Survival of human enteric and respiratory viruses on plastics in soil, freshwater, and marine environments

Vanessa Moresco[1*], David M. Oliver[1], Manfred Weidmann[2], Sabine Matallana-Surget[1], Richard S. Quilliam[1]

[1]Biological and Environmental Sciences, Faculty of Natural Sciences, University of Stirling, Stirling, FK9 4LA, UK

[2]Institute of Microbiology and Virology, Brandenburg Medical School Theodor Fontane, Senftenberg, D-01968, Germany

[*]Corresponding author

E-mail address: van.moresco@gmail.com (V. Moresco).

Accepted refereed manuscript of: Moresco V, Oliver DM, Weidmann M, Matallana-Surget S & Quilliam RS (2021) Survival of human enteric and respiratory viruses on plastics in soil, freshwater, and marine environments. Environmental Research, 199, Art. No.: 111367. https://doi.org/10.1016/j.envres.2021.111367

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ABSTRACT

The public health significance of plastics and microplastics in different environmental matrices has mainly focused on the toxicological effects of human ingestion. But these pollutants can also harbour pathogenic bacteria as the surfaces of plastics in the environment quickly become colonised by microbial biofilm. This novel microbial habitat has been termed the 'plastisphere' and could facilitate the survival and dissemination of important bacterial and fungal pathogens. Importantly, however, the role of plastic pollution as a secondary pathway for the transmission of human pathogenic viruses has never been addressed. Due to the high prevalence of both enteric and respiratory viruses in the population and in the environment, there is significant potential for human viruses to become associated with the plastisphere. In this review we critically evaluate current knowledge on the interaction of human enteric and respiratory viruses with plastic surfaces and identify the main environmental conditions and plastic characteristics that could affect virus survival and persistence in the environment. Our hypothesis is that the plastisphere can enhance the adhesion, survival and dissemination of human pathogenic viruses and potentially lead to more effective transfer and transmission of viral diseases within the environment. We identify key research questions needed to more fully assess the potential human health risks associated with viruses on plastic surfaces. These include understanding, (1) the mechanisms of viral attachment to either naked or biofilm-colonised plastic, (2) how the structural characteristics of viruses (e.g., enveloped, or non-enveloped) affect

their persistence in the plastisphere, (3) whether the plastisphere offers protection and increases the persistence of infectious viruses in soil, freshwater, and marine environments.

Keywords: biofilm; environmental virology; microplastics; plastic pollution; wastewater

Funding information

This work was supported by the UKRI Natural Environment Research Council (NERC) as part of the project, “Microbial hitch-hikers of marine plastics: the survival, persistence & ecology of microbial communities in the 'Plastisphere'” [grant number NE/S005196/1], the SPACES project [grant number NE/V005847/1] and the SE Asia microplastic project [grant number NE/V009621/1].

1. Introduction

The occurrence of emerging virus outbreaks has increased within the past few decades, exemplified by epidemics such as Severe Acute Respiratory Syndrome (SARS-CoV-1) in 2003, Influenza A (H1N1) in 2009, Middle East Respiratory Syndrome (MERS) in 2012 and the current SARS-CoV-2, declared a pandemic in March 2020. Other endemic viral illnesses, e.g., gastroenteritis caused by enteric viruses such as norovirus (NoV) and rotavirus (RV), continue to be highly prevalent worldwide (Ahmed et al., 2014; Bányai et al., 2018). Factors such as high population density and life expectancy, increased commercial trade and the ease of global travel can favour the transmission of viral diseases, particularly when person-to-person spread is the primary route of exposure (Wu et al., 2016; Lindsay et al., 2018; Tuite et al., 2020). In common, both emergent and non-emergent viral diseases highlight the significant role that the environment plays in disease transmission (Pica and Bouvier, 2012). For example, the contamination of surface waters by faecal waste, especially in developing countries, remains a common pathway for the contraction of preventable enteric viral illnesses either through direct contact with unsafe drinking or recreational water, or indirectly through contamination of food (Adelodun et al., 2021; Gall et al., 2015).

The increasing availability of plastic surfaces in the environment combined with a growing awareness of how important pollution is for generating novel pathways of disease transmission, highlights the multi-pollutant potential of plastics for human health. Plastics, and microplastics (defined as being less than 5mm) in aquatic or terrestrial environments become rapidly colonised by microorganisms (Oberbeckmann et al., 2018; Zettler et al., 2013). The microbial biofilm situated at the interface between the plastic surface and the environment has been termed the

‘plastisphere’ and can include bacterial taxa that contain species known to cause enteric human infections, e.g., *Vibrio*, *Campylobacter* and Enterobacteriaceae (Zettler et al., 2013; Kirstein et al., 2016; Rodrigues et al., 2019). Therefore, plastics could contribute to the dissemination of diseases in the environment and have as yet unquantified implications for human health (Bowley et al., 2020; Keswani et al., 2016). However, the potential for human viruses to attach to, and persist, in the plastisphere has not yet been addressed. In this review, we aim to critically assess the potential for plastic pollution in the environment to act as a reservoir for human viruses and identify key factors involved with viral survival and persistence in the plastisphere.

