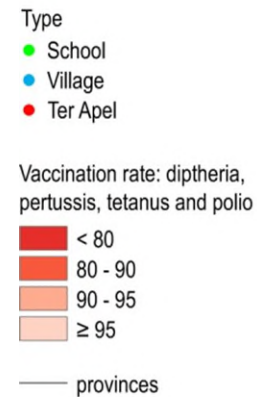
Ministry of Health/National Institute of Health (RIVM), municipalities

Action

Alert health system and community, suggest isolation, offer vaccination

POLIO GLOBAL ERADICATION INITIATIVE

Sewage sampling sites



Source: RIVM

Use case: hepatitis A virus outbreak at school

Confirmed cases of hepatitis A virus infection, all same school, all same HAV genotype

Wastewater collection in area near school and city area residence (passive samplers)

HAV was detected and confirmed as outbreak genotype by Next Generation Sequencing

Sewage data were used to indicate whether public health actions were effective and no silent transmission occurred in wider community.



Photo: Partners for Urban Water



GGD
Rotterdam-
Rijnmond

~~GGD~~
~~Amsterdam~~



Use case: mumps virus outbreak in region with low vaccination coverage

Sewage monitoring used because syndrome and clinical surveillance information are under-reporting.

Also works for mumps virus: found in sewage at pumping stations serving towns and at sewage near primary school.

Related to reported cases.

Also able to determine mumps virus types via molecular info from wastewater samples

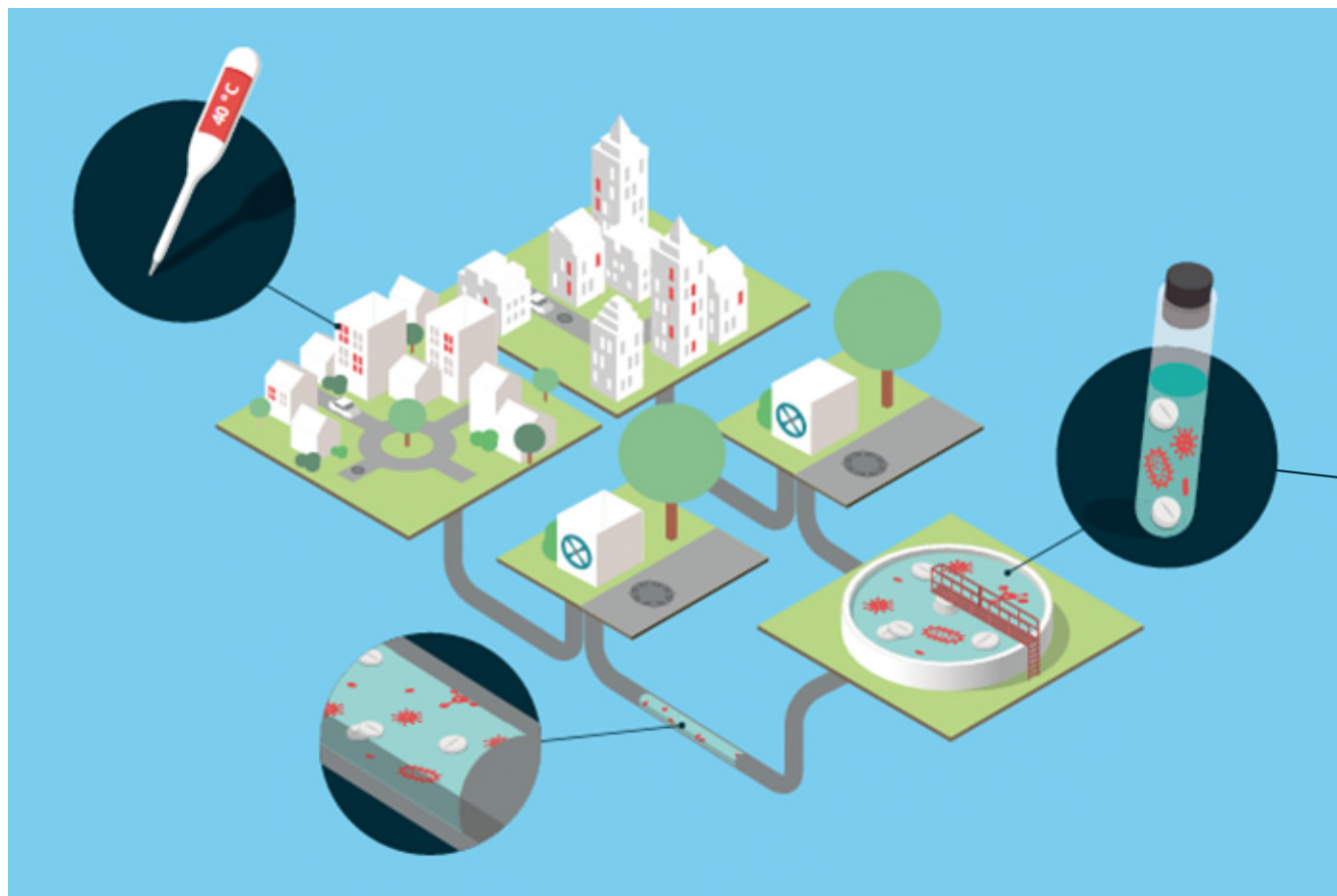
GGD vraagt aandacht voor uitbraak bofvirus Midden-Nederland



VELUWE RANDMEER - Sinds november 2023 is er een uitbraak van het bofvirus gaande in gebieden met een lage vaccinatiegraad in Midden-Nederland. De GGD vraagt aandacht voor het virus via de

Wastewater as a mirror of society

Antimicrobial resistance



Use case: AMR (RIVM NL)

Objective

prevalence/carriage of BRMO (types), distribution across the country, trends, hot spots

Design

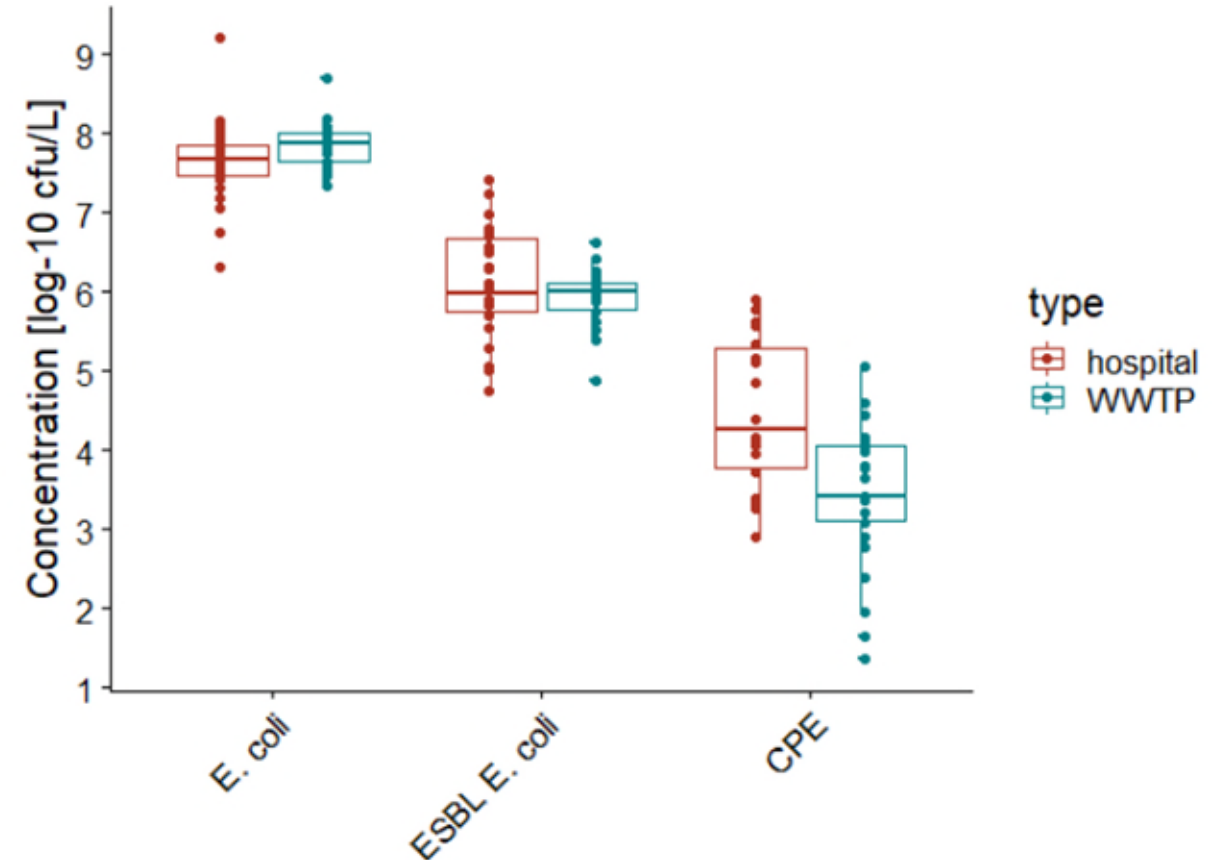
Periodic daily composite samples at the inlet of the wastewater treatment plants (hospitals)

