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Detection of enteric viruses and SARS-CoV-2 in beach sand

A. Robalo [a], J. Brandão [c], T. Shibata [d,e], H. Solo-Gabriele [f], R. Santos [a,b], S. Monteiro [a,b,*]

[a] Laboratorio Analises, Técnico Lisboa, Universidade Lisboa, Portugal
[b] Departamento de Engenharia e Ciências Nucleares, Técnico Lisboa, Universidade de Lisboa, Portugal
[c] Department of Environmental Health, National Institute of Health Dr. Ricardo Jorge, Lisboa, Portugal
[d] Institute for the Study of the Environment, Sustainability, and Energy, Northern Illinois University, DeKalb, IL, USA
[e] Center for Southeast Asian Studies, Northern Illinois University, DeKalb, IL, USA
[f] Department of Chemical, Environmental, and Materials Engineering, University of Miami, Coral Gables, FL, USA

HIGHLIGHTS

- Enteric viruses detected in high prevalence (89 %) in beach sand
- Aichi virus most frequently detected virus (74 %)
- Distinct viral distribution in intertidal and supratidal beach sand
- Higher viral diversity in the supratidal zone
- Beach events with high impact on sand quality

GRAPHICAL ABSTRACT
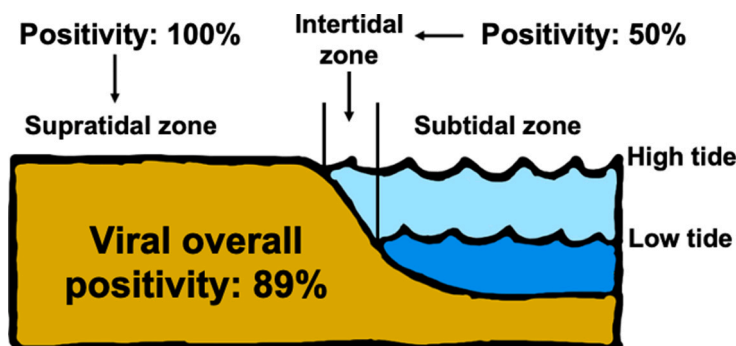
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ABSTRACT

Beach sand harbors a diverse group of microbial organisms that may be of public health concern. Nonetheless, little is known about the presence and distribution of viruses in beach sand. In this study, the first objective was to evaluate the presence of seven viruses (Aichi virus, enterovirus, hepatitis A virus, human adenovirus, norovirus, rotavirus, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)) in sands collected at public beaches. The second objective was to assess the spatial distribution of enteric viruses in beach sand. To that end, 27 beach sand samples from different beaches in Portugal were collected between November 2018 and August 2020 and analyzed for the presence of viruses. At seven beaches, samples were collected in the supratidal and intertidal zones. Results show that viruses were detected in 89 % (24/27) of the sand samples. Aichi virus was the most prevalent (74 %). Noroviruses were present in 19 % of the samples (norovirus GI – 15 %, norovirus GII – 4 %). Human adenovirus and enterovirus were detected in 48 % and 22 % of the samples, respectively. Hepatitis A virus and rotavirus were not detected. Similarly, SARS-CoV-2 in beach sand collected during the initial stages of the pandemic was also not detected. The detection of three or more viruses occurred in 15 % of the samples. Concentrations of viruses were as high as 7.2 log copies (cp)/g of sand. Enteric viruses were found in higher prevalence in sand collected from the supratidal zone compared to the intertidal zone. Human adenovirus was detected in 43 % of the supratidal and 14 % in the intertidal samples and Aichi virus in 57 % and 86 % of the

intertidal and supratidal areas, respectively. Our findings suggest that beach sand can be a reservoir of enteric viruses, suggesting that it might be a vehicle for disease transmission, particularly for children, the elderly, and immunocompromised users.

1. Introduction

Coastal watersheds are a source of public recreational amenities. The constant increase in population and subsequent urbanization has a negative impact in the ecosystems, resulting in the degradation of water quality. Point and non-point sources of fecal contamination, particularly from human origin, are an ever-increasing global health concern (Mallin et al., 2000). Particularly, recreational beaches polluted with point and non-point sources of fecal contamination can pose a significant health risk due to the exposure of enteric pathogens (Graczyk et al., 2007; Chase et al., 2012; Staley et al., 2012), through the accidental ingestion or inhalation of contaminated waters and therefore potentially causing enteric and nonenteric illnesses in beachgoers (Petri Jr. et al., 2008). Additionally, beach closures due to high levels of fecal contamination leads to losses in tourism and therefore towards significant economic losses (Rabinovici et al., 2004).

