Long-term wastewater monitoring of SARS-CoV-2 viral loads and variants at the major international passenger hub Amsterdam Schiphol Airport: A valuable addition to COVID-19 surveillance

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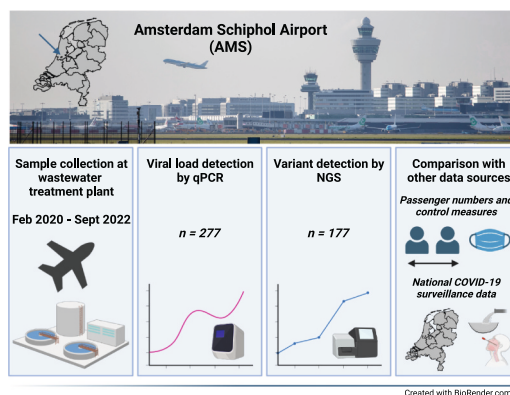[f] Department of BioInformatics and computational services (BIR), National Institute for Public Health and the Environment (RIVM), Antonie van Leeuwenhoeklaan 9, 3721, MA, Bilthoven, the Netherlands
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HIGHLIGHTS

- Wastewater surveillance for SARS-CoV-2 was performed at Amsterdam Schiphol Airport.
- Airport wastewater monitoring feasible throughout pandemic irrespective of measures
- Viral load trends parallel and sometimes predates Dutch national viral load trends.
- Viral loads rose simultaneously with the emergence of new variants.
- VOC emergence mirrors and at times predates those reported in clinical samples.

GRAPHICAL ABSTRACT

A B S T R A C T

Wastewater-based epidemiological surveillance at municipal wastewater treatment plants has proven to play an important role in COVID-19 surveillance. Considering international passenger hubs contribute extensively to global transmission of viruses, wastewater surveillance at this type of location may be of added value as well. The aim of this study is to explore the potential of long-term wastewater surveillance at a large passenger hub as an additional tool for public health surveillance during different stages of a pandemic. Here, we present an analysis of SARS-CoV-2 viral loads in airport wastewater by reverse-transcription quantitative polymerase chain reaction (RT-qPCR) from the beginning of the COVID-19 pandemic in Feb 2020, and an analysis of SARS-CoV-2 variants by whole-genome next-generation sequencing from Sep 2020, both until Sep 2022, in the Netherlands. Results are contextualized using (inter)national measures and data sources such as passenger numbers, clinical surveillance data and national wastewater surveillance data. Our findings show that wastewater surveillance was possible throughout the study period, irrespective of measures, as viral loads were detected and quantified in 98.6 % (273/277) of samples. Emergence of SARS-CoV-2 variants, identified in 91.0 % (161/177) of sequenced samples, coincided with increases in viral loads. Furthermore, trends in viral load and variant detection in airport wastewater closely followed, and in some cases preceded, trends in national daily average viral load in wastewater and variants detected in clinical surveillance. Wastewater-based epidemiology at a large international airport is a valuable addition to classical COVID-19 surveillance and the developed expertise can be applied in pandemic preparedness plans for other (emerging) pathogens in the future.

1. Introduction

A high transmissibility enabled the rapid worldwide spread of SARS-CoV-2 and has led to the COVID-19 pandemic which has caused over six million deaths to date (World Health Organization, 2023). Intensive efforts have been made to control virus spread and prevent hospitalizations, through vaccination campaigns and measures such as social distancing and travel restrictions (Han et al., 2022; Le Targa et al., 2022; Talic et al., 2021). Although natural immunity and vaccinations have improved COVID-19 health outcomes and have led to COVID-19 being declared endemic in the Netherlands as of Feb 22, 2023, the continued circulation and evolution of SARS-CoV-2 necessitate the continuation of diverse surveillance efforts (van Dissel, 2023).

It was shown early in the pandemic that infected persons excrete sufficient SARS-CoV-2 RNA particles through faeces to be detectable in wastewater (Lodder and de Roda Husman, 2020; Medema et al., 2020). Wastewater Based Epidemiology (WBE) for SARS-CoV-2 enables monitoring of asymptomatic, pre-symptomatic, and symptomatic cases and is independent of testing behaviour or capacity (Kilaru et al., 2023). Previous research has shown a clear relation between SARS-CoV-2 viral loads, i.e., virus concentrations in wastewater, and both incidence and hospitalization rates (Hetebrij et al., 2024; Shah et al., 2022). Furthermore, it has enabled the detection of circulating variants (Amman et al., 2022; Brunner et al., 2022; Karthikeyan et al., 2022). These findings have led to the implementation of wastewater surveillance programs for SARS-CoV-2 in various countries (Amman et al., 2022; Brunner et al., 2022; de Araujo et al., 2022; Guerrero-Latorre et al., 2022; Kirby et al., 2021; Kisand et al., 2023), including the Netherlands (Geubbels et al., 2023).