2. Sources of plastics in the environment

Sources of plastic pollution, and their accumulation in aquatic environments are well understood (Andrady, 2011; Avio et al., 2017; Cole et al., 2011; Li et al., 2016). However, reports of microplastic concentration and distribution vary according to geographical location, environmental factors that affect transport, and the methods employed for recovering and quantifying microplastics in the environment. It has recently been estimated that a global average of up to 10^{15} and 10^{16} microplastics (from treated wastewater effluents and untreated influent respectively) are released annually into the aquatic environment (Uddin et al., 2020).

Although there are relatively fewer studies evaluating the presence and impact of microplastic pollution in the terrestrial environment (Nizzeto et al., 2016), recent research has drawn attention to the increasing accumulation of microplastics in soils, especially those used for agriculture (Gao et al., 2020; Mohajerani and Karabatak, 2020). Sewage sludge can retain up to 60 - 99 % of the microplastics that enter wastewater treatment plants (WWTPs) (Gao et al., 2020), but is often treated and recycled back to land as a soil amendment and therefore considered one of the main

routes for microplastic contamination of soil (Nizzeto et al., 2016). Degradation of the plastic mulching films used in arable farming can also introduce microplastics in the soil and provide microorganisms (including those involved in plastic degradation) with suitable colonisation sites (Huang et al., 2020; Zhang et al., 2019). More recently, in response to the Covid-19 pandemic, there are increasing numbers of disposable personal protective equipment (PPE) such as face masks and gloves being released into the environment (Aragaw, 2020; Fadare and Okoffo, 2020). Microplastics can also occur in atmospheric fallout in major urban areas (Wright et al., 2020) which highlights the importance of atmospheric transport and deposition pathways, that are likely to favour interactions with respiratory viruses present as aerosol particles. Furthermore, the transport of viruses colonising marine microplastic could be facilitated by aerosolization in sea-spray (Allen et al., 2020). The pathways in which plastics can enter the environment (Figure 1) provide numerous opportunities for interactions with pathogenic microorganisms, including enteric, and respiratory viruses (both human and zoonotic).

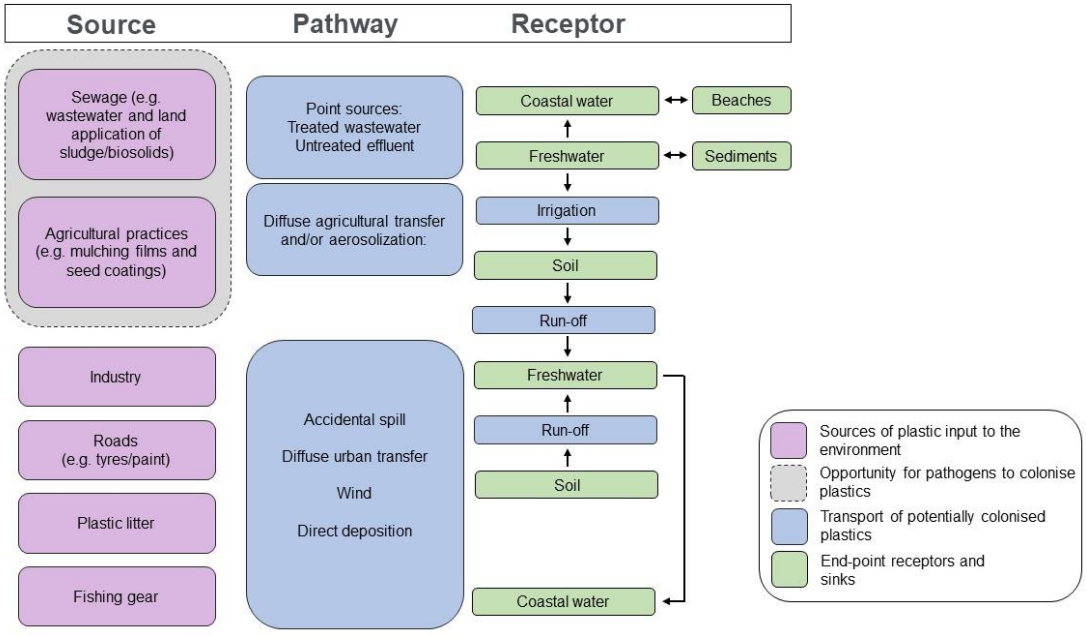

Figure 1. Sources of microplastic in the environment and potential pathways for virus interaction, colonisation, and dissemination

3. Viruses in the environment

The ubiquity and high prevalence of both enteric and respiratory viruses provides significant opportunity for their interaction with plastic surfaces in the environment, for example, during transit through wastewater systems, via faecal contamination of environmental plastic fomites, and deposition of respiratory viruses on plastic surfaces (Figure 1). Virus stability in the environment is strongly influenced by the size and structure of the virus particle (including the presence or absence of an envelope), type of genome (DNA or RNA), transmission route (e.g., faecal-oral, air droplets), and the viral concentration of the contamination source (Fong and Lipp, 2005; Pinon and Vialette, 2018). Therefore, these characteristics will impact and shape the potential for virus particles to associate with and be transferred via plastic surfaces (Table 1). Enveloped viruses, i.e. those with a bilipid membrane and glycoprotein receptors surrounding the viral protein capsid, are usually less thermostable in environmental samples (e.g., wastewater) compared to non-enveloped viruses; however, they can still persist for a considerable amount of time (hours), in ambient temperatures (~ 25 °C) or even days/weeks, at lower temperatures (4 - 10 °C) (Casanova et al., 2009; Gundy et al., 2009; Ye et al., 2016). This could represent enough time (e.g., residence time in wastewater) for enveloped viruses to interact with plastic waste or microplastics.

