

## Poliomyelitis and COVID-19: a repeated history

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### ABSTRACT

The measurement of the enterovirus and the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in sewage water is relevant in the early detection of the introduction or disappearance of these viruses in the ecosystem. We evaluated the co-circulation of the enteroviruses and SARS-CoV-2 in 81 sewage water samples collected between September 2021 and April 2023 from different regions of north and southeast Romania, at the border with Ukraine. We used, for the molecular detection of the pathogens, the multiplex real-time polymerase chain reaction (PCR) assay produced for respiratory samples and the Respiratory 2.1 Plus panel Biofire Film array. The isolation of enteroviruses was performed on cell culture lines, in accordance with the World Health Organization (WHO) recommendations. By molecular investigations, we detected the SARS-CoV-2 in 22 (27%) samples, and the human rhinovirus/enterovirus in 64 (79%) samples. By isolation on cell culture lines, 27 samples (33,33%) were positive for non-polio enteroviruses, and no poliovirus strains were isolated, proving the maintenance of the polio-free status in Romania. In an emergency situation, the molecular detection of the pathogens in sewage water using a PCR system integrating sample preparation, amplification, detection, and analysis in 1 h could be implemented.

**Key words:** COVID-19, poliomyelitis, poliovirus, risk assessment, SARS-CoV-2, sewage water

### HIGHLIGHTS

- During the pandemic, the co-circulation of non-polio enteroviruses and SARS-CoV-2 was recorded in sewage water samples collected in Romania, at the border with Ukraine.
- The surveillance of the pathogens in the sewage water can be a tool for mitigation of the potential outbreaks.

### INTRODUCTION

Prior to the coronavirus disease 2019 (COVID-19), sewage water surveillance was used to evaluate the circulation of the poliovirus in the framework of the Global Polio Eradication Strategy and to assess for chemicals in the communities (WHO 2003; Choi *et al.* 2020). While for poliovirus there is a methodology for surveillance in the sewage water, for the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) assays were developed to identify and quantify SARS-CoV-2 ribonucleic acid (RNA), or assays developed for identification of the virus in the respiratory samples were adapted or used (Medema *et al.* 2020; Băicuș *et al.* 2021). In 2016, the combined poliovirus surveillance in the sewage water and in healthy children was evaluated in Romania as a tool for risk assessment (Băicuș *et al.* 2020).

During the epidemic of poliomyelitis in the northeastern United States in 1916, the role of asymptomatic persons in the spreading of infection with poliovirus was recorded by the sewage water testing; a ratio of 100 subclinical infections for every paralytic case was estimated (Melnick 1947). In late 2019 in Wuhan, China, the first detection of SARS-CoV-2, an enveloped, positive-sense single-stranded RNA virus belonging to the Coronaviruses family, was recorded. The virus was responsible for COVID-19, a respiratory tract disease which ranged from mild illness to severe hypoxia requiring mechanical ventilation. It spread rapidly worldwide and became a pandemic in March 2020. The primary route of SARS-CoV-2 infection is respiratory, but fecal–oral transmission is possible as the virus can be detected in stool samples (Gu *et al.* 2020; Termansen & Frische 2023). The disease was controlled by vaccination (Hadj Hassine 2022).

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Because SARS-CoV-2 and enteroviruses may survive for up to several days outside the human body, their measurement in sewage water is relevant in the early detection of these viruses. Our goal was to implement the combined poliovirus and SARS-CoV-2 surveillance in the sewage water during the pandemic in Romania.