In the European Union, beach advisories are issued when threshold levels of fecal indicator bacteria (FIB) in bathing water are exceeded (EEC, 2006). However, beaches are complex ecosystems with beachgoers spending much of their time on sand-related activities, including digging and playing with sand (Ferguson et al., 2021). These two activities require special attention due to the close contact of children with sand. Heaney et al. (2009) demonstrated a positive correlation between digging in sand and the development of gastrointestinal illnesses, with stronger association for those reporting being buried in sand. Children given their play behavior and differences in the development of immune systems may be at higher risk than the general population from exposure to microorganisms in beach sand (Ferguson et al., 2017).

Despite being a harsh environment, beach sand may harbor many microorganisms including pathogens and be a vehicle for the transmission of disease (Tomenchok et al., 2020). Studies evaluating the microbiological quality of sand have mostly focused on FIB (Ghinsberg et al., 1994; Alm et al., 2003; Boehm and Weisberg, 2005; Whiley et al., 2018) and fungal contamination (Larrondo and Calvo, 1989; Ghinsberg et al., 1994; Shah et al., 2011; Brandão et al., 2020). Epidemiological studies have associated fecal contamination, sand contact and the development of illness (Heaney et al., 2009, 2012; Lamparelli et al., 2015). Contact with microbiologically contaminated sand has also been linked to skin problems (Esiobu et al., 2013; Praveena et al., 2016). In 2019 in Azores, Portugal, many people experienced an episode of skin rash and the epidemiological study conducted determined that symptoms were linked to the leakage of chlorine-disinfected sewage (Brandão et al., 2020). In Portugal, no gastrointestinal outbreak has been reported so far possibly because gastrointestinal illnesses are not of mandatory report. Regardless of the importance of beach sand as a vehicle for disease transmission and the low infectious dose, only a few studies have evaluated the presence of enteric viruses (Nestor et al., 1984; Pianetti et al., 2004; Goodwin et al., 2009; Abdelzaher et al., 2010; Shah et al., 2011; Monteiro et al., 2016; Carducci et al., 2022). Moreover, most studies focused on the detection of cultivable enteric viruses, implying that only a small, selected number of viruses could be evaluated. With the development of improved molecular technologies, many more viruses can now be detected in different types of matrices including beach sand.

Further studies are required to understand the prevalence and fate of enteric viruses in beach sand. In this study, we report results from virus measurements in beach sand collected from recreational beaches in different locations in Portugal. In total, twenty-seven samples of beach sand were collected and analyzed for six enteric viruses (Aichi virus, enterovirus, hepatitis A virus, human adenovirus, norovirus (GI and GII), and rotavirus) and for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) during the early epidemiological stages of the pandemic. In addition, the spatial distribution of the viruses in two beach zones, the supratidal and intertidal zones, where people spend most of their time and have longer contact with sand was also evaluated. To the best of our knowledge, this is the first study looking into the presence of such a wide variety of viruses in beach sand, and only one of the few addressing their spatial distribution.

2. Materials and methods

2.1. Sample collection

A total of twenty-seven beach sand samples were collected between November 2018 and August 2020, from thirteen beaches in the south of Portugal. To have a better knowledge on the presence of viruses in sand from beaches in Portugal, several beaches were selected instead of just having a single one tested several times. The majority of the beaches were sampled just once, except for samples 7 and 13 (with the wet counterpart samples 24 and 27) that are from the same beach. This beach was collected twice because it is one of the few beaches that allows for the presence of dogs during peak season. Air temperatures ranged between 12 °C and 38 °C at the sampled beaches. Samples were collected during dry weather and the samples collected during the bathing season were collected 1 h after high tide, to assure sample collection in the intertidal zone that was recently exposed to water. The collected beach sand was mostly composed of coarse sand, with a median size of 0.7 mm, with a high content of medium sand and a median percentage of gravel of about 10 %. For seven of the beach areas, samples were collected in the intertidal and supratidal zones, to determine differences and therefore the potential risks in the two zones (Supplementary Table S1). At the remaining beach areas, samples were collected only in the supratidal zone. Each sample was collected in duplicate and placed in sterile zip-locked bags. Samples were transported refrigerated to the laboratory within eight hours of collection and processed upon arrival.

