Wastewater and Environmental Surveillance Summary for Measles, Mumps and Rubella

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World Health Organization

This document provides information on wastewater and environmental surveillance (WES) for measles. Other vaccine preventable diseases rubella and mumps are also included. This pathogen-specific summary should be used together with the WES Overview document which includes general and cross-cutting information (available here).

WES for measles at a glance

- There is limited but growing evidence of utility as a complementary measles surveillance tool with pilot results and initiation of at-scale multi-pathogen WES programs which include measles.
- These support the operational as well as technical feasibility for measles as part of multi-pathogen WES in sewered settings.
- There is inadequate evidence from non-sewered settings to assess these criteria (not shown).
- Wild type specific WES assays are important to discriminate pathogenic measles virus from vaccine-associated RNA as live attenuated vaccines may, rarely, result in low level shedding.

Table 1: At a glance' assessment of key WES criteria for measles given current evidence in sewered settings [a, b]

Setting	Categorical Assessment (CA) / Strength of Evidence (SoE)	Public Health Significance	Actionability / Relative value	Technical Feasibility	Operational Feasibility	Acceptability	Optimisation	
							Integrated disease response	Multitarget WES
Sewered	CA							
	SoE							

Key:

1. Categorical Assessment (CA) of criteria

Category	Code	Description
High		Criteria is evaluated as met at the highest level
Intermediate		Criteria is evaluated as met at an intermediate level (it may be that not all sub-components of the criteria are met)
Low		Criteria is evaluated as low
Not-supported		Criteria is evaluated as not supported
Not applicable		Criteria is not applicable OR cannot assessed due to inadequate evidence

2. Strength of evidence (SOE)

Evidence level	Code	Description
Strong		High quality consistent evidence, including from multiple relevant studies/settings, at scale, over a prolonged period, with evidence from program settings, not only from research studies or short projects.
Moderate		Relevant evidence is available but does not meet criteria for 'Strong' classification. [c]
Inadequate evidence		Evidence is inadequate and further study/evaluation is needed

[a] Further description of the criteria used to assess the applicability of WES for a specific pathogen, as well as the methods used to evaluate them, is included in *WES Guidance for one or more pathogens*. The assessment in Table 1 provides a snapshot at the global level, but country level assessment may differ.

[b] Sewered settings refers to closed reticulated sewage systems. Non-sewered settings refers to the diverse settings which are not 'sewered', including open drains and community sampling points. Individual small septic tanks at residential or building level are not viable to sample individually and are not considered here separately. Most WES evidence to date is reported from reticulated sewered settings, often from high-income settings. Yet much of the global population is on heterogenous non-sewered systems and this has implications for assessment of various WES categories.

[c] Evidence classified as 'Moderate' meets one or more of the following criteria: not from numerous settings, for a short period, without program-level evidence, and/or where findings are not consistent or of high quality.

Summary

- Measles, mumps, and rubella viruses are human pathogens of major global importance.
- Vaccination with measles-containing vaccine (MCV) has averted millions of deaths globally.
- Measles has been named a target for elimination by 2030; however recent decline in vaccine coverage and increasing vaccine hesitancy have resulted in increased outbreaks worldwide.
- Measles is extremely infectious with a R0 among the highest for vaccine preventable diseases.
- Transmission of measles is primarily by respiratory droplets and aerosols. There is no zoonotic source. Measles outbreaks occur where vaccination coverage is suboptimal. High levels of vaccine coverage (>95%) are needed to prevent outbreaks.
- Shedding of measles virus (MeV) occurs in high concentrations during acute infection, especially in respiratory secretions as well as in urine. While shedding may be prolonged over weeks in an immunocompromised host, chronic shedding is not a feature of the standard clinical course.
- Low level shedding may also occur following vaccination with live attenuated measles vaccination.
- Vaccine derived measles is uniquely genotype A while pathogenic measles are genotypes B3 and D8.
- Evidence for detection of MeV RNA in WES is rapidly expanding; measles is included in multiple WES pilots and is integrated within at-scale multi-pathogen WES programs in diverse global settings.
- MeV RNA can be detected in wastewater. WES MeV results can distinguish vaccine- from disease-derived genotypes using RT-PCR and/or sequencing methods. PCR-based methods that target the specific viral genomic targets can be more sensitive, rapid, and at a lower relative cost than non-targeted whole genome sequencing of wastewater.
- MeV sequencing is feasible if viral load in the sample is adequate, and sequences from wastewater can be used with sequences from clinical cases to infer source and travel association. WES is not included in current WHO surveillance recommendations for measles, mumps or rubella.
- However, given expanding evidence for detection of MeV RNA in WES, **there is a potential role of WES to strengthen surveillance** over case-based surveillance alone as follows:
    - Routine WES as an early warning of incursion/local circulation
    - Agile responsive WES in outbreak contexts, including to assess the geographic extent of circulation, inform targeted responses such as the focus of communications, supplementary vaccination activities, to assess the effectiveness of the outbreak mitigation, and to help confirm the end of the outbreak.
- Use of WES for measles may be particularly relevant among populations with suboptimal vaccine coverage and heightened risk to measles exposure (through travel or local exposure) and/or adverse measles outcomes, especially if clinical surveillance is weak or when multiple genotypes circulate.
- Research priorities for measles WES include better understanding of viral shedding and detection in environmental waters; optimizing and validating highly sensitive sampling and laboratory methods; evaluating and modelling value-addition over case-based surveillance alone in varied contexts, including non-sewered settings; and to its potential future role in elimination certification.
- There is very limited evidence for WES applications for rubella or mumps and further research is needed. The technical feasibility of measles-rubella multiplex assays is established.

Contents

1. General information

1.1. The pathogens and associated disease

Each of the viruses causing measles, mumps and rubella (MMR) are **enveloped RNA viruses** (1–3)**.**

Measles virus (MeV) is a highly contagious virus of the family *Paramyxoviridae*, genus *Morbillivirus* (1). There has been a reduction in circulating genotypes from eighteen detected in 2003 to only two genotypes, B3 and D8, detected since 2021 (4). Attenuated vaccine measles strains are of genotype A. MeV causes measles, an acute febrile-rash illness characterized by fever, cough, coryza, conjunctivitis, and a maculopapular rash. Complications can be severe and include pneumonia, acute encephalitis, and death, particularly among young children and immunocompromised individuals (1,5,6). Beyond the acute illness, measles has two important long-term consequences:

- Sustained immunosuppression: measles infection can induce "immune amnesia," erasing previously acquired immunity to other pathogens and leaving individuals vulnerable to secondary infections for months to years (7).
- Subacute sclerosing panencephalitis (SSPE): a rare (approximately 1 in 10,000 cases) but fatal late complication that typically develops 7–10 years post-infection, causing progressive neurological deterioration (8).

Rubella virus belongs to the family *Matonaviridae* , genus *Rubivirus* (3,9). It causes rubella ("German measles"), a typically mild febrile-rash illness in children and adults, marked by low-grade fever, lymphadenopathy, and a transient maculopapular rash. While usually self-limiting, rubella poses major public health concern during pregnancy: maternal infection, especially in the first trimester, can lead to **congenital rubella syndrome (CRS)**. CRS results in miscarriage, stillbirth, or severe lifelong disabilities such as deafness, cataracts, microcephaly, and congenital heart disease (1,10,11).