Person-to-person spread, through small aerosol particles or droplets is the primary transmission route for respiratory viruses, e.g., coronavirus (CoV) and human rhinovirus (HRV), which are enveloped and non-enveloped respectively, whilst enteric viruses (e.g., RV and NoV) are transmitted mainly through the faecal-oral route: either

by person-to-person contact, or via contaminated food or water (Table 1). Enteric viruses are detected in high concentrations, i.e. up to 10^8 particles per gram of stool in infected individuals, or 10^8 genome copies per litre in wastewater or surface water samples (Barril et al., 2015; Farkas et al., 2018; Fong and Lipp, 2005; Wyn-Jones et al., 2011). Usually, enteric viruses have a low infective dose, for example, 1 FFU (Focus Forming Unit) of RV was enough to cause infection in 25% of healthy volunteers (Graham et al., 1987). The concentration of respiratory viruses can depend on the type of clinical sample analysed; concentrations of SARS-CoV-1, for example, can vary from 6.1 $\log_{10}$ copies per mL (copies/mL) in stool, to 2.4 $\log_{10}$ copies/mL in nasopharyngeal aspirate samples (Hung et al., 2004). The average concentration of SARS-CoV-2 detected in different clinical specimens, including faecal samples was < 2.6 x 10^4 copies/mL, and on average 1.4 x 10^6 copies/mL eluted from nasal swabs (Wang et al., 2020). Other important respiratory viruses, such as influenza virus (FluV) and HRV have also been detected in faeces of infected individuals suggesting the potential for faecal-oral transmission of some respiratory viruses (Chan et al., 2009; Harvala et al., 2012).

4. Attachment of virus particles to plastic surfaces

4.1 Naked plastic surfaces

The interaction of viruses with surfaces and the increased risk of subsequent transmission has always been a major public health concern (Doerrbecker et al., 2011). Virus adhesion to abiotic surfaces such as plastics is governed by non-specific electrostatic interactions and hydrophobic forces that surround the virus particle and the surface (Dika et al., 2015). Surface physicochemical characteristics, such as roughness, together with extrinsic environmental parameters, can also play a role during virus-plastic interactions (Dika et al., 2013; Langlet et al., 2008). The presence

of water or a liquid interface can also influence virus adhesion onto solid surfaces, as the virus surface charges may vary according to the pH and ionic strength in the fluid (Armanious et al., 2016; Mi et al., 2020). Virus attachment onto naked plastics (i.e. with no biofilm), such as polyethylene terephthalate (PET) and polypropylene (PP) are dominated by non-ionic forces and directly affected by the pH of the medium and the virus isoelectric point, which is defined as the pH at which the net surface charge surrounding the virus particle is considered neutral (Gassilloud et al., 2007; Gassilloud and Gantzer, 2005) (Figure 2).

4.2 Plastic surfaces colonised by biofilms

The presence of an ecocorona and the secretion of exopolymeric substances (EPS) containing lipopolysaccharides (LPS), proteins and nucleic acids by microorganisms can support the development of biofilms on plastic surfaces (Galloway et al., 2017; Rummel et al., 2017). The characteristics of biofilms allow microorganisms to persist under stressed conditions, e.g., desiccation or the depletion of nutrients; however, biofilms could also enhance the survival of viruses, particularly on wet surfaces, such as irrigation and drinking water distribution pipes (Storey and Ashbolt, 2003; Wingender and Flemming, 2011). Therefore, biofilm on the surfaces of plastics could also provide a novel platform for viral attachment.

The mechanisms by which viruses can interact with and bind to biofilms on plastic surfaces in the environment are poorly understood, highlighted by the paucity of data on the possible interactions occurring between enveloped viruses, such as SARS-CoV-2 and biofouled plastic surfaces. Non-enveloped enteric viruses, such as NoV genotypes GII.4 (Sydney and New Orleans strains) and GI.6, can interact with bacteria in the human gut by binding to the external cell surface and pili structures (Almand et al., 2017). Coxsackievirus B5 (CVB5) particles (which are closely related

to human rhinoviruses) are also capable of physically interacting with LPS and peptidoglycans, which facilitates virus aggregation and binding (Waldman et al., 2017).