Governance

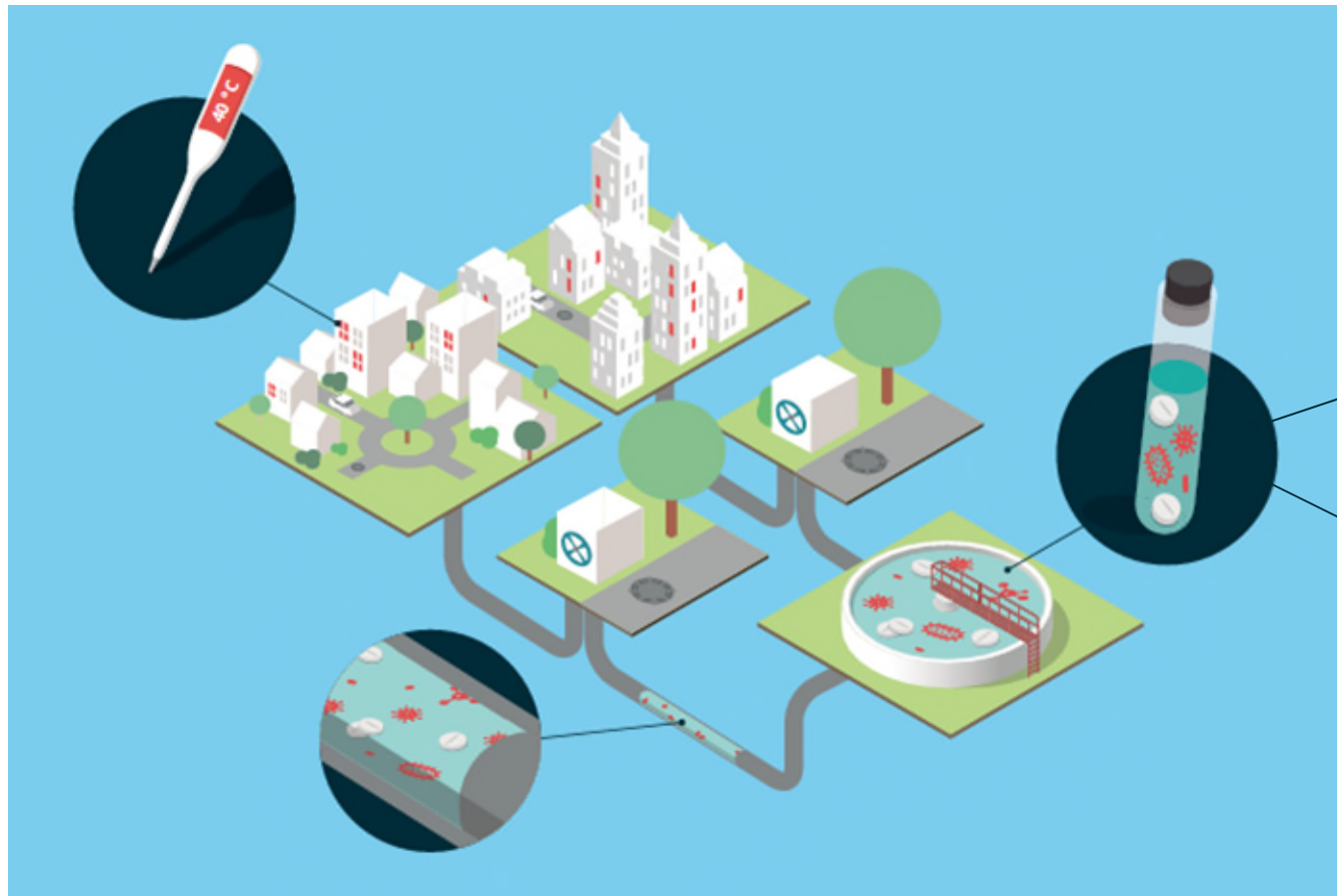
Ministry of Health, National Institute of Health (RIVM), joint wastewater authorities

Action

situational awareness, prevalence of rare antibiotic resistant bacteria (CPE) in the population, hospital do not have significant contribution compared to communal



Wastewater as a mirror of society



Chronic diseases/drug use



Use case: Illicit drugs (NL)

Objective

situational awareness cities, trends in use, hotspots

Design

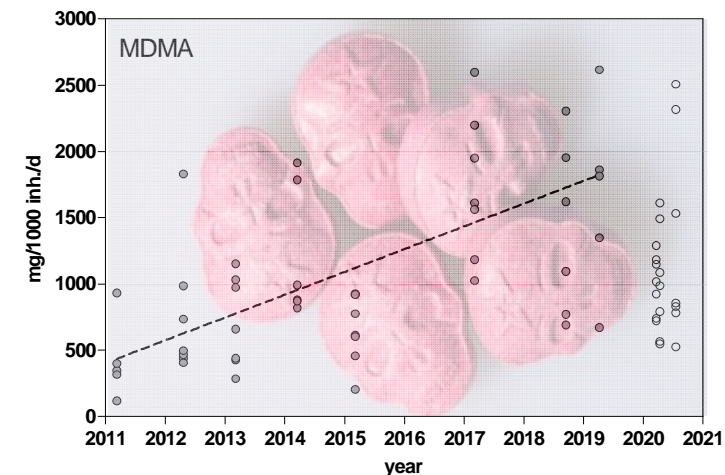
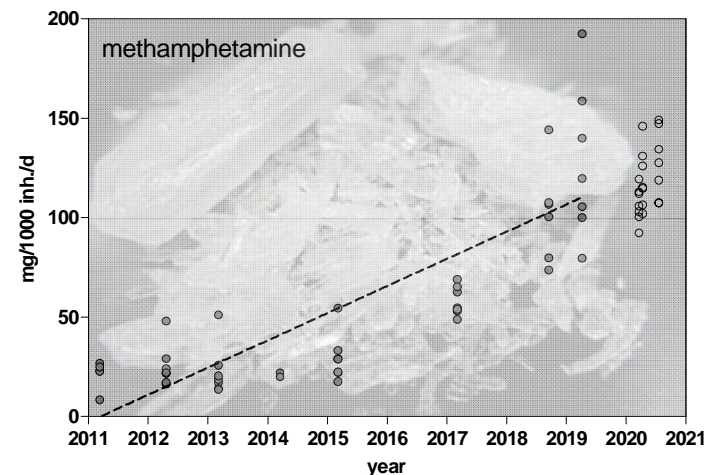
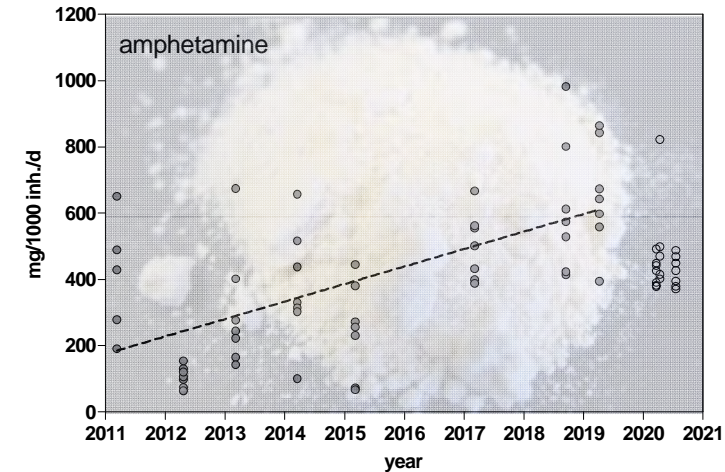
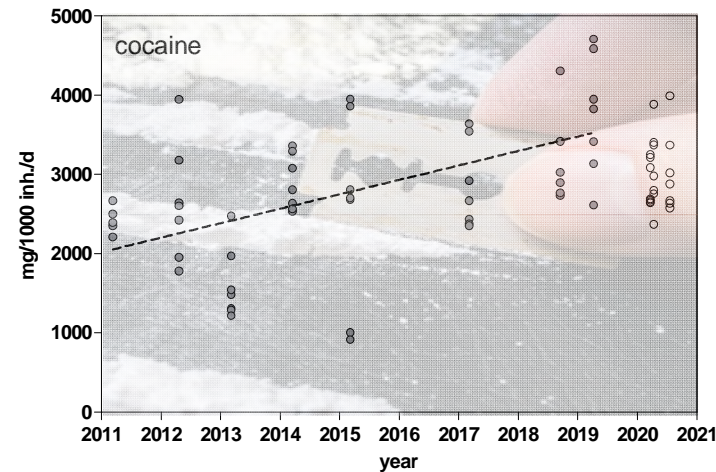
one uneventful week of daily composite samples at the inlet of the city's wastewater treatment plant in the same months every year

Governance

Municipalities, water authorities, lab

Action

Adapt policy, increase support and control





Illicit drugs (EU)

Objective

situational awareness cities, trends in use, hotspots

Design

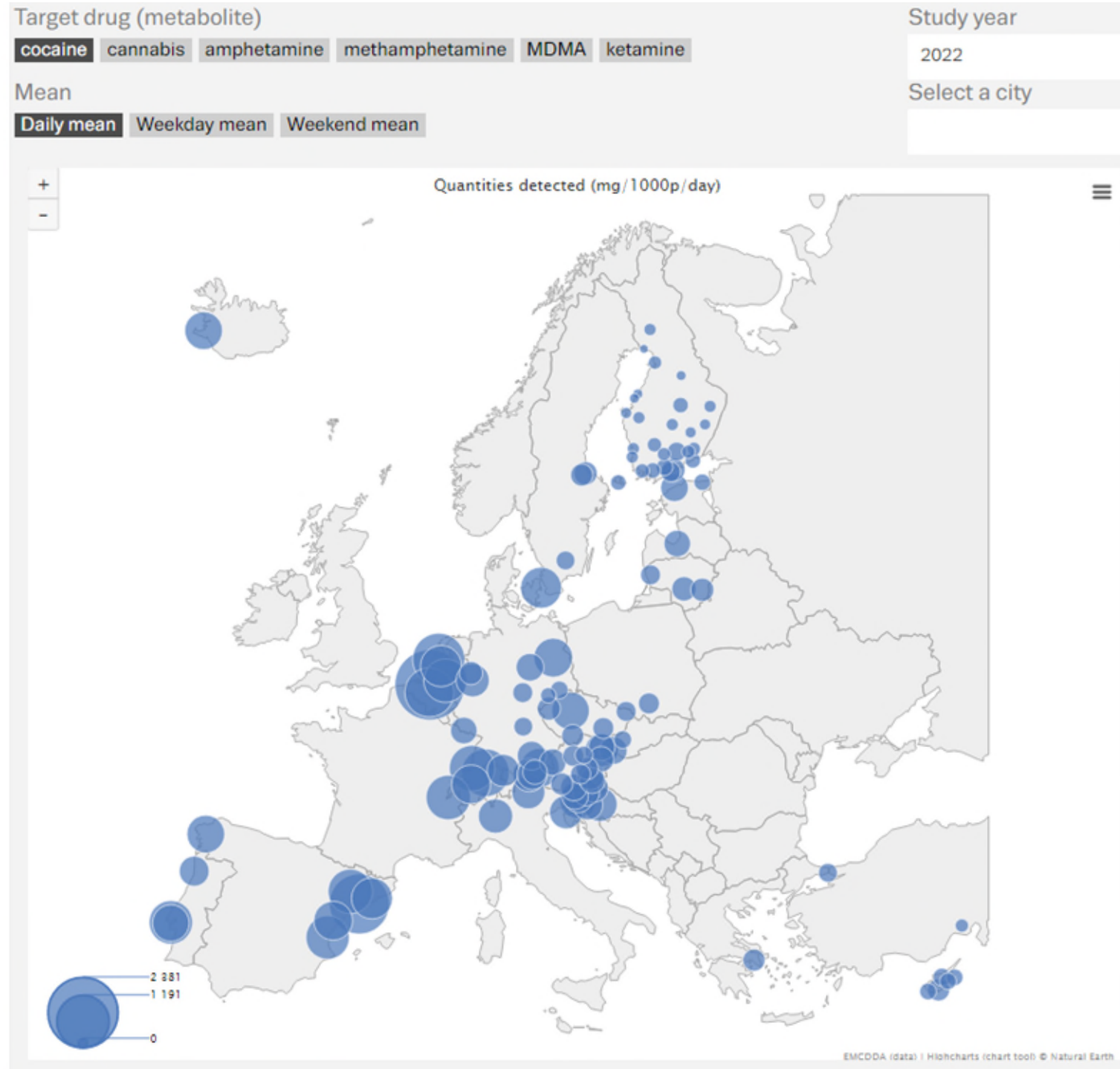
one uneventful week of daily composite samples at the inlet of the city's wastewater treatment plant in the same months every year