Mumps virus is a member of the family *Paramyxoviridae*, genus *Orthorubulavirus* (2) which causes mumps, a contagious disease most often recognized by parotitis (swelling of the salivary glands). Symptoms may also include fever, headache, malaise, and muscle aches. Complications, while less common, are clinically important including orchitis (which can impair fertility), oophoritis, aseptic meningitis, pancreatitis, and permanent sensorineural deafness (12) (13)**.**

1.2. Global burden, geographic distribution and risk factors

Measles remains a leading cause of childhood morbidity and mortality, with mortality highest in infants and young children and in sub-Saharan Africa (14). In 2023, WHO and US CDC estimated 10.3 million measles cases and >107,000 deaths worldwide, representing a 20% annual increase in cases compared to 2022 (1,15) After decades of significant progress, there has been a resurgence of cases and outbreaks in recent years threatening the long-held elimination status in some regions and countries and global progress disease toward elimination (16,17). Some high-income countries have experienced vaccine hesitancy that undermines adequate population immunity and cause recurrent measles outbreaks (1).

Rubella is endemic in many regions but has declined substantially with global scale-up of combined measles-rubella (MR) vaccination. Reported rubella cases dropped from ~670,000 in 2000 to ~17,800 in 2022 (3), though this underestimates true incidence due to weak surveillance in many low- and middle-income countries. While acute rubella is generally mild, the major burden is **congenital rubella syndrome (CRS).** WHO and partner modelling estimated that in the absence of vaccination, rubella caused ~100,000 CRS cases annually worldwide; by 2019, this had declined to ~32,000 CRS cases (95% CI: 13,000–60,000) (6). The highest risk remains in the WHO African and Eastern Mediterranean Regions, where rubella vaccine coverage remains below 50% (10). Risk factors include low vaccination coverage, delayed vaccine introduction, and susceptibility in women of childbearing age.

Mumps burden is less well quantified globally because it may not be a reportable disease with variable surveillance and under-reporting, and deaths are rare. However, outbreaks continue to occur worldwide in both vaccinated and under-vaccinated populations. In Europe and the US, where surveillance is established, cases and outbreaks are reported among adolescents and young adults with incomplete or waning vaccine-induced immunity (18,19).

1.3. Hosts and routes of transmission

Measles, rubella and mumps are human pathogens. While non-human primates can be infected with measles (20), zoonotic reservoirs are not known to exist. There are however, viruses which are closely related to rubella virus suggesting a likely zoonotic origin and potential for future zoonotic transmission (21).

- Measles: human pathogen; spread via airborne respiratory droplets and aerosols (1) (22). Measles is extremely infectious with a R_0 among the highest for vaccine preventable diseases, while the R_0 is often stated as between 12 – 18 in susceptible populations the R_0 varies widely depending on contextual factors and super-spreader events may occur (23,24). Transmission may occur prior to symptom onset.
- Rubella: human-only host; transmission by respiratory secretions with transplacental infection leading to congenital rubella syndrome (CRS) (3).
- Mumps: human-only host; transmission by respiratory droplets and saliva (2).

2. Information related to MMR and wastewater

2.1. Potential inputs to wastewater and environmental waters

Wild-type measles, rubella and mumps viruses have all been detected in urine and upper respiratory samples (but not stool) following acute infections with wide intraindividual variation. Measles, rubella and mumps all have attenuated live vaccines which means that RNA fragments from both the pathogenic wild-type virus and the vaccine may theoretically be shed. Vaccine associated RNA has been documented in urine and upper respiratory samples for both measles and rubella, noting this is at lower relative viral levels compared to that shed by acute infections with the pathogenic wild-type virus. Mumps vaccine virus has been detected in buccal swab samples from rare parotitis cases following vaccination (25).

Of note, overall shedding is expected to be lower into wastewater and environmental waters given: a) individual shedding results in much lower viral loads and of shorter duration compared to those seen in enteric and some respiratory pathogens (eg polio, SARS-CoV-2) and b) case-loads are also typically few in comparison to other respiratory pathogens such as SARS-CoV-2 and influenza (except in large outbreaks). However, there may be exceptions in large outbreaks such as those that occur in under-vaccinated communities such as refugee/displaced person settings.

Table 2. Summary of shedding evidence for measles, mumps and rubella (relevant to inputs into wastewater and environmental waters)

Pathogen	Urine	Faeces	Upper respiritory. specimens [1]	Vaccine derived shedding	Zoonotic Source	Prolonged shedding Wildtype/ vaccine
Measles	✓✓ (26–28)	?/✕	✓ (26–28)	✓ (29–32)	Humans main	✕/✕
Rubella	✓ (33)	✕	✓✓ (33)	✓ (34,35)	✕	✕/✓ (35)
Mumps	✓ (36–39)	✕	✓✓ (36–39)	✕	✕	✕/✕

[1] Upper respiratory specimens include saliva, throat swab, and nasopharyngeal swab.

Measles

- MeV is shed in respiratory secretions and urine in acute infection (27,28). Wide intraindividual variation occurs with not all acute cases shedding into urine. Of note, urinary shedding is of longer median duration than viremia.
- Immunocompromised individuals have been shown to have higher extended shedding rates compared to those with normal immune function (>28 days) (26) however chronic MeV shedding (>3 months) has not been documented.
- Modelling studies have estimated the viral loads in sputum, saliva and urine (40).
- There is an absence of studies to evaluate faecal shedding directly. It is known that measles infects lymphoid tissue including Peyer's patches (41,42). Characterization of faecal shedding represents a key knowledge gap in relation to WES applications.

- Shedding of measles vaccine virus (MeVV) RNA also occurs (29–31)
- Approximately 5% of vaccine recipients may develop a vaccine reaction 8-12 days after vaccination and measles virus RNA is detectable in these vaccine reactions (43,44).
    - MeVV RNA can be detected in nasopharyngeal swabs up to 29 days post measles containing vaccination; the amount of vaccine RNA shedding is low (31). A recent study showed MeVV shedding in the respiratory tract may be prolonged over 3 months (30).
    - MeVV RNA is detected in urine of vaccinated individuals over 14 days (45)

Rubella

- In acute rubella infection, rubella virus RNA is shed for a short duration in respiratory secretions and, less frequently in urine (33).
- Rubella vaccine virus has also been documented to be shed with rare case reports including transient shedding in healthy individuals and a single case report of prolonged shedding in immunocompromised individuals (34,35).

Mumps

- In acute mumps infection, mumps virus RNA is shed in saliva, respiratory secretions and, less frequently in urine, typically of short duration < 1 week (36–39).

2.2. Target persistence and degradation in wastewater

All three (measles, rubella and mumps) are enveloped RNA viruses, which theoretically means they are generally less stable outside the host and more susceptible to inactivation by temperature, detergents, and desiccation compared with non-enveloped viruses.

Laboratory evidence from one study reported limited viral decay of measles, mumps and rubella RNA; this involved spiking experiments at different concentrations and temperatures (4 degrees Celsius and at room temperature) over 28 days (46).