Large international passenger hubs are considered informative monitoring sites due to their contribution to the global transmission of SARS-CoV-2 (Li et al., 2023). Therefore, studies have been performed on the detection of SARS-CoV-2 viral loads and variants in samples from both aircraft and airport wastewater, in various countries (Agrawal et al., 2022; Ahmed et al., 2022; Albastaki et al., 2021; Bonanno Ferraro et al., 2022; Farkas et al., 2023; Le Targa et al., 2022; Morfino et al., 2023; Nkambule et al., 2023; Qvesel et al., 2023). Although most studies have shown the potential of WBE, these were either short term studies or studies with low sampling frequencies. Whereas de Araujo et al. (2022) did perform long-term monitoring of SARS-CoV-2 in wastewater from multiple transportation hubs, including airports, their study did not include identification of variants. To our knowledge, no extensive longitudinal studies have been performed on the monitoring of both viral load and variants of SARS-CoV-2 in wastewater at a major passenger hub.

Between 2019 and 2021, Amsterdam Schiphol Airport (AMS) was one of the three busiest airports in the world by international passenger traffic. The wastewater treatment plant (WWTP) situated at AMS (WWTP-AMS) exclusively processes wastewater from the terminal, aircrafts, and approximately 70 companies associated with the airport. In this study, we attempt to contextualize observed patterns in SARS-CoV-2 viral load and variant estimates from airport wastewater using passenger counts, (inter)nationally imposed control measures, clinical surveillance data, and country-wide wastewater surveillance data collected in the Netherlands. The aim of this study is to explore the potential of long-term wastewater surveillance during different stages of a pandemic at a large passenger hub as an additional tool for public health surveillance.

2. Methods

2.1. Sampling, RNA extraction and RT-qPCR

Samples included in this study comprised of 24 h flow-proportional influent samples taken at WWTP-AMS between Feb 16, 2020, and Sept 4, 2022 ($n = 277$). Initially samples were taken weekly, but sampling frequency was increased to four, by addition of one sample per week on Jun 1, 2021, Aug 30, 2021, and Nov 8, 2021. Samples were transported by cold-chain to the Dutch National Institute for Public Health and the Environment where all samples were processed and analysed (Geubbels et al., 2023). Direct total nucleic acid extraction was performed using a MagNA Pure 96 DNA and Viral NA Large Volume Kit (Roche Diagnostics, Mannheim, Germany). Samples were processed and analysed in duplicate for both CDC N1 and N2 gene targets by RT-qPCR (Lu et al., 2020). Viral RNA concentrations are obtained by using a seven-day rolling window standard curve as described by Nagelkerke et al. (2023). Flow-corrected viral loads (FC-VL) (in virus particles per 24 h) was calculated by averaging the obtained RNA concentrations, (in particles per ml denoted by c,) of N1 and N2 targets quantified in duplicate (resp. c_{N1}, c_{N2}), and correcting for flow, (in m^3 per day denoted by f) according to Eq. (1):

$$FC - VL = \frac{\sum\limits_{i=1}^{2}(c_{N1i} + c_{N2i})}{4} * f * 10^6 \qquad (1)$$

2.2. Next-generation sequencing analysis

2.2.1. Library preparation and sequencing

Storage of extracted nucleic acids at −80 °C enabled additional next-generation sequencing (NGS) analysis more than a year after initial sampling. Initially, nucleic acids were treated with TURBO DNase (Thermo Fisher Scientific, MA, USA) and consecutively cleaned and concentrated with the RNeasy MinElute Cleanup Kit (Qiagen, Hilden, Germany). Starting in Aug 2022, RNA was purified using Zymo Research RNA Clean & Concentrator Kits (Zymo Research, Leiden, Netherlands), which included an additional DNase step. Random hexamer primers (pdN6) (Roche Diagnostics, Mannheim, Germany) were used to generate cDNA libraries, which were amplified using either the Artic V3 or Artic V4.1 primer sets, based on availability (Supplementary Table 1), whereby a subset of samples was spiked with additional Artic V3 primers and the V4.1 primer set was employed for retrospective sequencing. A subset of samples was subjected to multiple rounds of sequencing in case of insufficient genome coverage. Sequencing libraries were prepared with the TruSeq Nano DNA kit (Illumina, CA, USA) and sequenced in 2 × 300 cycles on an Illumina MiSeq platform.

2.2.2. Bioinformatic analysis

The approach used in this study is based on and incorporates parts of the *SARS2seq* (v0.6.6) (https://github.com/RIVM-bioinformatics/SARS2seq) software. Briefly, demultiplexed reads are aligned to the Wuhan-Hu-1 index genome (RefSeq: NC_045512.2) with *Minimap2* (v2.24) (Li, 2018) using default settings for short genomic paired-end reads. Adapter sequences are removed through removal of soft-clipped regions after alignment. Sequence quality control was performed with *fastp* (v0.23.2) (https://github.com/OpenGene/fastp) by which reads were trimmed if the average base quality dropped below 20 for a window of five bases, and reads shorter than 100 bases were discarded. Subsequently, primer sequences were removed with *AmpliGone* (v1.1.0) (https://github.com/RIVM-bioinformatics/AmpliGone) and re-aligned to the index genome. Where required, aligned sequences were converted from SAM to BAM format, sorted, indexed or merged by *SAMtools* (v1.14) (https://github.com/samtools/).