2.2. Sand sample preparation

The method selected for the recovery of viruses from beach sand was a combination of previously published methods commonly used in water (Hill et al., 2005; Blanco et al., 2020). Mengovirus was added to each beach sand sample as a process control (ISO 15216-1, 2017) prior to the start of each experiment (3 log copies/g). Recovery of viral particles from 30 g of beach sand was performed in $1\times$ phosphate buffer (PBS) supplemented with 0.01 % sodium polyphosphate (NaPP) and 0.01 % Tween 80/0.001 % antifoam in a proportion of 1:3 (*w/v*). Samples were agitated at 100 rpm for 120 min at 5 ($\pm$3) °C and the supernatant clarified by centrifugation at 5445 $\times g$ for 10 min. To the supernatant, 20 % (w/v) of polyethylene glycol 8000 (PEG 8000), 1.33 % (w/v) of beef extract and 2.17 % NaCl (w/v) were added, and the samples were then incubated overnight on a rotating platform at 5 ($\pm$3) °C. Samples were centrifuged at 10,000 $\times g$ for 30 min at 4 °C, the supernatant discarded, and the pellet eluted in 2 mL of $1\times$ PBS with 0.01 % NaPP and 0.01 % Tween 80/0.001 % antifoam. Samples were stored at −80 ($\pm$10) °C until further processing. Data from the recovery trials and process control can be found in the Supplementary Materials.

2.3. Viral nucleic acid extraction, and quantification

Viral nucleic acids were extracted from 140 μL of concentrated samples using the QIAamp Viral RNA Mini kit (QIAGEN, Germany), according to the manufacturer's instructions. Viral nucleic acids were recovered in a final volume of 100 μL. The extraction kit can extract RNA and DNA, as described by the manufacturer.

One-step RT-qPCR assays (Luna® Universal One-Step RT-qPCR kit; NEB, US) were used for the quantitative detection of viral RNA. For the quantitative detection of viral DNA, the qPCR assay (Luna® Universal qPCR Master Mix; NEB, US) was used. Primers and probes used in this study are presented in Table S2. Limits of detection for each viral target are provided in Table S1. For the specific quantification of viral nucleic acids, 5 μL of 4-fold and 10-fold dilutions of each viral nucleic acid extract were assayed in parallel with crude extracts; dilutions were meant to overcome amplification inhibition due to the nature of the samples. The final volume of the reaction mixture was 25 μL, composed of 800 nM of each primer, 200 nM of probe, and 5 μL of extracted nucleic acid. RT-qPCR reactions were carried out at 55 °C for 10 min, 95 °C for 10 min, followed by 45 cycles of amplification at 95 °C for 15 s and 58 °C for 60 s for SARS-CoV-2 and 60 °C for 60 s for the remaining RNA viruses. qPCR reaction for human adenovirus was performed at 95 °C for 10 min, followed by 45 cycles of amplification at 95 °C for 15 s and 60 °C for 60 s. PCR reactions were performed on an Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems, US). SARS-CoV-2 was analyzed in the samples collected in 2020, during the first stage of the pandemic, following reopening of the country after the first complete lockdown. Additional information about the quantification of the viruses can be found in the Supplementary Materials.

2.4. Data analysis

Data analysis was conducted using SPSS 26 (IBM Software, US) and R Studio (version 2023.03.0) and the ggplot 2 package (3.4.2). Mann-Whitney tests were conducted to evaluate the difference in the proportion of viruses in samples collected from the intertidal and the supratidal zones.