Governance

Municipalities, water authorities, lab

Action

Adapt policy, increase support and control





European Union

Collaboratory: dashboards




























GLOWACON: global collaboratory

Sep 2024: testing airport wastewater samples from around the world for 5 pathogens

Home > Dashboards >

National

Dashboards listed here are owned and operated by National Health Authorities and the data displayed is taken into consideration in the respective national strategies to manage COVID-19.

 Austria (de)	 Belgium (en, nl, fr)	 Canada (en, fr)	 Czech Republic (cz)	 Denmark (dk, en)	 Finland (fi, en)
 France (fr)	 Germany (en, de)	 Greece (en, gr)	 Hungary (hu)	 Ireland (en)	 Italy (it, en)
 Japan (jp)	 Latvia (lv)	 Lithuania (lt, en)	 Luxembourg (en, fr)	 The Netherlands (en, nl)	 New Zealand (en)
 United Kingdom - Scotland (en)	 Slovakia (en, sk)	 Slovenia (sl)	 South Africa (en)	 Spain (es)	 Sweden (en, sv)
 Switzerland (en, fr, de, it)	 Turkey (en, tr)	 United States (en)			

WHO evaluation of scientific evidence and public health value

Status of environmental surveillance for SARS-CoV-2 virus

Scientific brief
5 August 2020



Background

Environmental surveillance by testing of wastewater for evidence of pathogens has a long history of use in public health, particularly for poliovirus¹ and more recently antimicrobial resistance (AMR).² In the context of the ongoing COVID-19 pandemic, it is being used for the detection of SARS-CoV-2 shed into wastewater from the upper gastrointestinal and upper respiratory system and via faeces.

Detection of non-infective RNA fragments of SARS-CoV-2 in untreated wastewater and/or sludge has been reported in a number of settings, such as Milan, Italy;³ Murcia, Spain;⁴ Brisbane, Australia;⁵ multiple locations in the Netherlands;⁶ New Haven, Connecticut⁷ and eastern Massachusetts,⁸ United States of America; Paris, France;⁹ and existing poliovirus surveillance sites across Pakistan.¹⁰ Researchers in the Netherlands,⁶ France⁹ and United States of America⁸ demonstrated a correlation between wastewater SARS-CoV-2 RNA concentrations and COVID-19 clinical case reports, the latter two further suggesting that the RNA concentrations could provide a 4- to 7-day advanced notice ahead of COVID-19 confirmed case data. Efforts are also ongoing to analyse historical wastewater samples for evidence of past SARS-CoV-2 circulation.

Most of these detections have been in the context of research studies. However, at least one country, the Netherlands, plans to incorporate daily sewage surveillance into its national COVID-19 monitoring.¹¹ A similar approach to using environmental surveillance as part of the routine COVID-19 surveillance package is being studied in Germany¹² and has been initiated in Australia and New Zealand.¹³

Most studies published to date on the use of environmental surveillance for SARS-CoV-2 have been from high-resource settings. However, approaches are needed that can be applied in lower-resource settings, where a greater proportion of the population is not connected to sewers and instead uses pit latrines or septic tanks. Possibilities include testing surface water contaminated by sewage.

To date, there have been no published studies demonstrating the use of environmental surveillance to identify SARS-CoV-2 in animal populations.

This brief explores potential use cases, considerations, and research needs for this emerging tool for SARS-CoV-2 detection that may be explored in close coordination with established public health surveillance for COVID-19. At present, there is not yet sufficient evidence to recommend environmental surveillance as a standard approach for COVID-19 surveillance. Recommended strategies for COVID-19 surveillance can be found in the WHO interim guidance "[Public Health Surveillance for COVID-19](#)".¹⁴

Major potential use cases for environmental surveillance for SARS-CoV-2

Early warning

Several studies have demonstrated that increases in SARS-CoV-2 RNA can be detected in environmental samples several days before detection of COVID-19 through clinical surveillance. Consequently, there is potential to use environmental surveillance for early warning, particularly of clusters or outbreaks in countries that have already contained transmission and are easing public health and social measures, or in the event of seasonality. Here, a cost-benefit assessment would need to be undertaken of the improvement in early warning. In addition, environmental surveillance would need to be closely linked to a plan for immediate action in the event of a positive signal or significant rise in cases from a non-zero baseline. Unanswered questions include what level of RNA rise is informative for action in the case of a significant rise.

Even in high-resource settings it is challenging to conduct widespread environmental surveillance for early warning because this approach requires frequent sampling to provide actionable data. Furthermore, a high sampling volume also is required because of low sensitivity. One potential approach could be to reserve environmental surveillance for pooled testing of particularly high-risk settings where response can be quickly implemented, such as closed residential settings (e.g. nursing homes, prisons, worker dormitories), large crowded workplaces or in the context of mass gatherings.

Environmental surveillance for SARS-COV-2 to complement public health surveillance

Interim Guidance
14 April 2022



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Environmental surveillance for SARS-CoV-2 to complement other public health surveillance

WHO: multipathogen wastewater surveillance

2024

-

2025

Under development

Package of WES decision support tools

1. Summary guidance for multipathogen WES implementation to support public health decision making
2. Pathogen specific WES summary sheets for priority targets
3. Preferred Pathway Characteristics

Initial Priority Targets/Diseases

- Polio
- COVID-19
- Influenza
- Mpox
- Cholera
- Typhoid

Intention is to support country-led decision making - while complementing and enabling other global, regional and local initiatives

Package will be available for pilot implementation in Oct 2024

Concept:

Planning for WES program must consider **current** and **future** threats

=> 3 inter-related components

Current threats

- Ongoing e.g. polio, SARS-CoV-2, seasonal influenza



Preparedness

- Sampling
- Laboratory
- Public Health response
- Partnerships & Capability



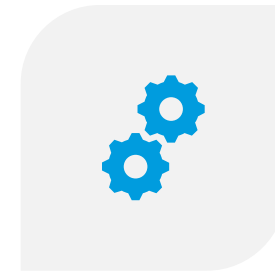
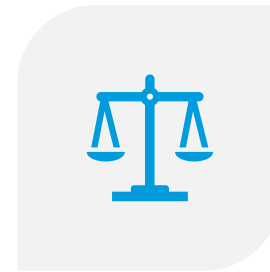
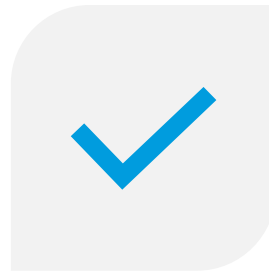
Emerging threats

- Trigger from existing ES/other, e.g. polio, mpox
- Novel threat, e.g. H5N1, pathogen X..

Concept:

The suggested process involves 4 broad **sequential** steps:

Consider potential targets



PUBLIC HEALTH:

PRIORITISE THOSE THAT ARE OF **PUBLIC HEALTH SIGNIFICANCE**, WHERE RESULTS WOULD BE **ACTIONABLE** AND WHERE WES WOULD **ADD VALUE** OVER EXISTING SURVEILLANCE

FEASIBILITY:

WHETHER WES CAN DELIVER RESULTS WITH BOTH **TECHNICAL** AND **OPERATIONAL FEASIBILITY**

ACCEPTABILITY:

WHETHER PROPOSED WES IS **ACCEPTABLE** CONSIDERING ETHICAL, SOCIAL AND ECONOMIC ASPECTS

OPTIMISATION:

HOW BEST TO **INTEGRATE** IN **SURVEILLANCE AND RESPONSE SYSTEMS** AS WELL AS IN **MULTITARGET WES**

Routine: Influenza A (NWSS USA)