The genome of NoV and enteroviruses, have been detected in naturally occurring biofilm on patches of polyethylene (PE) receiving treated wastewater (Skraber et al., 2009), and the persistence of viruses on plastic surfaces associated with either drinking water or wastewater is directly correlated with biofilm structure and composition (Helmi et al., 2008; Storey and Ashbolt, 2003). This supports the hypothesis that plastic surfaces colonised by biofilm provide a suitable environment for increased virus retention and could facilitate their persistence in the environment. Enteric viruses can also adhere to plastic without a visible biofilm, (e.g., on the inside of plastic PET drinking water bottles) (Butot et al., 2007). However, the inactivation of poliovirus type 1 (PV1) by surface adhesion to a hydrophobic polypropylene container used to store water (Gassilloud and Gantzer, 2005) indicates that, in some cases, viral adhesion to plastics, especially in the presence of biofilm, could reduce the environmental transport of viruses through aquatic environments.

5. Detection and persistence of viruses on plastic surfaces

The detection, survival, and transmission of pathogenic viruses (enteric and respiratory) via commonly touched surfaces (i.e. fomites) has been extensively described for enclosed environments such as hospitals and day-care facilities, by either quantifying anthropogenic viral contamination or employing lab controlled experiments (Firquet et al., 2015; Fischer et al., 2015; Guo et al., 2020; Verani et al., 2014). Similar studies have also been carried out in public areas, such as airports, where contamination by human respiratory viruses (HRV and CoV OC43) has been found on plastic toys at a children’s playground, hand-luggage trays, buttons in a payment terminal, and handrails (Ikonen et al., 2018).

The detection of viruses and their persistence on plastics surfaces, has mainly been studied by laboratory-based experiments under controlled conditions, which often only evaluate virus recovery and stability on artificially seeded plastic surfaces. Different human coronavirus strains have been shown to persist on commonly touched surfaces such as polyvinyl chloride (PVC) and Teflon® for a period of up to 5 days (Duan et al., 2003; Warnes et al., 2015). Both SARS-CoV-1 and SARS-CoV-2 can remain stable on plastic surfaces for a period up to 72h (van Doremalen et al., 2020). RNA from infectious murine norovirus (MNV-1) particles and NoV GII.4 were recovered by plaque assay and RT-qPCR respectively after being artificially seeded onto high density polyethylene (HDPE) surfaces and left to dry for 15 min at room temperature and 20-40 % humidity (Lee et al., 2018). Contamination by parainfluenza virus (respiratory) and NoV GII RNA have also been detected on plastic surfaces in open and closed office spaces (Stobnicka et al., 2018).

Detection methods allow the quantification of the amount of virus on a plastic surface, whereas survival experiments can assess how long viruses can persist on a surface when exposed to pre-determined conditions (e.g., temperature, disinfection agent), and calculated from inactivation rate *(-k*) values. Such an approach can give a recovery percentage and can include values for infectious vs non-infectious particles. A summary of studies demonstrating detection, recovery and survival of important viral pathogens potentially transmitted through associations with plastics or the plastisphere are summarised in Table 2.

Carefully selecting an appropriate method for analysing virus presence and survival in environmental plastic surfaces is important for quantifying and comparing different profiles of viral stability among distinct groups and environmental matrices. In the presence of biofilm, elution procedures often assume that viruses are strongly

attached to the biofilm (Skraber et al., 2009), but different buffers and elution procedures can influence recovery rates depending on the virus type and surface characteristics (Gassilloud et al., 2007; Julian et al., 2011). Although the detection of viral nucleic acid by methods such as real time PCR (qPCR) is commonly used to detect viruses in the environment, this approach does not necessarily represent the presence of infectious viral particles. Amplified nucleic acid could come from intact infectious particles or from fragments of damaged or 'dead' virus particles, which are non-infectious (Gassilloud et al., 2003). Therefore, the use of cell culture-based methods to evaluate viral infectivity is desirable to provide a more accurate estimation of the potential health risks associated with human contact with a contaminated plastic surface. However, most of the viruses present in environmental samples are not adapted to *in vitro* cell culture or fail to produce a notable cytopathic effect. Furthermore, some of the current cell culture-based methods, such as the plaque assay can be time consuming, which limits the number of samples that can be processed at the same time (Hamza et al., 2011). Using methods that ensure only the amplification of intact virus particles, such as Integrated Cell Culture-qPCR (ICC-qPCR), enzymatic treatment (e.g., RNAse/DNAse) prior to nucleic acid extraction, or the use of nucleic acid intercalants, such as prodidium monoazide (PMA) or ethidium monoazide (EMA), are reliable alternatives to overcome many of the obstacles imposed by classic cell culture or molecular methods (Hamza et al., 2011; Haramoto et al., 2018; Leifels et al., 2016).

6. Environmental factors influencing virus stability on plastic surfaces

Temperature, relative humidity, solar radiation, salinity, presence of particulate matter and naturally occurring microbiota can all play an important role in maintaining or affecting virus stability in the environment (Fong and Lipp, 2005; Hanley et al., 2018;

Wigginton and Kohn, 2012). Although the attachment of viruses to biofilms on plastic surfaces could facilitate virus survival and transfer in the environment, viral stability in the plastisphere could be compromised compared to those associated with non-plastic surfaces, such as organic material. The natural abiotic and biotic processes of plastic degradation in the environment can also influence virus persistence (Rummel et al., 2017), which will depend on the different behaviour of enteric and respiratory, enveloped or non-enveloped viruses (Figure 2).