Variant abundance estimates were calculated using *Freyja* (v1.3.11; mutation barcodes downloaded on 03-08-2023) (Karthikeyan et al., 2022), with a minimum lineage abundance of 0.001. Samples were considered of sufficient quality for typing if at least 50 % of the genome was covered by a minimum of 10 reads. A 100-fold bootstrapping procedure was employed to determine variability between lineage estimates. Lineage abundance estimates for Variant of Concern (VOC) sublineages were binned into monophyletic parent lineages (https://github.com/cov-lineages/pango-designation). Other lineages, including recombinant lineages were binned into *"Other"*.

2.3. Data analysis and external data sources

Data analysis and visualization were performed using *R* (v4.2.2) (R Core Team, 2022) with *Tidyverse* (v2.0.0) (Wickham et al., 2019). Schiphol Airport Services provided the passenger data. Evides Industriewater provided the flow-proportional influent wastewater with date and time-specific flow rates (in m^3). Hospital admission data was obtained from Our World in Data (2023). Pathogen surveillance data of SARS-CoV-2 in clinical samples in the Netherlands, as well as national average viral loads were obtained from the RIVM open data (Nationale Kiemsurveillance, 2021; Nationale Rioolwatersurveillance, 2021). Reported clinical cases per lineage were binned similarly to lineage estimates from wastewater.

3. Results and discussion

3.1. Study overview

Viral RNA was detected and quantified in 98.6 % (273/277) of the wastewater samples taken. The four non-detects were observed in samples taken in the first year of the pandemic at various passenger numbers. As a linear relation was observed for flow and passenger arrivals (Supplementary Fig. 1), viral loads were corrected for flow and presented here as flow-corrected viral loads (FC-VL). In total, 63.9 % (177/277) of the samples were analysed by short-read next-generation sequencing (NGS). Lineage estimates of sufficient quality were obtained for 90.9 % (161/177) of those samples and lineages were grouped by associated Variant of Concern (VOC). To facilitate interpretation of the results, the study period was divided into five predefined periods based on the stage of the pandemic and the circulating VOCs: (A) Early pandemic, (B) Alpha, (C) Delta, (D) Omicron BA.1 and BA.2, and (E) Omicron BA.4 and BA.5 (Fig. 1A).

3.2. Period A: the early pandemic stage

The early pandemic stage (Feb 2020 until Nov 2020), started with: (1) two negative wastewater samples taken on Feb 16 and 23, (2) the confirmation of the first clinical case in the Netherlands on Feb 27 (Alderweireld et al., 2020), and subsequently (3) the initial detection of SARS-CoV-2 in the third wastewater sample, on Mar 1 (Fig. 1C). Passenger numbers did not deviate from the 2017–2019 numbers until March, when a sharp decline was observed (Fig. 1B). Initial travel restrictions included flight cancellations from designated high risk areas, such as China, Iran and Italy by the Dutch Government on Mar 13 (Bruins and van Nieuwenhuizen Wijbenga, 2020). Subsequently, arrivals declined, while FC-VLs continued to increase until Mar 15 (Fig. 1B-C). An overall decrease in FC-VL was observed around Mar 18 when non-essential travel from non-EU countries into the EU was restricted (European Commission, 2023). The decline in passenger arrivals continued until April, when numbers were exceptionally low with an average of 4200 passengers per day while in 2017–2019 daily passenger arrivals were 23 times higher (97,740). During this month, the percentage of arrivals classified as transfers (32 %) was relatively high compared to the same period in the previous years (21 %). This might be explained by flights being scheduled as a means of COVID-19-related repatriation whereafter passengers took connecting flights to get home. From Apr 17 onwards, travel bans expired and health certificates became required when arriving from high-risk areas or with repatriation flights organized by the Dutch government (https://www.rijksoverheid.nl/onderwerpen/coronavirus-tijdlijn/april-2020-verlenging-maatregelen-aankondiging-en-uitbreiding-testbeleid). Whereas passenger arrivals increased slowly in the second half of April, FC-VL decreased until May (Fig. 1C). These results suggests that trends in viral loads can be monitored irrespective of measures and even at very low passenger numbers. Hence, wastewater monitoring can be used to detect SARS-CoV-2 at an airport indicating circulation of the virus.

Initial sequencing results were obtained for samples taken in September ($n = 2$) and October ($n = 1$), which identified the pre-Alpha lineages B.1.1.500 (78.9 %), B1.1.297 (97.6 %) and B.1.177 (47.9 %) as most abundant. These findings correspond well to those of Izquierdo-Lara et al. (2023), who compared sequence data from clinical and wastewater samples originating from the city of Rotterdam in the same period, and found B.1.177 to be one of the predominant lineages.