Given the typical transit time from host through wastewater to laboratory of hours to a few days, this supports the feasibility of wastewater monitoring for nucleic acid (not infectious virions) of all three pathogens. Correlation of WES results with measles and mumps cases provides additional supportive evidence and is described in the next section.

2.3. WES experience for measles, mumps and rubella

Measles

Published evidence includes multiple pilots and research studies summarized in Table 2.1 below and in the Case Studies at the end of the document. There are a growing number of at-scale WES national programs where measles has been integrated as part of ongoing multi-pathogen surveillance. These include South Africa since January 2025 with more than 60 sites sampled weekly (47,48) - see Case Study 1: Measles WES in Republic of South Africa – Informing Targeted Response. The large US National Wastewater Surveillance System with more than 400 sites nationally integrated wildtype measles WES with its multi-pathogen surveillance program since August 2025 (49)following vanguard work by the Wastewater SCAN Project across 150 sites since May 2025 with ongoing public-facing reports (50,51) - see Case Study 3: Initiation and integration of measles in multipathogen WES at scale – USA early adopter. Guinea also has a large-scale WES program involving predominantly non-sewered sites – see Case Study 4: Environmental surveillance for Measles in the Non-Sewered Setting of Conakry, Guinea (2022-2025).

Mumps and Rubella:

Evidence is extremely limited for rubella and mumps viral RNA detection in wastewater, but technical feasibility for multiplexing of measles, mumps and rubella together is demonstrated (52). A study in South Africa identified rubella (and measles) in wastewater samples from districts with no reported cases (53). Another study from the Netherlands demonstrated detection of mumps virus RNA in wastewater correlating with areas with known cases in an outbreak (54).

Table 3. Measles wastewater and environmental surveillance studies

Location	Research aim / methods	Sampling methods	Analytic methods	Period of sample collection (number of samples)	Key results	Contribution	Reference
Netherlands	*Polio ES pilot; measles tested retrospectively during outbreak*	*Grab samples from sewage pits at schools/residential areas*	*RT-PCR + Sanger sequencing (N-450 genotyping)*	*2013 {retrospective samples during measles outbreak}* *(56 samples)*	*6/56 (11%) MeV RNA positive, genotype D8; detections matched outbreak areas*	*First evidence of measles RNA in sewage; demonstrated correlation with cases*	*Benschop et al., 2017* (55)
South Africa	*Assess feasibility of measles WES during outbreak*	*Grab samples from 28 sentinel WWTPs + 19 Gauteng catchments*	*RT-dPCR assays (validated wild-type vs vaccine strain)*	*Feb 2021–Mar 2024* *(2,149 samples)*	*43/2,149 (2%) positive; wild-type and vaccine strains detected; wastewater found measles in 48% of district-weeks without clinical cases*	*Demonstrated outbreak monitoring potential with WES supplementing case data; first field test of wild-type vs vaccine differentiation informed public health response*	*Ndlovu et al., 2024 (preprint) (47)* *McCarthy et al., 2025 (53)*
France	*Validate multiplex RT-dPCR for measles detection in wastewater*	*24h composite influent samples from 3 WWTPs (pop. 150k–600k)*	*Multiplex RT-dPCR targeting N, P, M genes; vaccine-specific assay; controls*	*Jan–Jul 2024*	*18/40 (45%) samples positive; confirmed wild-type B3/D8; aligned with local case reports*	*First validated multiplex RT-dPCR for measles; robust detection and reduced false positives*	*Roman et al., 2025* (56)

Ottawa, Canada	*Investigate unexpected measles RNA detections; differentiate vaccine vs wild-type*	*24h composite sludge samples, main WWTP (~91% pop.)*	*RT-qPCR; sequencing (N450)*	*2020–24 (archived); detections in 2024*	*11/135 samples (8%) MeV positive; all genotype A (vaccine strain); correlated with immunization campaigns*	*Demonstrated importance of genotyping to avoid misinterpreting vaccine shedding as outbreak*	*Tomalty et al., 2025* *(32)*
Belgium	*Investigate measles circulation in wastewater; genotyping feasibility*	*24h influent samples, 5 WWTPs (Brussels, Leuven, Antwerp)*	*RT-qPCR; nested PCR + Sanger sequencing (N-450)*	*Feb–Mar 2024 (weekly)*	*MeV RNA in 3 consecutive samples at Brussels North; genotype D8; negatives elsewhere*	*Early-warning of circulation in Brussels; genotyping feasible during low incidence*	*Rector et al., 2024* *(preprint)* (57)
Switzerland	*Retrospective analysis of 2024 outbreak*	*64 × 24h composite influent samples, Lausanne WWTP (pop. 240k)*	*Duplex dPCR (Wu et al. assay) distinguishing WT vs vaccine*	*Jan–Mar 2024 (tested retrospectively Oct–Dec 2024)*	*MeV RNA in 9/64 samples (14%); viral loads peaked early; none in smaller later cluster*	*Showed WES alignment with outbreak curve; detection threshold depends on outbreak size and catchment*	*Gan et al., 2025* (58)
Texas, USA	*Test WES for outbreak detection vs clinical reporting*	*Weekly 24h composite influent samples from 2 cities (pop. 266k, 103k)*	*RT-PCR (N, M genes); confirmatory Sanger sequencing*	*Jan–Mar 2025 (22 samples)*	*MeV RNA detected 1–2 weeks before first confirmed case; genotype D8; detections in one city without cases*	*Demonstrated early-warning potential; revealed silent transmission*	*Joseph et al., 2025 (preprint)* (59)
Texas, USA	*Sequence-based WES during outbreak; importation vs endemic differentiation*	*2 Houston WWTPs (~218k residents)*	*Hybrid-capture sequencing (Illumina) + confirmatory RT-ddPCR*	*Jan 2025 (prospective); compared with 821 prior negatives*	*MeV RNA detected Jan 7; genotype B3; linked to 2 travelers; excluded endemic spread (D8 elsewhere)*	*Provided sequencing evidence for link to travelers relevant for elimination verification (imported vs endemic)*	*Javornik Cregeen et al., AJPH 2025* (60)

Texas, USA	*PCR-based WES during outbreak*	*Twice weekly 24h composite influent samples from seven cities (30-1400 kms from fixed point)*	*RT-qPCR, digital PCR and digital droplet PCR*	*Jan -June 2025*	*MeV and MeVV detections with spatial correlation to cases.*	*Demonstrated outbreak monitoring potential with WES supplementing case data; included assessment of LOD/LOQ and comparison of three PCR methods for wild type MeV and vaccine-derived MeVV*	*Langan et al, 2025* (61)

Note two other studies from Malawi (62) and South Korea (63) also reported measles WES as part of multi-pathogen WES pilot studies. However, these are not included in table above as neither reported any detections of measles RNA in wastewater and there were no reported measles cases in Malawi during the one month study period and few reported cases in South Korea with an unknown relationship to sampling period or locations.

