Wastewater sequencing as a powerful tool to reveal SARS-CoV-2 variant introduction and spread in French Guiana, South America

Marine Combe, Emira Cherif, Théo Deremarque, Georgina Rivera-Ingraham, Fatou Seck-Thiam, Fabienne Justy, Jean-Claude Doudou, Jean-François Carod, Thierry Carage, Angélique Procureur, et al.

Science of the Total Environment 924 (2024) 171645

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Wastewater sequencing as a powerful tool to reveal SARS-CoV-2 variant introduction and spread in French Guiana, South America

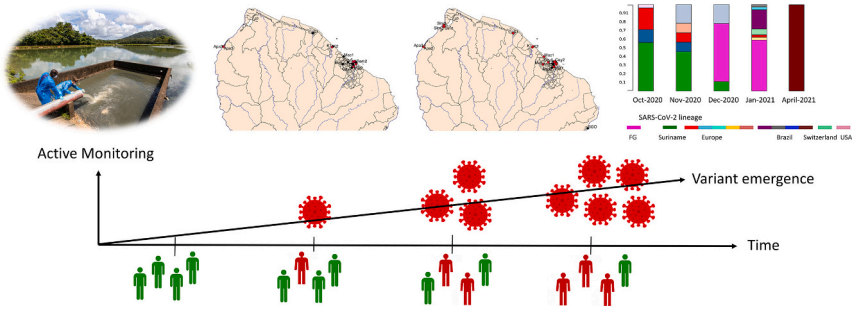
Marine Combe [a,*,1], Emira Cherif [a,1], Théo Deremarque [a], Georgina Rivera-Ingraham [a,b], Fatou Seck-Thiam [a], Fabienne Justy [a], Jean-Claude Doudou [b], Jean-François Carod [c], Thierry Carage [d], Angélique Procureur [d], Rodolphe Elie Gozlan [a]

[a] ISEM, Univ Montpellier, CNRS, IRD, Montpellier, France
[b] Centre IRD de Cayenne, Guyane Française, France
[c] Laboratoire et Pôle Appui aux Fonctions Cliniques, Centre Hospitalier de l'Ouest Guyanais (CHOG), 97320 Saint-Laurent du Maroni, Guyane Française, France
[d] Laboratoire de Biologie Médicale Carage de Kourou, 6 avenue Leopold Heder, 97310 Kourou, Guyane Française, France

HIGHLIGHTS

- WBE is a powerful tool to monitor pathogen circulation in tropical areas.
- Wastewater sequencing identified SARS-CoV-2 genetic diversity in French Guiana.
- A wide diversity of SARS-CoV-2 lineages were introduced in French Guiana.
- Wastewater sequencing identified cryptic transmission.
- Air travel is a significant risk factor for cross-border introduction of pathogens.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Warish Ahmed

Keywords:
Virus
Variant emergence
Cross-border effects
In-flight travel

ABSTRACT

The origin of introduction of a new pathogen in a country, the evolutionary dynamics of an epidemic within a country, and the role of cross-border areas on pathogen dynamics remain complex to disentangle and are often poorly understood. For instance, cross-border areas represent the ideal location for the sharing of viral variants between countries, with international air travel, land travel and waterways playing an important role in the cross-border spread of infectious diseases. Unfortunately, monitoring the point of entry and the evolutionary dynamics of viruses in space and time within local populations remain challenging. Here we tested the efficiency of wastewater-based epidemiology and genotyping in monitoring Covid-19 epidemiology and SARS-CoV-2 variant dynamics in French Guiana, a tropical country located in South America. Our results suggest that wastewater-based epidemiology and genotyping are powerful tools to monitor variant introduction and disease evolution within a tropical country but the inclusion of both clinical and wastewater samples could still improve our understanding of genetic diversity co-circulating. Wastewater sequencing also revealed the cryptic transmission of SARS-CoV-2 variants within the country. Interestingly, we found some amino acid changes specific to the variants co-circulating in French Guiana, suggesting a local evolution of the SARS-CoV-2 variants after their

* Corresponding author.
E-mail address: marine.combe@ird.fr (M. Combe).
[1] Equally contributing authors.

https://doi.org/10.1016/j.scitotenv.2024.171645
Received 27 November 2023; Received in revised form 19 January 2024; Accepted 9 March 2024
Available online 11 March 2024

introduction. More importantly, our results showed that the proximity to bordering countries was not the origin of the emergence of the French Guianese B.1.160.25 variant, but rather that this variant emerged from an ancestor B.1.160 variant introduced by European air plane travelers, suggesting thus that air travel remains a significant risk for cross-border spread of infectious diseases. Overall, we suggest that wastewater-based epidemiology and genotyping provides a cost effective and non-invasive approach for pathogen monitoring and an early-warning tool for disease emergence and spread within a tropical country.

1. Introduction

From where do pathogens arrive in a country during a pandemic? It's a question that may seem trivial or obvious, but on closer examination it involves a great deal of complexity (Hughes et al., 2010; Piret and Boivin, 2021). One might ask whether the dynamics of an epidemic within a country during a global pandemic are driven by its direct borders with neighboring countries (Laroze et al., 2021; Hossain et al., 2020), or by long-distance importations of cases by travelers from afar. In the same way, do the variant dynamics result from internal country dynamics or from the stochastic importation of variants by the various comings and goings of international travelers in the country? Answering these questions will help us to better understand the border effect, the trade route effect or the territorial effect, as in the case of countries with overseas territories. The particularity of countries with overseas territories, such as France, is that these territories are part of the same national unit and therefore do not fall under the scope of a border closure (Benwell et al., 2021). However, from an epidemiological point of view, these territories represent the same import or export risks as a foreign territory.

The spread of Emerging Infectious Diseases (EIDs) is often not limited in space. Once emerging locally, pathogens can spread rapidly and widely in the human population thanks to the intensity and speed of international interconnectivity in human mobility and trade, and thus risk developing into a global pandemic (Jagadesh, 2020). This is notably the case for air-borne RNA viruses, which are easily transmitted from infected to susceptible people via droplets. This was clearly seen in the SARS-CoV-2 pandemic, which spread rapidly around the world in just a few weeks, followed by the emergence of a wide variety of variants in time and space. Some variants remained highly localized, while others spread very quickly. There is undoubtedly an intrinsic effect linked to the nature of the variant itself, increased fitness (e.g. transmissibility) and virulence, changes in pathogenesis (clinical presentations), which makes it more or less contagious, but also undoubtedly to dispersal mechanisms in a territory that are still poorly understood (Siebenga et al., 2009; Eden et al., 2013; Majumdar and Niyogi, 2021; Khandia et al., 2022). In the case of SARS-CoV-2, a population genetic and phylodynamic analysis of >25,000 sequences from the UK showed that viruses carrying the mutation 614G in the spike glycoprotein had spread faster than viruses carrying the 614D (Volz et al., 2021) and the Omicron variant perfectly illustrated a higher transmissibility (Ito et al., 2022) while showing similar or milder clinical symptoms than the previous variants (Jansen et al., 2021).

Cross-border areas represent the ideal location for the sharing of viral variants between countries, with international air travel, land travel and waterways playing an important role in the cross-border spread of infectious diseases (Shingleton et al., 2023). Therefore, border effects could also affect the emergence of new variants through mutation or recombination (hybrids) with potentially higher fitness (e.g. transmissibility) and outperforming locally circulating variants, or through "founder events". Indeed, "founder effects" (the transmission of a limited number of individual viral particles) also shape viral evolution by retaining ancestral mutations that will increase in frequency in the population regardless of their effect on viral fitness (Lauring and Hodcroft, 2021). Phylogenetic analysis of SARS-CoV-2 whole-genome sequences showed multiple ($N = 62$) independent introduction events of the virus in Spain at the beginning of the epidemic, as well as a surprisingly high prevalence (40 %) of clade 19B (variant D614) compared with other European countries, pointing towards a founder effect followed by the local emergence of mutation G614 in the spike protein (Díez-Fuertes et al., 2021). It is also important to note that socially marginalized populations including migrants, refugees, asylum seekers, people living in camps and overcrowded environments with limited access to resources for hygiene or physical distancing, are particularly at risk of viral (co)infections, especially in low- and middle-income countries (LMICs) (Chaillon et al., 2022). For example, cross-border mobility has increased vulnerability to tuberculosis (TB) at the border region between the United States and Mexico (Deiss et al., 2009) with an increase in TB cases diagnosed in border regions with a high concentration of immigrants (Schneider et al., 2004). More recently, co-circulation and co-infection with Delta and Omicron variants of SARS-CoV-2 have led to the emergence of recombinant and hybrid variants that could alter the epidemiology of the disease (Kaiwan et al., 2023). Interestingly, Chaillon et al. (2022) found up to 10 distinct SARS-CoV-2 variants (lineages) co-circulating among people who inject drugs (PWID) and migrants in shelters on the California-Mexico border, illustrating the reservoir of viral genetic diversity that can co-infect people and thus promote viral evolution (i.e. the emergence of new variant). This also highlights that poverty remains a major socio-economic driver of bacterial and viral (neglected) disease emergence (Hotez, 2013a, 2013b; Hotez, 2020).

The extent of the SARS-CoV-2 pandemic illustrates the need to develop and validate tools that are easy to use, relatively inexpensive in a routine installation and that allow us, in near-real time, to closely monitor the evolutionary dynamics of viruses in space and time within local populations. Environmental and waste-water based epidemiology (WBE) are now well recognized as representing an invaluable predictive tool for addressing the epidemiology of disease outbreak (Polo et al., 2020; de Araújo et al., 2021, 2022; Saththasivam et al., 2021; Yousif et al., 2023; Barnes et al., 2023). The efficiency of WBE has been demonstrated during the global polio eradication programme to assess the circulation of the polio virus within populations and the effectiveness of immunization (Hovi et al., 2012; Ndiaye et al., 2014; Roberts, 2013; Hart and Halden, 2020). WBE has also been used to monitor and predict outbreaks of hepatitis A and norovirus-associated gastroenteritis (Hellmér et al., 2014), of Influenza A (H1N1) surveillance (Heijnen and Medema, 2011), or even measles and the emergence of antibiotic-resistant bacteria (Mallapaty, 2020). WBE approach has now extended to epidemics caused by enveloped viruses such as SARS-CoV, MERS-CoV, and more recently SARS-CoV-2 (Polo et al., 2020; Wurtzer et al., 2020; Medema et al., 2020; Yousif et al., 2023; Barnes et al., 2023) and although these viruses are considered to be typical respiratory viruses, they also exhibit gastrointestinal cell tropism, with SARS-CoV-2 infecting glandular epithelial cells for example and exhibiting viral shedding in faeces (Xu et al., 2020; Xiao et al., 2020a, 2020b). This approach has the advantage to *i)* capture the overall health status of the human community connected to a wastewater distribution network, *ii)* identifying hotspots of increasing disease incidence that could not be detected by individual clinical tests, *iii)* accounting for viral load not only in symptomatic individuals but also in pre-symptomatic and asymptomatic individuals (Gonzalez et al., 2020; Cariti et al., 2022) and *iv)* offers a more economically suitable disease epidemiology tool, particularly for LMICs where individual testing is limited and poor communities generally lack access to adequate sanitation and health facilities (Hart

and Halden, 2020; Zarza et al., 2022; Barnes et al., 2023). More interestingly, WBE combined with wastewater-based genotyping (WBG) has been shown to provide a promising system for identifying and tracking the evolutionary dynamics of viral variants in a matrix of pooled samples in near real-time (Adriaenssens et al., 2018; Lin et al., 2021; Karthikeyan et al., 2022; Yousif et al., 2023), predicting the emergence and spread of new variants of concern (VoCs) in the population (Lin et al., 2021; Barnes et al., 2023) such as up to 14 days earlier compared with clinical genomic surveillance (Karthikeyan et al., 2022), which can be notably associated with increased levels of transmission (Davies et al., 2021a), disease severity (Davies et al., 2021b) and/or immune escape (Wibmer et al., 2021), and also to identify the origin of the introduction of new viral variants into the population.

WBE-WBG has been widely used since the beginning of the Covid-19 pandemic as a early-warning indicator of the circulation and (re)-emergence of SARS-CoV-2 variants in the population (Wurtzer et al., 2020; de Araújo et al., 2021; Karthikeyan et al., 2022; Yousif et al., 2023). Although some studies have tested its efficacy in tropical areas (Ahmed et al., 2020a; Prado et al., 2020; Fongaro et al., 2021; de Araújo et al., 2022, 2023), *i*) none have been conducted in French Guiana and *ii*) few have tested potential border effects in the genesis of the emergence of new viral variants. Here we aimed to answer the following questions 1) whether WBE is an efficient tool for monitoring the epidemiology of the disease in tropical zones with alternating periods of highly contrasting rainfall and drought such as French Guiana (South America); 2) whether WBE-WBG can identify the overall diversity of SARS-CoV-2 variants co-circulating in a country and their evolutionary dynamics over time; and 3) the role of direct border effects and air travel as drivers of variant emergence at the country level.

2. Material & methods

2.1. Sampling site

We conducted our study in French Guiana (FG), a tropical overseas territory of France located in South America and characterised by cyclical wet and dry seasons. Despite its land area of 83,534 km^2, the territory has a low population density of 3.11/km^2 with 72.78 % (95 CI 0.726–0.728) of the total population living along the coastal region. Approximately 95 % of the total land area is classified as primary rainforest representing a large part of the Guiana shield, which is rich in biodiversity. FG has direct borders with Suriname to the west via the Maroni, Awa and Itany rivers, and with Brazil to the east via the Oyapock river.

Fig. 1. Sampling wastewater across the French Guiana territory (South America) from October 2020 to July 2021. @Thibault_Vergoz; @Emira_Cherif.