3.3. Period B: the first variant of concern

The second period (Dec 2020 until May 2021), included the introduction of the first VOC, Alpha (B.1.1.7 lineage), and started with several new travel related restrictions: (1) mandatory use of face masks for travellers from 1 Dec, (2) a required negative COVID-19 test when

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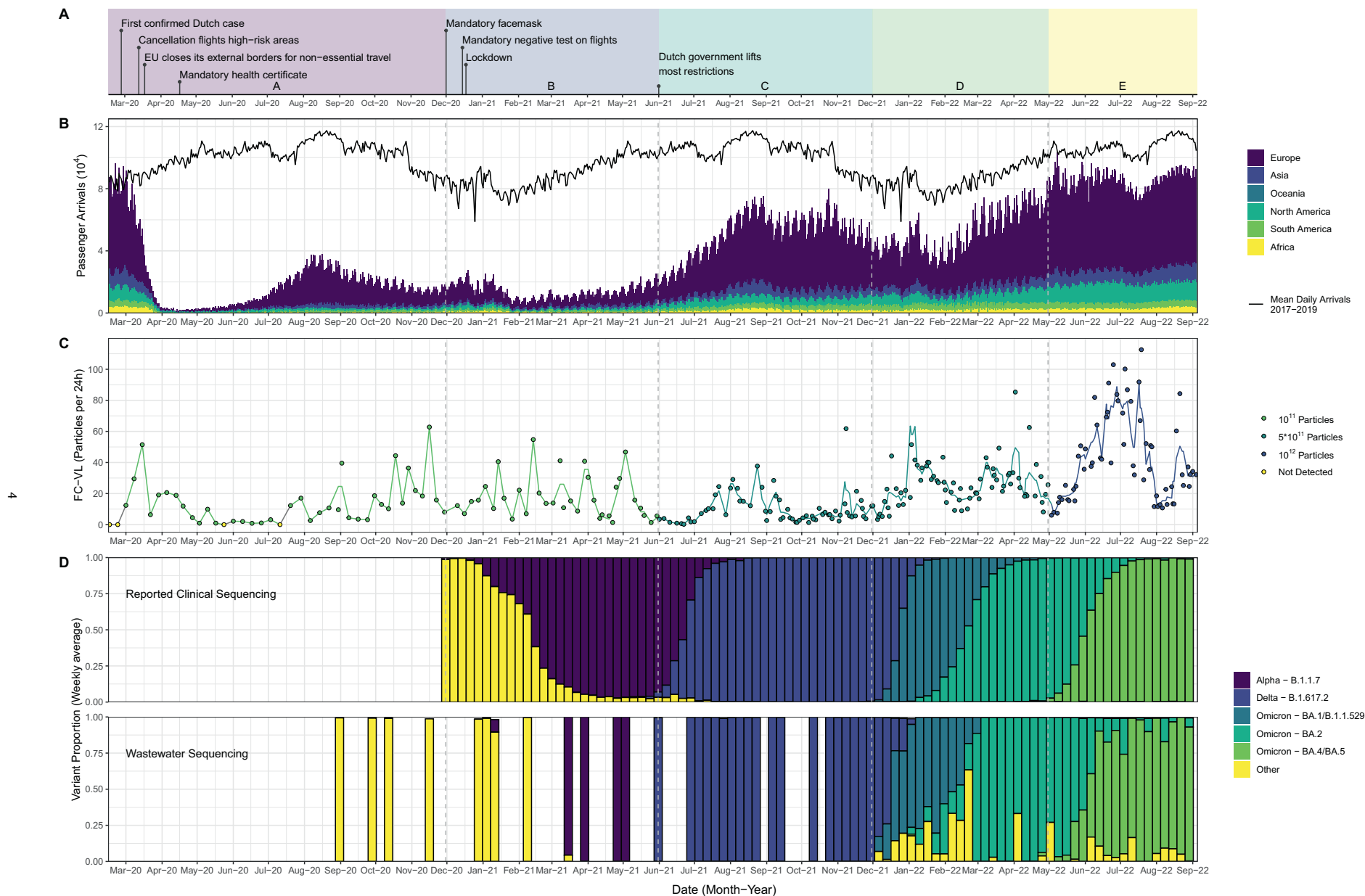
Fig. 1. Overview of investigated parameters. (A) A timeline of the predefined periods in this paper, main events and associated control measures related to travel taken during the pandemic to impair virus transmission. (B) Daily passenger arrivals at Amsterdam Schiphol Airport by continent of origin including transfers and transits. The black line depicts the 2017–2019 daily average of passenger arrivals. (C) Detected flow-normalized viral loads ($n = 277$). Positive measurements (scale colour) are distinguished from non-detects (yellow). A rolling average (7-day window) is depicted by the solid line shown in different colours for scaling purposes. (D) Variant of Concern abundance estimates of clinical (top) and wastewater (bottom) samples ($n = 161$). Data shown comprises weekly proportion and abundance averages of VOCs, respectively.

Table 1

Overview of first reported detection of SARS-CoV-2 Variants of Concern in the Netherlands. For initial detection in clinical samples, dates were taken from Han et al. (2022) and supplemented for VOC Omicron with week start dates of clinical open data. For initial detection in wastewater samples included in this study, VOC lineage estimates were calculated with Freyja (Karthikeyan et al., 2022), and bootstrap estimates are shown in brackets.