3. Results and discussion

3.1. Detection of enteric viruses and SARS-CoV-2 in beach sand

Enteric viruses were recovered from 24 sand samples (89 %). Norovirus GI was found in four samples (15 %), with concentrations ranging between 2.2 and 4.0 log copies (cp)/g, whilst norovirus GII was found in a single sample at a concentration of 3.4 log copies/g (Table S1). Enterovirus were positive in 22 % of the samples, with concentrations varying from 2.7 and 4.3 log cp/g. The detection of Enterovirus occurred only in beach sand samples collected during the off-season. Human adenovirus was recovered from 13 sand samples (48 %), at concentrations up to 7.2 log cp/g. Aichi virus, the most prevalent virus, was detected in 20 samples (74 %), with concentrations up to 4.7 log cp/g. The highest concentrations determined in this study for human adenovirus and Aichi virus were collected at a beach that had received in the prior days, a beach event that lasted for several days and where the participants camped out. Hepatitis A virus and rotavirus were undetected in all samples. Similarly, SARS-CoV-2 remained undetected in sand samples collected during the initial epidemiological stages of the pandemic. The introduction of SARS-CoV-2 in beach sand could occur via surface runoff, wastewater, seawater, or through infected people. Although SARS-CoV-2 RNA has been detected in raw wastewater throughout the world (Ahmed et al., 2020; Gonzalez et al., 2020; Kitajima et al., 2022; Monteiro et al., 2022), survival of this virus in raw wastewater, river water, and seawater is limited (Mahlknecht, 2022). In our study, wastewater did not impact the tested beaches. Moreover, the weather was dry during the peak season with no surface runoff (IPMA,

2023). Infected individuals could have contributed to the presence of SARS-CoV-2 in beach sand. However, our study took place during the initial phase of the pandemic when the number of reported cases in Portugal was low (DGS, 2023). Furthermore, the survival of SARS-CoV-2 is highly influenced by temperature, with higher decay observed at higher temperatures (Mahlknecht, 2022). In the summer of 2020, Portugal reached temperatures as high as 40 °C, at which SARS-CoV-2 would present low stability if introduced in beach sand (IPMA, 2023). The low environmental persistence of SARS-CoV-2 to high temperatures and in different water matrices, coupled with the lack of sources of contamination (point and non-point), might have contributed to the observed absence of SARS-CoV-2 in beach sand.

Fig. 1 displays the proportion of samples testing positive for a set number of enteric viruses species in beach sand. Most of the samples contained either one or two enteric viruses species (74 %), usually involving the detection of Aichi virus and human adenovirus. Detection of three or more viruses occurred in 15 % of the samples, generally with the presence of Aichi virus, enterovirus, and human adenovirus. Samples positive for four viruses were collected during the November 2018 campaign. These samples were collected following a festival that occurred at the beach, where the participants camped out.

Previous studies conducted in the USA and Australia have not documented the presence of human adenovirus and norovirus in this matrix (Goodwin et al., 2009; Abdelzaher et al., 2010; Hughes et al., 2017). A recent study by Carducci et al. (2022) was able to detect adenovirus in beach sand. On the other hand, a few studies have reported the presence of enterovirus in beach sand (Nestor et al., 1984; Pianetti et al., 2004; Shah et al., 2011). A multiplicity of factors might account for the differences in the prevalence of enteric viruses in beach sand, including the efficiency of the recovery methods used, and variability in PCR-based workflows.

Detection of viruses was performed using q(*RT*)PCR, which does not discriminate between infectious and non-infectious viral particles. However, several studies that were conducted in cell culture systems (Nestor et al., 1984; Pianetti et al., 2004; Shah et al., 2011), have already demonstrated the extended survivability and infectivity of enteric
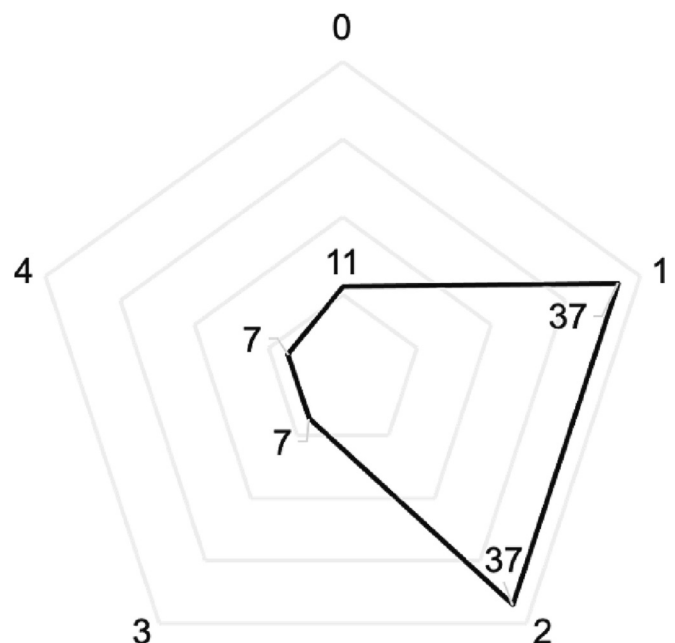