6.1 Organic matter

Virus particles in the water column often form aggregates with particulate natural organic matter (NOM) (Gerba and Betancourt, 2017), which can disrupt viral inactivation during disinfection processes, such as UV irradiation treatment. However, by catalysing the absorption of sunlight and the production of reactive oxygen species, NOM can also act as a sensitizer (i.e., through indirect photoinactivation), leading to nucleic acid damage, and thus reducing viral survival and infectivity (Kohn et al., 2007; Rosado-Lausell et al., 2013).

Plastics in the environment can quickly sorb a range of hydrophobic contaminants and pollutants, in addition to nutrients, biomolecules and organic matter (Galloway et al., 2017), which could favour virus interactions with microplastics (e.g., by facilitating aggregation). Dissolved organic matter (DOM) can impair non-enveloped viruses (e.g., MS2 phages) to interact with surfaces, by decreasing the hydrophobic forces required for virus adsorption and favouring the occurrence of electrostatic repulsion between naturally negatively charged virus particles and surfaces (Armanious et al., 2016). In contrast, within closed systems, such as drinking water networks, the presence of particulate matter (i.e. clay), can increase PV1

retention within biofilms, although this does not affect subsequent inactivation by chlorine (Quignon et al., 1997).

6.2 Salinity

The stability of enteric viruses is more greatly affected in environments with higher salinity, such as in marine or brackish waters (Moresco et al., 2015; Wetz et al., 2004). The aggregation of transparent exopolymer particles (TEP), a type of exopolymeric substance (EPS) generated by bacteria, is directly correlated to increasing salinity levels, and higher rates of MNV-1 genome recovery from TEP aggregates has been shown (Hanley et al., 2018). Salinity can also be an important factor involving the formation of specific bacterial assemblages on microplastics in the marine environment (Oberbeckmann et al., 2018), and EPS in the biofilm colonising microplastics in the marine environment, could enhance virus adhesion and survival within these aggregates. The presence of such aggregates on microplastics could also decrease the buoyancy of the plastic and increase their availability for shellfish uptake in the water column or facilitate their incorporation into the sediment.

6.3 Temperature and UV radiation

Temperature is one of the most critical factors affecting virus stability in the environment and on surfaces (Bertrand et al., 2012; Kim et al., 2012), either by directly affecting the structure of the virus or by facilitating processes that can affect its stability, e.g., by enhancing proteolytic enzyme activity of autochthonous microbial communities and subsequent degradation of exposed virus genomes (Casanova et al., 2009). Temperatures > 25° C for enveloped viruses such as Phi6 phage (Aquino De Carvalho et al., 2017; Casanova and Weaver, 2015) and > 50° C, for non-enveloped viruses such as feline calicivirus (FCV) and NoV GII.4 RNA (Topping et al., 2009) can directly impact virus infectivity by acting on viral structural proteins,

promoting capsid denaturation and exposing nucleic acid to degradation by extracellular enzymes (Topping et al., 2009; Ward et al., 1986).

Certain enteroviruses such as PV1 can form aggregates in the presence of lipopolysaccharides and peptidoglycan, which can increase their stability against thermal degradation and inactivation by UV radiation (Robinson et al., 2014; Waldman et al., 2017). Whilst the aggregation of viruses (i.e. MS2 phage) in solution does not significantly protect them from UV_{254} irradiation (Mattle and Kohn, 2012), being associated with a biofilm on the surface of plastic may offer some protection to viruses from solar and temperature inactivation and facilitate virus persistence and dissemination through the environment.

6.4 Physicochemical properties of the plastic surface

Plastic materials in the environment undergo aging processes that are catalysed by the action of solar radiation (photodegradation), mechanical (fragmentation) and microbial degradation (Oberbeckmann and Labrenz, 2020). Such modifications can alter the physical, chemical and mechanical characteristics of the plastic, and increase the surface area; whilst the appearance of small fissures and changes in the surface roughness can affect the potential for microbial adhesion and persistence (Dika et al., 2013). Adsorbed virus particles (or their genomes) can be efficiently recovered from smooth plastic surfaces, e.g., NoV and SARS-CoV-2 (Lee et al., 2018; van Doremalen et al., 2020). However, specific physicochemical characteristics of plastic polymers, such as porosity, surface charges, hydrophobicity and polymer composition will influence virus adhesion/detachment and stability, and consequently, the potential for transfer (Dika et al., 2013). Increased hydrophobicity of plastics, e.g., through aging, can also favour the adsorption and concentration of organic pollutants, antibiotics, pharmaceuticals, and heavy metals (Wang et al., 2020)

and thus increase the co-pollutant potential of plastics in the environment. However, elevated concentrations of copper ions within the plastisphere could affect virus stability by generating reactive oxygen species (ROS), and exposing the virus genome to degradation due to virion morphological changes (Warnes et al., 2015). The stability of viruses associated with plastics could also be affected by plastic additives, (e.g., plasticizers), which are often added to increase flexibility, but are released during plastic degradation. Some taxa of bacteria are able to degrade the most common plasticizers employed by the industry (Wright et al., 2020); however, the effect of these substances on virus integrity and infectivity has not yet been studied.