Table 4. Measles method development studies (with field samples)

Location	Research aim / methods	Sampling methods	Analytic methods	Period of sample collection (number of samples)	Key results	Contribution	Reference
Halifax, Canada	*Develop multiplex assay for SARS-CoV-2, RSV, flu, measles; field validation*	*24h composite samples (55k WWTP) + passive samplers from 3 sewer-sheds*	*Multiplex RT-qPCR vs monoplex*	*May–Jul 2022 (44 samples)*	*Multiplex sensitivity comparable to monoplex; measles assay validated with spiked wastewater and field-tested*	*Showed feasibility of multiplex including measles in routine surveillance*	*Hayes et al., 2023* (64)
USA (Houston lab validation)	*Develop multiplex RT-ddPCR assay for measles, mumps, rubella; persistence and partitioning*	*Raw influent wastewater (spiked + 1 outbreak sample); separated liquid/solids*	*Multiplex RT-ddPCR assays (WT vs vaccine probes); sequencing*	*2019–24 (validation); outbreak sample 2024*	*WT vs vaccine measles assay validated (B3 vs Edmonston); RNA persisted 40d at 4°C, 6–8d at RT*	*First multiplex covering MMR; persistence/partitioning insights; distinguished WT vs vaccine*	*Wu et al., 2024 (46)*

Table 5. Measles Wastewater Surveillance Programs – at scale as part of multi-pathogen surveillance

Location	Aims	Sampling methods	Analytic methods	Period of sample collection	Key results	Contribution	Reference
Republic of South Africa	*Integrated measles surveillance (clinical lab and WES)*	*Municipal WWTP influent /hybrid non-sewered across ~65 sites*	*RT-qPCR primer/probes for wild-type and vaccination + WGS (Ongoing method optimization)*	*Jan 2025 – ongoing*	*National program detections integrated into measles response*	*Vanguard disease and WES integration Method development Knowledge sharing/training*	*RSA NICD (public facing dashboard) (48)*
United States Wastewater SCAN Project	*Integration of measles into large-scale multi-pathogen WES*	*Municipal WWTP influent / settled solids across 147 WastewaterSCAN sites*	*Validated RT-digital PCR primer/probes for wild-type MeV*	*May–Aug 2025 (SCAN);*	*39 positive at 15 sites in 11 states; (Case Study 3)*	*Demonstrated scalability Method development*	*WEF, 2025 (report)(51) WastewaterSCAN website (50)*
United States National Wastewater Surveillance System (NWSS)	*Integration of measles into national multi-pathogen WES*	*Municipal WWTP influent across >200 NWSS sites*	*Validated RT-qPCR primer/probes for wild-type MeV*	*August 2025 ongoing (NWSS rollout)*	*NWSS detections with 24h alerts*	*National integration; validated assays for wild-type measles*	*NWSS website (public facing dashboard) (49) WEF, 2025 (report)* (65)

Collectively, these studies and evidence from implementation demonstrate the operational and technical feasibility of WES for measles in varied contexts. They demonstrate WES may provide:

- early warning or identify geographic extent of an outbreak above that provided by clinical surveillance alone (47,48,51,57–59,61);
- differentiation of pathogenic measles (including genotypes B3 and D8) from vaccine derived measles shedding (genotype A) (32,47);
- differentiation of pathogenic measles genotypes (ie B3 from D8) and additional evidence to link measles cases to source (55,56,59–61)
- information relevant to assess elimination evidence for certification (32,47,56,60).

3. Global strategies for surveillance and control of measles, mumps and rubella

3.1. Global Strategies for control of measles, mumps and rubella

Vaccination backbone. Measles, mumps and rubella (MMR) vaccines are live attenuated vaccines and are most commonly delivered as a combined Measles-Rubella (MR) or MMR vaccine or together with varicella (MMRV). WHO recommends two doses of measles-containing vaccine (MCV1+MCV2) in all national schedules. Sustained ≥95% two-dose coverage is required to prevent outbreaks and interrupt transmission (66).

Measles elimination target and rationale. The Measles & Rubella Strategic Framework 2021–2030 sets the goal to achieve and sustain regional measles and rubella elimination, aligned with Immunization Agenda 2030 (67). Because measles is one of the most contagious pathogens, elimination programmes hinge on equitable ≥95% MCV1/MCV2 access, uptake and coverage, high-quality case-based surveillance with laboratory confirmation and genotyping, rapid outbreak detection and response, and 'zero-dose/reach-the-missed' strategies. The latter strategies focus on finding children who have never received any vaccines and making special efforts to ensure they, and others who missed doses, are fully vaccinated. (68) Innovations to bridge current gaps including expanded use of rapid diagnostic tests are also prioritized (69).

Rubella control and elimination. WHO's position is to introduce rubella-containing vaccine (RCV) and to use wide-age-range MR (measles–rubella) catch-up campaigns**,** while ensuring strong routine coverage in both sexes and special focus on protecting women of reproductive age to prevent congenital rubella syndrome (70).

Mumps control. WHO recommends routine MMR with two doses in countries that can sustain high coverage within an effective childhood programme; this reduces mumps burden including complications (e.g., orchitis/infertility, aseptic meningitis and deafness). During outbreaks, some programmes may deploy a third MMR dose for at-risk groups to improve short-term protection to address waning immunity (2).

3.2. Surveillance for measles, mumps and rubella

Measles

- Case-based surveillance with laboratory confirmation (RT-PCR, IgM serology, genotyping) is the global standard with established key performance indicators. Laboratory support for case-based surveillance is provided by the WHO Global Measles and Rubella Laboratory Network consisting of more than 700 laboratories serving 190 countries (71).

- Because measles is highly contagious and symptoms overlap with other febrile rash illnesses, timely detection and laboratory confirmation are critical to enable effective timely responses.
- Case-based genotyping distinguishes wild-type from vaccine strains and helps characterise linkages between outbreaks.

- The WHO framework for verifying endemic measles elimination emphasizes the necessity of genotyping clinically confirmed measles cases to distinguish between measles illness from a circulating wildtype strain and a vaccine-associated illness and enabling the tracking of transmission pathways of wildtype strains (72).
- Full case definitions and protocols are detailed in WHO measles surveillance manuals (73).
- There is a challenge in the completeness and timeliness of measles case-based surveillance with laboratory confirmation particularly in the African and South-East Asian regions.
- The lag between infectivity and symptom onset also poses a challenge for surveillance, as measles is highly infectious and significant transmission can occur before or in the absence of case identification. Further viremia and shedding is of a short duration.

Rubella and Congenital Rubella Syndrome (CRS)

- Rubella surveillance is best integrated with measles, using shared rash/fever case definitions and laboratory confirmation (RT-PCR, IgM serology, genotyping).
- CRS surveillance targets infants with birth defects consistent with rubella infection.
- Both rubella and CRS surveillance are essential to track progress towards elimination and to protect women of reproductive age from infection during pregnancy.
- WHO provides detailed case definitions, classification systems, and laboratory guidance (74) (71).

Mumps

- In endemic or pre-vaccine settings, aggregate reporting of mumps cases may suffice; however, in countries with routine MMR vaccination, WHO recommends enhanced case-based surveillance with laboratory confirmation (RT-PCR, serology).
- Objectives are to monitor burden, detect outbreaks, and assess vaccine impact.
- IgM has limitations post-vaccination, making molecular methods preferred in elimination contexts (75).