Objective

situational awareness seasonal flu, early warning (H5N1?), trends, hotspots

Design

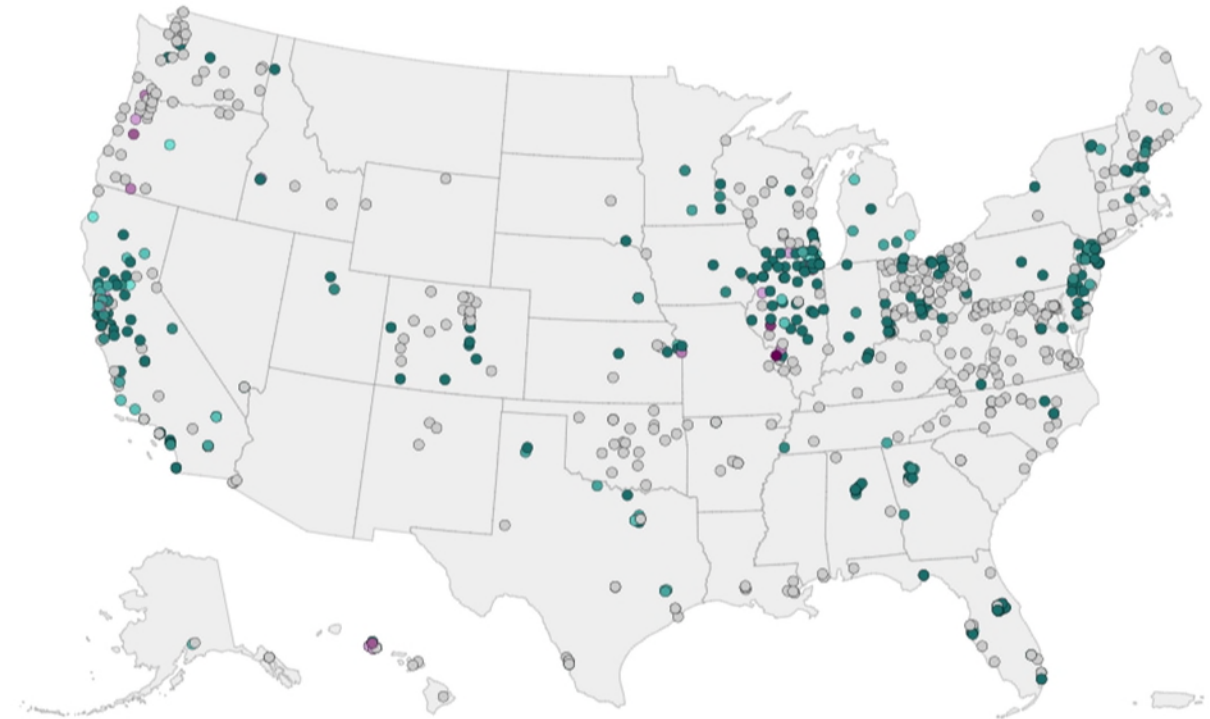
Frequent (several times per week) daily composite samples at the inlet of the wastewater treatment plants (all/sentinel)

Governance

Department of Health, Federal Health center (CDC), state/local health departments, wastewater utilities (+WEF), labs

Action

Early warning: alert health system, investigate cause of increase



All data are preliminary and may change as more reports are received. Wastewater data does not distinguish between human and animal waste or by-products.

Emergence: H5N1 (NWSS USA)

Objective

early warning H5N1?

Design

Frequent (several times per week) daily composite samples at the inlet of the wastewater treatment plants (all/sentinel)

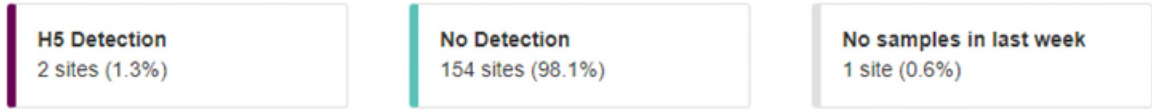
Governance

Department of Health, Federal Health center (CDC), state/local health departments, wastewater utilities (+WEF), labs

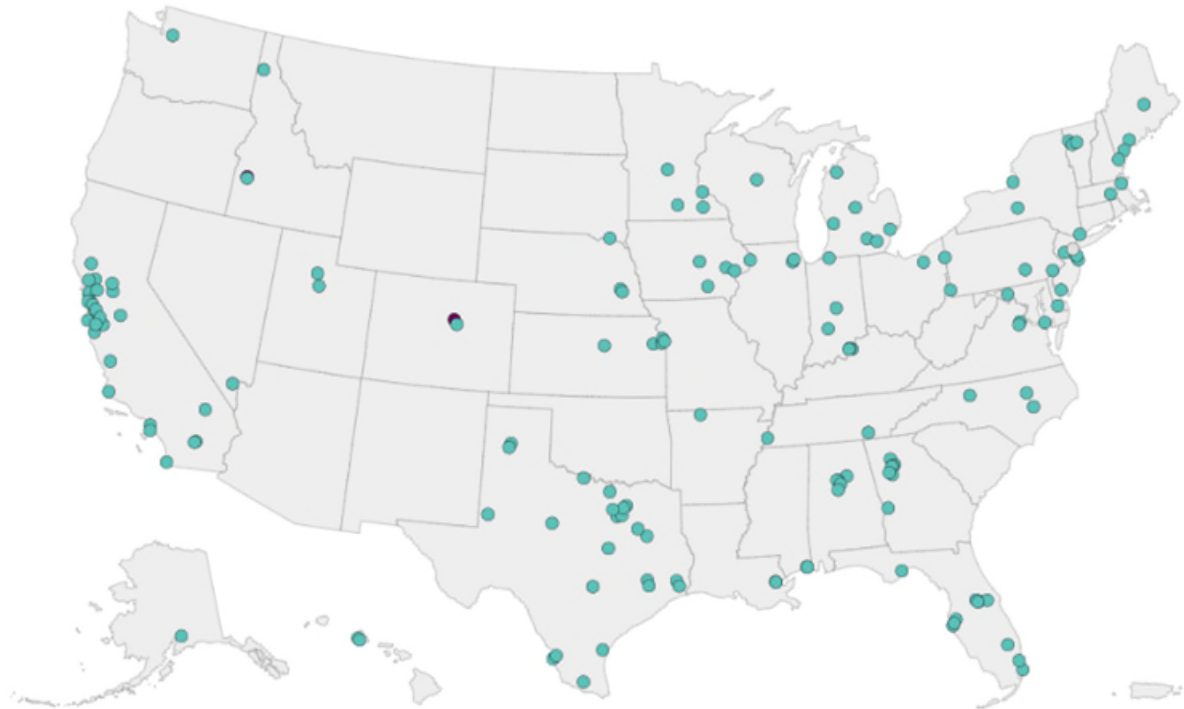
Action

Early warning: alert health system, investigate cause

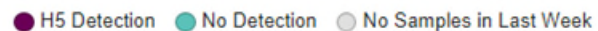
Time Period: August 25 - August 31, 2024



Click on the legend below to see detections.



Select a detection type below to add or remove it from the map.





Routine: mpox (NWSS USA)

Objective

situational awareness mpox, early warning (clade 1b), trends, hotspots

Design

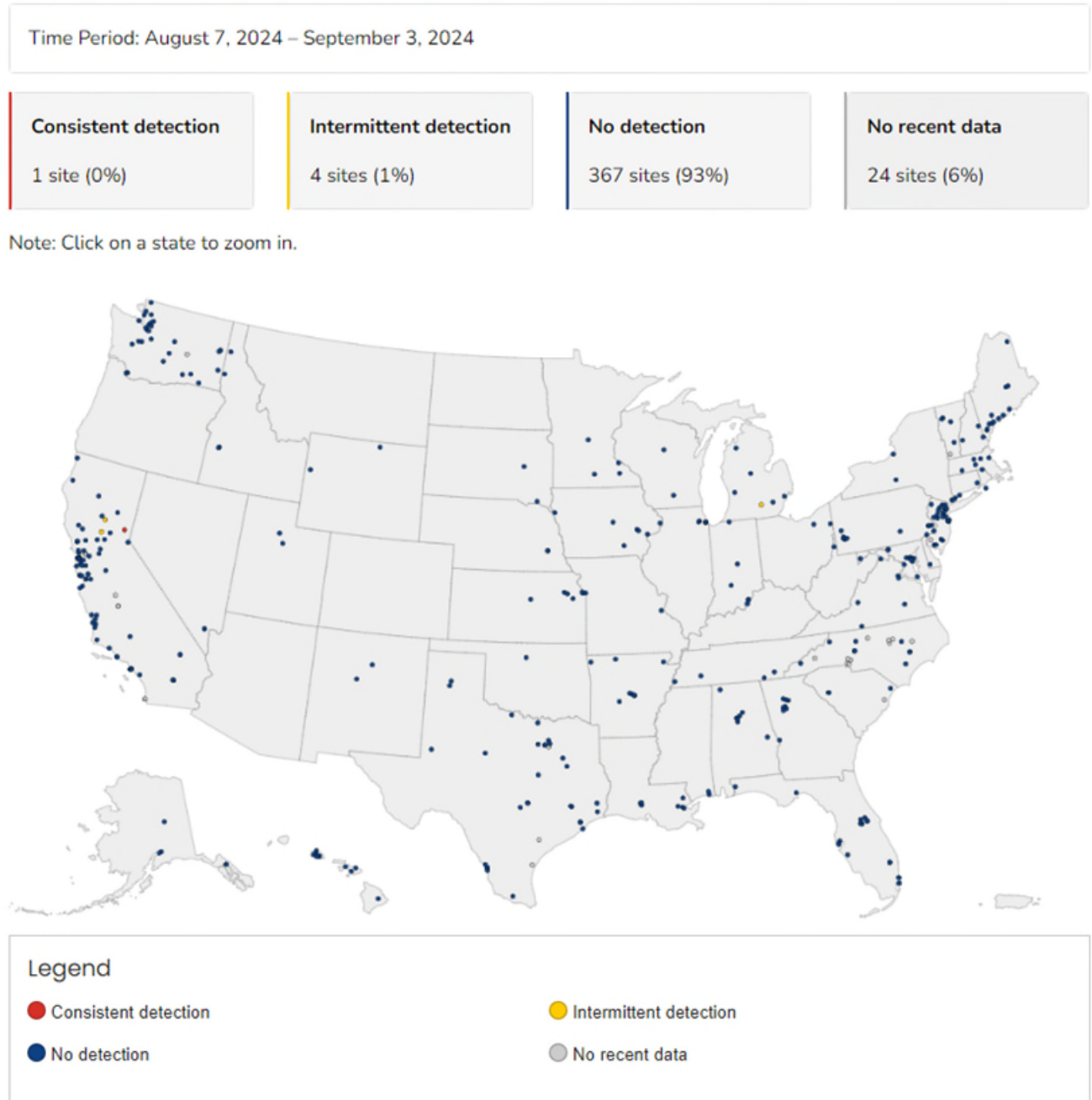
Frequent (several times per month) daily composite samples at the inlet of the wastewater treatment plants (all/sentinel)

Governance

Department of Health, Federal Health center (CDC), state/local health departments, wastewater utilities (+WEF), labs

Action

Early warning: alert health system, investigate cause of increase



EU4S coordination in EU Monthly routine updates from EU States + emergency bulletins

European Commission
MONTHLY BULLETIN
August 2024

EU Wastewater Observatory for Public Health

EXECUTIVE SUMMARY

- Updated data on SARS-CoV-2 wastewater surveillance is accessible at the national level for 18 out of the 27 EU countries.
- Information on other pathogens is up-to-date for five EU countries. Specifically, Finland is monitoring Influenza A, B, and RSV; Hungary is actively observing Influenza A; Ireland is testing for poliovirus 2; Slovenia has begun monitoring for poliovirus; and Sweden is conducting surveillance for Enteric and Influenza viruses.
- Information regarding updated data from wastewater surveillance for SARS-CoV-2 is available for one EU neighborhood country and nine countries outside the EU.