Variant of Concern (VOC)	Date initial detection in clinical samples	Date initial detection wastewater samples	Lineage frequency in corresponding wastewater sample
Alpha (B.1.1.7)	Dec 5, 2020	Jan 14, 2021	Q.1 0.087 (0.000–0.089)
Beta (B.1.351)	Dec 22, 2020	Jan 10, 2021	B.1.351 0.322 (0.315–0.333)
Delta (B.1.617.2)	Apr 15, 2021	Jun 1, 2021	Multiple[a] (10) 0.981 (0.889–0.986) AY.87 0.010 (0.006–0.014) AY.116 0.003 (0.000–0.008) AY.46.6 0.001 (0.000–0.003)
Omicron (BA.1/ B.1.1.529)	Week of Nov 22, 2021	Dec 11, 2021	BA.1.21/BA.1.7 0.221 (0.201–0.239) B.1.1.529 0.198 (0.148–0.219)
Omicron (BA.2)	Week of Dec 13, 2021	Jan 6, 2022	BA.2.43 0.041 (0.000–0.104) BA.2.3 0.026 (0.003–0.053) BA.2.42 0.004 (0.002–0.006) BA.2.44 0.003 (0.000–0.006) BA.2.18 0.001 (0.000–0.002)
Omicron (BA.4)	Week of Apr 4, 2022	Feb 20, 2022	BA.4.4 0.005 (0.000–0.015)
Omicron (BA.5)	Week of Jan 31, 2022	Jan 6, 2022	BA.5.1.22 0.043 (0.014–0.045) BA.5.1.33 0.005 (0.000–0.011) BA.5.9 0.001 (0.000–0.002)

[a] AY.4.11; AY.102; AY.92; AY.4; AY.39; AY.110; AY.118; AY.126; AY.111; AY.79.

traveling from outside the EU from 15 Dec and (3) a lockdown in the Netherlands issued by the Dutch Government discouraging international travel, starting Dec 18 (Van Nieuwenhuizen Wijbenga et al., 2020). The aim was to reduce transmission by mandating the use of facemasks when traveling, instead of limiting travel of infected persons. Therefore, we consider it to have limited effect on FC-VLs.

A clear up- or downward trend in FC-VLs was not observed until May (Fig. 1C). This could be attributed to the population at AMS being highly variable in (1) overall size and (2) composition, as a result of different policies for travellers from in and outside the EU and the lockdown in the Netherlands (Fig. 1A-B). Passenger numbers decreased after Jan 2020, reducing the number of people that could potentially shed viruses into the airport wastewater.

Initial detection of the Alpha variant in wastewater occurred 14 Jan 2021, after the first detection in clinical samples 5 Dec 2020 (Table 1; Fig. 1D). Concurrently, the Beta (B.1.351 lineage) and Gamma (P.1 lineage) VOCs were reported in clinical samples as well, but neither exceeded 4 % of weekly cases. The B.1.351 lineage was also observed in two wastewater samples from Jan 10 and Mar 17, 2021.

3.4. Period C: the Delta variant and the first major peak in viral load

The third period (Jun 2021 until Nov 2021) featured the emergence of the Delta variant (B.1.617.2 lineage). Between May and June, the

Dutch government lifted most restrictions (de Jonge, 2021), which has been suggested to contribute to the short interval between the introduction and dominance of the Delta variant in July (Han et al., 2022). During this period, both passenger arrivals and FC-VL at AMS increased markedly (Fig. 1B). Considering FC-VL is indirectly corrected for passenger arrivals by flow, this indicates that proportionally more virus particles were shed into the wastewater, possibly due to a higher proportion of infected persons among passengers and increased shedding by persons infected by the emerging Delta variant (Prasek et al., 2023).

In July, the first clear wave in FC-VL could be observed (Fig. 1C). From this moment onwards, trends in viral loads at WWTP-AMS were compared with (1) the daily average viral load in the Netherlands corrected for flow and inhabitants (FIC-VL) (Fig. 2), which is measured at municipal WWTPs (primarily serving households), and (2) hospital admissions in the countries from which most passengers arrived (Fig. 3). FC-VL at WWTP-AMS started to increase simultaneously with the national FIC-VL around Jul 1 (Fig. 2). In the preceding two weeks, most arriving passengers originated from Spain, the United States of America, Italy, France, Germany, and the United Kingdom. The latter was the first to observe a Delta wave in Europe and, likely as a result, hospital admissions in the United Kingdom already started to rise mid-June (Fig. 3). In the Netherlands, new hospital admissions only started rising mid-July, after an increase was observed in both FC-VL and FIC-VL around Jul 1.

The first detection of the Delta variant in wastewater occurred later than the initial detection in clinical samples (Table 1), possibly due to sparse sampling selection (Supplementary Table 1). However, the abundance of Delta lineages in the wastewater sample was estimated to exceed already 99.2 % while the Delta variant accounted for <5 % of clinical sequences. This high frequency of Delta lineages continued in all analysed samples from Jun until Nov 2021.