Fig. 1. Proportion of samples testing positive for enteric viruses species evaluated in this study. Numbers on the outside corners of the pentagon represent the number of virus species detected per sampling site. The numbers within the pentagon represent the proportion of samples positive in percent for a set number of virus species.

viruses in beach sand despite the severe environmental conditions.

Although the mechanisms through which enteric viruses attach and survive in beach sand and sediments are still not well understood, the main aspects influencing viral sorption and persistence on porous substances are varied and include temperature, pH, ionic strength, the type of viruses and substance, the presence of organic matter (Jin and Flury, 2002), and the possible formation of biofilms. Ionic strength plays a pivotal role on the adhesion of viruses into surfaces, that occur through the strengthening of van der Walls forces or hydrophobic interaction (Bitton et al., 1976; Farrah et al., 1981). High ionic strengths promote the decrease in the relevance of the virus electrostatic charges, increasing hydrophobic interactions favoring viral adhesion to surfaces (Dika et al., 2013; Lukasik et al., 2000). High ionic strengths have been shown to increase viral adhesion to surfaces but this is dependent on the virus (Dika et al., 2013, 2015; Lukasik et al., 2000). Dika et al. (2015) determined that adhesion of bacteriophages (phages; MS2 and PDR1) onto negatively-charged hydrophilic membranes increased with increasing ionic strength. However, such effect was not observable for Phi X 174. Ionic strength was shown to impact different parts of the viral structure (Samandoulgou et al., 2015). Increasing ionic strength induced change from β-strand to α-helix and unordered structures in norovirus GI.1 while maintaining the turns unchanged. On the other hand, in norovirus GII.4, the α-helix and turn structures remained stable whereas the β-strands shifted to unstructured forms. In the case of feline calicivirus, at higher ionic strengths changes in turn content were also observed. Lance and Gerba (1984) found that enhanced adsorption occurred in the presence of divalent cations but also that the nature of the anion present influences the adsorption of poliovirus in soil column with Cl⁻ promoting the highest attachment. Concomitantly, da Silva et al. (2011) demonstrated that norovirus GI.1 and GII.4 were capable of attaching more efficiently under higher ionic strength. Ionic strength, in parallel with viral hydrophobicity and the balance between hydrophobicity and hydrophilicity of the surfaces are paramount in determining the efficiency of viruses onto surfaces such as beach sand. Studies focusing on the sorption of enteric viruses have shown that they readily attach to a wide variety of sediments, with rates reaching up to 100 % (Carlson et al., 1968; Gerba et al., 1977; LaBelle and Gerba, 1979; Gerba et al., 1980; Bitton et al., 1982; Tsai et al., 1983; Johnson et al., 1984) with the highest sorption rates obtained in marine and estuarine sediments. Conversely, the lowest sorption rate was found in freshwater sediments. Bitton et al. (1982) reported sorption levels of 99 % for viruses in marine sediments against 37 % for freshwater sediments. The different sorption rates can be attributed to the hydrophobicity of the sediments and organic content (Chrysikopoulos and Aravantinou, 2012). Other factors such as the physico-chemical properties of the water and viral particles and the viral capsid properties may play an important role in the sorption of viruses to sand (Carlson et al., 1968; LaBelle and Gerba, 1979; Gerba et al., 1980; Bitton et al., 1982; Dowd et al., 1998). High temperature and pH have been shown to change the attachment of norovirus from electrostatic to mainly hydrophobic due to the disappearance of an ordered molecular structure in the viral capsid resulting in increased hydrophobicity and resultant sorption of the viruses to the organic phase in sediments (Samandoulgou et al., 2015). Gerba et al. (1980) detected differential viral sorption to sediment depending on the species/strain, suggesting that capsid properties may play an important role in the attachment of viruses. Hydrophobicity, isoelectric point and the capsid structure are relevant for the attachment of viruses to porous surfaces (Dowd et al., 1998; Farkas et al., 2015). The composition of the capsid itself may influence the viral sorption as studies demonstrated that viral surrogates with similar zeta potential, size, and hydrophobicity adhered differently (Pang et al., 2014; Farkas et al., 2015). High levels of salinity also strengthen the hydrophobic bonds between proteins and sediments. In addition, Liew and Gerba (1980) have shown that attachment of viruses to sediments enhances viral thermostability.