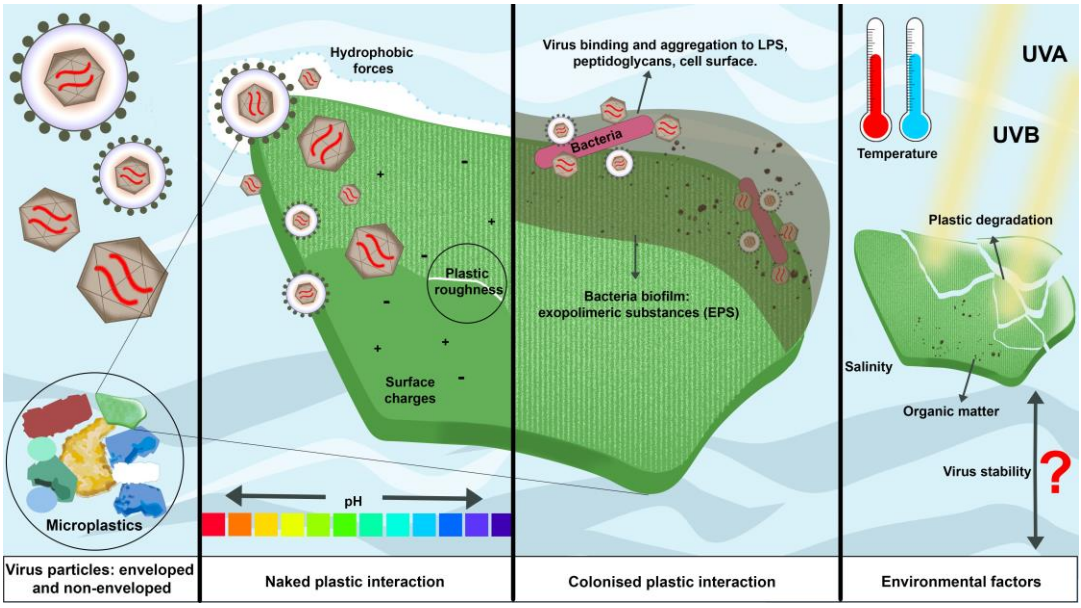

Figure 2. The interactions of enveloped and non-enveloped viruses with naked and biofilm-colonised microplastics and the environmental factors influencing virus stability on plastic surfaces.

7. Evolutionary perspectives on viral survival in the plastisphere

The population of any virus in the environment, particularly those containing RNA, is composed of subpopulations of viral quasispecies that contain considerable

genomic variation, which can lead to differential responses to selective pressures on virus survival and/or infectivity (Andino and Domingo, 2015). Bacterial biofilms colonising plastic surfaces are characterised by a heterogenic structure, with microcolonies intercalating with void spaces, which can allow viruses to penetrate and accumulate within different layers and sub-habitats (Lacroix-Gueu et al., 2005; Storey and Ashbolt, 2003). The interaction of human NoV with sugar residues belonging to histo-blood group antigens (HBGAs), which are present in high quantities on cell surfaces, can facilitate virus-cell binding and enhance virus infection (Harrington et al., 2004). Similar sugar residues are also found on the surface of certain bacteria from the gastrointestinal tract (Harrington et al., 2004). The presence of bacterial LPS in the mammalian gut can increase the stability of human enteric viruses and enhance virus binding to the host cell (Almand et al., 2017; Erickson et al., 2018). Bacterial LPS can also promote stability of poliovirus at higher temperatures and in the presence of diluted chlorine, whereas a mutant strain with a reduced ability to bind to LPS quickly lost viability in the environment (Robinson et al., 2014). This emphasises the potential for the selection of virus subpopulations that have been exposed to different levels of environmental stress and could lead to a competitive advantage for viruses to associate with biofilm in the environment.

Enveloped viruses are usually less resistant than non-enveloped viruses to wastewater treatment, as detergents and solvents tend to degrade the lipid envelope (Conley et al., 2017). However, relatively few studies have focused on evaluating the persistence and inactivation mechanisms of enveloped virus in the environment and on environmental surfaces, and yet, they represent a large percentage of human infections worldwide (Aquino De Carvalho et al., 2017; Gundy et al., 2009; Wigginton and Boehm, 2020; Ye et al., 2016). Therefore, there is an urgent need to understand

the interactions between both enteric and respiratory viruses (enveloped and non-enveloped) and biofilm on environmental plastic surfaces. The investigation of how these interactions might contribute to increased viral survival when exposed to different environmental conditions or inactivation treatments, are needed to fully understand the mechanisms by which viruses can persist and be transmitted through this secondary environmental pathway.