4. Potential public health actions arising from addition of WES

Wastewater and environmental surveillance (WES) is **not currently recommended** for the surveillance of measles, mumps, or rubella. Currently there is inadequate evidence to assess mumps and rubella WES applications.

However, several potential public health roles for **routine and agile measles WES** emerge from field and laboratory evidence.

4.1. Routine WES surveillance

- **Enhancing system sensitivity**
  WES can complement measles case-based surveillance by detecting measles cases missed clinically – whether as incursions or in delineating the temporal and spatial contours of an outbreak. This is demonstrated in South Africa, where WES identified MeV RNA in ~48% of districts without confirmed cases (47) and in multiple other country settings. (44,48–50).

- **Providing (some) reassurance in the absence of detections**

  Absence of WES detection does not equal absence of transmission, particularly in large catchments where dilution effects may mask low-level circulation. However negative WES signals (when using sensitive WES methods) may help confirm the absence of broader spread and the containment of an outbreak, particularly in settings with patchy vaccine coverage or under-performing case-based surveillance systems. However more data are needed to assess the negative predictive value of a single or repeated 'absence of' measles detection with optimised methods in various settings and catchment sizes.

- **Elimination contexts and equity**

  WES could be especially valuable in underserved or hard-to-reach populations where clinical surveillance is limited, and in countries close to elimination, to identify residual or reintroduced circulation. At present genotyping capacity for clinical specimens is essential to distinguish vaccine strain shedding from wild-type virus, discriminate travel-associated from local circulation of measles and to provide elimination verification evidence. Given the wide variability in genotyping coverage for clinical specimens with very low rates in sub-Saharan Africa and South-East Asia, there may be opportunities to strengthen surveillance and improve equity through wider use of WES in these regions(69,76).

4.2. Agile WES (responsive – time limited WES)

- **Agile outbreak response**

  Time-limited, intensive sampling (expanding locations and/or increasing frequency) can be deployed during outbreaks to confirm or exclude local transmission and track geographic spread and outbreak containment. This is likely to be most effective when outbreaks exceed the threshold of detection in wastewater catchments (40).

- **Situational targeting**

  Targeted WES could support surveillance during high-risk events (e.g. mass gatherings) or in areas of low vaccine coverage and increased risk to provide rapid intelligence. Measles was one of the six pathogens included during the Paris Olympics WES program (65).

4.3. Key considerations

- Implementing systematic nationwide WES for measles would require substantial effort and resources (even when integrated within existing multi-pathogen WES) and may have limited sensitivity for detecting small, localized clusters or in large catchments. There are tradeoffs between increasing WES sensitivity for detections and resource requirements; both in terms of intensity of sampling (site nos, catchment size, sampling frequency) and laboratory methods (number of technical replicates, use of digital droplet PCR versus RT-qPCR etc).
- Global measles surveillance already faces major constraints — including incomplete representativeness of case-based reporting, limited laboratory capacity in resource-constrained settings, and under-reporting of virologic data to the MeaNS database (77). WES should be positioned to complement rather than compete with these scarce resources, by filling gaps (e.g. silent transmission, populations with poor health-seeking) without diverting capacity away from essential case-based and virologic surveillance.
- Recent innovations, including rapid diagnostic tests (RDTs) and expanded molecular tools for clinical samples (e.g. extended sequencing, WGS), aim to address these gaps. WES should be considered within this broader innovation landscape as a complementary tool, not a replacement, to enhance both epidemiological and virological surveillance. Notably, WES may uniquely contribute by detecting asymptomatic or presymptomatic infections, and infections in individuals who do not present to clinical care or access RDTs.
- A priori consideration of what actions would be proportionate and appropriate in the specific context is required. A generic decision-aid summary to consider possible WES results, interpretation and actions is presented on the following page.

Table 6: Measles Wastewater Surveillance : Signal, Interpretation and Actions to Consider

Trigger / Signal	Interpretation / Caveat	Actions to consider
Single positive sample in a catchment	*Could reflect a local case +/- transmission, importation, or a vaccine-strain detection. Differentiation of MeV from vaccine derived MeVV may be needed (if RT-PCR not wild-type specific).*	*Alert public health surveillance team and triangulate with case data; report on public-facing website - consider further action such as intensified WES (same or additional sites), clinical alerts or other. (Differentiation by RT-PCR and/or sequencing is not wild type specific primary assay).*
Two or more consecutive detections from the same catchment	*Higher likelihood of sustained presence of a local case or cases than a transient case – higher likelihood of local transmission if repeated signals with consideration of quantitative level and trends.*	*As above plus :* *Escalate to outbreak investigation; alert immunisation programme; assess coverage gaps and consider targeted vaccination activities such as provider-based outreach or supplementary immunisation activities (SIAs).*
Detection of vaccine genotype A only	*Likely linked to recent immunisation, not wild-type circulation [Ottawa WES case study showed this scenario].*	*Communicate carefully to avoid false outbreak alarms; no outbreak declaration without supporting case evidence or MeV genotyping results.*
Detection of wild-type genotype during outbreak (e.g., B3, D8)	*Triangulate with case genotype data and consider evidence for linkage to local transmission or imported case/s.*	*Integrate with case-based and any outbreak data; consider relevance for elimination documentation and outbreak response.*
No detections during a known outbreak	*Negative predictive value uncertain; sensitivity depends on methods used, outbreak size and catchment scale.*	*Reinforce that "absence ≠ absence"; consider methods, catchment size and adjustments to WES; combine with case surveillance and laboratory reporting.*
Detections in non-sewered or small sub-catchments	*Evidence still limited; may provide highly targeted signals. [Guinea WES case study showed this scenario].*	*Treat as pilot data; escalate cautiously as above.*

Notes for use

- Wastewater surveillance adds to, but does not replace, case-based surveillance.
- Results should be contextualized with available clinical and epidemiological data.
- Specific assays which identify pathogenic MeV (distinct from vaccine derived MeVV by targeted PCR) or sequencing are critical. Such differentiation is also be relevant for any future role in elimination verification and certification (requiring further research and supportive evidence).

5. WES additional methodological considerations for measles

This section should be read in conjunction with general methodological consideration in Section 5 of *Wastewater and environmental surveillance for one or more pathogens: Guidance on prioritization, implementation and integration* (available here). At the time of writing there are no standard methods for WES for measles. Therefore, this section does not provide examples of, or recommendations for, specific methodological protocols or procedures. Rather, this section summarizes the key considerations that are specific to undertaking WES for measles that are worthy of consideration when designing and selecting methods.