Adding to the complexity of sorption, bacteria, and other organisms,

are known to attach to particles through extracellular polymeric substances, forming biofilms (Piggot et al., 2012; Weiskerger et al., 2019). The formation of biofilms may constitute a barrier against external aggression, contributing to the retention and survival of pathogens. Enteric viruses have been shown to colonize biofilms, suggesting biofilms may confer protection against environmental stressors, including inactivating agents and UV radiation. Additionally, substances with inactivating characteristics, such as enzymes, might also be trapped in the particles, preventing viral inactivation (Gerba and Schaiberger, 1975). On the other hand, humic substances and microbial activity might be factors conditioning the survivability of viruses in sediments. For example, enteric viruses are capable of surviving for longer periods in the absorbed state than in suspension. If they are absorbed to humic acids they may survive longer (Bitton and Gerba, 1984). Additionally, humic acids may also hinder the attachment of viruses, since they may compete with the virus reducing the level of adsorption to the sand.

3.2. Sampling location: supratidal zone vs intertidal zone

Microorganisms in the two zones (supratidal and intertidal) are affected by different factors (biotic, and abiotic such as UV radiation and temperature) that may contribute to their survival in beach sand. Several routes may contribute to the presence of viruses in beach sand, which include point and non-point sources. The presence of enteric viruses in bathing waters, discharge of treated wastewater or illegal spillage, infected or asymptomatic beachgoers, carry-over of enteric viruses in the shoes of beachgoers, wild animals, and surface run-off to the beach, are factors largely contributing the presence of enteric viruses in beach sand. To indirectly assess the effects that these factors have on the presence of viruses, we collected samples from each of these zones.

In this study, viruses were detected more frequently in the supratidal zone. In the paired sampling, norovirus GI was present only in the supratidal zone (14 %), whereas norovirus GII was detected only in the intertidal zone (14 %) (Fig. 2).

Aichi virus was detected in 86 % of the sand samples collected at the supratidal zone and in 57 % of the samples from the intertidal zone (Fig. 2). The concentration of Aichi virus in the supratidal zone varied between 2.3 and 4.2 log cp/g (difference of approximately 2-logs). For the samples collected in the intertidal zone, the variation in concentration was lower, ranging from 3.2 and 4.0 log gc/g (difference of less than 1-log).

Human adenovirus was more frequent in samples from the supratidal zone, 43 %, than in the intertidal zone, with 14 % of positive detection. Concentration of human adenovirus in both zones were similar, ranging from 2.1 to 3.2 log cp/g.

Fig. 3 represents the percentage of samples with positive detection of viruses species in beach sand collected within the supratidal and intertidal zones. Supratidal sand contained a greater variety of enteric viruses.

Within the supratidal zone, every sample contained at least one type of virus, while two enteric viruses were detected in 43 % of the samples. On the other hand, in the intertidal zone, viruses remained undetected in up to close to half of the samples (42 %), with an equal percentage of samples having one and two types of viruses (29 %). None of the paired samples were positive for three or more viruses. The number of virus species detected in the supratidal zone and in the intertidal zone was not statistically different ($p = 0.26$).

