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Discussion

Plastic pollution and fungal, protozoan, and helminth pathogens – A neglected environmental and public health issue?

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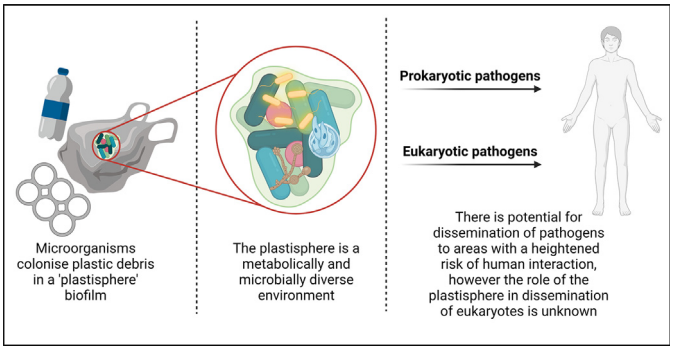
HIGHLIGHTS

- The plastisphere is a microbially and metabolically diverse environment.
- Relative to prokaryotes, the eukaryotic component of the plastisphere is understudied.
- Eukaryotes encompass some of the major disease-causing microorganisms worldwide.
- There is an urgent need to assess the plastispheres role in dissemination of eukaryotic pathogens.

GRAPHICAL ABSTRACT



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ABSTRACT

Plastic waste is ubiquitous in the environment and can become colonised by distinct microbial biofilm communities, known collectively as the 'plastisphere.' The plastisphere can facilitate the increased survival and dissemination of human pathogenic prokaryotes (e.g., bacteria); however, our understanding of the potential for plastics to harbour and disseminate eukaryotic pathogens is lacking. Eukaryotic microorganisms are abundant in natural environments and represent some of the most important disease-causing agents, collectively responsible for tens of millions of infections, and millions of deaths worldwide. While prokaryotic plastisphere communities in terrestrial, freshwater, and marine environments are relatively well characterised, such biofilms will also contain eukaryotic species. Here, we critically review the potential for fungal, protozoan, and helminth pathogens to associate with the plastisphere, and consider the regulation and mechanisms of this interaction. As the volume of plastics in the environment continues to rise there is an urgent need to understand the role of the plastisphere for the survival, virulence, dissemination, and transfer of eukaryotic pathogens, and the effect this can have on environmental and human health.

1. Introduction

The 'plastisphere' is the distinct microbial biofilm that colonises environmental plastic debris (Zettler et al., 2013). Plastisphere communities are highly variable, diverse, and genetically distinct from the free-living communities that surround them, suggesting that the surface of environmental plastics can provide a novel niche for colonisation (Kirstein et al., 2019; Wu et al., 2020; Wang et al., 2021). Research on the plastisphere has increased considerably in recent years and advanced our understanding of its role as a potential reservoir for pathogenic bacteria and viruses (Galafassi et al., 2021; Kelly et al., 2021; Zhang et al., 2021; Moresco et al., 2022). However, the majority of research on the plastisphere has focused on the prokaryotic species that make up these communities, with comparably little consideration of the potential for the plastisphere to harbour and disseminate pathogenic eukaryotes.

Pathogenic prokaryotes in the plastisphere have been extensively reported (Metcalf et al., 2022a), with bacteria such as *Vibrio* (Kirstein et al., 2016; Laverty et al., 2020; Rasool et al., 2021), *Salmonella* spp. (El-Liethy et al., 2020), *Escherichia coli* and intestinal enterococci (Rodrigues et al., 2019; Metcalf et al., 2022b) known to associate with plastics in the

