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Monitoring of SARS-CoV-2, noroviruses and adenoviruses in wastewater in the Czech Republic

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Abstract Monitoring SARS-CoV-2 presence in wastewater has been carried out since April 2020, when the new disease COVID-19 began to spread rapidly in the Czech Republic. The aim of this study was to detect viruses in order to evaluate the possibility of using wastewater analysis as one of the tools for the establishment of an early warning system. As a complement to SARS-CoV-2 RNA detection, noroviruses and adenoviruses were detected, as well. In the first round of monitoring, which took place from April to June 2020, a total of 122 samples of untreated wastewater were collected from 35 WWTPs of different size within the Czech Republic. Viral RNA was concentrated from wastewater and RT-PCR was used to detect RNA of viruses. SARS-CoV-2 RNA was found in 15 % of all samples, noroviruses in 43 % and adenoviruses in 95 %. It was confirmed that even low amounts of SARS-CoV-2 RNA in wastewater might be detected. Our data indicate that wastewater analysis may be a suitable tool for the establishment of early warning system. However, for unambiguous interpretation of data, it is necessary to evaluate other factors that might significantly influence the concentration of viral RNA in wastewater.

Keywords: SARS-CoV-2, wastewater, PCR, WWTP, WBE, adenoviruses, noroviruses

1. Introduction

The SARS-CoV-2 coronavirus is a representative of the viruses that cause respiratory diseases, which are primarily spreads by respiratory secretions. However, in many cases, SARS-CoV-2 RNA was also detected in faeces and urine of infected individuals (more than 50 % of the infected) and was secreted 3 to 33 days after a negative respiratory test (Mirjalali et al., 2020; Xiao et al., 2020; Wiktorczyk-Kapischke et al., 2021). Clinical studies have shown that the gut may be another target organ of the SARS-CoV-2 virus, the possibility of virus multiplication in faeces has been demonstrated by cell cultures (Lamers et al., 2020). Gastrointestinal symptoms such as abdominal pain, vomiting, and often diarrhea (in approximately 2 to 50 % of those infected) often occurred before the outbreak of the disease and persisted even long (about 10-14 days) after recovery from respiratory problems and negative detection of the virus in respiratory tract (Tian et al., 2020).

This information suggests the possibility of using the coronavirus SARS-CoV-2 as a biomarker to develop an early epidemic warning system. The wastewater based epidemiology (WBE) approach is a relatively new approach, using the quantitative measurement of human biomarkers in wastewater for targeted assessments of lifestyle, health or exposure of the population to a variety of substances. In the Czech Republic, it is currently used for the detection of drugs, polioviruses and enteroviruses (Očenášková, 2018; Kožíšek, 2020).

The detection of SARS-CoV-2 RNA as a biomarker in wastewater provides unique epidemiological information on the presence of the virus in a population or community (monitoring its presence, absence, seasonal or other trends and fluctuations). With a properly set up wastewater monitoring system, it will be possible to monitor the onset and development of the number of infectious diseases in future periods and use monitoring as an early warning tool to set up an effective surveillance system (Mao et al., 2020; Orive et al., 2020; Barcelo, 2020; Hart and Halden, 2020; Sims and Kasprzyk-Hordern, 2020).

Due to an extensive network of sewers and wastewater treatment plants (WWTPs) in the Czech Republic, it is suitable to use this system for systematic epidemiological monitoring. According to the information from the Czech Ministry of Agriculture, there are 3 166 WWTPs in the Czech Republic, of which nine are over 100 000 equivalent inhabitants (PE), 122 are over 10 000 PE, 417 are over 2 000 PE and 2 618 are up to 2 000 PE. According to the number of inhabitants connected to the WWTP, monitoring of their wastewater enable to observe the status of about 80 % of the population of the Czech Republic; monitoring of wastewater in WWTPs over 10 000 PE would enable to focus on more than 50 % of the population of the Czech Republic.

This study aimed at monitoring SARS-CoV-2 RNA in wastewater in order to evaluate the possibility of using wastewater analysis as one of the tools for the establishment of early warning system. As a complement, noroviruses and adenoviruses (as index viruses of human feacal contamination) were detected.