According to the most recently updated data, four countries (AT, BE, DK, NL) have shown a decreasing trend in SARS-CoV-2 levels in wastewater, while four countries (DE, HU, LV, LU) have shown an increase. For six countries, either the national trend has not been specified, or the information has not been updated to reflect the current period.

This bulletin offers insights derived from the latest wastewater surveillance data primarily obtained from EU national dashboards and websites owned and operated by National Health Authorities. Additionally, data from existing regional/local, and non-EU sources are included. The summarised information in this bulletin is based on online access, with the most recent retrieval performed on 1 August 2024.

AUSTRIA
Last data update: 28 July 2024
Austria's monitoring of SARS-CoV-2 involves 48 strategically chosen sewage treatment plants, encompassing over 58% of the country's population.
SARS-CoV-2: Based on the most recent information collected in week 30, the trend indicator is below one, showing a decrease in viral loads. The most frequently identified variant in week 29 is KP.3 (FLIRT variant), which represents around 60% of reported cases.
Other pathogens: no information available.
Source: [Austria - Dashboard](#)

Person-weighted trends in the federal states (and Austria as a whole)
Source: [https://www.assessmonitoring.at/data/#board/](#)

BELGIUM
Last data update: 22 July 2024

JOINT RESEARCH CENTRE

European Commission
AD-HOC BULLETIN
May 2024 v.3

(Waste)water surveillance for bird flu (A/H5N1)

HIGHLIGHTS

- (Waste)water surveillance for bird flu is useful complementary information source regarding the recent bird flu outbreak reported by the United States.
- 12 countries run preliminary studies which can be upgraded.
- At present, the no HPAI A(H5N1) has been detected in European (waste)waters.
- JRC has developed a suitable PCR assay.
- 13 EU countries expressed their interest for a coordinated action targeting European (waste)waters.
- Seven non-EU countries are willing to join such an effort.

In late March 2024, the HPAI A(H5N1) virus was found in dairy herds in Texas and Kansas. This is the second time the virus has been detected in a human in the United States and the first time it appears to have been acquired from contact with an infected mammal. However, other influenza subtypes have been known to be acquired from mammals.

animal and human health, as well as the poultry industry.

The H5N1 virus primarily affects birds, especially domesticated and wild aquatic birds, such as ducks, geese, swans, and chickens. However, it can also infect other animal species, including cats, dogs, and even humans, although human-to-human transmission is rare.

The following information has been gathered since May 5 using the community of practice of the EU Wastewater Observatory for Public Health and the Global Consortium for Wastewater and Environmental Surveillance for Public Health (GLOWACON).

Both initiatives are part of the pandemic preparedness efforts of the European Commission. The respective policies with regard to wastewater surveillance are overseen and led by the European Commission's Health Emergency Preparedness and Response Authority (HERA). The European Commission's Joint Research Centre builds and maintains the EU Wastewater Observatory for Public Health and assists HERA by providing scientific, technical and logistical support.

THE SITUATION

Bird flu, also known as avian influenza, caused by the influenza A virus subtype H5N1 (A(H5N1)), is a highly contagious and deadly disease affecting various bird species. The virus has been detected in several countries worldwide, posing a significant threat to

JOINT RESEARCH CENTRE

European Commission
AD-HOC BULLETIN
Last update: 23 August 2024

Wastewater surveillance for Mpox

HIGHLIGHTS

- Wastewater Surveillance is used successfully in the surveillance of Mpox.
- Currently, 4 EU Member States have such surveillance in place, and another 6 EU Countries have methods in stand-by.
- 2 Member States perform WES surveillance at airports.
- The Global Consortium GLOWACON is preparing an International Test on the aviation sector.
- involving two dozen of International Airports, while several countries increase entry point surveillance.
- Capacities are focussed on High Income Countries, but capacity development in LMICs is ongoing.
- Available evidence suggests that WES for Mpox can close information gaps.

On 13 August 2024, the Africa Centers for Disease Control and Prevention (Africa CDC) proclaimed mpox a Public Health Emergency of Continental Security (PHECS), marking the organization's inaugural declaration since its establishment in 2017.

Following an inquiry by the Community of Practice on Wastewater Surveillance for Public Health and in accordance with the European Commission's Health Emergency Preparedness and Response Authority (HERA), the EU Wastewater Observatory for Public Health gathered information on (waste)water-related surveillance initiatives. This ad hoc bulletin encapsulates the feedback received and updates on ongoing or forthcoming activities.

THE SITUATION

Mpox (formerly known as monkeypox), caused by the Mpox virus, is rare and milder than smallpox, belonging to the same virus family. Originating in monkeys, it's now known to infect humans, primarily in central and western Africa. The 2022 outbreak involved Clade 1b of the virus, with a low fatality rate, especially in healthy individuals. However, those with weakened immune systems or specific conditions face higher risks of severe illness or death.

From the end of 2023, a significant epidemic of Mpox has been ongoing in the Democratic Republic of Congo (DRC). On August 14, 2024, the World Health Organization (WHO) once again declared Mpox a "Public Health Emergency of International Concern" (PHEIC - Public Emergency of International Concern) under the International Health Regulations (IHR, 2005), following a surge in cases reported in the Democratic Republic of Congo over the past year and the expansion of the epidemic to an increasing number of African countries.

The following information has been gathered since August 5 using the community of practice of the EU Wastewater Observatory for Public Health and the Global Consortium for Wastewater and Environmental Surveillance for Public Health (GLOWACON).

Both initiatives are part of the European Commission's pandemic preparedness efforts. The respective policies with regard to wastewater surveillance are overseen and led by the European

JOINT RESEARCH CENTRE

Target the monitoring targets to public health use

Public health use

- **Routine:** surveillance for situational awareness and response to diseases and AMR, drug use, for which there are gaps in conventional surveillance that hinder adequate response
- **Emergency:** determine spread of outbreaks, molecular epidemiology, evaluation of outbreak control actions; early warning of diseases following disasters
- **Pandemic preparedness:** global observatory of infectious diseases and AMR circulation with early warning of emerging diseases/agents



LOCAL
Emergency

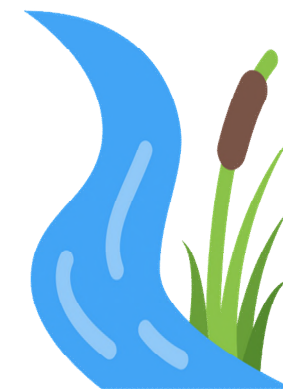
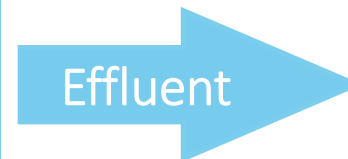
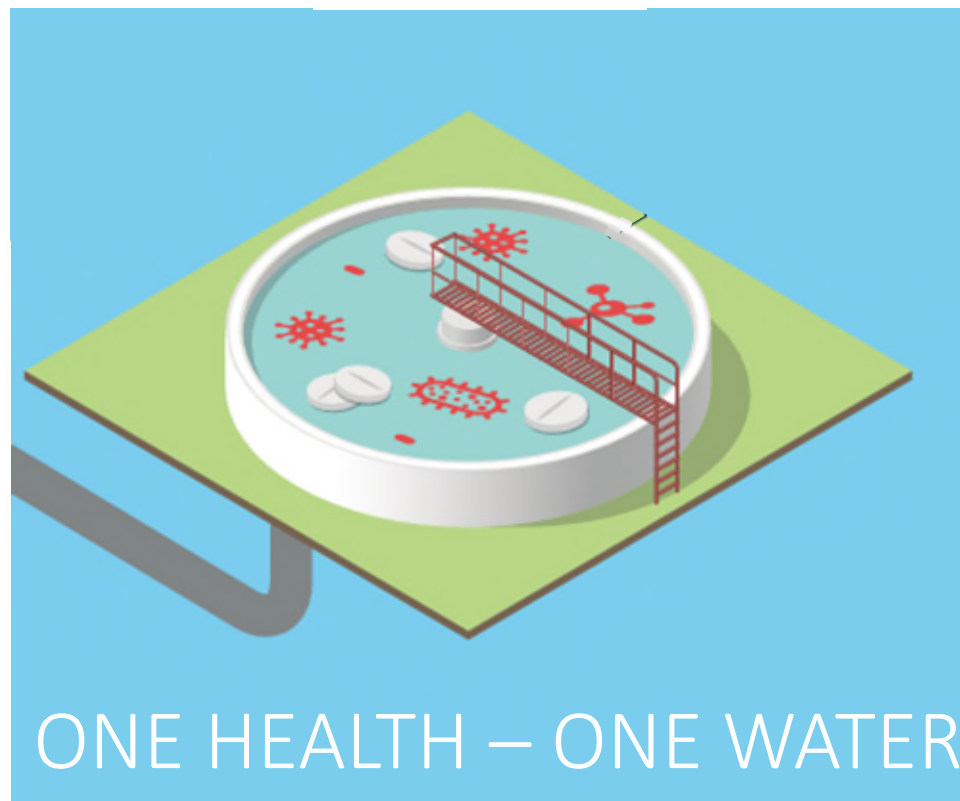
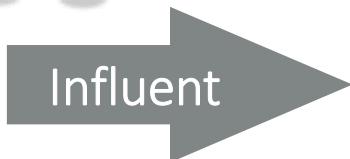


NATIONAL
Routine



GLOBAL
Pandemic
preparedness

~
New EU Urban Wastewater Treatment Directive:
Monitoring wastewater for public health and for the water sector: win-win



Monitoring for **public health**:

Viruses

Antimicrobial resistance

Pharmaceuticals

...