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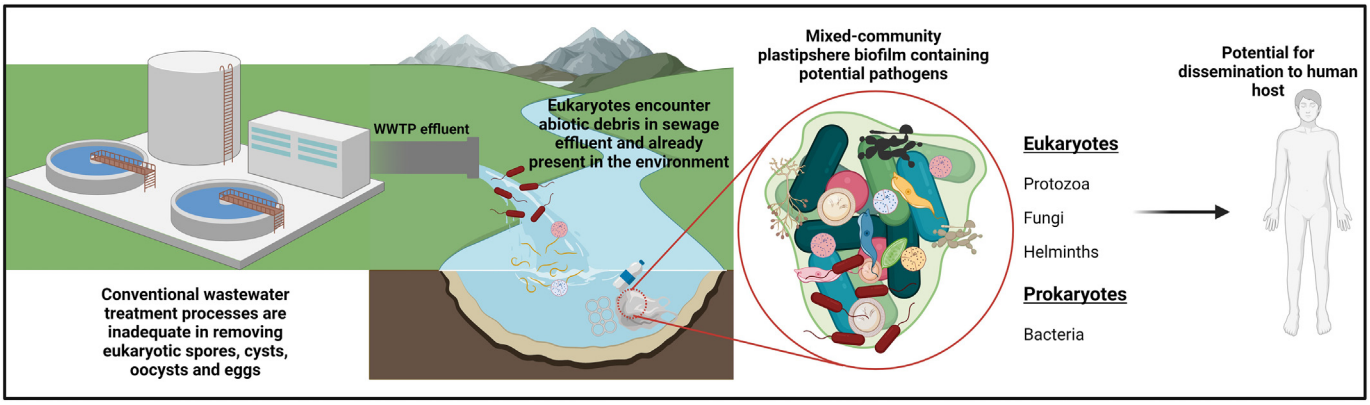

Fig. 1. In the environment pathogenic microorganisms can associate with plastic debris and increase the potential for human interaction. Organisms with pathogenic potential can enter the environment in multiple ways, including in WWTP effluent. There is potential for both prokaryotic organisms such as bacteria; and eukaryotic organisms including protozoa, fungi and helminths to associate with plastic debris and be disseminated within the environment with an increased risk of exposure for humans.

environment and present a potential risk to human health. Human pathogenic viruses can also adhere to plastic debris and bind to the plastisphere (Moresco et al., 2021), with rotavirus known to retain its virulence as demonstrated through cell culture and plaque assays (Moresco et al., 2022).

While metagenomics and taxonomic studies are commonly used for identification, culture-based methods and microscopy have also been employed allowing for a greater understanding of the association between bacterial pathogens and different plastic types (Wright et al., 2020). Importantly however, these studies often lack information on the retention of virulence of plastisphere-associated bacterial pathogens, and their ability to disseminate to the human host (Beloe et al., 2022).

Eukaryotes are ubiquitous in natural environments such as soil and freshwater and represent some of the most important disease-causing agents worldwide. Opportunistic fungal pathogens including members of the genera *Candida*, *Aspergillus*, *Cryptococcus*, and *Pneumocystis* are global in distribution (Firacative, 2020); human pathogenic protozoan parasites such as *Toxoplasma*, *Cryptosporidium* and *Giardia* can survive in both aquatic and terrestrial environments (Fayer et al., 2004); and helminths such as nematodes, trematodes and cestodes produce eggs that can remain viable in the environment for several years (Brooker et al., 2006). Despite the recognition that eukaryotic pathogens can often survive conventional wastewater treatments and persist outside the host under harsh environmental conditions (e.g., extremes in temperature, pH, salinity, UV irradiation) (Chahal et al., 2016; Guadagnini et al., 2013) (Fig. 1), research identifying pathogenic eukaryotes associated with the plastisphere has been limited to large scale metagenomic studies or in vitro experiments that attempt to replicate environmental settings (Gkoutselis et al., 2021; Zhang et al., 2022). High-throughput sequencing studies reveal that eukaryotic reads often dominate the plastisphere community (Bryant et al., 2016), being much more abundant than the more well-studied prokaryotes.

Table 1
Selected examples of eukaryotic pathogen interaction with different plastic polymers in nosocomial/clinical settings, and in environmental studies.