2.2. Wastewater sampling

From October 2020 to July 2021, water samples were taken every 4 weeks at 31 wastewater sites comprising lift stations and treatment lagoons collecting wastewater from coastal communities in French Guiana (South America) as well as rainwater (Fig. 1, Table 1). Whilst October and November 2020 and then July 2021 corresponded to the wet season, all other month from December 2020 to June 2021 belonged to the rainy season. Local inland communities were not sampled because most of them do not have a wastewater distribution system and because of the logistical difficulties of accessing the sites. It has been suggested that the SARS-CoV-2 genome is rapidly degraded at elevated temperatures and by UV irradiation from sunlight, and some studies have suggested a detection limit of 20 h or less after its excretion by infected individuals (Hart and Halden, 2020; Hovi et al., 2012). Therefore, we decided to collect unexposed and untreated water samples. A single water sample of 200 ml was collected from each site using a telescopic aluminum sampling rod and beaker. The sample collection was performed the first ten days of each month and between 8:00 am and 11:00 am for each station. The water collected was immediately and carefully transferred into a 500 ml sterile container. Although we are aware that usually composite wastewater samples of 4 h–24 h are used for wastewater surveillance (de Araújo et al., 2021), such experimental design was not available in French Guiana and the timing of the project and the accessibility of the stations and lagoons did not allow us to install such equipment. Also, we acknowledge that our limited financial and human resources allowed us to collect only one wastewater sample per station per month, which have obviously biased our results and limited the detection of viral variants. The outer surface of each container was immediately disinfected with a chemical spray and transported in the dark at 4 °C in ice to the laboratory (IRD Centre in Cayenne). On arrival, a second external disinfection of the containers was carried out before introducing the samples into the laboratory. The water samples were stored at 4 °C until the RNA was extracted. The time elapsed between sampling and analysis (i.e. virus inactivation and RNA extraction) was between 12 and 48 h.

Table 1
Study sites analyzed from October 2020 to July 2021 in French Guiana (South America). A total of 31 wastewater sites, including lift stations and sewage lagoons, collecting sewage of coastal communities in French Guiana and rainwater were collected for unexposed and untreated water samples every 4 weeks. Cities, wastewater site names and type, identification (id) and the geographic locations are presented. LS: lift stations; SL: sewage lagoon. The population size (Pop: inhabitants) as of January 1, 2019 of each area sampled is indicated, although these numbers do not include the number of illegal immigrants that can be quite high in French Guiana (https://www.insee.fr/fr/statistiques/6012651#tableau-figure1).

City (Pop)	Site name (type)	Site id	Latitude	Longitude
Apatou (9482)	Colombia (LS)	Apa1	5.1559444	−54.344499
	Office de Tourisme (LS)	Apa2	5.1554444	−54.343833
	Albertine Sida (LS)	Apa3	5.1538333	−54.341944
Saint-Laurent-du-Maroni (47621)	Pôle Isnar (LS)	Slm1	5.467427	−54.021866
	Lac Bleu (LS)	Slm2	5.472167	−54.035834
	Charbonière (LS)	Slm3	5.495775	−54.029091
Sinnamary (2875)	Sinnamary (SL)	S1	5.37157	−52.954112
Kourou (24903)	General (SL)	K1	5.154444	−52.657222
	Hospital (LS)	K2	5.166111	−52.645555
Macouria (16219)	Macouria (LS)	Mac1	5.009932	−52.476370
Soula (176)	Soula (SL)	Mac2	4.920994	−52.409570
Montsinéry (2957)	Montsinery (LS)	Mon	4.888954	−52.493212
Cayenne (65493)	Felix Eboué (LS)	Cay1	4.927824	−52.319028
	Zéphir (LS)	Cay2	4.936933	−52.307600
	PUG (LS)	Cay3	4.934718	−52.303481
	Mont Lucas (LS)	Cay4	4.922334	−52.301958
	Suzini (LS)	Cay5	4.925750	−52.292134
	Galmot (LS)	Cay6	4.931708	−52.329085
	Concorde (LS)[a]	Cay7	4.836146	−52.347155
	Novaparc (LS)	Cay8	4.923527	−52.313937
Rémire-Montjoly (26358)	Atilla-Cabassou (LS)	Rem1	4.890054	−52.288347
	Ames-Claires (LS)	Rem2	4.911216	−52.285081
	Lagune Morne Coco (SL)	Rem3	4.900933	−52.288477
Matoury (33458)	Scirie (LS)	Mat1	4.896528	−52.333136
	Barbadines (LS)[a]	Mat2	4.852175	−52.322667
	Matoury2 (LS)	Mat3	4.850888	−52.325319
	Lamirande (LS)	Mat4	4.878233	−52.327833
	Balata (LS)	Mat5	4.888313	−52.338039
	Lagune Concorde (LS)	Mat6	4.837439	−52.355513
	Lagune Barbadines (SL)	Mat7	4.852332	−52.320033
Saint-Georges (4245)	Saint-Georges (SL)	SGO	3.894165	−51.798593

[a] Sites sampled only in October 2020, thus not represented on the maps.

2.3. Viral particle concentration and RNA extraction from wastewater samples

Viral concentration and RNA extraction procedures were recommended by OBEPINE (Observatoire Épidemiologique dans les Eaux Usées), a French network of physicians and researchers monitoring SARS-CoV-2 in wastewater at national level, but also as part of international collaborations (https://www.reseau-obepine.fr). For practical reasons, the polyethylene glycol (PEG) based separation method was used. For each sample, 1.5 g NaCl, 1.5 g of beef extract powder (Sigma B4888), 0.2 g glycine and 50 ml of wastewater were mixed in a Falcon tube (50 ml) and inverted manually until the powder was dissolved and the sample homogenized. In particular, NaCl and beef extract pretreatment have been shown to promote SARS-CoV-2 viral particles and nucleic acids detachment and recovery from organic matter, while allowing the viral particles to bind and therefore precipitate (Zarza et al., 2022; Farkas et al., 2023). Next, 10 g of PEG 6000 was added and the outer surface of the tube was disinfected. Indeed, the use of NaCl/PEG has been shown to recover higher SARS-CoV-2 viral particles (1227 gc/ml) after the BE-$NaNO_3$/PEG (1858 gc/ml) protocol (Farkas et al., 2023). The tubes were shaken for 2 h at 4 °C using a magnetic stirrer until the powder was completely dissolved. After removing the magnetic stirrer, samples were centrifuged at 4500 x*g* for 45 min at 4 °C and the pellet obtained was resuspended in 2 ml of distilled water by pipetting up and down. Each sample was then incubated for 5 min with 5 ml of lysis buffer (NucliSENS® bioMérieux SA) to inactivate the virus. This incubation step was repeated 2 times. The outer surface of the tubes was again disinfected. The nucleic acids were purified in separation tubes previously filled with 4 g of a mixture of silicon grease warmed at 40 °C and silica powder (ratio 90:10 g) and centrifuged at 4000 ×*g* for 2 min. The tubes were then filled with 7.5 ml of phenol-chloroform-isoamyl alcohol (ratio 25:24:1) and 12 ml of concentrated viral particles. The tubes were centrifuged at 3500 ×*g* for 5 min and the aqueous phase collected for nucleic acids extraction using the NucliSENS® Extraction kit (bioMérieux SA) according to the manufacturer's instructions. To remove any contaminants that may inhibit downstream enzymatic reactions, the OneStep PCR inhibitor removal kit (ZymoResearch, Leiden, Netherlands) was used according to the manufacturer's protocol. Nucleic acids were immediately stored at −80 °C.

2.4. SARS-CoV-2 RNA detection in wastewater samples

Extracted nucleic acids were used to detect the presence of the SARS-CoV-2 RNA genome in the samples by targeting a small fragment of the nucleocapsid (N) and RNA-dependent RNA-polymerase (RdRP) genes (Table 2). The RT-qPCR reaction mix consisted of 5 µl TaqMan® Fast Virus 1-Step Master Mix (LifeTechnologies), specific primers at 400 nM final concentration each, probes (FAM) at 200 nM final concentration

Table 2
Primers and probes used to detect SARS-CoV-2 RNA by RT-qPCR in wastewater in French Guiana (South America). These primers and probed were provided by the OBEPINE network (https://www.reseau-obepine.fr/).

Target gene	Primer/Probe	Sequence (5′-3′)
N	2019-nCov_N1-F	GACCCCAAAATCAGCGAAAT
	2019-nCov_N1-R	TCTGGTTACTGCCAGTTGAATCTG
	2019-nCov_N1-P	FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1
RdRp	nCov_IP4-14059Fw	GGTAACTGGTATGATTTCG
	nCov_IP4-14059Rv	CTGGTCAAGGTTAATATAGG
	nCov_IP4-14059probe (+)	FAM-TCATACAAACCACGCCAGG-BHQ1

each and 10 μl of RNA template. Reverse transcription was performed at 50 °C for 5 min and was followed by qPCR reactions consisting of 1 cycle at 95 °C for 20 s, 45 cycles at 95 °C for 5 s and 45 cycles at 58 °C for 40 s. Enzymatic reactions were performed using a QuantStudio5 Real-Time PCR System (ThermoFisher Scientific Inc). Previous studies considered samples positive for Ct-values up to 40 (Wang et al., 2020). However, based on our standard curves (Supplementary Fig. S1) and in order to avoid any potential overestimations of detection we decided to set our cycle threshold at Ct-value 38. Samples were then considered positive when at least 1 out of 2 target genes had a Ct-value ≤38. Positive controls consisting of a plasmid containing the target gene were included in each RT-qPCR run. Three negative controls in which the RNA template was replaced by distilled water were included in each RT-qPCR run.

2.5. Clinical samples

Clinical samples consisted of nasopharyngeal samples collected by the Laboratoire du Centre Hospitalier de l'Ouest Guyanais (CHOG) at Saint-Laurent du Maroni (Dr. Jean-François CAROD) and the Laboratoire de Biologie Médicale CARAGE (LBM CARAGE) in Kourou (Dr. Thierry CARAGE, Dr. Angélique Procureur). Clinical samples were collected and analyzed according to ethical protocols and standard detection methods as part of the detection of SARS-CoV-2 cases in French Guiana. The following statement was written on all examination reports: "biological samples will be eliminated or transferred for scientific, epidemiological or quality control purposes (except human genetics), unless opposed to our secretariat, in accordance with the texts in force and in compliance with medical confidentiality".

Positive samples for which patient consent has been obtained in advance, with a Ct-value ≤30 and living in the same areas (Kourou, Sinnamary, Saint-Laurent du Maroni, Rémire-Montjoly, Apatou, Cayenne, Montsinéry, Macouria) as the wastewater sampling sites and tested positive during the same period as our wastewater screening were used for analysis and compared with the variants detected in wastewater (See Supplementary Table). Patient samples from localities not covered by the wastewater sampling were not used. Because of a new French regulation on the use of clinical samples patient samples were obtained from October, November and December 2020 and January 2021 but not for the remaining month covered by the wastewater sampling. SARS-CoV-2 RNA was extracted using the same protocol as described above for wastewater samples (NucliSENS® Extraction kit) and PCR inhibitors were also removed using the same protocol (OneStep PCR inhibitor removal kit, ZymoResearch).

2.6. Nanopore sequencing technology combined with ARTIC's multiplex PCR-based protocol

Wastewater samples with Ct-value ≤ 38 and clinical samples with Ct-value ≤ 30 were treated with DNase I to remove any residual DNA from the RNA samples.

Briefly, a nucleic acids subsample was incubated at 37 °C for 10 min with 1× DNase I reaction buffer and 2 units of DNase I (NEB). EDTA (0.5 M) was added at a final concentration of 5 nM and incubated at 75 °C for 10 min to stop the reaction. The RNA was then purified using the Monarch RNA cleaning kit (NEB T2030L), according to the manufacturer recommendations. RTs consisted of a mix of 2 μl of LunaScript RT SuperMix (5×) and 8 μl of RNA template incubated at 25 °C for 2 min, 55 °C for 10 min and then 95 °C for 1 min. The amplicons were sequenced using the ARTIC nCov-2019 sequencing protocol (Quick et al., 2017) previously implemented for clinical samples (https://protocols.io/view/ncov-2019-sequencing-protocol-v3-locost-bh42j8ye). This protocol was initially developed for sequencing single-stranded RNA viruses from high cycle threshold (Ct) clinical samples (Quick et al., 2017), and several versions have been released since January 2020, consisting of 98 primer pairs spanning the 30 kb of the SARS-CoV-2 genome, excluding the 3′ and 5′ regions (Lambisia et al., 2022), with amplicons of approximately 400 bp in length. The V3 primer was released in March 2020 and included additional alternative primers added to the V1 primer sets, providing over 50× coverage in all amplicons compared to the V1 and V2 primer-sets (Quick et al., 2017). In particular, the V3 protocol (LoCost) allowed to avoid the high cost of sequencing. For these reasons (already established protocols, low cost of reagents, availability of MinIon devices in our laboratory) we used the ARTIC nCov-2019 sequencing protocol V3 (LoCost) to analyse both clinical and wastewater samples. Clinical samples were processed using the published protocol while for wastewater samples exhibiting lower viral loads and a higher RNA degradation some adjustments were performed: 1) the denaturation step during the PCR cycle occurred at 95 °C; 2) 35 PCR cycles were set up; 3) the incubation time of the DNA end-prep step was increased by 5 min; 4) the ligation step was increased by 10 additional minutes of incubation.

We used the ONTdeCIPHER, an amplicon-based Oxford Nanopore Technology (ONT) sequencing pipeline to analyse and assess the genetic diversity of SARS-CoV-2 from clinical and wastewater samples, as implemented by Cherif et al. (2022). This pipeline allowed us to identify and assign the consensus lineage (reference genome Wuhan-Hu-1 NC_045512.2) found in each wastewater or clinical sample analyzed using the Pangolin database (pangoLEARN) (last update of the 31/07/2022). Consensus sequences with >54 % unidentified nucleotides (N) were removed from the analysis. SNP calling was performed with *artic medaka*, mutations were annotated with *SnpEff* (Cingolani et al., 2012) implemented in *decipher* and Coronapp (Mercatelli et al., 2021). The SARS-CoV-2 consensus sequences from French Guiana were deposited on the Global Initiative on Sharing All Influenza Data (GISAID) database.