2. Methods

2.1. Wastewater sampling

Monitoring for the presence of viruses in wastewater has been carried out since April 2020. In total, 122 composite samples from 35 wastewater treatment plants (WWTPs) of different size within the Czech Republic were collected in 2020. The share of infected persons in the catchment of WWTP was in the spring (April-June 2020) around 0.2 %. Immediately after sampling the samples were cooled and stored at 5 ± 3 °C until analyses. The analysis was performed within 48 hours after the sampling. In 2021, 12 WWTP were monitored on a regular basis, with a total of 134 composite samples being collected. All samples from 2021 were stored frozen at -70 °C. Out of these collected samples, 10 samples were analyzed for SARS-CoV-2.

2.2. Sample concentration and RNA extraction

Sample concentration and RNA extraction was conducted as described by Mlejnková et al. (2020).

2.4. RT-qPCR analysis

The presence of SARS-CoV-2 genome in wastewater was detected by real time reverse transcription polymerase chain reaction (RT-qPCR) using EliGene COVID19 Basic A RT kit (Elizabeth Pharmacon, the Czech Republic) according to manufacturer's instructions. The incidence of human noroviruses (NoV GI and NoV GII) was determined using a one-step duplex RT-qPCR assay according to ISO/TS 15216-1 (Anonymous, 2017). Detection of human adenoviruses was performed by qPCR assay using primers and probe adopted from the literature (Wong et al., 2008). The RT-qPCR/qPCR reactions were carried out by LightCycler 480 (Roche Molecular Diagnostics, Germany), further analysis was done using "Fit point analysis" of the LightCycler 480 Software release 1.5.0 (version 1.5.0.39). The process control virus (PCV) was chosen to be transmissible gastroenteritis coronavirus from infected pigs (Mlejnková et al. 2020).

3. Results

In this study wastewater samples from 35 WWTPs were collected and subjected to SARS-CoV-2, noroviruses and adenoviruses RNA detection. Out of the total analysed samples from 2020 (122), 18 samples (15 %) were found positive for SARS-CoV-2 RNA, from 35 WWTPs was positive 11 (31 %). The presence of noroviruses (NoV GI or NoV GII) was observed in 43 % resp. in 27 WWTP (77 %) and the adenoviruses in 95 % of all samples and in all WWTPs (see Fig. 1). All analyzed samples in 2021 were tested positive for SARS-CoV-2 (Cq<36). Based on the determination of PCV, the mean efficiency of virus detection and quantification was 35.53% with a standard deviation of 13.04. Adenoviruses were found in all WWTPs positive for SARS-CoV-2 RNA, noroviruses was found in almost all WWTPs positive for SARS-CoV-2 RNA (only 2 were negative). More detailed overview can be found in Table 1.

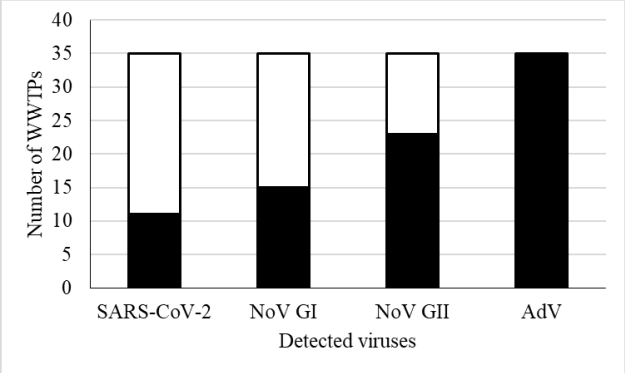

Figure 1. Occurrence of selected viral RNA in wastewater from 35 WWTPs in the Czech Republic (■ RNA positive samples, □ RNA negative samples)

Regions of the Czech Republic where SARS-CoV-2 RNA was detected in wastewater in spring 2020 are depicted the Figure 2. Prevalence of COVID-19 cases in those regions varied between 0 to 1.2 % of inhabitants.

SARS-CoV-2 RNA was found even in WWTP catchment where few or no infected persons were reported. Our findings indicate a very high sensitivity of the detection method and suitability for the development of early warning system.