There is a lack of information on the distribution of enteric viruses in the distinct areas of the beach. Shah et al. (2011) detected enteroviruses in one dry sample from the supratidal zone and one inundated sand sample from the subtidal zone, with higher concentration in the former. Beach sand collected in the intertidal zone remained undetected for enteroviruses. These results are in agreement with our current study, as enterovirus were only detected in sand samples collected form the supratidal zone. The distribution of enteric viruses, and other microorganisms, in the different areas of the beach is a complex equilibrium
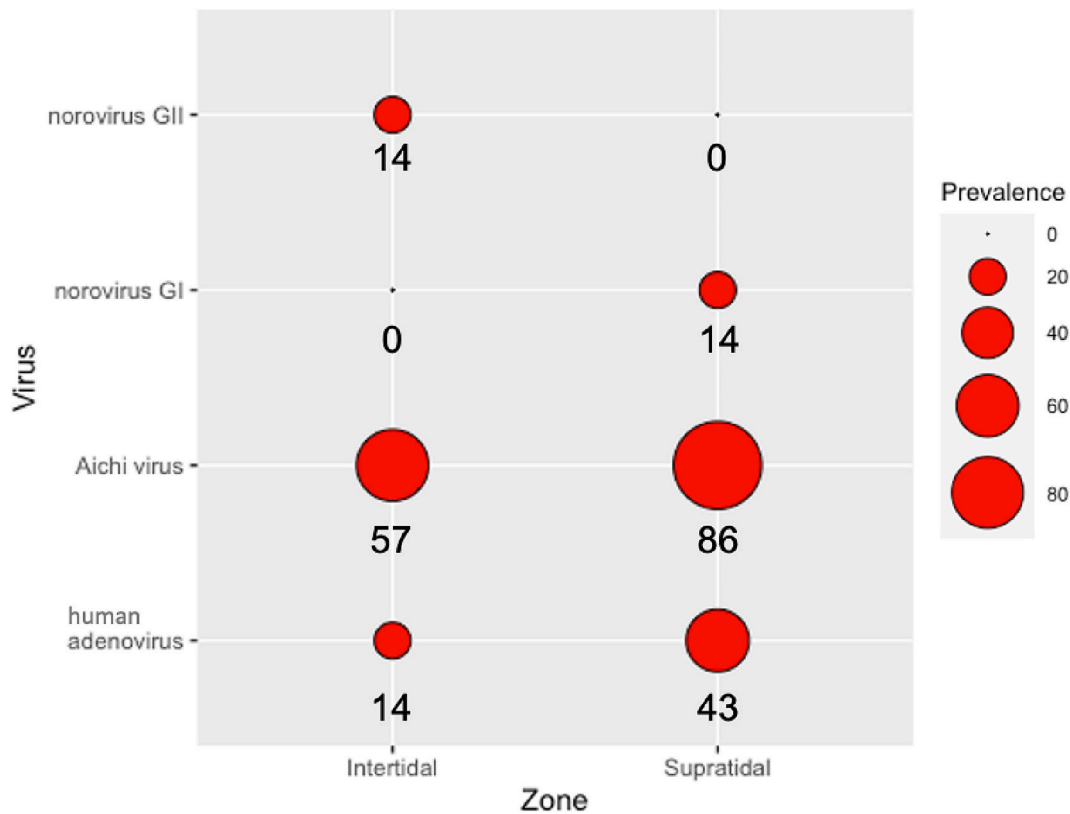
Fig. 2. Detection of viruses in beach samples from the supratidal and intertidal zones. The size of the circle is related to the relative percentage of detection of the viruses in each zone.
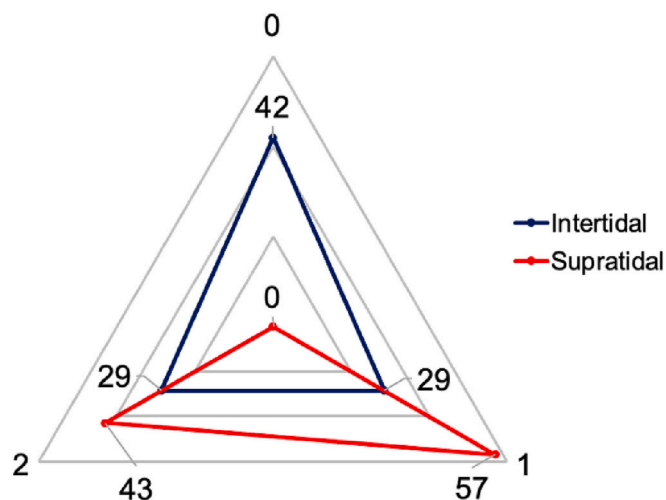

Fig. 3. Proportion of enteric viruses in beach sand collected in the supratidal and intertidal zones. Numbers on the outside corners of the triangle represent the number of virus species detected per sampling site. The numbers within the triangle represent the proportion in percent of samples positive for a set number of virus species whether within the intertidal or supratidal zones. Number of samples collected in the supratidal area, $n = 7$ and number of samples collected in the intertidal area, $n = 7$.

between several factors, including moisture content, resuspension into the water column, predation, rainfall, radiation, temperature, virus species/strain, viral capsid, and integration into biofilms, among others, as discussed previously (Whitman et al., 2014; Hassard et al., 2016).