8. Evaluating the potential human health risks associated with viruses on plastic surfaces

The increased global production of plastics and their subsequent accumulation in the environment together with the evidence that plastic pollution can act as a vector for the dissemination of potentially hazardous substances, highlights a growing portfolio of research questions regarding virus adhesion and dissemination through this possible additional route of exposure. In response to this, the following key research recommendations are suggested:

8.1 Mechanisms of virus adhesion to different naked plastic polymers

There remains a lack of consensus regarding the main forces (hydrophobic interactions, surface charges) and/or factors (pH, isoelectric point, surface roughness) governing virus particle interactions with abiotic surfaces. Due to the structural nature of virus particles (soft multi-layered), it is challenging to determine exactly how viruses interact and adhere to solid surfaces (Dika et al., 2013). Understanding the nature and the strength of these interactions, would provide evidence to support more robust assessment of the risk posed by plastics debris in acting as fomites (or vectors) for virus persistence and dissemination through the environment.

8.2 Virus interactions with microplastics colonised with biofilm

The development of bacterial communities on environmental plastics is likely to occur regardless of the polymer type (Wu et al., 2020); and such assemblages are usually distinct and less diverse in comparison to those communities in the surrounding environment, e.g., water column or sediment (McCormick et al., 2014; Zettler et al., 2013). Pathogenic species of *Vibrio crassostreae* associate with polystyrene particles as a secondary coloniser (Foulon et al., 2016), which suggests that the presence of an ecocorona composed of primary colonisers could moderate the absorption of microbial pathogens and other substances. Therefore, the potential for viruses to bind to bacterial biofilm may be driven by the specific characteristics of the microbial community, such as the stage of succession or type of bacterial assemblage.

Although there is only limited evidence demonstrating the presence of human viral pathogens (genome or infectious particle) in biofilms on plastic surfaces (Skraber et al., 2009; Storey and Ashbolt, 2003), the fact that some enteric viruses, such as NoV, are able to interact with cell receptors commonly found on the surface of enteric bacteria (Harrington et al., 2004) highlights the need to investigate the possible interactions between different viruses and the biofilm components. Specifically, a greater understanding is needed on the role of LPS on virus adhesion to the plastisphere and the influence of this interaction on virus survival in the environment and resistance to inactivation processes.

8.3 Virus structural characteristics influencing adhesion and persistence on naked or colonised plastic surfaces

Although non-enveloped enteric viruses are usually assumed to be more resistant to inactivation factors in comparison to enveloped viruses (e.g., SARS-CoV-2), little is known about the influence of viral structural characteristics (genetic material,

size, and presence or absence of a bilipid envelope) on viral stability when associated with the plastisphere. Evaluating virus survival against environmental conditions and inactivation factors will help to determine target viruses with higher probability of transmission when associated with plastics (colonised or not) and establish efficient and specific inactivation treatments.

8.4 Virus accumulation, stability, and detachment from plastics

Bacterial biofilms are diverse and dynamic structures highly influenced by environmental and intrinsic conditions that determine their establishment and maintenance (Lobelle and Cunliffe, 2011; Oberbeckmann et al., 2018). The accumulation of viral pathogens within the plastisphere could offer protection against inactivation by disinfectants (i.e., chlorine in drinking water systems) or environmental parameters (e.g., temperature), and increase the risk of virus transfer once detached from the biofilm into, e.g., water or soil. Future studies of virus attachment, survival, and detachment from biofilms on plastic surfaces will provide important data regarding virus concentration, presence and quantification of infectious particles, inactivation decay rates and T_{90} values. These could be used to inform Quantitative Microbiological Risk Assessments (QMRA) and modelling studies to estimate the risk of infection associated with this novel pathway.

9. Conclusion

The benefits provided by plastics are undeniable; however, the indiscriminate use and disposal of plastics is causing significant impacts to both environmental (aquatic, terrestrial and atmospheric) and public health. In this review, we have critically evaluated the present knowledge regarding virus interactions with plastics in the environment and raised concerns about the co-pollutant potential of important viral pathogens becoming associated with plastic surfaces. Once in the environment,

plastic surfaces become readily colonised by microorganisms; our hypothesis is that the development of such a biofilm on plastics can subsequently enhance the adhesion, survival, and dissemination of human pathogenic viruses within the environment.

Data are urgently needed to confirm the role of plastic contaminants as a secondary pathway for the transmission of important viral diseases. Despite some successes in implementing new regulatory policies for controlling plastic use and discharge to the environment, microplastics released from treated or untreated wastewater effluents, or from biosolids or agricultural practices continue to be the main sources of plastic pollution in the environment. Tackling these sources in a more effective way, for example, through the implementation of more efficient methods for wastewater treatment and inactivation of pathogenic viruses in WWTPs, would decrease the potential for infectious viruses to become bound to the plastisphere and be transported through the environment.

10. Acknowledgements

This work was supported by the UKRI Natural Environment Research Council (NERC) as part of the project, “Microbial hitch-hikers of marine plastics: the survival, persistence & ecology of microbial communities in the 'Plastisphere'” [grant number NE/S005196/1], the SPACES project [grant number NE/V005847/1] and the SE Asia Microplastic project [grant number NE/V009621/1]. We would like to thank Natalie Boyd Williams for helping with Figure 2.