2.7. Climatic parameters

Rainfall (millimeters/month) data were collected from the Météo France website (https://donneespubliques.meteofrance.fr/?fond=produit&id_produit=129&id_rubrique=29), France's official meteorological and climatological service, at 4 stations and then averaged for the whole French Guiana territory (Supplementary Table).

2.8. Statistics

Statistics were performed with Rstudio (V 1.1.453). First, we used the Shapiro-Wilk normality test and then applied the Anova test to look for correlations between the number of samples analyzed and the number of SARS-CoV-2 lineages identified or the number of SARS-CoV-2 lineages and the distance to the Surinamese border.

3. Results

3.1. SARS-CoV-2 RNA detection in wastewater in French Guiana

As only one wastewater sample (one time point) was collected for

each month, we were unable to calculate the average daily viral concentration (viral particles/24 h) excreted by asymptomatic, pre-symptomatic and symptomatic carriers. As a result, we were only able to provide maps of the number of positive (detection of SARS-CoV-2 RNA) versus negative wastewater sites across French Guiana over time (Supplementary Fig. S2). These maps were shared with France Public Health (Santé Publique France), the Regional Health Agency (Agence Régionale de Santé, ARS) and the Prefecture at Cayenne (i.e. governmental representation). The results of this spatio-temporal monitoring show some fluctuations in positivity (number of positive wastewater sites/total number of sites analyzed) over time, with a minimum of 8.33 % of positive wastewater sites recorded in November 2020 and a maximum of 78.57 % of positive wastewater sites recorded in June 2021 (Fig. 2; Supplementary Table). Overall, we found similar trends between the number of positive wastewater sites, the number of confirmed Covid-19 cases and the number of hospitalizations for Covid-19 provided by Santé Publique France for 6/9 month analyzed, except between February and March 2021 where the number of positive wastewater sites increased (up to 60.71 %) while the number of Covid-19 confirmed cases and hospitalizations decreased. The opposite trend was observed between April and May 2021, with a drastic decrease in the number of positive sites (29.63 %), while the number of cases and hospitalisations reached the highest values recorded during this period. In addition, the highest numbers of positive wastewater sites, observed in January and June 2021, correspond to curfew and lockdown periods in French Guiana (Fig. 2; Supplementary Table).

3.2. Genetic diversity of SARS-CoV-2 in French Guiana

3.2.1. SARS-CoV-2 variant co-circulation in wastewater and in clinical samples

We compared the diversity of SARS-CoV-2 lineages (i.e. variants) from wastewater and clinical samples. Using the ONTdeCIPHER pipeline (Cherif et al., 2022), we were able to identify and assign the consensus lineages found in each wastewater ($N = 20$) and clinical ($N = 54$) sample based on the Pangolin database (Fig. 3A, B). During the period from October 2020 to April 2021, we found a large diversity ($N = 13$) of SARS-CoV-2 lineages co-circulating in French Guiana. In clinical samples, the French Guianese lineage (B.1.160.25) was the main lineage (37.74 % prevalence, Fig. 3A), whereas in wastewater samples, the Surinamese lineage (B.1.219) was the most prevalent one (35 %) (Fig. 3B) during this period. Interestingly, although a higher number of SARS-CoV-2 lineages were found in clinical samples ($N = 10$) compared to wastewater samples ($N = 7$), only four lineages were found in both types of samples such as lineages from French Guiana (B.1.160.25), Surinam (B.1.219), Europe (B.1.160) and Brazil (B.1.1.33). The remaining lineages (from USA, Brazil, Switzerland and Europe) were found either in wastewater or in clinical samples. For example, two lineages from Brazil (P.2 and P.1) were found either in clinical samples (P.2) or in wastewater samples (P.1) but we were not able to detect those lineages in both samples. Unfortunately, wastewater samples from February–March 2021 and from May–July 2021 were of poor nucleotide sequence quality and we were unable to assign any SARS-CoV-2 lineage.

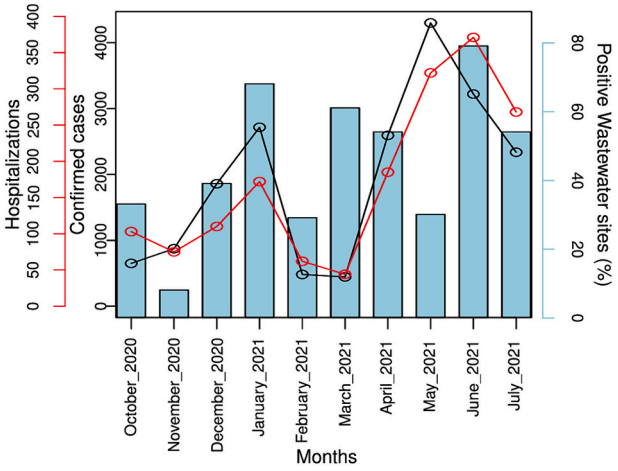


Fig. 2. Temporal follow-up of number of hospitalizations, confirmed cases of Covid-19 and wastewater sites for which SARS-CoV-2 RNA was detected by RT-qPCR in French Guiana (South America). Data on the number of hospitalizations and confirmed cases of Covid-19 were obtained from the monthly report of Santé Publique France in French Guiana and are representative of the whole territory. Wastewater positive sites were obtained from the present study.

3.2.2. SARS-CoV-2 genetic diversity in space and time

By combining both clinical (N = 54) and wastewater (N = 20) samples, we mapped the spatial genetic diversity of SARS-CoV-2 across French Guiana (Fig. 4). We observed the highest α-genetic diversity (SARS-CoV-2 lineages) in Saint-Laurent du Maroni (N = 7), Kourou (N = 5), Cayenne (N = 5), Apatou ($N = 4$) and Sinnamary (N = 4) compared with the other communes, with the number of assigned SARS-CoV-2 lineages being positively correlated with the number of samples analyzed in each of these localities (p-value: 0.000273; Supplementary Table). Moreover, these communes, together with Rémire-Montjoly, correspond to the locations where we found the Surinam lineage (B.1.219) to be highly prevalent, and some of these localities are at the border with Surinam (Apatou, Saint-Laurent du Maroni) or on the west part of French Guiana (Kourou, Sinnamary). However, we did not find any correlation between the diversity of SARS-CoV-2 lineages and the distance with the Surinamese border (p-value = 0.267; Supplementary Table).

Then, due to the low quality of wastewater samples from May to July 2021, we focused on the dynamics of SARS-CoV-2 genetic diversity over time, specifically from October 2020 to April 2021 (Fig. 5). We observed a higher frequency of the Surinamese lineage (B.1.219) in October and November 2020, followed by the European (B.1.160) lineage and less frequent lineages from the USA (B.1), Brazil (B.1.1.33) and Europe (B.1.177). Then, from October to November 2020 the frequency of the Surinamese lineage (B.1.219) decreases drastically and this lineage is replaced by the French Guianese lineage (B.1.160.25), which became the main SARS-CoV-2 lineage circulating in French Guiana, while other less frequent lineages were found to co-circulate throughout the territory, notably with the arrival of the Brazilian P.2 lineage in January 2021 and the Brazilian P.1 lineage in April 2021. Overall, the highest genetic diversity of SARS-CoV-2 variants co-circulating in French Guiana was recorded in January 2021, with at least 7 lineages detected in clinical and wastewater samples.

3.2.3. Mutational patterns of SARS-CoV-2 in French Guiana

SNP calling allowed us to identify for each sample the type of mutation, the mutated genes and the potential effects of these SNPs on the protein. The results showed SNPs occurring in several proteins with the 5'UTR region, NSP3 non-structural replicative protein, NSP12 non-structural RdRp protein and S Spike protein showing SNPs in all samples analyzed (Supplementary Fig. S3). The structural matrix (M) protein and the nucleocapsid (N) protein were also the most mutated proteins, with 93 % of the samples showing SNPs in those proteins. No SNPs were found in the structural envelop E protein. When all samples were combined, we identified several amino acids that were the most frequently mutated and we notably found 4 amino acids that were frequently changed in the samples, such as NSP12b:P314L, S:D614G, 5'UTR:241, NSP3:F106F (Supplementary Fig. S4). Among the other amino acid changes detected in our samples, we found 5 amino acid changes such as N:P13L, NSP12b:A88V, NSP3:T1198K, S:Y789Y, 3'UTR:29870 not frequently found in the SARS-CoV-2 genome. These changes could be more specific to the lineages circulating in French Guiana between October 2020 and April 2021 (Table 3). Among the

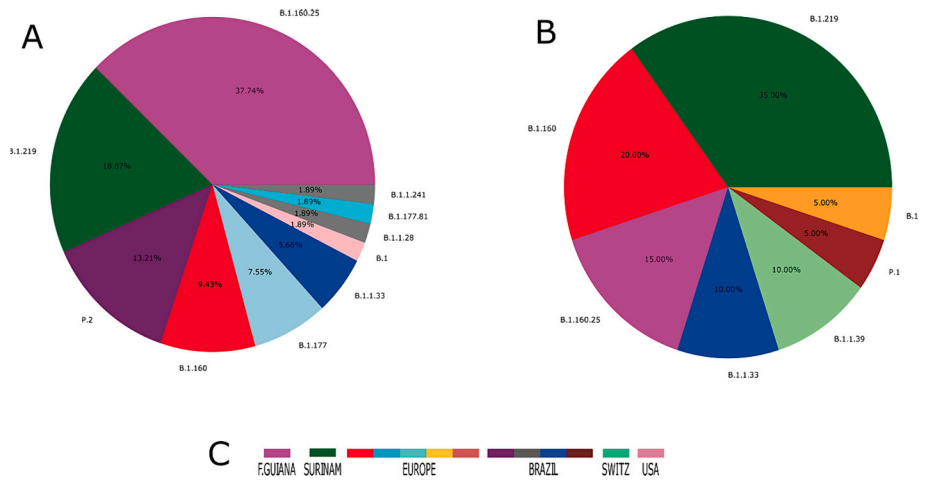


Fig. 3. Prevalence of SARS-CoV-2 lineages identified in French Guiana (South America) from October 2020 to April 2021 based on the Pangolin database. A: clinical samples ($N = 54$ corresponding to 100 % of selected positive samples sequenced); B: wastewater samples ($N = 20$ corresponding to 24.39 % of selected positive samples sequenced); C: SARS-CoV-2 lineages.

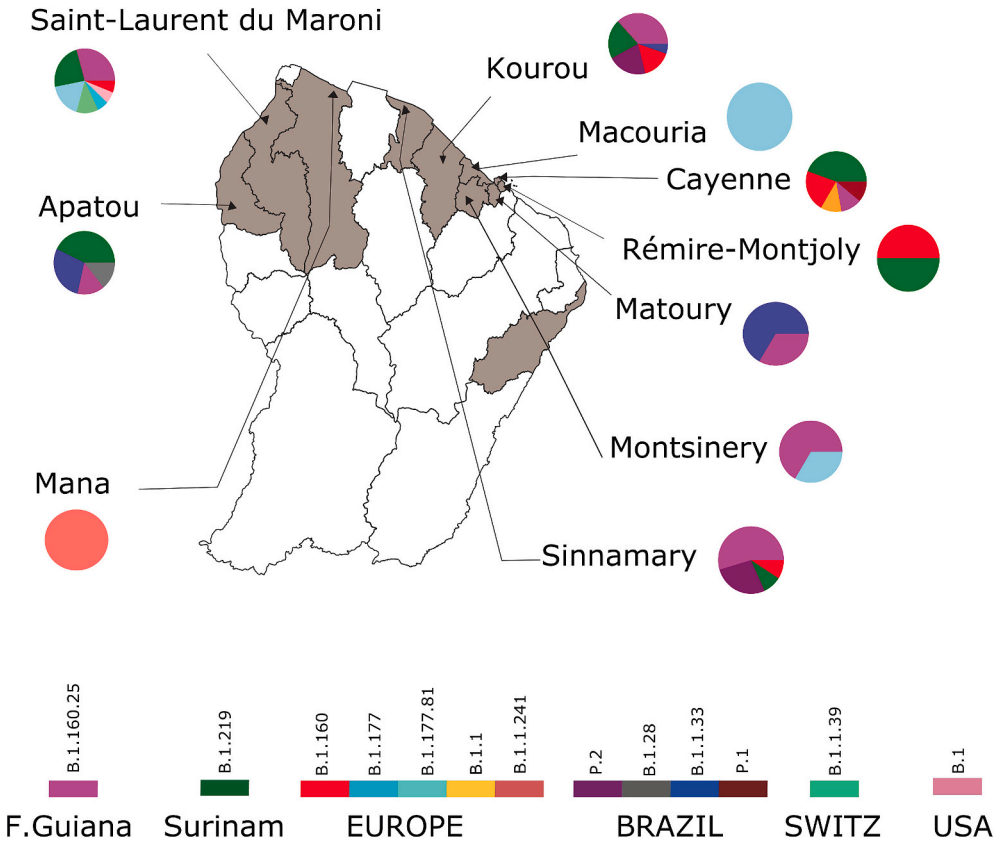


Fig. 4. Spatial distribution of SARS-CoV-2 lineages identified from each sampling area across French Guiana (South America). Sampling areas are indicated in grey. Clinical (N = 54) and wastewater (N = 20) samples were grouped together. SARS-CoV-2 lineages were identified based on the Pangolin database and are represented by a specific color for simplicity.

mutations found in our samples, the SNP, SNP_silent and extragenic ones were the most frequent compared to the deletion and SNP_STOP (Supplementary Fig. S5). Regarding at the impact of those mutations on the function of the proteins (*SnpEff* tool, Cingolani et al., 2012) for each lineage we found that most of them had a LOW (SNP_silent), MODERATE (SNP) or MODIFIER (extragenic, notably in lineage P.1) impact on the protein, while very few SNP_stop had a HIGH impact on the protein and these were only found in the B.1.160.25 lineage from French Guiana and in the P.2 lineage from Brazil (Supplementary Fig. S6, S7).