3.5. Period D: rapid succession of the Delta variant by Omicron lineage BA.1 and BA.2

The fourth period (Dec 2021 until Apr 2022) featured the displacement of the Delta variant by the BA.1 and BA.2 Omicron lineages (Fig. 1D; Fig. 4, top panel). In the first week of December, FC-VL at AMS increased almost concurrent with the emergence of the BA.1 lineage (Fig. 4, top panel), while the national daily average FIC-VL did not increase until the beginning of January (Fig. 2). Although BA.1 lineage abundance displayed large fluctuations, the overall patterns closely resembled those observed through clinical surveillance. The BA.2 lineage rapidly surpassed the BA.1 lineage, which was shown to be more abundant in wastewater samples compared to clinical samples in Jan 2022 (Fig. 1D; Fig. 3, top panel). The circulation of the Omicron BA.1 and BA.2 lineages was associated with increased detection of recombinant lineages in wastewater (Fig. 4, top panel). These recombinant lineages had been included in clinical surveillance reports, but never exceeded 6 % of total cases per week in the study period. As these comprise recombinants of lineages circulating in that period, namely B.1.617.2 (Delta), BA.1 and BA.2 (Omicron), these detections in wastewater samples could potentially be attributed to the methodology used.

3.6. Period E: Omicron BA.5 and the largest peak in viral load

In the fifth period (May until Sep 2022) the BA.2 lineage was overtaken by the BA.5 lineage (Fig. 1D). The BA.4 lineage arose concurrently and appeared to co-circulate at a low abundance (Fig. 4, bottom panel). Whereas both the BA.4 and BA.5 lineages were detected in wastewater samples already in the first months of 2022, ahead of clinical reporting (Table 1), the relative abundance of either lineage did not show an increase until May, when their frequencies increased in both clinical and wastewater samples (Fig. 4, bottom panel). Although these initial detections were not further validated, overall trends of BA.5 and BA.4
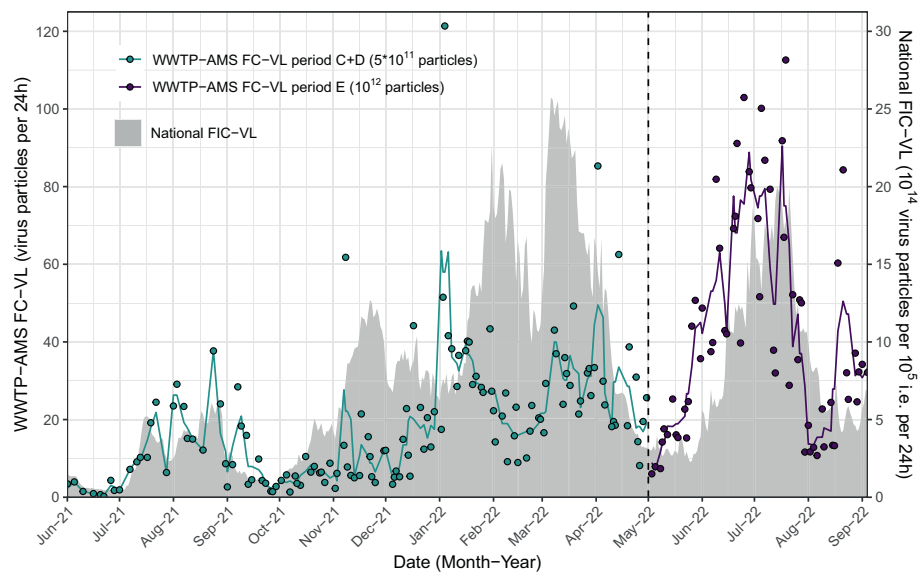
Fig. 2. A comparison of trends in viral load at Amsterdam Schiphol Airport (FC-VL shown in green and purple) with the Dutch national daily average viral load (FIC-VL shown in grey area). The measured FC-VLs in periods C and D (June 1, 2021, up until April 30, 2022, in teal) are split from those of period E (May 1 until Sep 4, 2022, in purple) due to the much higher FC-VLs at AMS during the latter period. The dashed vertical line depicts the split between period D and E. The national trend of FIC-VL is additionally corrected for inhabitant equivalents (i.e.) compared to the FC-VL. The green and purple lines depict a 7-day centred moving average.
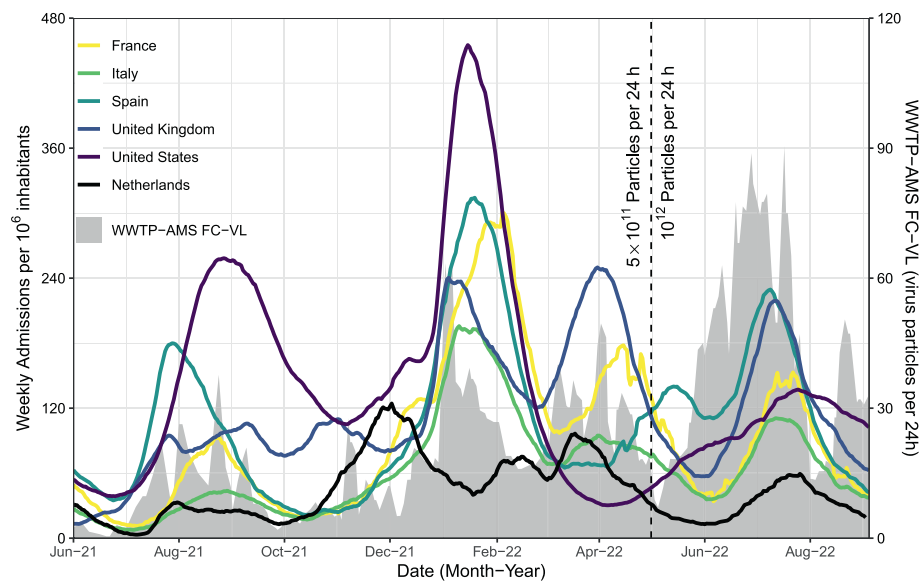
Fig. 3. Hospital admissions per million inhabitants per week in six countries over time compared to the trend in viral load at Amsterdam Schiphol Airport (FC-VL shown in grey area). Weekly hospital admissions per million are given per country. A selection of countries is made based on where most passengers are arriving from at Amsterdam Schiphol Airport (France, Italy, Spain, the United Kingdom and the United States of America) as well as the country where the study is performed (the Netherlands). The trend in FC-VL at Schiphol Airport is scaled separately for the period before and after May 1, 2022, shown by the dashed vertical line.