Monitoring for **environmental health**:

Viruses (bathing, reuse)

Antimicrobial resistance (bathing, reuse)

Pharmaceuticals (reuse, environment)

...



In

= wastewater treatment control

Out

Our activities would not have been possible without our partners, collaborators, funders



Bijlage

Supplementary information

This supplementary material is hosted by Eurosurveillance as supporting information alongside the article 'Agile, on-demand wastewater surveillance of virus infections to support pandemic and outbreak response in Rotterdam-Rijnmond, the Netherlands, 2020 to 2022' on behalf of the authors, who remain responsible for the accuracy and appropriateness of the content. The same standards for ethics, copyright, attributions and permissions as for the article apply. Supplements are not edited by Eurosurveillance and the journal is not responsible for the maintenance of any links or email addresses provided therein.

Table S.1 Evaluation of the wastewater surveillance case studies of Rotterdam Rijnmond against the attributes of a quality surveillance system.

Attributes	Case study 1	Case study 2	Case study 3	Case study 4	Case study 5	Case study 6
Objective	Passive sampling is a feasible method that could reliably detect SARS-CoV-2 in wastewater in different community contexts.	Evaluation of community transmission after the first case of the Omicron variant was identified in the region	Early warning of viral introduction in displaced populations.	Determine if student houses are hot-spots for introduction of SARS-CoV-2 in Rotterdam after the summer holidays	Determine if neighbourhoods with low vaccination coverage result in more SARS-CoV-2 infections	Detection of local transmission of MPXV
Sensitivity	Similar positivity rate and (normalized) concentrations of SARS-CoV-2 RNA in 24h volume-proportional samples and composite samples, similar concentration-trends over time observed in both sampler types.	Higher concentrations of SARS-CoV-2 were detected in round 1 (7-9 Dec 2021) than in round 2 (28-30 Dec 2021). No Omicron was identified during week 1 (ddPCR and sequencing) but its presence was confirmed in week 2 in both passives and sewage water. Numerical values of the Omicron proportion were variable, probably due to relatively low proportions of Omicron.	Due to potential stigmatization of this population, and the complexity of obtaining consent from all stakeholders the case study was cancelled.	SARS-CoV-2 was detected at each student home, but at different positivity rates and (normalised) concentrations. CrAssphage indicated similar amounts of human faeces were sampled in each sample.	Virus circulation was shown amongst all the populations measured. Distribution of viral circulation was equal in most neighbourhoods, except for one of the neighbourhoods with high vaccine coverage, which had a high amount of SARS-CoV-2 present in October 2022. There was no explanation for why this area had higher virus-circulation.	MPXV was found in the sewage water of the area in 20-80 gene copies per passive sample in the wastewater in the street of a known case. The presence of MPXV was shown in the sewage water, in low copy numbers in 2 samples, which were positive for both the Orthopox qPCR and MPXV qPCR and ddPCR assay.
Representativeness	A large COVID-19 peak was detected both in clinical data from the last week of October and in wastewater tested on 27 of October 2021. In areas with low circulation, trends were more variable. The overall trend observed with passive samplers was similar to the	Omicron BA.1 was detected in round 2 at each site in the area		The student houses surveyed were representative of larger student residences, common in the Netherlands, that house 80-150 students in single-person studios, or shared apartments within the same building.	All neighbourhoods were adequately measured, with the passives distributed across 8 sewer pumping stations to cover the complete neighbourhoods with all inhabitants. One neighbourhood needed to be sampled at 3 pumping stations, one at 2 and the	MPXV was found in the wastewater in the street of a known case.

	trend observed with a 24h volume-proportional sampler.				other 3 at one pumping station each.	
Completeness	Sites were selected from the sewer network to cover the different city area populations and matched with the clinical surveillance via zip code. Deployment of passive samplers was feasible at each selected site.	Sites were selected from the sewer network to cover the area of the case residence and the wider community via zip code. Deployment of passive samplers was feasible at each selected site and 24h volume composite samples were also available for the wastewater treatment plant. This allowed comparison between passive and composite samplers.		Proof of concept, using 5 student houses as sentinel. Sites were selected from the sewer network to cover the 5 student houses. Deployment of passive samplers was feasible at each selected site.	Sites were selected from the sewer network to cover the city areas with different vaccination rates. Deployment of passive samplers was feasible at each selected site. All identified neighbourhoods were sampled for 2.5 months.	Sites were selected from the sewer network to cover the area of the case residence and the wider community via zip code. Proof of concept: shown for only one local case. Repeated for a second case (data not shown).
Validity	4/80 passives could not be recovered from the sampling site (3 as the suspending strings became entwined due to variable water flow and the strings broke, 1 for reasons unknown). Passives were no longer installed in parallel & no more went missing. 72/76 yielded a valid result. 4 results were considered invalid because CrAssphage	All samples were successfully recovered. The recovery was high for all passives. Duplicate wastewater samples showed very little variation. The concentrations in passive samples correlated well (after normalization) with 24-hour volume-proportional wastewater samples.		134 of 140 samples provided valid results. The SARS-CoV-2 PCR control indicated lab errors in 4 samples. The CrAssphage assay indicated 2 samplers had captured very little human faecal material.	229/238 passive samples produced a valid result. 1 passive was lost because the rope was cut, 1 did not contain cotton tips. SARS-CoV-2 PCR control indicated that 7 samples did not produce a valid result due to lab errors.	All 9 passive samplers that were deployed produced valid results. Quality controls including positive and negative PCR controls, blank samples and PCR inhibition controls, gave good results. Combining RT-PCR assays of both Orthopox and MPXV assays as well as an additional ddPCR assay for West African MPXV provided confirmation. CrAssphage

	<p>indicated very low recovery of human faecal material on the passive sampler. The other passive sampler results were reliable and correlated well with 24-hour sewage samples. Triplicates, measured daily for 1 week, provided full, valid results on 3 days and agreed well with 24 hour-sampling (they deviated substantially on 1 day). Averaged results from passives compared well with results from the pumping station on 4/5 days but deviated by a factor of 3 on day 5.</p>					<p>was used to determine amount of human faecal material captured on the passive samplers, as previously described.</p>
Timeliness	<p>Although not critical here, appropriate sampling sites in the sewer network were identified within a week. Sites were sampled at the planned timing.</p>	<p>Passives reached and were processed in the lab on the same day as they were recollected from the test location. N2 and CrAssphage measurements were available in 1 day. Results were available within 5 days after the request the ddRT-PCR results on day 6 & sequencing on day 14.</p>		<p>Preplanned 2 months before start. The samples of a week were analysed as one batch, making the time-to-result 1-5 days.</p>	<p>Preplanned 2 months before start. The samples of a week were analysed as one batch, making the time-to-result 1-5 days.</p>	<p>Implementation was executed within days of the availability of information surrounding the first cases in Rotterdam-Rijnmond. The first series of passive samplers were installed in the sewer mains that drained the area of the mpox case residences within 2 days of receipt of the request. The samples of the week were analysed as one batch, making the time-to-result 1-5 days.</p>

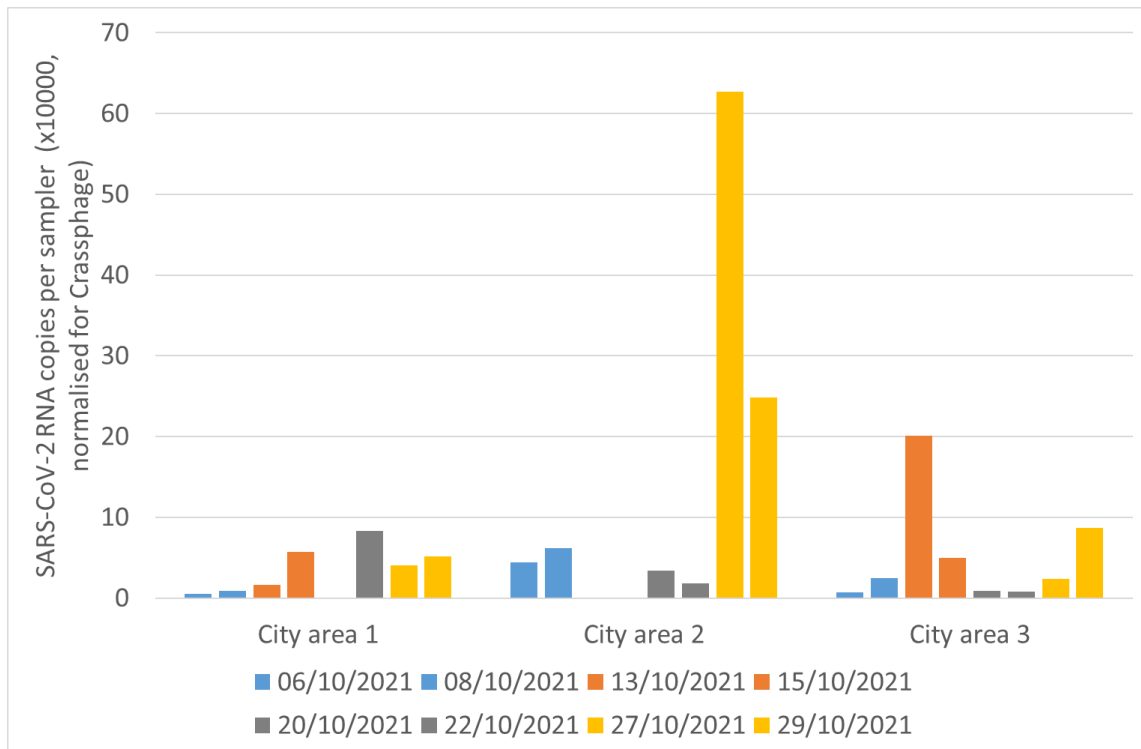
Acceptability	The city areas were large enough for the sampling to be anonymous, but it was recognized that smaller areas could result in potential identification. Also the potential for stigmatization was indicated.	Even though this was at a small scale, the area of the case was sampled without the address disclosed, and with sufficient other addresses to ensure the case was not identifiable.		Letters were sent out to the housing organisations of the student locations to inform them of the research. They were facilitated to easily inform the residents. As described in the main text, informed consent was not required.	Areas were sufficiently large not to result identifiable cases.	Even though this was at a small scale, the area of the case was sampled without the address disclosed, and with sufficient other addresses to ensure the case was not identifiable.
Simplicity	All sites were readily identified and could be sampled without obstructing traffic. Processing of passive samples in the lab was conducted without problems.	Within 1 hour and 45 minutes of request receipt, the above-ground location was matched to the underground sewer infrastructure. The first passive was installed on the next working day.		Samplers were readily deployed to selected manholes. One manhole was not reachable at sampling day 10, due to construction work. All samplers were recovered.	Identification of appropriate sampling sites in areas of different vaccination rates was rapid. Passive samplers were easy to deploy at sewer pumping stations, only 1 of 238 samplers was lost.	Manholes were readily identified and sampled in the sewer network in the area of the case (without disclosing the address, and with sufficient other addresses to ensure the case was not identifiable).
Flexibility	All desired locations were sampled, both via manholes and pumping stations, and reference samples were collected at the pumping stations at the same time.	Samples could be taken close to the case, as well as a larger surrounding area via a pumping station, and a larger area via the wastewater treatment plant		Student homes sampled in planned period.	All desired locations in the different city areas were sampled.	All desired locations sampled.
Consistency	At small (6600-21000) population scale daily fluctuations were substantial and trend extraction was more difficult and less reliable than observed in earlier research at larger population scale (>75,000-128,000).	Not evaluated as only 1 case was reported at the time in this region		The three student locations showed different and dynamic patterns of SARS-CoV-2 concentrations in their wastewater, also after normalization for CrAssphage. The concentration in student homes was relatively low compared to the city areas and overall city wastewater data, indicating the virus was	The different city areas showed dynamic patterns of SARS-CoV-2 concentrations in their wastewater, also after normalization for CrAssphage. High variation per site hampered trend detection. One of the areas with high vaccination coverage showed a clear peak in concentrations in October 2022, so more prominent	Not evaluated.