3.3. On the origin of SARS-CoV-2 introduction pathways in French Guiana

Here we analyzed the phylogenetic relationships between the different SARS-CoV-2 lineages co-circulating in French Guiana between October 2020 and April 2021, in order to assess the origin of the French Guianese B.1.160.25 lineage. We used the RAxML tool implemented in decipher and visualized in *FigTree* (Cherif et al., 2022). This phylogenetic analysis confirmed that some SARS-CoV-2 lineages are shared by

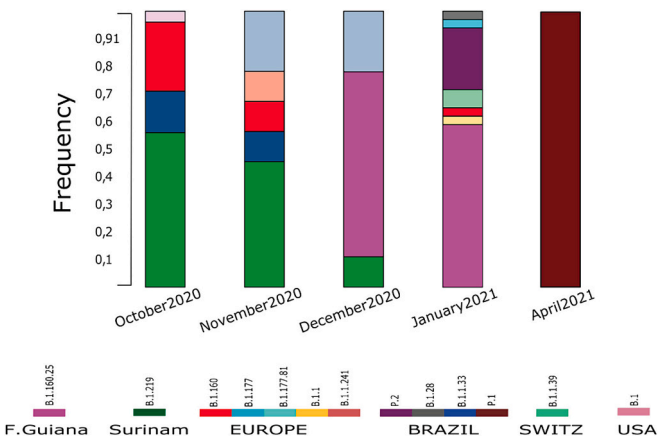


Fig. 5. Temporal dynamics of SARS-CoV-2 lineages in French Guiana (South America). Clinical (N = 54) and wastewater (N = 20) samples were grouped together. SARS-CoV-2 lineages were identified based on the Pangolin database and are represented by a specific color for simplicity.

Table 3
Mutational pattern found frequently in the B.1 lineage (Mercatelli and Giorgi, 2020) and specifically found in the French Guianese lineage.

	Nucleotide change	Gene:Amino acid change
Known SNPs in B.1 lineage	C14408T	NSP12b:P314L
	A23403G	S:D614G
	C241T	5'UTR:241
	C3037T	NSP3:F106F
	G11083T	NSP6:L37F
	C13730T	N:P13L
Specific SNPs to French Guianese lineage (B.1.160.25)	C23929T	NSP12b:A88V
	C28311T	NSP3:T1198K
	C6312A	S:Y789Y
	A24862G	3'UTR:29870

clinical (indicated by 'p') and wastewater (indicated by 'e') samples, as discussed previously (Fig. 6). Interestingly, our results show that the oldest SARS-CoV-2 lineage circulating in French Guiana appears to be the Surinamese lineage (B.1.219) which appears to be a common ancestor for more recently emerging lineages. We also observe some European lineages clustering together, for example lineages B.1.177.81, B.1.177, B.1.1.241 and B.1.1. These European lineages gave rise to the Brazilian variant P.1, while the Brazilian lineage B.1.1.28 gave rise to the Brazilian P.2 variant. The P1 lineage was particularly worrying for the WHO. From our results we can also confirm that the French Guianese and Brazilian lineages have appeared in French Guiana from December 2020, replacing the previous Surinamese and European lineages. Another interesting cluster we found is the one formed by the B.1.160 lineage from European countries (France, Spain, Italy) with the French Guianese B.1.160.25 lineage. The structure of the phylogenetic tree allows us to conclude that this later lineage (B.1.160.25) derives from these European lineages rather than from Brazilian or Surinamese lineages despite the geographical proximity of Brazil and Surinam with French Guiana.

4. Discussion

4.1. WBE as a powerful tool to monitor disease epidemiology in tropical areas

Importantly, our results show that SARS-CoV-2 RNA was successfully detected by RT-qPCR in wastewater samples across the territory of French Guiana and across tropical wet (October and November 2020, July 2021) and dry (December 2020–June 2021) seasons. Previous studies have suggested that SARS-CoV-2 RNA degrades more rapidly at high water temperatures up to 25–37 °C (Ahmed et al., 2020a, 2020b), but should remain detectable in wastewater for several days in tropical countries (de Araújo et al., 2021). For instance, experimental infections of river water and wastewater with SARS-CoV-2 showed that the persistence (measured as the time to achieve 90 % reduction in viral titer, T_{90}) of the virus at 24 °C was 1.9 days in river water vs 1.2 days in wastewater. However, at 4 °C this time (T_{90}) was increased to 7.7 days in river water and 5.5 days in wastewater (De Oliveira et al., 2021). In Tapachula, a migratory hub in Southern Mexico, although Zarza et al. (2022) found a negative correlation between the SARS-CoV-2 RNA copy number/mL and wastewater temperature, they successfully detected and quantified viral loads in wastewater up to 29 °C. Although we did not measure wastewater temperature at each sampling point, our results are consistent with previous findings and suggest thus that detection of SARS-CoV-2 RNA in wastewater is a relevant approach to monitoring Covid-19 epidemiology in tropical areas such as French Guiana. Interestingly, the trends observed between the number of positive wastewater sites, the number of Covid-19 cases and hospitalizations were consistent for the 6/9 month analyzed. However, between February and March 2021, we observed opposite trends between the dynamics of the epidemic in wastewater samples (including asymptomatic, pre-symptomatic and symptomatic individuals) and in clinical samples (biased towards symptomatic individuals). These different patterns could be explained either by 1) a higher proportion of asymptomatic people infected with a less virulent variant circulating during this period and therefore not individually tested, 2) a lower effort of clinical testing or 3) an early detection of the resurgence of the epidemic in wastewater, as previously proposed in The Netherlands, France, Sweden, Spain, Germany, Australia, India, etc. (Mallapaty, 2020; Medema et al., 2020; Wurtzer et al., 2020; Randazzo et al., 2020; Hart and Halden, 2020; Ahmed et al., 2020a; de Araújo et al., 2021; Sarkate et al., 2021). For example, a study conducted in the canton of Ticino in Switzerland analyzed wastewater samples collected during the onset of the first wave of the Covid-19 pandemic and showed that SARS-CoV-2 virus was already widespread by February 29th, 2020, while only a few and localized cases where reported (Cariti et al., 2022). A retrospective analysis of preserved wastewater in Santa Catarina state (Florianopolis city), Brazil, also detected SARS-CoV-2 RNA as early as 27th November 2019, while the first reported cases in the Americas did not occur until only the 21st January 2020 (Fongaro et al., 2021). Karthikeyan et al. (2022) detected the presence of new variants of concern (VoCs) in wastewater up to 14 days in advance compared with clinical genomic surveillance. Similar early peaks of SARS-CoV-2 detection in wastewater compared with clinical testing were observed in Malawi (Barnes et al., 2023). In contrast, as Covid-19 cases and hospitalizations in French Guiana peaked in May 2021, we can rule out a decline in the epidemic between April and May 2021, as our wastewater survey might suggest (Fig. 2). Rather, we propose that the lower detection of RNA in wastewater between April and May 2021 could be attributed to strong flooding events occurred in French Guiana during this period (Supplementary Table; Supplementary Fig. S8), resulting in a strong dilution of viral RNA in wastewater as well as in a potential change in physico-chemical properties that could affect RNA stability and thus detection (Zarza et al., 2022). In fact, in French Guiana in almost all wastewater networks, extraneous water enters the system, especially during rainfall. When comparing different wastewater catchments or regions over space and time, it is then necessary to normalize for the dilution of viral RNA copies in the wastewater system, which remains challenging and would require flow meters to be installed across each catchment in order to calculate the volume of water in the system at any time (Wilde et al., 2022). In French Guiana, we were not able to measure such wastewater flow and thus the impact of rainfall on virus dilution. Alternatively, our sampling strategy, mainly constrained by financial and human resources, could have impacted the detection of SARS-CoV-2 RNA in wastewater. Indeed, a 24 h composite sample has been recommended

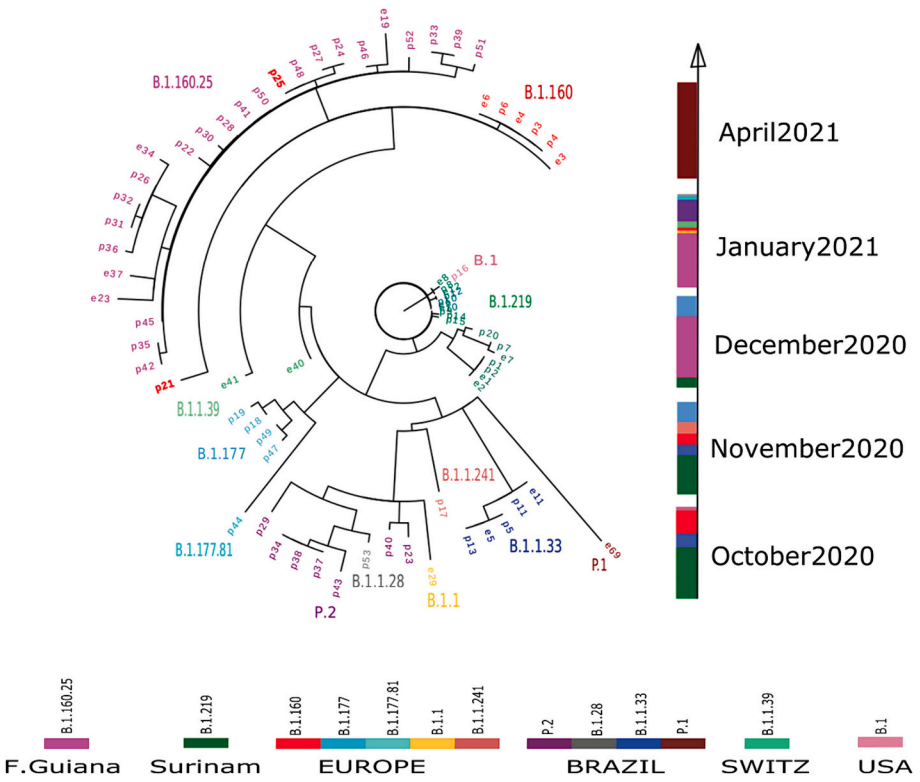


Fig. 6. Phylogenetic tree of SARS-CoV-2 lineages found in clinical and wastewater ($N = 74$) samples in French Guiana (South America) and identified by the Pangolin database. Clinical samples are indicated by "p" while wastewater samples are indicated by "e". The temporal evolution of each lineage is also represented. The phylogenetic tree was built based on 1000 boostraps.

for wastewater surveillance, with the collection of 500 mL of sewage every 20 min for at least 3 h in Low-Income countries, and repeated between 2 and 4 times per month (reviewed in de Araújo et al., 2021). However, despite these inherent biases of the French Guiana context, our spatio-temporal monitoring of SARS-CoV-2 RNA in wastewater strongly suggests that WBE provides a powerful tool for monitoring a broad diversity of pathogens – including zoonotic and non-zoonotic – and disease emergence in this French tropical overseas territory.

4.2. *WBE-WBG allows to identify the overall diversity of SARS-CoV-2 variants co-circulating across the French Guiana territory*

Interestingly, our results confirmed that WBE-WBG allows for the overall detection and spatial and temporal monitoring of SARS-CoV-2 variant co-circulation in French Guiana. However, we found that neither the clinical nor wastewater analysis alone was sufficient enough to cover the full diversity of SARS-CoV-2 lineages co-circulating in French Guiana. In fact, our sampling bias towards clinical samples ($N = 54$) resulted in higher genetic diversity compared to wastewater samples ($N = 20$) (p-value: 0.000273), but still some SARS-CoV-2 lineages were only detected in wastewater samples (i.e. lineage B.1.1.39 from Switzerland, lineage B.1 from Europe, lineage P.1 from Brazil). Such patterns have been previously observed by Haver et al. (2023) who have detected a lineage (AY.43) of the Delta SARS-CoV-2 variant during a period of Omicron variant (BA.5) dominance in wastewater samples in the Netherlands whilst this variant was not detected in clinical samples, strongly suggesting cryptic transmission of some variants. In Malawi, Barnes et al. (2023) also found cryptic transmission of the Beta, Delta and Omicron variants within the same period. It could also be hypothesized that lineages found only in wastewater could be carried by asymptomatic individuals and therefore be less virulent in terms of disease symptoms, potentially resulting in a lack of individual testing (Xu et al., 2023). In fact, the B.1.1 SARS-CoV-2 Variant of Interest (VOI) (Fig. 3) harbors, in addition to the D614G mutation, the E484K mutation in the spike (S) protein, which has been shown to confer reduced neutralizing activity of convalescent and post-vaccination sera against replication-competent and pseudoviruses (Álvarez-Díaz et al., 2022), while having no impact on viral stability and host cell entry (Jangra et al., 2021). As for the P.1 (gamma) variant, it emerged in Manaus, Brazil, in mid-November 2020 and has acquired 17 mutations, including a trio in the S protein (K417T, E484K and N501Y) associated with increased binding to the human ACE2 (angiotensin-converting enzyme 2) receptor. This variant was therefore classified as a Variant of Concern (VOC) (Boehm et al., 2021) and was estimated to be 1.7- to 2.4-times more transmissible than previous (non-P.1) variants (Faria et al., 2021). Since 26 clinical samples were analyzed in January 2021 we suggest that if these variants had an increased virulence we should have detected them in clinical samples. Rather, we can hypothesize that these variants circulated mainly in communities not tested at the CHOG and Kourou centers, or not tested at all. Also, no clinical samples were analyzed in April 2021, explaining a sampling bias in the detection of the P.1 (gamma) variant in wastewater samples only. Similarly, the Brazilian P.2 lineage, classified as variant of interest (VOI) and also harboring the E484K mutation (Silva et al., 2021), was found in clinical samples in January 2021 at a relatively high prevalence (13.21 %) and should therefore have been detected in wastewater samples. These observations could be explained either by a low number of wastewater ($N = 6$) samples analyzed or by the carriage of this P.2 variant by communities not connected to the sewage network sampled. It is also important to keep in mind that we may have missed the detection of some lineages because we identified the consensus sequence for each sample. Indeed, wastewater samples contain a mixture of fragmented nucleic acids that belong to different infected individuals. Consequently, when working on consensus sequences only does not reflect the presence of a single viral haplotype (Yousif et al., 2023). Moreover, the Pangolin tool used for SARS-CoV-2 lineage classification was initially designed for

clinical samples harboring a single dominant viral variant compared with wastewater samples composed of a mixture of viral variants (Karthikeyan et al., 2022). Whilst sample deconvolution using for instance the Freyja tool could remove such bias (Karthikeyan et al., 2022; Xu et al., 2023), allowing the recovery of VOC frequencies from mixed wastewater samples (Barnes et al., 2023), this tool was not used in the present analysis. Thus, variants co-circulating at a low prevalence in wastewater samples may have disappeared during the bioinformatic consensus sequence assembly (Xu et al., 2023). The use of the Freyja deconvolution tool associated with the analysis of signature mutations in the Spike protein efficiently allowed the genomic characterization of SARS-CoV-2 lineages co-circulating in wastewater in South Africa between April 2021 and January 2022 (Yousif et al., 2023) and also in Malawi (Barnes et al., 2023).