	Pathogen	Environmental form	Mode of infection	Interaction with plastic polymers[a]			
				Nosocomial/lab based	References	Environmental	References
Fungi	*Aspergillus* spp.	Hyphal/Conidia	Skin contact; Ingestion (aflatoxin); Respiratory	PO; PE; PU	(Neely and Orloff, 2001)	PE	(Zahra et al., 2010)
				60%Cotton-40%PE	(Koca et al., 2012)	PE; PA; PU; PP; PS; CA	(Lacerda et al., 2020)
	Candida spp.	Yeast cell	Skin contact; Ingestion	PO; PE; PU	(Neely and Orloff, 2001)	HDPE	(Oliveira et al., 2022)
				PVC acrylic alloy	(Welsh et al., 2017)	LLDPE; PA	(Wallbank et al., 2022)
				PS	(Falanga et al., 2022)	PET; PE	(Marsay et al., 2022)
				60%Cotton-40%PE	(Koca et al., 2012)		
	Cryptococcus spp.	Encapsulated yeast cell	Respiratory	60%Cotton-40%PE	(Koca et al., 2012)	Mixed polymers	(Gkoutselis et al., 2021)
				PS	(Korem et al., 2017)		
				PS	(Yang et al., 2022)		
	Fusarium spp.	Filamentous	Skin contact; Ingestion (mycotoxins)	PO; PE; PU	(Neely and Orloff, 2001)	PE	(Zahra et al., 2010)
				PS	(Falanga et al., 2022)		
Protozoa	*Cryptosporidium* spp.	Oocyst	Ingestion			PO; PE	(Zhang et al., 2022)
						PC	(Helmi et al., 2008)
						PVC	(Skraber et al., 2007)
	Giardia spp.	Cyst	Ingestion	Mulitple polymeric substances	(Pickering et al., 2012)	PO; PE	(Zhang et al., 2022)
						PC	(Helmi et al., 2008)
	Toxoplasma gondii	Oocyst	Ingestion			PO; PE	(Zhang et al., 2022)
Helminths	*Ascaris* sp.	Egg	Ingestion	PS	(Capizzi-Banas et al., 2002)	–	–
	Trichuris trichuria	Egg	Ingestion	–	–	–	–
	Ancylostoma sp.	Egg	Skin contact	–	–	–	–
	Necator sp.	Egg	Skin contact	–	–	–	–

[a] Polyethene, PE; Polyurethane, PU; Polypropylene, PP; Polystyrene, PS; Polyester, PO; Polyamide, PA; Polyvinylchloride, PVC; Polycarbonate, PC; Cellulose acetate, CA.

Sequencing of eukaryotic rRNA has shown that diatoms, dinoflagellates, red, green, and brown algae, parasitic ciliates and apicomplexans are also present in the plastisphere (Dudek et al., 2020), with microplastic-associated dinoflagellates associated with harmful algal blooms (HABs) (Maso et al., 2003; Kettner et al., 2019). However, there is a general lack of understanding of whether environmental plastic pollution could facilitate the survival, persistence, and dissemination of eukaryotic human pathogens. Therefore, in this paper we address this knowledge gap by critically reviewing the currently available information on the potential for fungal, protozoan, and helminth pathogens to associate with the plastisphere, and pose a risk to human health.

2. Fungi

Fungal infections account for approximately 1.7 million deaths per year. This is considerably more than from diseases such as tuberculosis (1.5 million deaths per year) or malaria (405,000 deaths per year) (Bongomin et al., 2017; Kainz et al., 2020). Furthermore, over one billion people are affected each year by fungal infections, of which >150 million cases are classified as severe or life-threatening. Studies on the interactions between fungi and abiotic surfaces have historically focused on nosocomial settings, with pathogenic fungal species such as *Aspergillus*, *Candida* and *Fusarium* known to form biofilms and persist on surfaces of medical, industrial, and household appliances (Wirth and Goldani, 2012; Vethaak and Leslie, 2016; Neu et al., 2018) (Table 1). In the environment, most opportunistic fungal pathogens are naturally saprophytic organisms; however, they can become opportunistic human pathogens particularly when the immune system of the host is compromised (Rokas, 2022). Despite the ubiquity of fungi in the environment, their ability to grow on a wide variety of natural substrates, and their significant potential to cause disease, research on human pathogenic fungi in the plastisphere of environmental plastic debris has received relatively little attention.