lineage emergence were shown to greatly mirror clinical reporting (Fig. 4, bottom panel).

Once more, the emergence of a new VOC lineage coincided with an increase in FC-VL at AMS, starting from May 9 (Fig. 4, bottom panel) while passenger arrivals remained relatively stable (Fig. 1B). In the first week of June, three weeks after the rise in FC-VL at AMS, the national daily average FIC-VL started to rise as well. In the two weeks preceding the increase in FC-VL at AMS, most passengers originated from the United Kingdom, Spain, the United States of America, Italy, Germany, and France. During this period, hospital admissions decreased in these countries, except for the United States of America and Spain. Here, a rising trend could be observed indicating increased virus circulation. The rising trend together with the increase in FC-VL at AMS three weeks

before the increase in the daily average FIC-VL in the Netherlands, indicates that AMS might have played a role in seeding this wave in the Netherlands.

3.7. Perspectives and challenges

In this study, we explored the potential of long-term wastewater surveillance at a large international passenger hub as an additional tool for public health surveillance, by analysing wastewater samples taken at the WWTP situated at Amsterdam Schiphol Airport during different phases of the COVID-19 pandemic. We aimed to contextualize our findings using other data sources and travel related control measures. We did not aim to evaluate such measures, which would require a
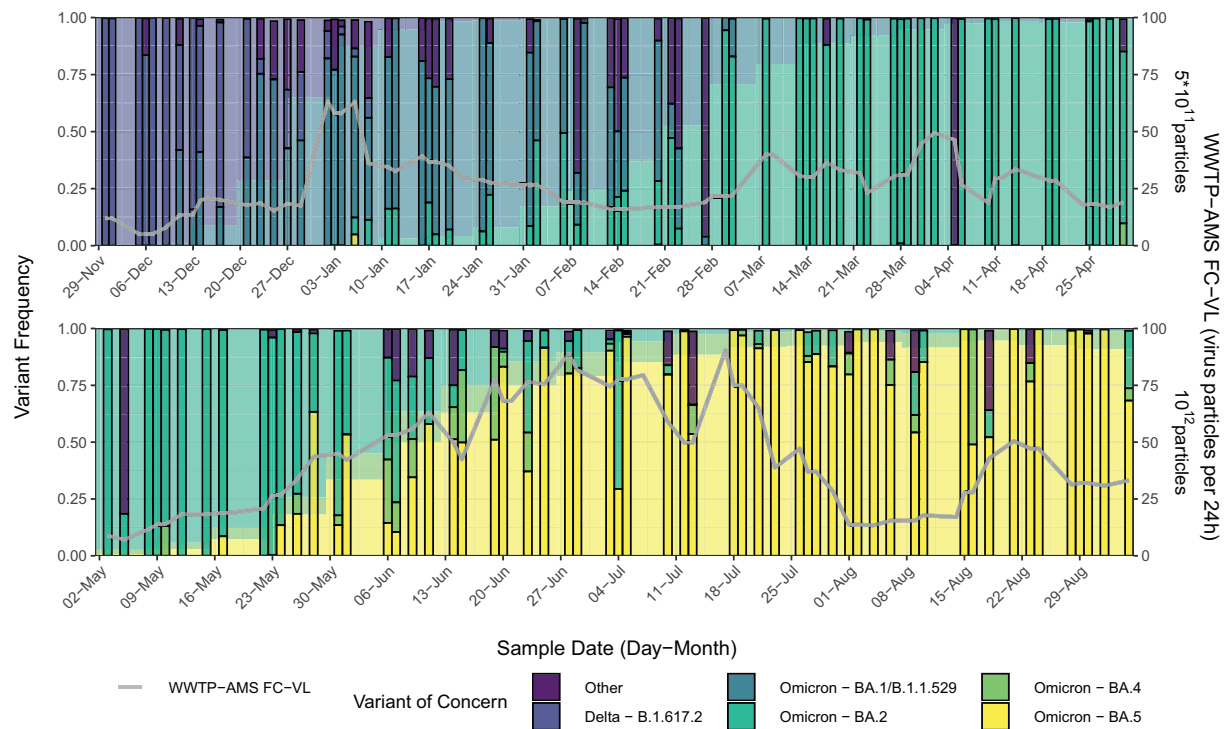
Fig. 4. A comparison of estimated variant of concern (VOC) of wastewater samples with reported weekly clinical frequencies and viral loads (FC-VL). The two panels represent period D (top) and E (bottom), denoting the periods between Dec 2021 and Apr 2022, and between May 2022 and Sep 2022 respectively. Estimated lineage frequencies from wastewater variant analysis are shown as single bars and reported clinical frequencies are shown as a semi-transparent background. Grey lines depict a 7-day centred average of FC-VLs in wastewater.