Our results of the evolution of SARS-CoV-2 variant over time are consistent with previously published data on the emergence of different lineages worldwide. For example, we found that the Surinamese lineage (B.1.219) circulated at high frequency throughout the territory at the beginning of our study and was subsequently replaced by the French Guianese lineage (B.1.160.25). Genomic data from the Pangolin database show that the Surinamese lineage was identified and intensively sequenced worldwide from March 2020 to August 2020 (Supplementary Fig. S9). Our results then show that this lineage was still circulating in French Guiana in October and November 2020, and we can hypothesize that it was actively circulating in the territory the month before. Furthermore, the French Guianese lineage was first sequenced from clinical samples and was reported in the Pangolin database from November 2020 (Supplementary Fig. S10), whereas we detected its presence in French Guiana from December 2020. However, as we only sampled one time point per month, we can hypothesize that this lineage was already circulating in French Guiana in November 2020 at a low prevalence compared to other lineages and we may have missed its detection during the consensus sequence assembly. Whilst the P.1 VOI emerged in Manaus (Brazil) in January 2021 (Silva et al., 2021), we could not use nucleotide sequence data from February and March 2021, a methodological bias that could explain its late detection in French Guiana from April 2021.

Whilst our results confirm that wastewater sequencing is a powerful tool to monitor variant introduction and evolution within a country (Lin et al., 2021; Karthikeyan et al., 2022; Barnes et al., 2023), comparison of both clinical and wastewater samples could still improve our understanding of genetic diversity co-circulating at the country level, especially when only a small number of wastewater samples can be processed. We acknowledge that only 20/82 wastewater samples could be efficiently sequenced and assigned to a specific SARS-CoV-2 lineage (Supplementary Table). Interestingly, Barnes et al. (2023) followed a similar Nanopore sequencing pipeline and showed that only 20/86 samples sequenced had >50 % genome coverage. By comparing their results with the EasySeq method and an Illumina MiSeq or NovaSeq they obtained similar results and concluded that low viral loads might have affected the different sequencing methods similarly (Barnes et al., 2023). Consequently, our sequencing is biased towards clinical samples, with most wastewater samples having unusable nucleotide sequences. Several methodological biases, other than the Pangolin tool, could be considered such as the choice of the sampling sites (e.g. spatially segregated cultural communities with specific variants), the presence of specific compounds such as residual chlorine or metals in raw wastewater that could affect the integrity of the viral particles, the RNA extraction protocol, the potential presence of PCR inhibitors, or even the ARTIC amplicon-based sequencing technology (Karthikeyan et al., 2022; Xu et al., 2023). Importantly, it was shown that the highest SARS-CoV-2 RNA concentrations and detection rates were obtained for wastewater collected from sewers close to the source, while samples collected further away from the source and at the receiving wastewater treatment plant showed lower detection rates (Farkas et al., 2023). Such observations may, for example, be due to viral decay and/or viral accumulation in the biofilm in the sewer (Petrovich et al., 2019; Morales Medina et al., 2022). In addition, it has been suggested that SARS-CoV-2 RNA is rapidly degraded under UV irradiation from sunlight (Hart and Halden, 2020; Hovi et al., 2012). Therefore, in French Guiana, we chose to collect wastewater from unexposed and untreated wastewater treatment plants and from town of relatively small size, which allowed us to collect wastewater close to the source of viral release (approx. 5–15 km away). Although some studies recommended the adsorption-direct RNA extraction method using electronegative membrane (de Araújo et al., 2021), we decided to use the beef extract elution method coupled with PEG precipitation to extract RNA from wastewater, as this method has been shown to recover most of the spiked SARS-CoV-2 Wuhan and Alpha variants from composite wastewater and was also used for SARS-CoV-2 surveillance at Edinburgh airport (Farkas et al., 2023). Indeed, it has been shown that up to 25 % of SARS-CoV-2 RNA can be retained in the solid matrix of wastewater (Forés et al., 2021; Ahmed et al., 2021), and pre-treatment methods such as application of beef extract enhanced the detachment of viral particles and viral nucleic acids from solids and significantly increased recovery (Farkas et al., 2023). Also, Sapula et al. (2021) compared the efficiency of PEG precipitation and adsorption-extraction to concentrate the SARS-CoV-2 virus and found that PEG precipitation resulted in the highest and most reproducible recovery rates (46.6–56.7 % depending on the matrix) compared with the adsorption-extraction (recovery rate of 0–21.7 %) one. PCR inhibitors were removed from extracted nucleic acids using a specific removal kit (OneStep PCR inhibitor removal kit, ZymoResearch) and an internal PCR control (IPC) was used to test for the presence of PCR inhibitors. Unfortunately, it was not possible to measure the presence of certain compounds (soap, chlorine, metals, etc.) in the raw wastewater that could have damaged the viral particles and viral RNA (Greaves et al., 2022; Chin et al., 2020). Finally, either metagenomic or amplicon-based approaches have been used for whole genome sequencing (WGS) of SARS-CoV-2, but the most widely used targeted amplicon approach is the ARTIC protocol (Lambisia et al., 2022). However, the ability to generate near complete genomes using the ARTIC V3 primers is still dependent on sample quality and viral load quantity (Lambisia et al., 2022). Consequently, the potential presence of harmful compounds in raw wastewater (soap, chlorine, metals, etc.), the dilution of viral particles during rainfall or by water used for other purposes than toilets, and the potential fragmentation and rapid degradation of RNA (Wood et al., 2020; Jo et al., 2022) in a complex wastewater matrix (Giroux et al., 2022; Farkas et al., 2023) could explain the high proportion of unidentified nucleotides (N) and thus unusable nucleotide sequences from wastewater samples collected in French Guiana (Xu et al., 2023). For instance, de Araújo et al. (2023) found that the SARS-CoV-2 concentration in three Brazilian hospital' wastewater did not correlate with the number of Covid-19 cases reported by these same hospitals, trends potentially explained by the different amount of viral shedding between infected individuals, the decay and/or the dilution of the virus in sewage pipes. Also, Holm et al. (2022) found that the concentration of the SARS-CoV-2 RNA in wastewater relied on the sewershed size, with smaller community sewershed areas showing a higher variability in the abundance of the virus.

Based on the results of this study, we strongly suggest that future wastewater surveillance studies should use composite sample of at least 4 h and sample the same site every week (4 times per month) (de Araújo et al., 2021). Also, while Lin et al. (2021) found that 400 bp amplicons provided better amplifications of viral RNA compared with 150 bp and 1200 bp amplicons, we suggest that further eRNA-based (environmental RNA) amplicon sequencing approaches starting from a wastewater or environmental complex matrix should consider the use of shorter amplicon sizes <200 bp (Deiner et al., 2017; Takahashi et al., 2023; Xu et al., 2023), which may still improve viral RNA amplification and sequencing. Evaluation of the type of degrading compounds in raw water may also be essential to assess the quality of collected RNAs. Finally, the implementation of sample deconvolution using for instance

the Freyja tool could remove biases attributed to samples with a mixture of variants and enabling the detection of variants circulating at a low frequency (Karthikeyan et al., 2022; Xu et al., 2023).

4.3. Specific amino acid changes of SARS-CoV-2 in French Guiana

Looking at the mutational profile of SARS-CoV-2 variants co-circulating in French Guiana between October 2020 and April 2021, we found the 5'UTR region and the NSP3, NSP12 and S proteins were mutated in all ($N = 74$) samples analyzed, while the M and N proteins were mutated in 93 % of samples and no mutations were found in the E protein. Interestingly, the 4 most frequent amino acid changes detected in our samples corresponded to two transversion mutations affecting the protein sequence (NSP12b:P314L, S:D614G) and two silent mutations not affecting the protein sequence (5'UTR:241, NSP3:F106F). These amino acid changes were among the four most frequently detected mutations in an analysis of 48,635 SARS-CoV-2 genomes worldwide in 2020 (Mercatelli and Giorgi, 2020). Similarly, amino acid mutation NSP6:L37F found in our samples was among the top 20 most frequent amino acid changes reported by the same analysis (Mercatelli and Giorgi, 2020). We found a massive prevalence of single nucleotide polymorphisms (SNPs) over insertion/deletion (indel) events, with SNPs generating a stop codon remaining very rare and insertion events completely absent in our samples, trends that were also observed on a global scale and in every continent affected by Covid-19 (Mercatelli and Giorgi, 2020).

The 5′ two thirds of the SARS-CoV-2 genome encode non-structural proteins associated with viral RNA synthesis, while the 3′ one third encodes all structural and accessory proteins (Marra et al., 2003; Rota et al., 2003; Thiel et al., 2003). The silent SNPs may not directly affect the protein sequence, but they may alter codon usage and translation efficiency (Mercatelli and Giorgi, 2020). For example, mutations occurring in the 5'UTR extragenic region could affect the transcription and replication rates of the virus, or the folding of the genomic ssRNA (Kim et al., 2020). Non-structural proteins (NSPs) are involved in viral replication. For example, NSP3, also known as papain-like protease, is the largest non-structural protein of SARS-CoV-2. It is a transmembrane, glycosylated, multidomain protein that, together with NSP4 and NSP6, prevent the dsRNAs from immune degradation (Gosert et al., 2002; Prentice et al., 2004). In SARS-CoV-2, NSP3 has two main functions, *i)* cutting and releasing other viral proteins to do their own job and *ii)* to remove tags from old and damaged proteins, thereby altering the balance of proteins and compromising the host cell immune system to clear the viral infection (Corum and Zimmer, 2020). Some residues within the NSP3 proteins showed high mutation rates during the Covid-19 pandemic, while NSP3 is considered a promising vaccine candidate after the S protein (Gorkhali et al., 2021). The SARS-CoV-2 genome is thought to contain two RNA-dependent RNA polymerase (RdRp) proteins. One is the NSP12 protein, which plays an important role in the assembly of the entire RNA polymerase replicative machinery. Together with NSP7 and NSP8 co-factors, NSP12 enables the nucleic acids binding (polymerization) and efficient RNA synthesis (Kirchdoerfer and Ward, 2019). During the pandemic, NSP12 was one of the most mutated proteins (Gorkhali et al., 2021), with the second most common amino acid change P314L affecting NSP12 (Mercatelli and Giorgi, 2020). However, genomic surveillance of SARS-CoV-2 variants has mainly focused on SNPs that occur in the Spike (S) structural glycoproteins, which are involved in host-virus interactions during viral entry into the host cell (Tortorici and Veesler, 2019). S proteins are composed of two subunits: S1 binds to host cell receptors (ACE2), while the S2 subunit promotes membrane fusion of the virus with the host cell (Gorkhali et al., 2021). As spike proteins are exposed on the surface of the virus, they are a major target for neutralizing antibodies (Lauring and Hodcroft, 2021) and the major viral antigen in current vaccines (Gorkhali et al., 2021). There is therefore great interest in the mutations that have arisen in this protein and their potential escape from host immunity, thereby compromising vaccine efficacy. For example, the D614G amino acid change (aspartate to glycine in protein position 614) caused by the A23403G transversion mutation in the spike glycoprotein (Mercatelli and Giorgi, 2020) was first detected at a significant level in early March 2020 and spread globally over the next months due to its increased infectivity (Korber et al., 2020). This mutation was identified in several Chinese provinces in late January 2020 (Lauring and Hodcroft, 2021) and was associated with faster transmission (Volz et al., 2021; Plante et al., 2021; Hou et al., 2020). The main function of the capsid (N) protein is to protect the genomic RNA, while the membrane (M) protein is implicated in the assembly of the virion. During the course of the SARS-CoV-2 pandemic, the N protein remained the second most mutated protein after the spike (S) (Gorkhali et al., 2021), while the matrix (M) and envelope (E) proteins showed very low mutation rates (Vilar and Isom, 2021). Thus, our results are consistent with previous findings on SARS-CoV-2 mutational patterns and amino acid changes, and also support the hypothesis that SARS-CoV-2 tends to maintain its genomic integrity throughout its global spread (Mercatelli and Giorgi, 2020). However, we also found 5 amino acid changes (N:P13L, NSP12b:A88V, NSP3:T1198K, S:Y789Y, 3'UTR:29870) that appear to remain more specific to the variants co-circulating in French Guiana, as we did not find them as frequently in the SARS-CoV-2 reference genome (B.1.1) or in previously analyzed samples worldwide (Mercatelli and Giorgi, 2020). Similar findings were reported by Yousif et al. (2023) from South African wastewater samples from where they found amino acid mutations commonly found in the literature and other uncommon or rarely reported amino acid changes, also highlighting the complementarity of sewage genomic surveillance and clinical sequencing. In the case of French Guiana, we hypothesize that these amino acid changes occurred locally after the genetic bottlenecks imposed by the introduction of viral variants into French Guiana from bordering countries and/or air travel.