				not particularly circulation among students at the time of the study.	trends were more discernible.	
Usefulness	Definition of minimal time from request to result: 3 days, but only if site selection is fast. A large COVID-19 peak was detected both in clinical data from the last week of October and in wastewater tested on 27 of October 2021.	Samples processed from passives could be sequenced just as well as from sewage water samples. Omicron was identified through ddPCR and sequencing in round 2 of testing at each of the sampling sites, indicating the VOC had spread through the community and quarantining and contact tracing was not a feasible option to contain further spread.		Variability of the observed concentration in sewers from the student houses obscured firm conclusions about the students as 'introducers' after the summer holidays.	Results were different than expected, and appear not to align with reports that vaccination protects contacts from infection. But differences in vaccination coverage are not very large and no other information on frequency and diversity of contacts of the communities is available.	The presence in the wastewater in the street of the case, but not in the surrounding area, showed proof of concept of MPXV detection in wastewater and supported no further local spread of MPXV.



Figure S.1 Passive and pensive sampler (A) and installation in manhole (B) and of the quality control of the samplers, showing large amounts of wipes deposited on the sampler (C) or the sampler moved outside of the wastewater flow (D).

Left



Right

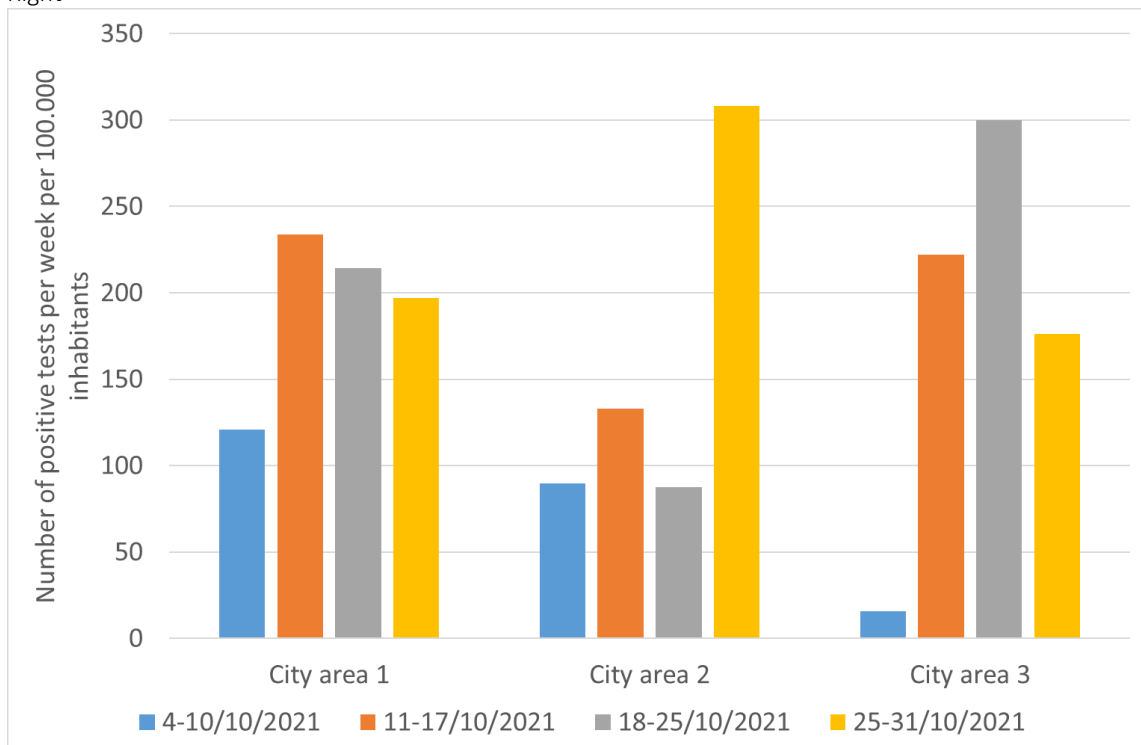


Figure S.3 Dynamics of the wastewater concentration of SARS-CoV-2 with passive samplers in small city areas (normalised for Crassphage, left) and the positive cases reported in the same areas and weeks (right). Two passive samples were taken each week, each sampling for 24 hours. The number of positive cases was reported on a weekly basis and the data reported in the week that encompassed the dates of the wastewater samples were used (see the corresponding colours in the left and right graph). In city area 2, no SARS-CoV-2 RNA was detected in the samples of 13 and 15 October 2021. In

city area 1, SARS-CoV-2 RNA was detected in the sample of October 13, 2021, but the Crassphage analysis was not reliable, so no normalized concentration could be calculated.

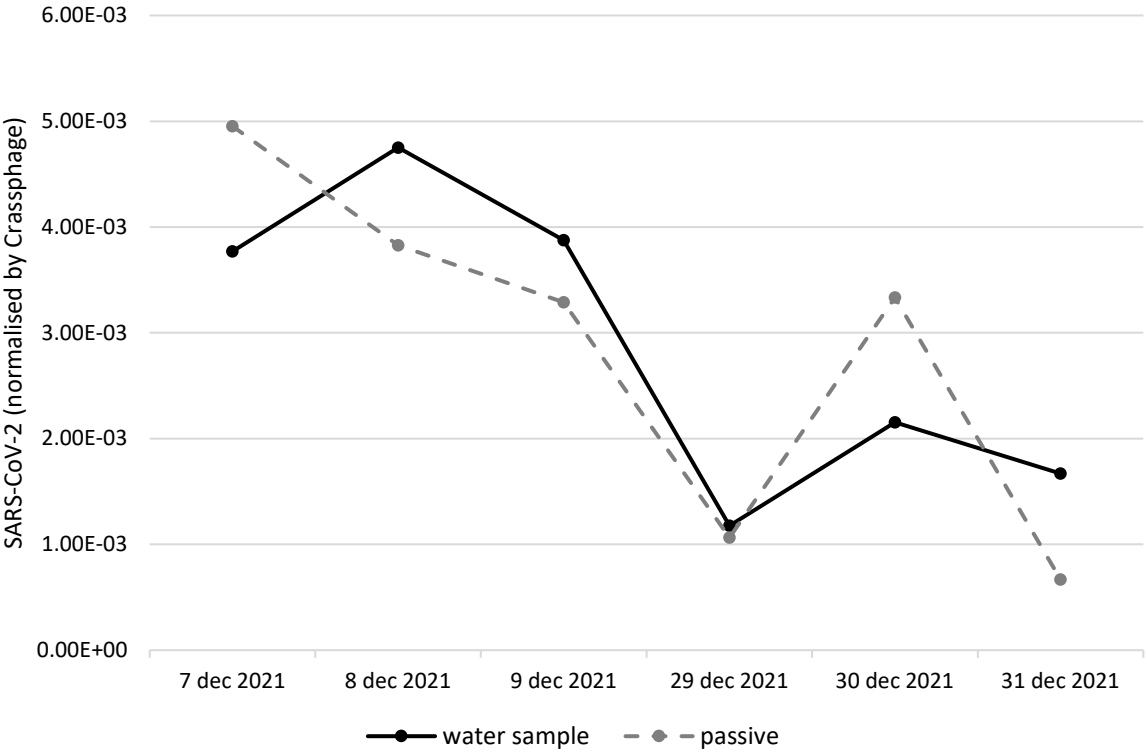


Figure S.4. Comparison of normalized SARS-CoV-2 RNA concentrations (SARS-CoV-2 gc/ml divided by Crassphage GC/ml) in 24-hour water sampling versus in passive samples in Case Study 2, detection of omicron variant in wastewater after the first diagnosis in the region, Rotterdam-Rijnmond, the Netherlands, December 2021.