Studies of fungi in the plastisphere have so far been limited to taxonomic characterisation through DNA metabarcoding and examination of fungal succession (Kettner et al., 2017; de Tender et al., 2017; Lacerda et al., 2020), with several studies identifying fungal pathogens in the plastisphere using 18S ribosomal RNA (rRNA) sequencing (Oberbeckmann et al., 2016; Kirstein et al., 2018; Kettner et al., 2019). The limitations of using 18S rRNA sequencing are well documented, as the taxonomic resolution for some fungal groups is relatively limited (Stoeck et al., 2010; Richards et al., 2012*)*. *S*equencing of the internal transcribed spacer (ITS) region of nuclear DNA is more commonly employed to analyse fungal diversity in environmental samples and has been used to identify pathogenic fungal species such as *Cladosporium* and *Rhodotorula* on plastic debris in freshwater (Xue et al., 2021), and *Candida* and *Cladosporium* in biofilms on nylon-6 (PA) and linear low-density polyethylene (LLDPE) in the marine environment (Wallbank et al., 2022). To date, only two high throughput sequencing studies have simultaneously analysed prokaryotes and eukaryotes on the same environmental plastic sample (Bryant et al., 2016; Debroas et al., 2017). In an environmental setting, single species biofilms are highly unlikely, and so understanding the interactions and associations between different groups of organisms in the plastisphere, including pathogenic prokaryotic and eukaryotic species (such as fungi), would provide important information as to how they influence each-others survival and virulence. For example, in vitro studies have shown that interactions between an opportunistic fungal species (*Cryptococcus neoformans*) and the soil bacterium *Acinetobacter baumannii*, resulted in the fungus producing more robust biofilms, a thicker capsule and releasing more capsular polysaccharide; consequently, the pathogenicity of this opportunistic human pathogen could become enhanced (Abdulkareem et al., 2015). Furthermore, free living amoeba (FLA) in biofilms can promote the survival and proliferation of pathogenic fungi in hospital and domestic water supply systems (Vanessa et al., 2012; Hubert et al., 2021). Therefore, although FLA have not yet been found associated with the plastisphere, there is potential for FLA to facilitate the persistence of pathogenic microorganism in biofilms colonising the surfaces of plastic debris in the environment.

Fungal species are well adapted for life in the plastisphere due to their adsorptive nutrition mode, apical and invasive growth forms, melanisation, thigmotropism, and their ability to form and associate with established biofilms (Kumamoto, 2008; Harding et al., 2009; Harms et al., 2011; Gkoutselis et al., 2021). Microscopy has demonstrated that microbial eukaryotes are well-represented on the surface of environmental plastic debris (Carson et al., 2013), and scanning electron microscopy (SEM) has shown that potentially pathogenic fungal species are readily able to colonise microplastics (Gkoutselis et al., 2021). The mechanisms through which pathogenic fungi bind to either plastics or established plastisphere biofilms are complex and yet to be fully elucidated. But as fungal propagules, i.e., vegetative and reproductive hyphae, and asexual fungal spores, are all present in large clusters and mats in plastisphere biofilms (Gkoutselis et al., 2021) it is likely that pathogenic fungi utilise a secreted polymer matrix for adherence to plastics in the environment. In common with bacteria, microalgae, cyanobacteria (Parikh and Madamwar, 2006; Boonchai et al., 2015) and protists (Jain et al., 2005; Lee Chang et al., 2014), pathogenic fungi secrete exopolysaccharides (EPS) which play important structural and functional roles in the development and maintenance of microbial biofilms (Hwang et al., 2004; Elisashvili et al., 2009). The filamentous hyphae of the medically significant pathogenic fungus *Aspergillus fumigatus* grows embedded within an EPS, which mediates adherence to inorganic substrates (Mowat et al., 2007; Loussert et al., 2010). Understanding whether environmental plastic debris encourages the secretion of EPS to aid in fungal adhesion would provide key information on how fungi associate with plastics and the plastisphere.