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Virus	Genome type	Enveloped (EV) Non-enveloped (NE)	Excretion	Concentration	Primary transmission route	Secondary transmission route	References
Enteric Viruses							
Rotavirus (RVA)	dsRNA (segmented)	NE	Faeces	10^4 - 10^7 (genome copies/g)	Faecal-oral (person to person)	Contaminated food, water, and fomites Plastics in the environment?	(Gutierrez et al., 2020)
Poliovirus (PV)	ssRNA (+)	NE	Faeces	10^8 - 10^{11} (virus particles/g)**	Faecal-oral (person to person)		(Fong and Lipp, 2005)
Hepatitis A (HAV)							
NoVGI NoVGII	ssRNA (+)	NE	Faeces	10^4 - 10^{10} (copies/g)	Faecal-oral (person to person)		(Chan et al., 2006)
Adenovirus	dsDNA (linear)	NE	Faeces Upper respiratory tract secretion	10^3 - 10^{10} DNA/g 10^2 - 10^{10} DNA copies/µg/DNA	Faecal-oral and aerosol (person to person)		(Berciaud et al., 2012)
Polyomavirus JC BK	dsDNA (circular)	NE	Urine	10^2 -10^4 viral particles/4mL of sewage 10^1 -10^3	Faecal-oral Respiratory*		(Bofill-Mas et al., 2000) (Boothpur and Brennan, 2010)

Respiratory Viruses							
Influenza Virus (H1N1)	ssRNA (-) (segmented)	EV	Respiratory	10^7 - 10^8 copies/mL	Aerosol and droplets (person to person)	Fomites	(To et al., 2010)
			Stool	10^4 copies/mL			
SARS-CoV-1	ssRNA (+)	EV	Stool	6.1$\log_{10}$ copies/mL		Plastics in the environment?	(Hung et al., 2004)
			NPA	2.4 $\log_{10}$ copies/mL			
SARS-CoV-2			Stool	6.8 - 8.1 $\log_{10}$/g		Faecal-oral?	(Lescure et al., 2020)
			NPA	6.78 $\log_{10}$			(Jacot et al., 2020)

*suggested; **average concentration for enteric viruses in stool samples
ssRNA+ (single stranded RNA positive sense); ssRNA- (single stranded RNA negative sense); dsRNA (double stranded RNA)
dsDNA (double stranded DNA)
NPA (nasopharyngeal aspirate)

Table 1. Potential transmission of enteric and respiratory viruses through the “plastisphere”

Virus (strain or serotype)	Plastic	Detection method	Detection or recovery (log_{10} or %)	Reduction (log_{10}) Stability (days or hours)	References
EV	PE colonised biofilm (primarly treated wastewater)	RT-qPCR	50%	ND	(Skraber et al., 2009)
NoV GI			46%		
NoV GII			37%		
PV1	PVC colonized biofilm (drinking water)	MPNCU/mL	0.6% (0.02 to 0.04 free chlorine/L)	ND	(Quignon et al., 1997)
			27.8% (without chlorine)		
PV1 (infectious)	PC colonised biofilm (wastewater)	MPNCU/mL	2.60 log units/cm^2	ND	(Helmi et al., 2008)
PV1 (genome)		RT-qPCR	2.65		
PV1 (infectious)	PC colonised biofilm (drinking water)	MPNCU/mL	1.49 log units/cm^2	Up to 6 days	
PV1 (genome)		RT-qPCR	1.74	34 days	
PhiX174		PFU/mL	1.81	Up to 6 days	
MS2		PFU/mL	2.04	Up to 6 days	
HAV MNV-1 NoV GI NoV GII	PP	RT-qPCR	2.5 to 3.8log_{10} (adhesion average)	ND	(Deboosere et al., 2012)

Phi6 (EBOV surrogate)	Tyvek® (HDPE)	PFU/mL	ND	2.3log_{10} (up to 6h) 40% RH	(Brown et al., 2016)
CVB4	Plastic (Petri dish lids)	$TCID_{50}$/mL	ND	0.5log_{10} (6 weeks)	(Firquet et al., 2015)
MVM				4log_{10} (6 weeks)	
H1N1				Up tp 3 days	
HSV-1				Up to 5 days	
SARS-CoV-1	Plastic	$TCID_{50}$	ND	$10^{3.4}$ to $10^{0.7}$ (up to 72h)	(van Doremalen et al., 2020)
SARS-CoV-2				$10^{3.7}$ to $10^{0.6}$ (up to 72h)	
SARS-CoV-2	Plastic	$TCID_{50}$/mL	ND	3.8log_{10} (7 days)	(Liu et al., 2020)
SARS-CoV-2	Plastic	$TCID_{50}$/mL	ND	Up to 7 days	(Chin et al., 2020)
CoV (229E)	PVC	$TCID_{50}$/mL	ND	5 days	(Warnes et al., 2015)

PE: polyethylene; PVC: polyvinyl chloride; PP: polypropylene; HDPE: High Density Polyethylene; PC: polycarbonate
MPNCU: Most Probable Number Cytopathic Units; $TCID_{50}$: Median Tissue Culture Infectious Dose; PFU: Plaque Forming Units
CVB4: Coxsackievirus B4; MMV: Mouse minute virus; EV: Enterovirus; EBOV: Ebolavirus RH: relative humidity

Table 2. Detection and survival of bacteriophages and viruses on plastic surfaces or the plastisphere