4.4. Impact of European in-flight travel on the emergence of new variants in French Guiana

Interestingly, we did not find a correlation between SARS-CoV-2 genetic diversity, such as the number of lineages identified in clinical and wastewater samples for each community, and proximity to the Surinamese border (p-value = 0.267). We also found that the highest SARS-CoV-2 genetic diversity co-circulating in French Guiana occurred in January 2021, during the period when the French Guianese variant (B.1.160.25) was intensively circulating in the area (Fig. 5). Together with the phylogenetic analysis of the different SARS-CoV-2 lineages co-circulating in French Guiana during the study period, our results suggest that proximity to bordering countries such as Suriname and Brazil, where in particular two VOI (P.2) and VOC (P.1) were reported, was not the origin of the emergence of the French Guianese variant B.1.160.25. Furthermore, although this B.1.160.25 variant has been reported in the Amapá region of Northern Brazil (Zalona Fernandes et al., 2022) and on the border with French Guiana, it has not spread to other Brazilian regions and has been found at low prevalence in clinical samples from Brazil (Camargo et al., 2021), suggesting a likely introduction of this variant into Brazil from French Guiana. Rather, air travel between Europe and, in particular, Paris airport (mainland France) should have introduced the European lineages and, in particular, the B.1.160 lineage, from which viral evolutionary processes such as mutation, genetic drift and selection occurred locally, allowing the emergence of a new variant in French Guiana. This B.1.160.25 variant may have been selected for mutations conferring a fitness advantage (i.e. increased viral replication, increased transmission or escape immunity) over previous ones (Lauring and Hodcroft, 2021) and thus increased in frequency in the French Guianese population between December 2020 and January 2021.

Indeed, mutations that confer a competitive advantage are selected for, and those that reduce viral fitness are removed from the viral population. However, mutations can also increase or decrease in frequency through random events such as "founder effects", which occur when a

limited number of individual viral particles form a new population during transmission. In this case, the mutations present in the founder viruses will dominate the population regardless of their effect on viral fitness. Such interplay between natural selection and random events shapes viral evolution from the intra-host level to the country level (Lauring and Hodcroft, 2021). For example, the D614G mutation in the spike glycoprotein initially appeared to emerge independently and spread simultaneously across multiple geographic regions (Lauring and Hodcroft, 2021). This convergent evolution suggested natural selection and thus a fitness advantage for variants harboring the D614G mutation. However, subsequent sequencing efforts identified this mutation in several Chinese provinces in late January 2020, raising the possibility that the global spread of D614G variants may have resulted from founder events in which viral populations harboring D614G initiated the majority of early transmission events in multiple locations (Lauring and Hodcroft, 2021). However, in the present case, we propose to rule out the possibility of founder effects and thus the introduction of an ancestral lineage in French Guiana, as the B.1.160.25 variant has not been sequenced in other countries before November 2020, despite the large number of samples sequenced worldwide during this pandemic. Based on our results we therefore suggest that air travel from Europe was the main entry point of SARS-CoV-2 variants into French Guiana during this period, allowing the French Guianese variant to emerge from an ancestral lineage and spread rapidly across the territory.

During the Covid-19 pandemic, French Guiana's borders with Brazil and Suriname were extremely strict. In particular, from March 2020 the French authorities banned all transport by canoes between French Guiana and Brazil along the Oyapock River (https://www.guyane.gouv.fr/Actualites/Salle-de-presse/2020/Avril-2020/Controle-de-mesures-dans-la-frontiere-franco-bresilienne), and from the beginning of April 2020, the border between French Guiana and Suriname along the Maroni river was closed (https://www.guyane.gouv.fr/Actualites/Salle-de-presse/2020/Avril-2020/Controle-de-la-frontiere-France-Surianme). The French authorities (army, police) were permanently present on these two rivers and strict measures were taken to confiscate canoes in order to minimize transport between these countries. On arrival at Cayenne airport, passengers had to present a negative RT-qPCR test carried out within 72 h of travel, as was the case at Paris airport, in order to gain access to the plane. However, in line with other studies (Farkas et al., 2023; de Araújo et al., 2022), our results show that even with important mesures to reduce virus transmission, such as wearing a mask and having a negative Covid-19 test result prior to travel, airborne transmission continued to occur locally and globally. For instance, for the year 2020, 191,252 flight passengers between Paris and Cayenne airport were reported, while this number reached 251,654 passengers in 2021 (https://www.insee.fr/fr/statistiques/6454546?sommaire=6324691#:~:text=A%20l'inverse%20le%20trafic,%2C5%20%25%20sur%20Air%20Guyane), number well above the migratory flows from Brazil and Suriname.

Indeed, it is well recognized that international air travel had a significant impact on the rapid spread of SARS-CoV-2 between countries and was thus the main driver of the pandemic (Farkas et al., 2023). For example, in early 2020, travelers arriving from in the UK from mainland Europe were responsable for the introduction of approximately 1300 SARS-CoV-2 lineages (Du Plessis et al., 2021). Farkas et al. (2023) found high frequencies of SARS-CoV-2 in effluent from terminal sewers and aircraft in England, suggesting that passengers arriving at airports had ongoing SARS-CoV-2 infection despite negative Covid-19 tests. They also observed no differences in SARS-CoV-2 loads in wastewater before and after the lifting of Covid-19 restrictions. While airplane are perfect confined spaces for efficient viral transmission (Yan and Lan, 2020), these results suggest that mask use and individual testing (negative test result) did not allow asymptomatic and pre-symptomatic travelers to be filtered out and may not be an efficient preventive measure against pathogen transmission. Air travel therefore remains a significant risk for cross-border spread of infectious diseases (Farkas et al., 2023; Shingleton et al., 2023). Indeed, travel restrictions have been proposed to be particularly useful in the early stage of an outbreak (notably when the outbreak is localized), but may be less effective once the outbreak is more widespread (Kraemer et al., 2020).

5. Conclusions

Does WBE represent an efficient tool for pathogen monitoring in tropical zones such as French Guiana? The answer is yes. Whilst some biases might have hampered the detection of viral RNA (low sampling frequency, use of grab samples, etc.) our results strongly suggest that WBE can provide a cost effective and non-invasive approach for pathogen monitoring and an early warning tool for disease emergence within a population. Although WBE is not suitable for the identification and contact tracing of individual infections, this approach could be actively developed worldwide as it requires minimal sampling equipment and laboratory facilities, while providing a snapshot of pathogen circulation within a population (even at low prevalence levels) by testing only wastewater. Can WBE-WBG identify the overall diversity of SARS-CoV-2 variants co-circulating in a country and their evolutionary dynamics over time. Our data clearly support its potential and the identification of cryptic transmission of some variants. However, we also showed that combining both clinical and wastewater samples can enhance our understanding of the overall genetic diversity co-circulating. However, we are confident that the implementation and use of deconvolution tools for complex and mixed wastewater samples as well as a higher sampling effort of wastewater will in part remove this bias in the identification of viral variants. Finally, we found that European air travels were the main drivers of SARS-CoV-2 variant introduction in French Guiana compared with the role played by bordering countries such as Suriname and Brazil.

As previously suggested, our results provide further confirmation that WBE-WBG should be considered by public health authorities as a powerful tool to *i)* monitor the co-circulation and the prevalence of variants of infectious human pathogens, *ii)* follow the dynamics of epidemics in space and time, and *iii)* provide public health authorities with information on the cross-border movements of pathogens, such as the time of entry of a new pathogen (or its variants) into a country or a region within a country. Such early epidemiological data would be essential for disease risk modelling (identification of areas at risk of disease emergence) and for public health authorities' disease prevention strategies and intervention strategies (e.g. local lockdown, targeted interventions). However, to optimize the universal use of WBE-WBG, future studies should for instance address the issues related with the baseline for non-risky (normal) pathogen circulation in wastewater and thus the threshold to raise an alarm for public health.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171645.

CRediT authorship contribution statement

Marine Combe: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Emira Cherif:** Supervision, Software, Methodology, Formal analysis, Data curation. **Théo Deremarque:** Methodology. **Georgina Rivera-Ingraham:** Methodology. **Fatou Seck-Thiam:** Methodology. **Fabienne Justy:** Methodology. **Jean-Claude Doudou:** Methodology. **Jean-François Carod:** Resources, Investigation. **Thierry Carage:** Resources, Investigation. **Angélique Procureur:** Resources, Investigation. **Rodolphe Elie Gozlan:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

Acknowledgements

We acknowledge Dr. Stéphane Calmant, Director of the IRD Centre at Cayenne, French Guiana, for his precious support, including for all the logistic associated with the project and for dedicated a full laboratory for this project. We also want to thank Dr. Mathieu Chouteau, researcher at CNRS Guyane and Dr. Vincent Goujon, Director of the UMS LEEISA at CNRS Guyane, for their logistical support and for access to some facilities of their laboratory. We thank all our local collaborators in French Guiana for their precious help to access wastewater samples and thus for supporting the project, including the Cayenne Prefecture, the CTG, the CACL and the town hall of Kourou, Sinnamary, Saint-Laurent du Maroni, Apatou and Saint-Georges de l'Oyapock. We thank the local Public Health Agencies such as the Agence Régionale de Santé (ARS) de Guyane and Santé Publique France for their support, discussions and communication of your results. We also acknowledge Marie-Ka Tilak for her precious advices, experience and discussions about viral RNA extractions and ONT library preparation and sequencing. Finally, we acknowledge the help and advices of the OBEPINE network (Dr. Sébastien Wurtzer, Dr. Christophe Gantzer) who shared with us their protocols for wastewater sampling, the protocols for nucleic acid extraction and RT-qPCR RNA amplifications (including SARS-CoV-2 plasmids that served as positive controls).

Funding

This work was funded by the Agence Nationale de la Recherche (ANR-20-COV5-000) and the Institut de Recherche pour le Développement (IRD). EC received a postdoctoral fellowship from IRD. GRI received a postdoctoral fellowship from ANR. FST received a master student fellowship from ANR.

References

Adriaenssens, E.M., Farkas, K., Harrison, C., Jones, D.L., Allison, H.E., McCarthy, A.J., 2018. Viromic analysis of wastewater input to a river catchment reveals a diverse assemblage of RNA viruses. MSystems 3 (3), 10–1128. https://doi.org/10.1128/mSystems.00025-1.

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J.W., et al., 2020a. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. Sci. Total Environ. 728, 138764 https://doi.org/10.1016/j.scitotenv.2020.138764.

Ahmed, W., Bertsch, P.M., Bibby, K., Haramoto, E., Hewitt, J., Huygens, F., et al., 2020b. Decay of SARS-CoV-2 and surrogate murine hepatitis virus RNA in untreated wastewater to inform application in wastewater-based epidemiology. Environ. Res. 191, 110092 https://doi.org/10.1016/j.envres.2020.110092.

Ahmed, W., Bivins, A., Simpson, S.L., Smith, W.J., Metcalfe, S., McMinn, B., et al., 2021. Comparative analysis of rapid concentration methods for the recovery of SARS-CoV-2 and quantification of human enteric viruses and a sewage-associated marker gene in untreated wastewater. Sci. Total Environ. 799, 149386 https://doi.org/10.1016/j.scitotenv.2021.149386.

Álvarez-Díaz, D.A., Laiton-Donato, K., Torres-García, O.A., Ruiz-Moreno, H.A., Franco-Muñoz, C., Beltran, M.A., et al., 2022. Reduced levels of convalescent neutralizing antibodies against SARS-CoV-2 B. 1+ L249S+ E484K lineage. Virus Res. 308, 198629 https://doi.org/10.1016/j.virusres.2021.198629.

de Araújo, C.J., Gavazza, S., Leao, T.L., Florencio, L., da Silva, H.P., Albuquerque, J.D.O., et al., 2021. SARS-CoV-2 sewage surveillance in low-income countries: potential and challenges. J. Water Health 19 (1), 1–19. https://doi.org/10.2166/wh.2020.168.

de Araújo, J.C., Mota, V.T., Teodoro, A., Leal, C., Leroy, D., Madeira, C., et al., 2022. Long-term monitoring of SARS-CoV-2 RNA in sewage samples from specific public places and STPs to track COVID-19 spread and identify potential hotspots. Sci. Total Environ. 838, 155959 https://doi.org/10.1016/j.scitotenv.2022.155959.

de Araújo, J.C., Madeira, C.L., Bressani, T., Leal, C., Leroy, D., Machado, E.C., et al., 2023. Quantification of SARS-CoV-2 in wastewater samples from hospitals treating COVID-19 patients during the first wave of the pandemic in Brazil. Sci. Total Environ. 860, 160498 https://doi.org/10.1016/j.scitotenv.2022.160498.

Barnes, K.G., Levy, J.I., Gauld, J., Rigby, J., Kanjerwa, O., Uzzell, C.B., et al., 2023. Utilizing river and wastewater as a SARS-CoV-2 surveillance tool in settings with limited formal sewage systems. Nat. Commun. 14, 7883. https://doi.org/10.1038/s41467-023-43047-y.

Benwell, M.C., Clegg, P., Pinkerton, A., 2021. COVID-19 and the British overseas territories: a comparative view. The Round Table 110 (1), 159–170. https://doi.org/10.1080/00358533.2021.1875722.

Boehm, E., Kronig, I., Neher, R.A., Eckerle, I., Vetter, P., Kaiser, L., 2021. Novel SARS-CoV-2 variants: the pandemics within the pandemic. Clin. Microbiol. Infect. 27 (8), 1109–1117. https://doi.org/10.1016/J.CMI.2021.05.022.

Camargo, C.H., Gonçalves, C.R., Pagnoca, E.V.R.G., Campos, K.R., Abbud, A., Bugno, A., et al., 2021. Um ano de pandemia da COVID-19: diversidade genética do SARS-CoV-2 no Brasil. BEPA. Boletim Epidemiol. Paulista 18 (207), 12–33.