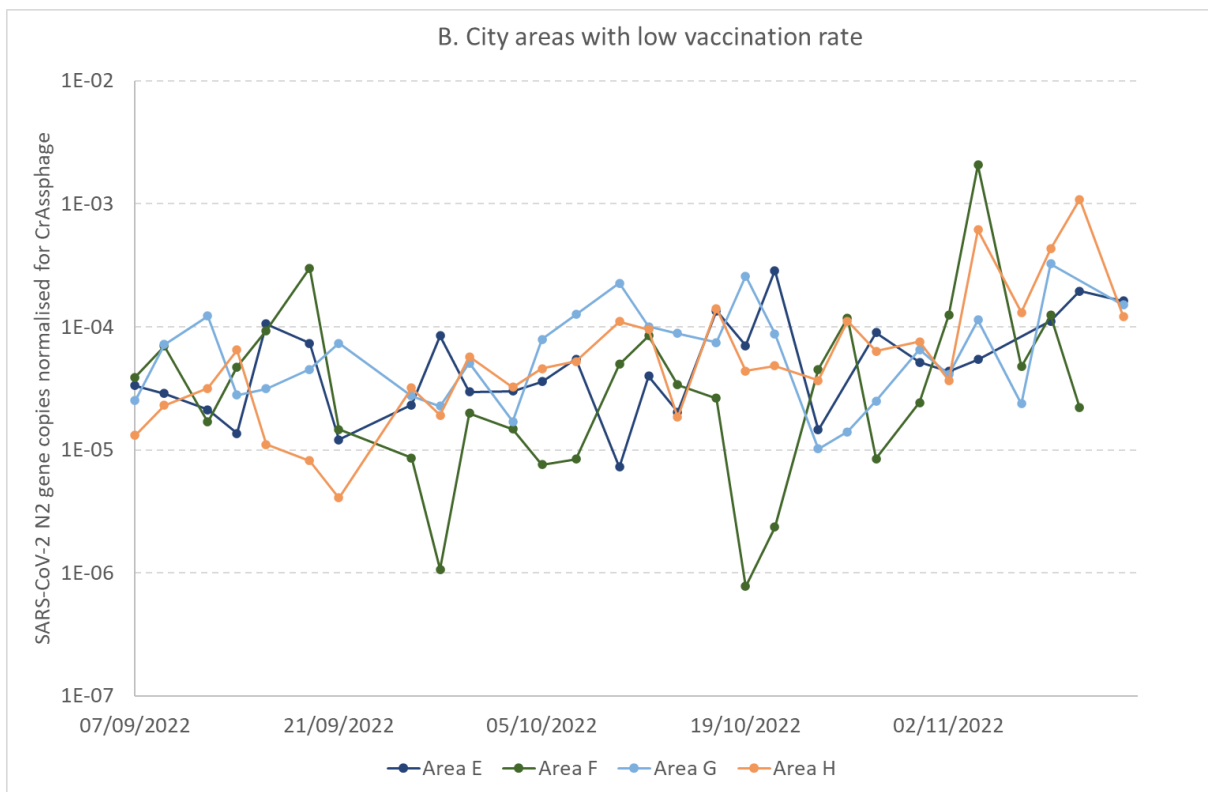
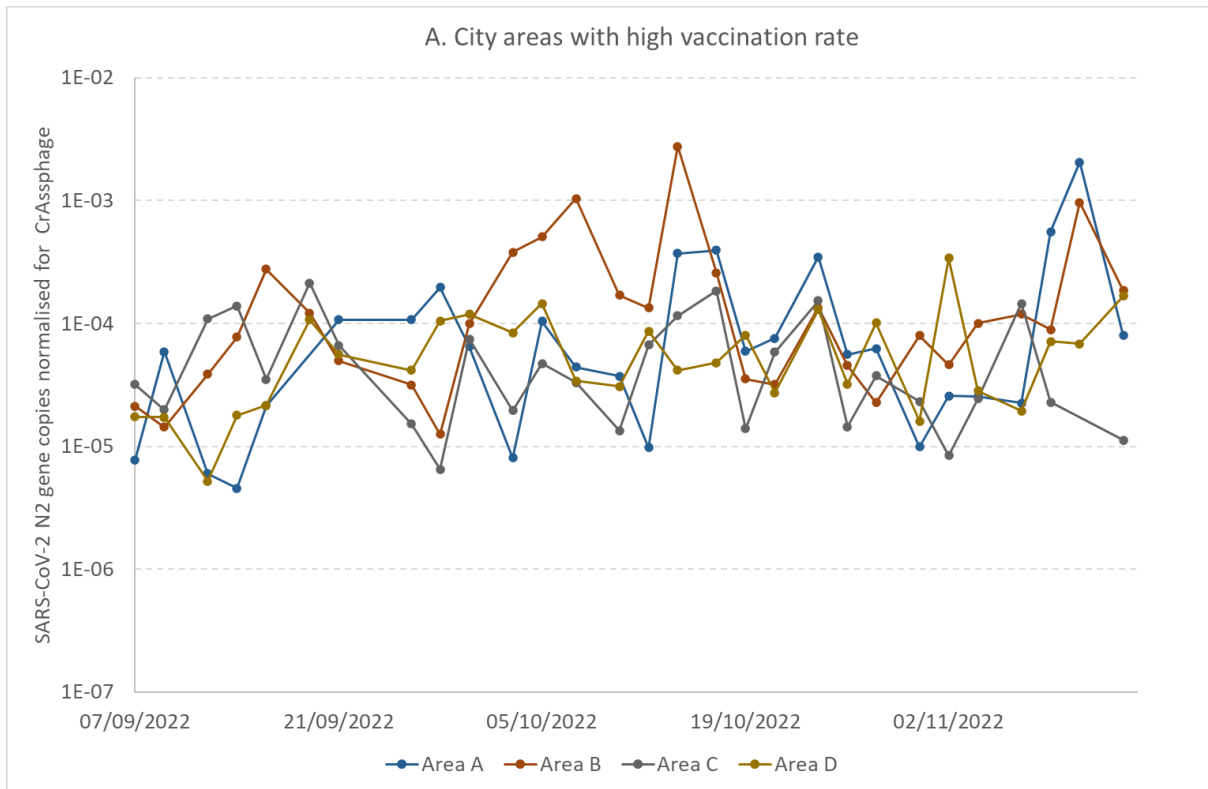


Figure S.5. Concentration of SARS-CoV-2 RNA in wastewater (normalized for CrAssphage) in city areas with high (A) and low (B) vaccination rates in September – November 2022.

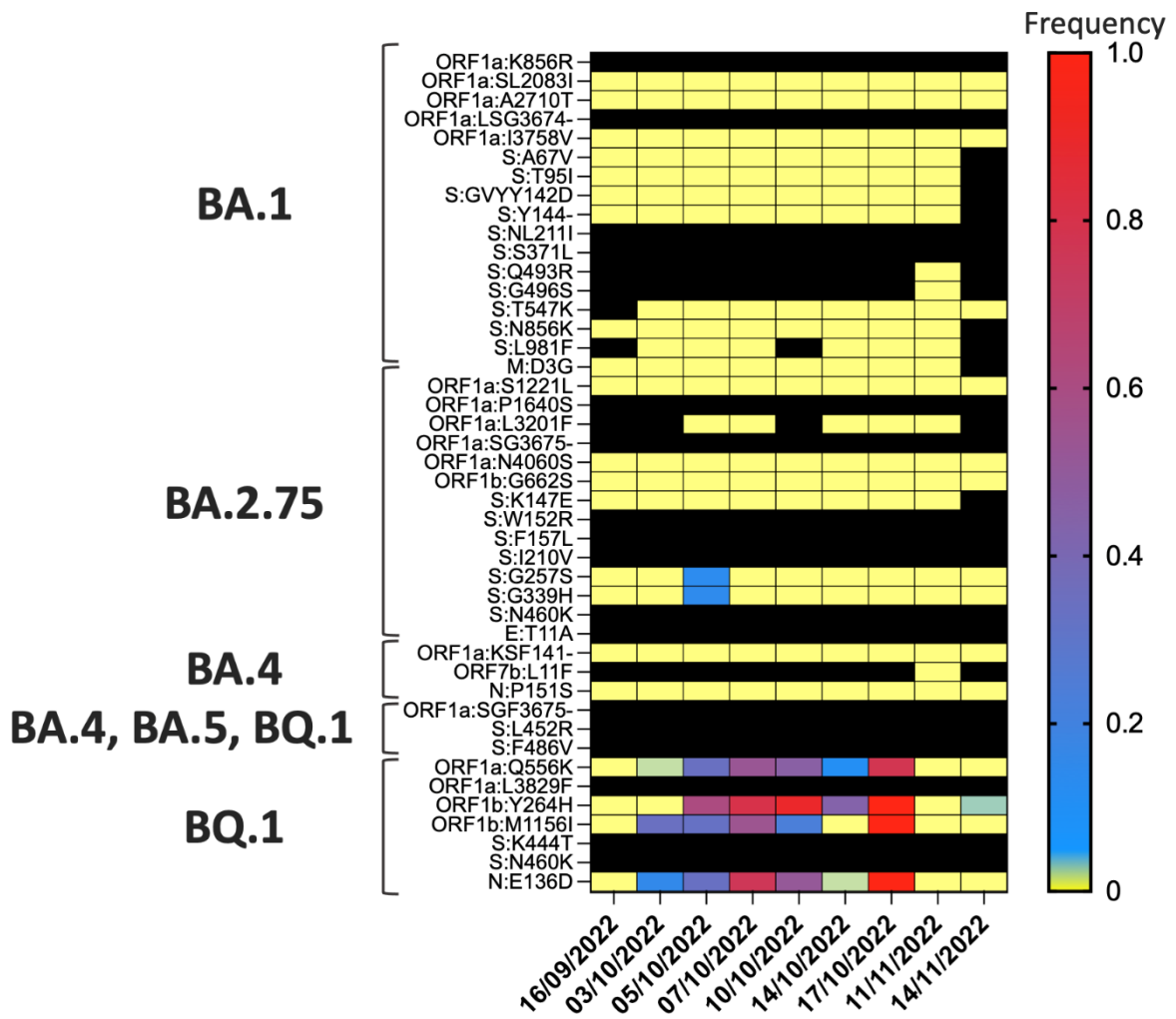


Figure S.6. Relative abundance of SARS-CoV-2 major lineages in the wastewater samples in Case study 5. Heatmap showing the frequency of the unique signature mutations per lineage.