5.1. Sampling methods

Grab and composite sampling have been used with MeV detections reported. Mumps viral RNA was detected with passive samplers (54). However optimal sampling methods have not been assessed noting the viral levels are expected to be much lower due to fewer cases and viral shedding levels and duration (in comparison to enteric viruses and other respiratory viruses such as SARS-CoV-2 and influenza A virus) (64)

5.2. Laboratory methods

- For clinical diagnosis, WHO has provided a manual for diagnostic testing and molecular characterization of circulating viruses for measles and rubella (33) and hosts an international network of diagnostic laboratories for these viruses, as well as surveillance and genetic databases (78). The RT-PCR primers and probes used in WES are similar to those for clinical diagnostics (see below).
- The concentration and isolation of virus and RNA extraction for wastewater/environmental samples require additional steps for processing and for analysis compared to clinical samples given the complex population pooled matrix.
- Sample processing volumes and methods are comparable to those used for other viruses in wastewater and environmental samples: centrifugation, filtration or precipitation for concentration of the viruses followed by viral RNA extraction using commercial kits.
- For measles, methods are available for detection of virus RNA in wastewater, and dedicated RT-PCR primers or probes have been tested for wild-type MeV and MeVV.
- Methods used include RT-PCR in single, duplex or, multiplex format, RT-digital droplet PCR. RT-PCR targeting measles virus N or H genes is standard in clinical labs. The same or similar primers have been applied for WES, but some results indicate that these insufficiently exclude vaccine strains. New primer-sets have been developed that target wild-type strains and vaccine strains specifically (79).
- Molecular epidemiology is important to understand when considering the circulation of wild-type measles viruses (4). Several WES studies have incorporated the entire N-450 sequence, as used for clinical samples, to determine the genotype (see below).

- The first study used ongoing polio-WES in regions with low vaccination coverage (55) and tested the feasibility of measles detection in wastewater during a measles outbreak in the same region. Tomalty et al used the same PCR in a WES scheme in Ottawa in 2024 as a response to the increase in measles globally (32). Rector et al used the same approach in Belgium, where MeV was detected in wastewater collected from a catchment where measles cases were also reported; sequencing confirmed MeV was homologous to a patient isolate from the same area(57). Other protocols have proven effective (52), (58), (60), (79), and (61).
- Separate initiatives in the USA (65), France (56) and South Africa (47) have developed PCR primers that are specific to wild-type strains and to vaccine strains. (47,56)
- Langan et al compared RT-qPCR, digital PCR and digital droplet PCR in the context of a wildtype outbreak and showed that the qPCR yielded a higher number of gene copies in wastewater samples than digital PCR (61).
- Sequencing of viruses in wastewater using virus enrichment using the Twist Comprehensive Virus Research Panel detected wild-type measles in wastewater in Texas (60). The RT-ddPCR of wastewater and reporting of two unvaccinated travel-associated cases of measles in the sewershed aligned with this finding.

5.3. Reporting and communications:

Measles

- WES results, case and other relevant data should be triangulated (as for any pathogen) to inform both interpretation and proportionate and contextual public health action
- It is expected that WES will not always be concordant with case data; positive WES MeV results have been reported in the absence of case reports and may reflect undetected case or cases of pathogenic MeV.
- It has been reported that vaccine derived MeVV RNA can also yield positive WES results so WES PCR assays specific to wild type MeV is important. Discrimination through RT-PCR or digital-PCR require optimization of the assay specificity. Sequencing of measles RNA detected in wastewater can also provide differentiation between wild type and vaccine strains, however these would be less timely as a separate step compared to specific assays.
- WES sequencing information can also provide the genotype and be helpful to link to source (ie ongoing outbreak, travel associated new incursion or other), but experience with MeV WES sequencing is limited. As above, case and WES data would always be triangulated and combined intelligence reported in a format which is understandable to the target audience and linked to a call to action.

5.4. Acceptability:

- Overall WES for infectious diseases in large catchments appears to have high acceptability.
- No specific ethical concerns identified unique to WES for measles/rubella/mumps.
- However general surveillance and WES issues apply including considerations related to trust and confidence in public health authorities and interventions including vaccines.

6. Integrated surveillance and multitarget WES considerations

6.1. Integration of measles WES into existing measles surveillance and response

- Evidence for WES for measles is relatively recent and consequently its place in multimodal measles surveillance is evolving.
- There has been recent large scale integration (in 2025) in various settings including frontrunner examples such as the USA (49–51), South Africa (48) and Guinea (see Case Study 4), all of which have had measles outbreaks and which are expected to provide highly relevant programmatic evidence.
- There is potential for improved integration at the national and subnational levels, including at the planning stage to optimize complementary multimodal surveillance, as well as at the analysis and reporting stage to better visualize and enable use of combined measles information to inform timely public health policy and practice decisions.
- There is also potential for strengthening cross-border, regional and global surveillance, and strengthening genotypic surveillance. This may include integration within multi-pathogen transport hub/strategic site surveillance involving multiple countries.

6.2. Integration of targeted WES into existing fever-rash surveillance and response

- Noting there are multiple fever-rash diseases with similar presentations, multi-pathogen WES may also have potential to complement syndromic and laboratory confirmed case surveillance and assist to identify which pathogens are circulating in a community; these may include measles, rubella, varicella (chickenpox), mpox, chikungunya, dengue, zika, coxsackie and others. WES has been shown to be feasible for multiple of these targets.

6.3. Integration of measles as part of multi-target WES surveillance

- Existing polio, SARS-CoV-2 or other WES activities allow the integration of measles or additional targets at low marginal cost with substantial alignment with some or many of the existing WES work-flows. Trade-offs may need to be considered between optimal methods for individual pathogen sensitivity and resource allocations.
- Likewise, routine WES activities for measles provide local capability to which agile WES can be initiated in response to an outbreak.
- In many high-income settings, multitarget WES surveillance already combines multiple respiratory and other pathogen targets from the same samples with publicly accessible WES dashboards – and with a design which allows additional targets to be added (e.g. the US National Wastewater Surveillance Program (81). The South Africa program provides an integrated disease approach with a public facing dashboard which includes clinical laboratory and WES results together (48).

7. Key knowledge gaps and applied research priorities

- In which contexts does wastewater environmental surveillance provide the greatest **added value**, and how should it complement **case-based surveillance**?
  (e.g., outbreak response, weak case reporting, vulnerable populations, elimination verification)
- What are the **magnitude, duration, and variability** of human shedding of wild-type measles RNA into wastewater — and of vaccine-derived RNA?
- Which **validated**, **standardized**, **sensitive**, and **specific** laboratory methods are required for reliable environmental detection?
- How feasible is wastewater surveillance in **non-sewered** or **low-infrastructure** sanitation systems, particularly in low- and middle-income countries?
- What **evidence-based thresholds** or criteria should guide **proportionate public-health actions** in response to detections?
- What is the **cost-benefit** or **cost-effectiveness** of wastewater surveillance compared to case-based surveillance alone, and how does this vary across different programmatic or elimination stages?
- How do these knowledge gaps and research priorities apply to rubella, mumps, and other vaccine-preventable diseases with potential for wastewater detection?

Annex 1. Case Studies

Case Study 1: Measles WES in South Africa – Informing Targeted Response [(48)2]

Background

Measles remains a leading cause of vaccine-preventable childhood mortality in low- and middle-income countries, despite progress toward the WHO 2030 elimination goal. Clinical surveillance is essential but has limitations, including under-reporting, incomplete diagnostic sampling, and delayed detection. Wastewater and environmental surveillance (WES), proven effective in polio and SARS-CoV-2 monitoring, has not been widely applied to measles.