Cariti, F., Tuñas Corzon, A., Fernandez-Cassi, X., Ganesanandamoorthy, P., Ort, C., Julian, T.R., et al., 2022. Wastewater reveals the spatiotemporal spread of SARS-CoV-2 in the Canton of Ticino (Switzerland) during the onset of the COVID-19 pandemic. ACS Es&T Water 2 (11), 2194–2200. https://doi.org/10.1021/acsestwater.2c00082.

Chaillon, A., Bojorquez, I., Sepúlveda, J., Harvey-Vera, A.Y., Rangel, M.G., Skaathun, B., et al., 2022. Cocirculation and replacement of SARS-CoV-2 variants in crowded settings and marginalized populations along the US-Mexico border. Salud Publica Mex. 65 (1), 10–18.

Cherif, E., Thiam, F.S., Salma, M., Rivera-Ingraham, G., Justy, F., Deremarque, T., Breugnot, D., Doudou, J.-C., Gozlan, R.E., 2022. Marine Combe, ONTdeCIPHER: an amplicon-based nanopore sequencing pipeline for tracking pathogen variants. Bioinformatics 38 (7), 2033–2035. https://doi.org/10.1093/bioinformatics/btac043. March.

Chin, A.W., Chu, J.T., Perera, M.R., Hui, K.P., Yen, H.L., Chan, M.C., et al., 2020. Stability of SARS-CoV-2 in different environmental conditions. Lancet Microbe 1 (1), e10. https://doi.org/10.1016/S2666-5247(20)30003-3.

Cingolani, P., Platts, A., Wang, L.L., Coon, M., Nguyen, T., Wang, L., et al., 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly 6 (2), 80–92. https://doi.org/10.4161/fly.19695.

Corum, J., Zimmer, C., 2020. Bad news wrapped in protein: inside the coronavirus genome. In: The New York Times, vol. 3. https://www.nytimes.com/interactive/2020/04/03/science/coronavirus-genome-bas-news-wrapped-in-protein.html.

Davies, N.G., Abbott, S., Barnard, R.C., Jarvis, C.I., Kucharski, A.J., Munday, J.D., et al., 2021a. Estimated transmissibility and impact of SARS-CoV-2 lineage B. 1.1. 7 in England. Science 372 (6538), eabg3055. https://doi.org/10.1126/science.abg3055.

Davies, N.G., Jarvis, C.I., Edmunds, W.J., Jewell, N.P., Diaz-Ordaz, K., Keogh, R.H., 2021b. Increased mortality in community-tested cases of SARS-CoV-2 lineage B. 1.1. 7. Nature 593 (7858), 270–274. https://doi.org/10.1038/s41586-021-03426-1.

De Oliveira, L.C., Torres-Franco, A.F., Lopes, B.C., da Silva Santos, B.S.Á., Costa, E.A., Costa, M.S., et al., 2021. Viability of SARS-CoV-2 in river water and wastewater at different temperatures and solids content. Water Res. 195, 117002 https://doi.org/10.1016/j.watres.2021.117002.

Deiner, K., Bik, H.M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., et al., 2017. Environmental DNA metabarcoding: transforming how we survey animal and plant communities. Mol. Ecol. 26 (21), 5872–5895. https://doi.org/10.1111/mec.14350.

Deiss, R., Garfein, R.S., Lozada, R., Burgos, J.L., Brouwer, K.C., Moser, K.S., et al., 2009. Influences of cross-border mobility on tuberculosis diagnoses and treatment interruption among injection drug users in Tijuana, Mexico. Am. J. Public Health 99 (8), 1491–1495. https://doi.org/10.2105/ajph.2008.142166.

Díez-Fuertes, F., Iglesias-Caballero, M., García-Pérez, J., Monzón, S., Jiménez, P., Varona, S., Cuesta, I., et al., 2021. A founder effect led early SARS-CoV-2 transmission in Spain. J. Virol. 95, e01583-20 https://doi.org/10.1128/JVI.01583-20.

Du Plessis, L., McCrone, J.T., Zarebski, A.E., Hill, V., Ruis, C., Gutierrez, B., et al., 2021. Establishment and lineage dynamics of the SARS-CoV-2 epidemic in the UK. Science 371 (6530), 708–712. https://doi.org/10.1126/science.abf2946.

Eden, J.S., Tanaka, M.M., Boni, M.F., Rawlinson, W.D., White, P.A., 2013. Recombination within the pandemic norovirus GII.4 lineage. J. Virol. 87, 6270–6282. https://doi.org/10.1128/JVI.03464-12.

Faria, N.R., Mellan, T.A., Whittaker, C., Claro, I.M., Candido, D.D.S., Mishra, S., et al., 2021. Genomics and epidemiology of the P. 1 SARS-CoV-2 lineage in Manaus, Brazil. Science 372 (6544), 815–821. https://doi.org/10.1126/science.abh2644.

Farkas, K., Williams, R., Alex-Sanders, N., Grimsley, J.M., Pântea, I., Wade, M.J., et al., 2023. Wastewater-based monitoring of SARS-CoV-2 at UK airports and its potential role in international public health surveillance. PLOS Glob. Publ. Health 3 (1), e0001346. https://doi.org/10.1371/journal.pgph.0001346.

Fernandes, H.M.Z., Miranda, K.R., da Silva Dias, R.C., Alviano, D.S., Duarte, R.S., da Silva Carvalho, A.C., 2022. The challenges of education in a continental country in the face of new severe acute respiratory coronavirus virus 2 (SARS-CoV-2) variant circulation. Infec. Control Hosp. Epidemiol. 43 (10), 1537–1539. https://doi.org/10.1017/ice.2021.291.

Fongaro, G., Stoco, P.H., Souza, D.S.M., Grisard, E.C., Magri, M.E., Rogovski, P., et al., 2021. The presence of SARS-CoV-2 RNA in human sewage in Santa Catarina, Brazil, November 2019. Sci. Total Environ. 778, 146198 https://doi.org/10.1101/2020.06.26.20140731.

Forés, E., Bofill-Mas, S., Itarte, M., Martínez-Puchol, S., Hundesa, A., Calvo, M., et al., 2021. Evaluation of two rapid ultrafiltration-based methods for SARS-CoV-2 concentration from wastewater. Sci. Total Environ. 768, 144786 https://doi.org/10.1016/j.scitotenv.2020.144786.

Giroux, M.S., Reichman, J.R., Langknecht, T., Burgess, R.M., Ho, K.T., 2022. Environmental RNA as a tool for marine community biodiversity assessments. Sci. Report. 12 (1), 17782 https://doi.org/10.1038/s41598-022-22198-w.

Gonzalez, R., Curtis, K., Bivins, A., Bibby, K., Weir, M.H., Yetka, K., et al., 2020. COVID-19 surveillance in southeastern Virginia using wastewater-based epidemiology. Water Res. 186, 116296 https://doi.org/10.1016/j.watres.2020.116296.
Gorkhali, R., Koirala, P., Rijal, S., Mainali, A., Baral, A., Bhattarai, H.K., 2021. Structure and function of major SARS-CoV-2 and SARS-CoV proteins. Bioinfo. Biol. Insights 15, 11779322211025876. https://doi.org/10.1177/11779322211025876.
Gosert, R., Kanjanahaluethai, A., Egger, D., Bienz, K., Baker, S.C., 2002. RNA replication of mouse hepatitis virus takes place at double-membrane vesicles. J. Virol. 76 (8), 3697–3708. https://doi.org/10.1128/jvi.76.8.3697-3708.2002.
Greaves, J., Fischer, R.J., Shaffer, M., Bivins, A., Holbrook, M.G., Munster, V.J., Bibby, K., 2022. Sodium hypochlorite disinfection of SARS-CoV-2 spiked in water and municipal wastewater. Sci. Total Environ. 807, 150766 https://doi.org/10.1016/j.scitotenv.2021.150766.
Hart, O.E., Halden, R.U., 2020. Computational analysis of SARS-CoV-2/COVID-19 surveillance by wastewater-based epidemiology locally and globally: feasibility, economy, opportunities and challenges. Sci. Total Environ. 730, 138875 https://doi.org/10.1016/j.scitotenv.2020.138875.
Haver, A., Theijn, R., Grift, I.D., Raaijmakers, G., Poorter, E., Laros, J.F.J., et al., 2023. Regional reemergence of a SARS-CoV-2 Delta lineage amid an Omicron wave detected by wastewater sequencing. Sci. Rep. 13, 17870. https://doi.org/10.1038/s41598-023-44500-0.
Heijnen, L., Medema, G., 2011. Surveillance of influenza A and the pandemic influenza A (H1N1) 2009 in sewage and surface water in the Netherlands. J. Water Health 9 (3), 434–442. https://doi.org/10.2166/wh.2011.019.
Hellmér, M., Paxéus, N., Magnius, L., Enache, L., Arnholm, B., Johansson, A., et al., 2014. Detection of pathogenic viruses in sewage provided early warnings of hepatitis A virus and norovirus outbreaks. Appl. Environ. Microbiol. 80 (21), 6771–6781. https://doi.org/10.1128/AEM.01981-14.
Holm, R.H., Mukherjee, A., Rai, J.P., Yeager, R.A., Talley, D., Rai, S., et al., 2022. SARS-CoV-2 RNA abundance in wastewater as a function of distinct urban sewershed size. Environ. Sci. Water Res. Technol. 8, 807–819. https://doi.org/10.1039/D1EW00672J.
Hossain, M.P., Junus, A., Zhu, X., Jia, P., Wen, T.H., Pfeiffer, D., Yuan, H.Y., 2020. The effects of border control and quarantine measures on the spread of COVID-19. Epidemics 32, 100397. https://doi.org/10.1016/j.epidem.2020.100397.
Hotez, P.J., 2013a. NTDs V.2.0: "Blue Marble Health"—neglected tropical disease control and elimination in a shifting health policy landscape. PLoS Negl. Trop. Dis. 7 (11), e2570 https://doi.org/10.1371/journal.pntd.0002570.
Hotez, P.J., 2013b. The disease next door. In: Foreign Policy March 25, 2013, accessed April 20, 2013. https://foreignpolicy.com/2013/03/25/the-disease-next-door/#cookie_message_anchor.
Hotez, P.J., 2020. Poverty and the Impact of COVID-19: The Blue-Marble Health Approach. Johns Hopkins University Press, Baltimore. https://doi.org/10.1353/book.75688.
Hou, Y.J., Chiba, S., Halfmann, P., Ehre, C., Kuroda, M., Dinnon III, K.H., et al., 2020. SARS-CoV-2 D614G variant exhibits efficient replication ex vivo and transmission in vivo. Science 370 (6523), 1464–1468. https://doi.org/10.1126/science.abe8499.
Hovi, T., Shulman, L.M., Van Der Avoort, H., Deshpande, J., Roivainen, M., De Gourville, E.M., 2012. Role of environmental poliovirus surveillance in global polio eradication and beyond. Epidemiol. Infect. 140 (1), 1–13. https://doi.org/10.1017/S095026881000316X.
Hughes, J.M., Wilson, M.E., Pike, B.L., Saylors, K.E., Fair, J.N., LeBreton, M., et al., 2010. The origin and prevention of pandemics. Clin. Infect. Dis. 50 (12), 1636–1640. https://doi.org/10.1086/652860.
Ito, K., Piantham, C., Nishiura, H., 2022. Relative instantaneous reproduction number of Omicron SARS-CoV-2 variant with respect to the Delta variant in Denmark. J. Med. Virol. 94 (5), 2265–2268. https://doi.org/10.1002/jmv.27560.
Jagadesh, S., 2020. Biogeography of emerging infectious diseases. Université de Guyane. https://doi.org/10.13140/RG.2.2.19882.54720.
Jangra, S., Ye, C., Rathnasinghe, R., Stadlbauer, D., Alshammary, H., Amoako, A.A., et al., 2021. SARS-CoV-2 spike E484K mutation reduces antibody neutralisation. Lancet Microbe 2 (7), e283–e284. https://doi.org/10.1016/S2666-5247(21)00068-9.
Jansen, L., Tegomoh, B., Lange, K., Showalter, K., Figliomeni, J., Abdalhamid, B., et al., 2021. Investigation of a SARS-CoV-2 B. 1.1. 529 (omicron) variant cluster—Nebraska, November–December 2021. In: Morbidity and Mortality Weekly Report, 70(51–52), p. 1782. https://doi.org/10.15585/mmwr.mm705152e3.
Jo, T., Tsuri, K., Hirohara, T., Yamanaka, H., 2022. Warm temperature and alkaline conditions accelerate environmental RNA degradation. In: Envir. DNA. https://doi.org/10.1002/edn3.334.
Kaiwan, O., Sethi, Y., Khehra, N., Padda, I., Chopra, H., Chandran, D., et al., 2023. Emerging and re-emerging viral diseases, predisposing risk factors, and implications of international travel: a call for action for increasing vigilance and imposing restrictions under the current threats of recently emerging multiple Omicron subvariants. Int. J. Surg. 109 (3), 589–591. https://doi.org/10.1097/JS9.0000000000000176.
Karthikeyan, S., Levy, J.I., De Hoff, P., Humphrey, G., Birmingham, A., Jepsen, K., et al., 2022. Wastewater sequencing reveals early cryptic SARS-CoV-2 variant transmission. Nature 609, 101–108. https://doi.org/10.1038/s41586-022-05049-6.
Khandia, R., Singhal, S., Alqahtani, T., Kamal, M.A., Nahed, A., Nainu, F., et al., 2022. Emergence of SARS-CoV-2 Omicron (B. 1.1. 529) variant, salient features, high global health concerns and strategies to counter it amid ongoing COVID-19 pandemic. Environ. Res. 209, 112816 https://doi.org/10.1016/j.envres.2022.112816.
Kim, D., Lee, J.Y., Yang, J.S., Kim, J.W., Kim, V.N., Chang, H., 2020. The architecture of SARS-CoV-2 transcriptome. Cell 181 (4), 914–921. https://doi.org/10.1016/j.cell.2020.04.011.
Kirchdoerfer, R.N., Ward, A.B., 2019. Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. Nat. Comm. 10 (1), 2342. https://doi.org/10.1038/s41467-019-10280-3.
Korber, B., Fischer, W.M., Gnanakaran, S., Yoon, H., Theiler, J., Abfalterer, W., et al., 2020. Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell 182 (4), 812–827. https://doi.org/10.1016/j.cell.2020.06.043.
Kraemer, M.U., Yang, C.H., Gutierrez, B., Wu, C.H., Klein, B., Pigott, D.M., et al., 2020. The effect of human mobility and control measures on the COVID-19 epidemic in China. Science 368 (6490), 493–497. https://doi.org/10.1126/science.abb4218.
Lambisia, A.W., Mohammed, K.S., Makori, T.O., Ndwiga, L., Mburu, M.W., Morobe, J.M., et al., 2022. Optimization of the SARS-CoV-2 ARTIC network V4 primers and whole genome sequencing protocol. Front. Med. 9, 836728 https://doi.org/10.3389/fmed.2022.836728.
Laroze, D., Neumayer, E., Plümper, T., 2021. COVID-19 does not stop at open borders: spatial contagion among local authority districts during England's first wave. Soc. Sci. Med. 270, 113655 https://doi.org/10.1016/j.socscimed.2020.113655.
Lauring, A.S., Hodcroft, E.B., 2021. Genetic variants of SARS-CoV-2—what do they mean? Jama 325 (6), 529–531. https://doi.org/10.1001/jama.2020.27124.
Lin, X., Glier, M., Kuchinski, K., Ross-Van Mierlo, T., McVea, D., Tyson, J.R., et al., 2021. Assessing multiplex tiling PCR sequencing approaches for detecting genomic variants of SARS-CoV-2 in municipal wastewater. Msystems 6 (5), e01068-21. https://doi.org/10.1101/2021.05.26.21257861.
Majumdar, P., Niyogi, S., 2021. SARS-CoV-2 mutations: the biological trackway towards viral fitness. Epidemiol. Infect. 149 https://doi.org/10.1017/S0950268821001060.
Mallapaty, S., 2020. How sewage could reveal true scale of coronavirus outbreak. Nature 580 (7802), 176–177. https://doi.org/10.1038/d41586-020-00973-x.
Marra, M.A., Jones, S.J., Astell, C.R., Holt, R.A., Brooks-Wilson, A., Butterfield, Y.S., et al., 2003. The genome sequence of the SARS-associated coronavirus. Science 300 (5624), 1399–1404. https://doi.org/10.1126/science.1085953.
Medema, G., Heijnen, L., Elsinga, G., Italiaander, R., Brouwer, A., 2020. Presence of SARS-Coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in the Netherlands. Environ. Sci. Technol. Lett. 7 (7), 511–516. https://doi.org/10.1101/2020.03.29.20045880.
Mercatelli, D., Giorgi, F.M., 2020. Geographic and genomic distribution of SARS-CoV-2 mutations. Front. Microbiol. 11, 1800. https://doi.org/10.3389/fmicb.2020.01800.
Mercatelli, D., Triboli, L., Fornasari, E., Ray, F., Giorgi, F.M., 2021. Coronapp: a web application to annotate and monitor SARS-CoV-2 mutations. J. Med. Virol. 93 (5), 3238–3245. https://doi.org/10.1002/jmv.26678.
Morales Medina, W.R., D'Elia, S., Fahrenfeld, N.L., 2022. Accumulation of SARS-CoV-2 RNA in sewer biofilms. ACS Es&T Water 2 (11), 1844–1851. https://doi.org/10.1021/ACSESTWATER.1C00345.
Ndiaye, A.K., Diop, P.A.M., Diop, O.M., 2014. Environmental surveillance of poliovirus and non-polio enterovirus in urban sewage in Dakar, Senegal (2007-2013). Pan African Medizinhist. J. 19 (1) https://doi.org/10.11604/pamj.2014.19.243.3538.
Petrovich, M.L., Ben Maamar, S., Hartmann, E.M., Murphy, B.T., Poretsky, R.S., Wells, G.F., 2019. Viral composition and context in metagenomes from biofilm and suspended growth municipal wastewater treatment plants. Microb. Biotechnol. 12 (6), 1324–1336. https://doi.org/10.1111/1751-7915.13464.
Piret, J., Boivin, G., 2021. Pandemics throughout history. Front. Microbiol. 11, 631736 https://doi.org/10.3389/fmicb.2020.631736.
Plante, J.A., Liu, Y., Liu, J., Xia, H., Johnson, B.A., Lokugamage, K.G., et al., 2021. Spike mutation D614G alters SARS-CoV-2 fitness. Nature 592 (7852), 116–121. https://doi.org/10.1038/s41586-020-2895-3.
Polo, D., Quintela-Baluja, M., Corbishley, A., Jones, D.L., Singer, A.C., Graham, D.W., et al., 2020. Making waves: wastewater-based epidemiology for COVID-19–approaches and challenges for surveillance and prediction. Water Res. 186, 116404 https://doi.org/10.1016/j.watres.2020.116404.
Prado, T., Fumian, T., Mannarino, C.F., Maranhão, A.G., Siqueira, M.M., Miagostovich, M.P., 2020. Preliminary results of SARS-CoV-2 detection in sewerage system in Niterói municipality, Rio de Janeiro, Brazil. Memórias Instituto Oswaldo Cruz, Rio de Janeiro 115, e200196. https://doi.org/10.1590/0074-02760200196.
Prentice, E., Jerome, W.G., Yoshimori, T., Mizushima, N., Denison, M.R., 2004. Coronavirus replication complex formation utilizes components of cellular autophagy. J. Biol. Chem. 279 (11), 10136–10141. https://doi.org/10.1074/jbc.M306124200.
Quick, J., Grubaugh, N.D., Pullan, S.T., Claro, I.M., Smith, A.D., Gangavarapu, K., et al., 2017. Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. Nat. Prot. 12 (6), 1261–1276. https://doi.org/10.1038/nprot.2017.066.
Randazzo, W., Cuevas-Ferrando, E., Sanjuán, R., Domingo-Calap, P., Sánchez, G., 2020. Metropolitan wastewater analysis for COVID-19 epidemiological surveillance. Inter. J. Hygiene Enviro. Health 230, 113621. https://doi.org/10.1101/2020.04.23.20076679.
Roberts, L., 2013. Israel's Silent Polio Epidemic Breaks All The Rules. https://doi.org/10.1126/science.342.6159.679.
Rota, P.A., Oberste, M.S., Monroe, S.S., Nix, W.A., Campagnoli, R., Icenogle, J.P., et al., 2003. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 300 (5624), 1394–1399. https://doi.org/10.1126/science.1085952.
Sapula, S.A., Whittall, J.J., Pandopulos, A.J., Gerber, C., Venter, H., 2021. An optimized and robust PEG precipitation method for detection of SARS-CoV-2 in wastewater. Sci. Total Environ. 785, 147270 https://doi.org/10.1016/j.scitotenv.2021.147270.