In our study, most of the viruses were detected in sand collected from the supratidal zone. Although further studies are required, with a larger number of samples collected at both areas, the differential distribution of viruses shows that the distribution and persistence of viruses in beach sand is dependent on the viral species/strain and capsid properties. The potential for resuspension of viral particles into the water column might also have contributed to the differences in distribution between the two studied areas, implying that as a result, water may herewith become a vehicle for disease transmission. One particular source that might have contributed to the higher prevalence of enteric viruses in the supratidal area is beachgoers that may carry with them fecally contaminated shoes or that may have been infected but still shedding viruses or may be asymptomatic, allowing for the transfer of enteric viruses to the sand. Additionally, contaminated bathing water may also be a contributing factor for the presence of enteric viruses in the intertidal area. Further studies are necessary to determine the main drivers behind the presence, distribution, and persistence of viruses in beach sand, as recommended recently by the World Health Organization's Guidelines on recreational water quality (WHO, 2021).

4. Conclusion

Most of the samples were positive for at least one type of viruses, while in 15 %, more than three viruses were found. Among the seven viral species evaluated, rotavirus and hepatitis A viruses were not recovered from beach sand and SARS-CoV-2 RNA remained undetected in the samples collected. Moreover, enteric viruses displayed marked localization patterns in our sampling locations, with most being detected in higher prevalence within the supratidal zone. The beaches chosen for this study are not impacted by wastewater effluents or other environmental waters such as creeks, and therefore the presence of enteric viruses might be attributed to beachgoers and bathing water.

Data obtained in this preliminary study indicate that further sampling should be conducted to assess the fate of enteric viruses once they enter the beach environment. The factors that most influence viral fate

in beach sand should be evaluated, and microbial risk assessment should be conducted to support risk management, for a better understanding of the recurring nature of enteric viruses in beach sand and the risks to human health.

4.1. Study limitations

A total of 27 samples were collected and monitored for the presence of enteric viruses and SARS-CoV-2, collected mostly during the bathing season. Additionally, seven samples were collected and available for paired comparisons between intertidal and supratidal zones. Therefore, the sample size of this study was small and so descriptive statistics were used. The samples were collected mostly during dry weather, which may not be representative of the viral profile after rainfall events. Detection by q(RT) PCR does not confirm the infectivity of detected enteric viruses. Cell culture is still the gold standard for the detection of infectious enteric viruses. However, for several enteric viruses the cell culture systems are fastidious, highly expensive, difficult to perform, or nonexistent. The application of capsid integrity q(RT)PCR procedures could be applied to better infer infectivity through PCR.

Due to the structure of the chosen beaches, not impacted by wastewater discharges, it is shown that non-point sources of fecal contamination contribute largely to fecal inputs in beach sand. More studies would also require the analysis of potential physical sources of contamination as well as the detection of source tracking markers.

Despite these limitations, this study addresses an important knowledge gap by measuring the quantity of several enteric viruses in beach sand. We demonstrate that beach sand harbors multiple enteric virus species at high concentrations and that they are unevenly distributed throughout the beach. Thus, the results from this study demonstrate the potential for the transmission of disease. Risk assessment studies are needed that evaluate children, the elderly, and immunocompromised people through potential contact with beach sand and water, or through the resuspension of viral particles to the water column.

CRediT authorship contribution statement

Adriana Robalo: sample collection, investigation, writing – review and editing. João Brandão: sample collection, formal analysis, Writing - Review & Editing, visualization; Tomoyuki Shibata: formal analysis, Writing - Review & Editing, visualization; Helena Solo-Gabriele: formal analysis, Writing - Review & Editing, visualization; Ricardo Santos: conceptualization, methodology, resources, formal analysis, writing – reviewing and editing, visualization. Silvia Monteiro: conceptualization, methodology, validation, formal analysis, investigation, writing – original draft, writing – review and editing, visualization.

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Declaration of competing interest

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

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.165836.

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