## METHODS

In this study, 81 sewage water samples (1,000 mL/sample) were collected by the grab sampling method, by the public health authorities, between September 2021 and April 2023, from regions of north and southeast Romania, namely, Maramures, Satu Mare, Suceava, Tulcea, Constanta, Iasi, Vaslui, and Galati, at the border with Ukraine. The sample concentration analysis and virological investigation were performed at the Enteric Viral Infections Laboratory, Cantacuzino Medico Military National Institute for Research and Development (MMNIRD), Bucharest, Romania. The samples were concentrated using the Polyethylene glycol (PEG) two-phase separation method and decontaminated by chloroform extraction (WHO 2003; Barril *et al.* 2021). The enteroviruses were isolated by cultivation on L20B cell lines (a genetically engineered mouse cell line expressing the human poliovirus receptor PVR) and RD cells (derived from a rhabdomyosarcoma) (WHO 2004). Positive samples on RD cell lines and negative samples on L20B cell lines were reported as non-polio enterovirus (NPEV), and negative samples on RD and on L20B cell lines were reported as negative for poliovirus. For the molecular detection of enteroviruses with other respiratory pathogens, 300 µL of concentrated sewage water from each of the sites was tested with the Respiratory 2.1 Plus panel Biofire Film array, a multiplex polymerase chain reaction (PCR) system that integrates sample preparation, amplification, detection, and analysis into one system that requires a total runtime of about one hour. The panel tests for 19 viruses and four bacteria that cause respiratory tract infections were carried out, with an overall sensitivity and specificity of more than 97% for the nasopharyngeal samples. The pathogens detected are Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus OC43, Coronavirus NL63, Middle East Respiratory Syndrome Corona Virus (MERS-CoV), SARS-CoV-2, Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, Influenza A/H1, Influenza A/H1-2009, Influenza A/H3, Influenza B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, Respiratory syncytial virus (RSV), *Bordetella pertussis*, *Bordetella parapertussis*, *Chlamydomphila pneumoniae*, and *Mycoplasma pneumoniae*. The panel does not differentiate between Rhinovirus and Enterovirus, as they belong to the same family, *Picornaviridae*.

## RESULTS

Of the 81 sewage water samples investigated, 27 samples (33.3%) were positive for non-polio enteroviruses by isolation on cell culture lines. No poliovirus strains were isolated. By molecular investigations, the SARS-CoV-2 was detected in 22 (27%) samples collected from Maramures county (Sighetu Marmatiei, Somcuta Mare, Baia Mare, Borsa, Viseu towns), Satu Mare county (Agris town), Suceava county (Suceava, Siret towns), Iasi county, Tulcea county (Babadag town), and Constanta and Galați counties. Adenovirus was detected in 75 (93%) samples, and Human Rhinovirus/Enterovirus in 64 (79%) samples. Other respiratory pathogens such as RSV, Coronavirus 229E, Coronavirus HKU1, Coronavirus OC43, Parainfluenzae virus 3 and 4, Influenza A virus, *Bordetella parapertussis*, and *Bordetella pertussis* were detected in few samples collected from Maramures county (Sighetu Marmatiei, Somcuta Mare, Borsa towns), Suceava (Siret town) Constanta, Iasi, and Galati (Supplementary material, Table S1).

## DISCUSSION

The worldwide eradication of poliomyelitis was proposed by the World Health Organization (WHO) to its member states in 1988. In 2021, wild poliovirus types 2 and 3 were declared globally eradicated. In 2023, endemic wild poliovirus type-1 remained in two countries: Pakistan and Afghanistan. Reports of a vaccine-derived poliovirus (VDPV) type-3 paralytic poliomyelitis case in an unvaccinated young child in Israel, a VDPV type-2 paralytic poliomyelitis case in an unvaccinated adult in the US, and the isolation of a VDPV type-3 from sewage water in Poland proved the risks for polio importation (Krzysztozsek *et al.* 2022; Link-Gelles *et al.* 2022; WHO 2022). The imported VDPV type-2 was detected in sewage water in New York, London, and Montreal (Hill & Pollard 2022; Government of Canada 2023; Thompson *et al.* 2023) and a case of poliomyelitis was recorded in 2022 in an unvaccinated patient in the US (Ryerson *et al.* 2022).

Owing to the risk of importation of the circulating vaccine-derived poliovirus (cVDPV) types 1 and 2 from Ukraine, a country that had been previously identified as at risk because of its low vaccination coverage, the environmental poliovirus surveillance was enhanced in the north and southeast of Romania, along the border regions with Ukraine, since 2015.

A recent study reported the correlation of SARS-CoV-2 in the sewage water with COVID-19 clinical disease diagnosed in the monitored population (Parkins *et al.* 2023).

Between October and December 2020, the co-circulation of the human rhinovirus/enterovirus and SARS-CoV-2 was recorded by molecular detection in sewage water in Romania (Băicuș *et al.* 2021).

## CONCLUSIONS

The absence of the isolation of poliovirus strains during the pandemic proved the maintainance of the polio-free status of Romania. As SARS-CoV-2 was present in the sewage water samples collected from the regions that border Ukraine, sewage treatment protocols and sanitation practices should be implemented.

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## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

## CONFLICT OF INTEREST

The author declares there is no conflict.

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