Intervention

During South Africa's 2022–2025 measles outbreak (>4,000 clinical confirmed cases), the National Institute for Communicable Diseases (NICD) piloted digital RT-PCR (RT-dPCR) assays for measles virus (MeV) detection in wastewater. Samples were retrospectively and in real-time tested from 28 national sentinel wastewater sites and 19 local sewer catchments in Gauteng Province. Assays differentiated wild-type strains (B3, D8, H1) from vaccine genotype A.

Findings

- During the entire period (16 February 2021 to 21 November 2025) 6,375 wastewater samples were tested; 540 (8%) were MeV positive with a mean concentration of 0.4043 genome copies/ml.
- Initial testing (16 February 2021 to 8 March 2024) was performed on stored samples. Of the 2,149 samples tested, 43 were positive for MeV (2%).
- Real time testing began on 19 February 2024 and continues to date (21 November 2025). A total of 4,629 wastewater samples have been tested, of which 502 (11%) were positive.
- Concordance with clinical surveillance: Using the entire dataset comparison of wastewater and clinical fever-rash surveillance data by epidemiological week and district identified in 246 district-week instances where wastewater was positive, 170 (69%) also had confirmed clinical cases. Importantly, wastewater detected MeV in 76 district-weeks (31%) where clinical surveillance failed to identify cases.
- No consistent correlation was observed between wastewater viral load and case counts, which may be due to storage degradation or dilution effects.
- In the period between 6 December 2023 to 9 February 2024, 267 samples were tested for genotype differentiation. wild-type strains predominated, while vaccine strain was detected in 6 samples, usually after supplementary immunization campaigns.

Public Health Significance

This study demonstrated that measles virus can be reliably detected in wastewater, even at low concentrations. Findings suggest:

- Early warning potential: WES may identify ongoing transmission in communities missed by clinical systems.
- Programmatic value: Real-time testing could guide supplementary immunization activities and outbreak response.
- Integration need: WES should complement, and does not replace, clinical fever-rash surveillance, especially in under-resourced settings.

Lessons Learned

- Timely processing is critical to prevent RNA degradation.
- Improved concentration and extraction methods would enhance sensitivity.
- Optimization of assays to discriminate wild-type measles genotypes from vaccine-derived RNA is critical – the initial assay was not specific enough and further optimization was required. Genotyping capacity is essential to distinguish between vaccine-derived and wild-type strains.

Conclusion

South Africa's experience illustrates the feasibility and added value of measles wastewater surveillance as a complementary tool for elimination efforts. WES could provide critical intelligence in contexts where health-seeking behaviour is low, or diagnostic capacity is limited, advancing progress toward the WHO 2030 measles elimination target.

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Case Study 2: Differentiating Vaccine-Derived and Wild-Type Measles in Canada: WES Insights [32][3]

Background

The resurgence of measles in 2023–2024, following COVID-related declines in immunisation coverage, prompted renewed attention to WES as a complementary tool. In April 2024, Ottawa Public Health partnered with researchers to monitor measles virus (MeV) RNA in wastewater as part of multi-pathogen WES. Unexpectedly, MeV signals were detected despite no reported clinical cases. Genetic analysis was undertaken to differentiate between wild-type and vaccine-derived strains.

Intervention

Daily 24-h composite primary clarified sludge samples were collected at the WWTP which serves approximately 91% of Ottawa's population. RT-qPCR targeting the MeV nucleoprotein gene was performed. Positive samples were sequenced (N450 region) and compared to reference databases. Retrospective testing of archived RNA samples (2020–2024) was also conducted. Statistical analysis assessed temporal associations between vaccine distribution data and WES detections.

Findings

- MeV RNA was detected in low concentrations in 8% of samples (11/135), mostly in 2024.
- No active clinical measles cases were reported locally during period of wastewater detections.
- Sequencing confirmed genotype A, identical to vaccine strains noting wild-type strains belonging to genotype A are extinct.
- Statistical analysis revealed an association between increased vaccine distributions and wastewater detections, with an ~8-day lag.

Public Health Significance

- Accurate interpretation: Immediately differentiating vaccine vs. wild-type strains prevents misallocation of resources and avoids false outbreak alarms.
- Outbreak preparedness: Correct strain identification ensures timely and proportionate response, avoiding unnecessary campaigns triggered by vaccine-derived detections.
- Global relevance: Similar observations of vaccine RNA in wastewater have been made for polio and rotavirus vaccines, reinforcing the need for WES methods specific to wild-type strains.

Lessons Learned

- Methods such as targeted PCR (wild-type versus vaccine strains) or genotyping is essential for MeV to differentiate pathogenic strains (non-A) from vaccine strains (A genotype) in wastewater.
- Vaccine shedding can (rarely) persist for weeks to months post-immunization, resulting in detectable RNA signals in wastewater.
- Integration of vaccine distribution and clinical data strengthens interpretation of WES results.
- Storage conditions and RNA degradation may affect retrospective analyses.

Conclusion

This Ottawa study demonstrated that measles RNA detected in wastewater may derive from vaccine strain shedding, not community transmission. Integrating routine sequencing and vaccination data strengthened interpretation. Specific assays (and/or timely differentiation) is critical to ensure WES informs, rather than confounds, public health responses. Accurate interpretation of WES data contributes to the broader measles elimination goal by ensuring surveillance remains aligned with WHO's Strategic Framework 2021–2030.

[3] Contribution from Professor Robert Delatolla, University of Ottawa. Contact: robert.delatolla@uottawa.ca

Case Study 3: Integration of measles in multi-pathogen WES at scale - USA early adopter[4]

Background

Measles was declared eliminated in the United States in 2000, yet 2025 has seen the highest measles burden since 1992; with more than 1,723 confirmed cases reported by Nov 12, of which 1,505 (87%) were linked to 45 outbreaks, compared to 285 cases and 16 outbreaks in 2024 (82). Clinical surveillance remains central to detection, but underreporting and diagnostic delays create blind spots. WastewaterSCAN is a large-scale frontrunner program for WES operating in the United States of America; wild-type measles virus (MeV) was added in 2025 (50).

Intervention

WastewaterSCAN integrated measles into multi-pathogen WES with a systematic process. It first developed and validated a digital droplet RT-PCR assay specific to wild-type MeV RNA, in response to stakeholder input to aid interpretation and avoid confusion with vaccine strains. The assay is described in a protocol on protocols.io (83). It then validated multiplex MeV with other targets prior to implementation at scale; to all 147 WastewaterSCAN sites in 40 states from May 2025 as part of multipathogen WES. By mid-October, 52 MeV detections were reported from 10,069 samples, including 21 sites across 16 states with at least 1 MeV detection. Detections trigger rapid alerts to jurisdictional epidemiologists and wastewater coordinators within 24 hours. Subsequently, the US National Wastewater Surveillance System added MeV in July 2025 and scaled across >400 sites by Nov 2025 (49).