different study setup. Instead, these measures enabled us to explain trends in SARS-CoV-2 viral load and variants that we identified in our study.

Firstly, our results show that, even when SARS-CoV-2 circulation is reduced due to e.g. measures taken, FC-VLs could be detected throughout the study period including during the early phase of the pandemic when passenger numbers were low. Whereas initial detection of viral RNA in wastewater during this phase occurred after the first reported clinical case, these results are consistent with a similar study of SARS-CoV-2 RNA detection at WWTP-AMS at the start of the pandemic, by Medema et al. (2020). We consider the relatively low sampling frequency (≤4 monthly samples) in both their and our study to have limited the chance of an earlier detection in wastewater. As we showed that wastewater surveillance is feasible at AMS, it should be noted that the WWTP at this large passenger hub is different from most municipal WWTPs, since the sampled population is more variable in size and composition over time. However, as mentioned earlier, flow was indirectly used to account for fluctuating population sizes. Even though employees are not included in passenger numbers, they do contribute to the total population. We assume that the ratio between passengers and employees remains relatively stable over time, due to national control measures affecting both groups and therefore will also be accounted for by flow correction. Furthermore, viral detection in wastewater depends on toileting behaviour. Passengers might decide to use the toilet before, during or after their flight, which is also dependent on the flight duration (Jones et al., 2023). In our study, this detection probability is variable over time due to changing ratios between long and short flights, as well as between flight types. This is partly the results of control measures, underlining the importance of contextualising our findings.

Secondly, trends in viral load coincided, and sometimes even preceded the national trend in viral load and hospitalizations in the Netherlands, which could be related to hospitalizations in countries from which passengers arrived. A comparable, earlier, surge in airport viral load in Dec 2021 and May 2022 relative to municipal wastewater,

was observed by de Araujo et al. (2022). This highlights the need for international collaboration, which as Li et al. (2023) notes, could greatly enhance the potential of WBE at passenger hubs. This would require flexibility to scale up sampling frequencies in future pandemics as well as rapid data generation and sharing. Additionally, as we have shown, contextualization of findings with multiple data sources is crucial to realize the potential of WBE in overall surveillance of pathogens.

Lastly, the emergence and proliferation of variants of concern in wastewater, which coincided with increases in viral load, greatly resembled and in some cases preceded those reported by clinical samples. For emerging pathogens, air travel is one of many routes into the Netherlands and research by Han et al. (2022) showed that introductions of SARS-CoV-2 variants into the Netherlands generally occurred from neighbouring countries, and well ahead of travel restrictions. Nevertheless, the ability to confirm variant trends with a limited number of wastewater samples from a single location, makes WBE at a major international airport a very cost-effective additional surveillance tool for detection of (emerging) pathogens of concern. WBE has already been applied for other targets, such as antibiotic resistance genes, poliovirus, and enteric viruses (Kilaru et al., 2023), but the feasibility of such surveillance is dependent on the shedding route (Ahmed et al., 2020), sensitivity of sampling strategy and, methods of detection and identification.

4. Conclusion

In conclusion, our results show that wastewater surveillance at AMS has the potential to detect trends ahead of other national COVID-19 surveillance tools. Furthermore, it can provide a snapshot of international circulation that reaches its full potential when performed in an international framework, whereby timely detection of emerging variants and waves can be used to track circulation worldwide. Overall, wastewater surveillance at Amsterdam Schiphol Airport is a valuable addition to national as well as global COVID-19 surveillance during

different phases of the pandemic and can be of great value for monitoring (emerging) pathogens which pose a public health emergency of international concern.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All code used in this study is available on GitHub at: https://github.com/rivm-syso/WW_AMS_SC2. Raw sequencing data is available upon request. Processed passenger and viral load data are available on Zenodo at: https://zenodo.org/records/11282406

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