Table 1. Overview of selected WWTPs involved in the study according to the population served and the results of viral RNA detection in their wastewater samples

WWTP capacity according to the population served	number of WWTP involved in the study	number of samples/number of samples with positive detection of SARS-CoV-2 RNA	number of samples/number of samples with positive detection of NoV GI	number of samples/number of samples with positive detection of NoV GII	number of samples/number of samples with positive detection of AdV
< 2 000	3	7/1	6/2	6/3	6/4
2 000–10 000	6	24/4	23/4	23/6	23/22
10 000–100 000	22	70/9	77/13	77/29	77/75
> 100 000	4	17/4	16/1	16/5	16/15

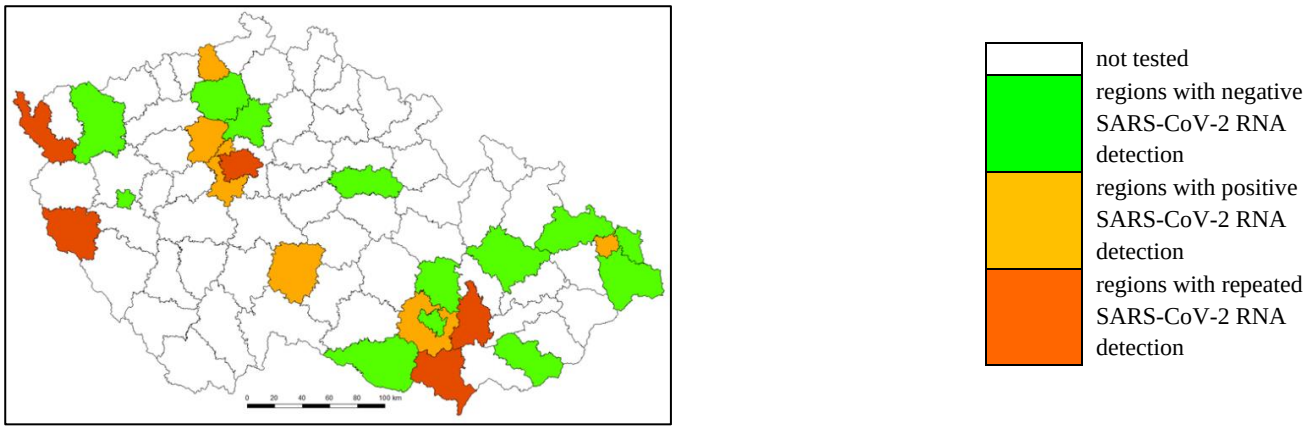

Figure 2. Occurrence of SARS-CoV-2 RNA in wastewater in the Czech Republic – spring 2020

4. Discussion

Testing of wastewater for the presence of SARS-CoV-2 RNA is actively underway in many countries around the world with the same goal of contributing to effective measures to combat the spread of insidious disease and in particular to exploit the epidemiological system in other possible epidemics. Following successful research by Dutch researchers, similar research from Australia, Spain, Austria, the USA, Israel, Brazil, Turkey, India, Italy and France was published (Medema et al., 2020; Ahmed et al., 2020; Wu et al., 2020; La Rosa et al., 2020; Wurtzer et al., 2020; Randazzo et al., 2020; Sharif et al., 2020).

A large number of clinical scientists from all over the world are currently studying the presence of coronaviruses in the gut and urine and the possibility of their transmission by the faecal-oral route. Many follow the research of previous important representatives of the coronaviruses SARS-CoV-1 and MERS. A major discovery was made by researchers at the Hubrecht Institute in Utrecht, who confirmed that SARS-CoV-2 viruses can infect and multiply in intestinal cells (Lamers et al., 2020). This finding significantly increases the importance of wastewater monitoring and the study of possible contamination of the aquatic environment. However, evidence of fecal-oral transmission has not been demonstrated yet. It is not known whether fragments of the virus are present in excreta of all infected individuals or whether it is occurrence is dependent on the course and symptoms, virus dose or stage of the disease. It is not confirmed, whether the virus is excreted by asymptomatic individuals, as well.

In our study, SARS-CoV-2 RNA was determined in wastewater from WWTPs, in whose catchment area only units of COVID-19 infected persons were currently registered (Mlejnková et al., 2020). These very low numbers indicate a very high sensitivity of the detection method and suitability for the development of early warning tool for effective surveillance of epidemics.

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