Findings

Validated primer-probe sets successfully distinguished wild-type genotypes (B, D) from vaccine strain A, preventing misinterpretation of vaccine-related shedding as outbreaks. Integration with SARS-CoV-2/RSV/influenza pipelines reduced marginal costs and supported near-real-time reporting. Measles RNA concentrations were generally low, consistent with expected shedding patterns, but detection was feasible. Early detections preceded case surges on multiple occasions, demonstrating early-warning potential. Results reinforced WHO priorities: surveillance must be timely, accurate, and linked to actions.

Public Health Significance

- Validation: Wild-type-specific assays increased confidence among public health users.
- Multipathogen integration: Measles was feasibly integrated alongside other targets.
- Scalability: Integration achieved at large subnational scale within existing systems. Early warning: Wastewater community level signals complemented case reporting.
- Sustainability: Aligned with Measles & Rubella Strategic Framework 2021–2030

Lessons Learned

Assay validation and primer-probe design are critical to differentiate wild-type MeV from vaccine strains. Validation and use of multiplex assays with streamlined workflows result in low marginal costs to add MeV. Leveraging existing large-scale systems through WastewaterSCAN built confidence with multiple stakeholders. Clear communication protocols (24h alerts, coordination with epidemiologists and public health actors) are essential to translate WES detections into action. Integration with clinical and immunization surveillance maximises interpretive value and prevents duplication.

Conclusion

The U.S. experience shows that measles WES can scale rapidly and systematically from pilots to large-scale multipathogen surveillance systems. With validated methods, cross-jurisdictional coordination, and real-time data sharing, WES offers early-warning capacity and programmatic value for measles alongside other high-priority pathogens.

[4] Contribution from WastewaterSCAN by Professor Ali Boehm and colleagues. Contact: aboehm@stanford.edu

Case Study 4: Environmental surveillance for Measles in the Non-Sewered Setting of Conakry, Guinea (2022-2025)[5]

Background

Guinea's health system remains stretched post Ebola and COVID-19 public health emergencies and has had recurring measles outbreaks (in 2021/2022 and 2024/2025) amid low vaccination coverage, estimated <50% in 2021. Clinical IgM-based surveillance has known limitations of under-reporting and ongoing logistical challenges. The capital Conakry is on a peninsula. Only Kaloum of it's five communes has a limited sewer network, elsewhere, wastewater is discharged via septic tanks or informal connections to stormwater systems and waterways. In an effort to strengthen measles surveillance, environmental surveillance (ES) was launched in Conakry in January, 2022.

Approach

Stepwise expansion to Conakry's five communes. Phase one sampling from Jan 2022–Mar 2024 focused on Kaloum's limited sewer network and a major hospital. Phase 2 sampling from Aug 2024 (ongoing) expanded citywide with sampling points from open drains and septic discharges identified via satellite imagery. 200ml grab samples were taken once or twice weekly and pooled by geographic area. Samples were analyzed by RT-qPCR targeting the nucleoprotein region that encompasses both wild-type and vaccine genotypes. Selected, low Ct, positive samples were further characterized with a B3 genotype-specific PCR, the prevalent circulating strain in West Africa (84).

Key Findings

- Phase 1 : 22% (74/343) samples positive; similar between hospital (24%) and community (19%) sites. Detections in Jan – July 2022 coincided with the '21-'22 outbreak.
- ES measles detections were identified in late 2023 December, 10 weeks prior to the first clinical case reports in week 6 of 2024, demonstrating early-warning potential.
- Phase 2 : 32% (345/1,072) pooled samples positive; heterogeneity by commune R: (8% – 42%).
- Genotype B3 confirmed in 26 of 37 strongly positive (Ct < 32) samples.
- Temporal spatial trends showed an expanding epidemic aligned with case reports (2025).

Public Health Impact

Measles ES provided early outbreak signals, revealed geographic hotspots, and strengthened the national alert system given known under-reporting of clinical cases. Results have been integrated into weekly epidemiological reports by the National Health Security Agency, improving local response planning which is ongoing.

[5] Contribution from Institute Pasteur Guinea by Pierre Roques pierre.roques@pasteur.fr, Yan Le Pennec lpnc.yann@gmail.com and Issiaga Toure issiaga.toure@pasteur-guinee.org with support from the Agence Nationale de Securité Sanitaire de Guinée (ANSS) and WHO country office. With recognition of financial support from the French Development Agency (AFD) project ATLANTES #CZZ3246.

Case Study 5: Passive wastewater sampling in a mumps outbreak[6]

Background

Mumps, a vaccine-preventable disease, continues to cause outbreaks in Europe despite high MMR coverage. In 2023–2024, a mumps outbreak occurred in a region of the Netherlands with suboptimal vaccination uptake (33–88%). Local health services suspected that the outbreak was larger than notified cases indicated, prompting exploratory use of wastewater and environmental surveillance (WES) for mumps.

Intervention

To study the outbreak spread, passive samplers (85) were deployed at the local school; local pumping stations in affected and neighbouring towns; wider area catchment; and a control site with no reported cases. Samples were analysed by RT-PCR targeting the mumps F-gene. SH gene sequencing was undertaken to genotype strains.

Findings

- Clinical surveillance: 24 confirmed cases and 57 GP-reported "possible" cases.
- Wastewater results: Passive samplers at the town's school and pumping station showed rising mumps virus RNA concentrations as reported cases increased, then declined as cases subsided. Genotype in wastewater matched that for cases. A neighbouring town yielded positive WES signals despite no reported clinical cases, suggesting silent transmission. The passive sampler downstream was negative, consistent with signal dilution. Control samples were consistently negative.

Public Health Significance

- Situational awareness: WES provided evidence of mumps circulation beyond notified cases with genotype matching. Wastewater trends aligned with clinical case trends.
- Complementary value: inform municipalities and the public about outbreak risks, and to strengthen syndromic surveillance.
- Policy implications: while no vaccination campaign was implemented (due to local religious context), WES guided geographic targeting of interventions.
- Alignment with WHO: outbreaks occur and highlight the need for ≥95% MMR coverage to prevent age-shifted outbreaks with higher complication rates (2).

Lessons Learned

- Passive samplers provide a low-cost, practical tool for local outbreak monitoring.
- Sequencing of wastewater samples confirmed identical genotypes as patient samples.
- Ethical framework for targeted WES in small settings needs further development.

Conclusion

This Netherlands proof-of-concept study demonstrated the feasibility of mumps virus WES in wastewater using passive samplers. WES captured both confirmed and unreported transmission, with sequence confirmation, supporting public health situational awareness where underreporting obscures the true burden.

[6] Contribution from Regional Public Health Services of North-East Gelderland (Dr Loes Jaspers ljaspers@ggdghor.nl and Dr Aart Dijkstra a.dijkstra@ggdnog.nl) Amsterdam (Maarten de Jong maadjong@ggd.amsterdam.nl); Utrecht (Dr Ewout Fanoy efanoy@ggdru.nl) and Rotterdam Rijnmond (Dr George Sips gj.sips@rotterdam.nl), Erasmus Medical Centre (Dr Miranda de Graaf m.degraaf@erasmusmc.nl) and Partners4UrbanWater (Dr Remy Schilperoort remy.schilperoort@urbanwater.nl). With financial support from Topsector Watertechnology Netherlands.

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