Sarkate, P.P., Bera, P., Kaundal, N., Sood, S., Singh, S.K., Prakash, C., 2021. Sewage analysis as a tool for environmental surveillance of SARS-CoV-2: experience from Delhi. India. J. Commun. Dis. 53 (2), 1–13. https://doi.org/10.24321/0019.5138.202119.

Saththasivam, J., El-Malah, S.S., Gomez, T.A., Jabbar, K.A., Remanan, R., Krishnankutty, A.K., et al., 2021. COVID-19 (SARS-CoV-2) outbreak monitoring using wastewater-based epidemiology in Qatar. Sci. Total Environ. 774, 145608 https://doi.org/10.1016/j.scitotenv.2021.145608.

Schneider, E., Laserson, K.F., Wells, C.D., Moore, M., 2004. Tuberculosis along the United States-Mexico border, 1993-2001. Rev. Panam. Salud Publica 16, 23–34.

Shingleton, J.W., Lilley, C.J., Wade, M.J., 2023. Evaluating the theoretical performance of aircraft wastewater monitoring as a tool for SARS-CoV-2 surveillance. PLOS Glob. Publ. Health 3 (6), e0001975. https://doi.org/10.1371/journal.pgph.0001975.

Siebenga, J.J., Vennema, H., Zheng, D.P., Vinjé, J., Lee, B.E., Pang, X.L., et al., 2009. Norovirus illness is a global problem: emergence and spread of norovirus GII. 4 variants, 2001–2007. J. Infect. Dis. 200 (5), 802–812. https://doi.org/10.1086/605127.

Silva, M.S.D., Demoliner, M., Hansen, A.W., Gularte, J.S., Silveira, F., Heldt, F.H., et al., 2021. Early detection of SARS-CoV-2 P. 1 variant in Southern Brazil and reinfection of the same patient by P. 2. Rev. Inst. Med. Trop. Sao Paulo 63, e58. https://doi.org/10.1590/S1678-9946202163058.

Takahashi, M., Saccò, M., Kestel, J.H., Nester, G., Campbell, M.A., Van Der Heyde, M., et al., 2023. Aquatic environmental DNA: a review of the macro-organismal biomonitoring revolution. Sci. Total Environ. 873, 162322 https://doi.org/10.1016/j.scitotenv.2023.162322.

Thiel, V., Ivanov, K.A., Putics, A., Hertzig, T., Schelle, B., Bayer, S., et al., 2003. Mechanisms and enzymes involved in SARS coronavirus genome expression. J. General Virol. 84 (9), 2305–2315. https://doi.org/10.1099/vir.0.19424-0.

Tortorici, M.A., Veesler, D., 2019. Structural insights into coronavirus entry. In: Advances in Virus Research, vol. 105. Academic Press, pp. 93–116. https://doi.org/10.1016/bs.aivir.2019.08.002.

Vilar, S., Isom, D.G., 2021. One year of SARS-CoV-2: how much has the virus changed? Biology 10 (2), 91. https://doi.org/10.3390/biology10020091.

Volz, E., Hill, V., McCrone, J.T., Price, A., Jorgensen, D., O'Toole, Á., et al., 2021. Evaluating the effects of SARS-CoV-2 spike mutation D614G on transmissibility and pathogenicity. Cell 184 (1), 64–75. https://doi.org/10.1016/j.cell.2020.11.020.

Wang, Y., Song, W., Zhao, Z., Chen, P., Liu, J., Li, C., 2020. The impacts of viral inactivating methods on quantitative RT-PCR for COVID-19. Virus Res. ISSN: 0168-1702 285, 197988 https://doi.org/10.1016/j.virusres.2020.197988.

Wibmer, C.K., Ayres, F., Hermanus, T., Madzivhandila, M., Kgagudi, P., Oosthuysen, B., et al., 2021. SARS-CoV-2 501Y. V2 escapes neutralization by South African COVID-19 donor plasma. Nat. Med. 27 (4), 622–625. https://doi.org/10.1038/s41591-021-01285-x.

Wilde, H., Perry, W.B., Jones, O., Kille, P., Weightman, A., Jones, D.L., et al., 2022. Accounting for dilution of SARS-CoV-2 in wastewater samples using physico-chemical markers. Water 14 (18), 2885. https://doi.org/10.3390/w14182885.

Wood, S.A., Biessy, L., Latchford, J.L., Zaiko, A., von Ammon, U., Audrezet, F., et al., 2020. Release and degradation of environmental DNA and RNA in a marine system. Sci. Total Environ. 704, 135314 https://doi.org/10.1016/j.scitotenv.2019.135314.

Wurtzer, S., Marechal, V., Mouchel, J.M., Moulin, L., 2020. Time course quantitative detection of SARS-CoV-2 in Parisian wastewaters correlates with COVID-19 confirmed cases. MedRxiv 2020-04. https://doi.org/10.1101/2020.04.12.20062679.

Xiao, F., Sun, J., Xu, Y., Li, F., Huang, X., Li, H., et al., 2020a. Infectious SARS-CoV-2 in feces of patient with severe COVID-19. Emerg. Infect. Dis. 26 (8), 1920. https://doi.org/10.3201/eid2608.200681.

Xiao, F., Tang, M., Zheng, X., Liu, Y., Li, X., Shan, H., 2020b. Evidence for gastrointestinal infection of SARS-CoV-2. Gastroenterology 158 (6), 1831–1833. https://doi.org/10.1053/j.gastro.2020.02.055.

Xu, Y., Li, X., Zhu, B., Liang, H., Fang, C., Gong, Y., et al., 2020. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. Nat. Med. 26 (4), 502–505. https://doi.org/10.1038/s41591-020-0817-4.

Xu, X., Deng, Y., Ding, J., Zheng, X., Wang, C., Wang, D., et al., 2023. Wastewater genomic sequencing for SARS-CoV-2 variants surveillance in wastewater-based epidemiology applications. Water Res. 244, 120444 https://doi.org/10.1016/j.watres.2023.120444.

Yan, Z., Lan, Y., 2020. Modeling COVID-19 infection in a confined space. Nonlin. Dynam. 101 (3), 1643–1651. https://doi.org/10.1007/s11071-020-05802-4.

Yousif, M., Rachida, S., Taukobong, S., Ndlovu, N., Iwu-Jaja, C., Howard, W., et al., 2023. SARS-CoV-2 genomic surveillance in wastewater as a model for monitoring evolution of endemic viruses. Nat. Commun. 14, 6325. https://doi.org/10.1038/s41467-023-41369-5.

Zarza, E., Diego-García, E., García, L.V., Castro, R., Mejía, G., Herrera, D., et al., 2022. Monitoring SARS-CoV-2 in the wastewater and rivers of tapachula, a migratory hub in Southern Mexico. Food Envir. Virol. 14 (2), 199–211. https://doi.org/10.1007/s12560-022-09523-2.