Melanins are natural pigments synthesized by members of all biological kingdoms, including fungi, bacteria, and helminths (Nosanchuk and Casadevall, 2003). While melanin is known to play important roles in fungal pathogenesis through alteration of host cytokine responses, decreasing phagocytosis, and reducing the toxicity of microbial peptides, it also has a significant function in fungal cell wall mechanical strength (Gómez and Nosanchuk, 2003; Nosanchuk and Casadevall, 2006; Nosanchuk et al., 2015) In addition, melanin can aid in adhesion to surfaces, and provide a physical protective barrier (Pouliot et al., 2005). The fungal pathogen *C. neoformans* undergoes melanisation in response to nutrient starvation; melanisation can also protect fungi against environmental stressors including temperature, antimicrobial compounds, and ionizing radiation (Cordero et al., 2020). Most fungal species that are associated with plastics are melanised (Gkoutselis et al., 2021), which may facilitate survival capabilities and virulence of pathogenic fungi once associated with the plastisphere (and could even be responsible for triggering the transition from saprophyte to opportunistic fungal pathogen).

Pathogenic fungi have many well-defined adhesion strategies (Weig et al., 2004; de Groot et al., 2008) that are recognised as major virulence factors (Calderone and Fonzi, 2001; Sundstrom, 2002; Verstrepen and Klis, 2006). Most known fungal adhesins are GPI-modified cell wall proteins, with the best described adhesins being from the agglutinin-like sequence (ALS) family, which encodes cell-surface glycoproteins involved in adhesion of fungal cells to host and abiotic surfaces (Hoyer and Cota, 2016), including polypropylene, polyvinyl chloride, polystyrene, and borosilicate glass (Aoki et al., 2012; de Groot et al., 2013; Demuyser et al., 2014). Hydrophobicity is key in microbe-plastic adhesion, as the hydrophobic nature of plastics stimulates biofilm formation and allows the establishment of a succession of prokaryotic and eukaryotic micro- and macro-organisms (Oberbeckmann et al., 2014; Reisser et al., 2014). It is thought that the hydrophobicity of ALS proteins is important in influencing ALS-mediated attachment to abiotic surfaces (Frank et al., 2010; Garcia et al., 2011).

Solid hydrophobic surfaces, such as polystyrene films, are known to induce morphological differentiation and formation of invasive structures in pathogenic fungi, including *Aspergillus fumigatus*, *A. terreus* and *Fusarium solani* (Kumamoto, 2008). Altering morphology in response to stress is a key strategy used by pathogenic fungi to cope with different conditions. The dimorphic fungus *Histoplasma capsulatum for example,* grows as a filamentous mould at ambient temperature, and switches to a yeast form at

elevated host temperature (Maresca and Kobayashi, 1989); whereas at ambient temperatures, *Candida albicans* favours the yeast form, and elevated temperature induces the filamentous growth (Gow et al., 2002). Pathogenic fungal species bound to abiotic surfaces in the environment will likely traverse many different environmental matrices (e.g., terrestrial-freshwater-marine), each providing different abiotic challenges. The morphological changes induced by the stress of these conditions may affect numerous phenotypic characteristics, including the ability of fungi to adhere to, or be released from the plastisphere. Furthermore, the specific influence of different plastic polymers on fungal morphology and virulence has not yet been elucidated and may provide important information on fungal survival, dissemination, and virulence strategies.

3. Protozoa

Major protozoan enteric parasites such as *Toxoplasma* spp. (causing toxoplasmosis), *Cryptosporidium* spp. (causing cryptosporidiosis), and *Giardia* spp. (causing giardiasis) can survive in both aquatic and terrestrial environments and infect a wide range of hosts in different ecological niches (Fayer et al., 2004). Toxoplasma is estimated to infect between 30 and 90 % of the population in Central America, South America, and Europe (Dubey and Jones, 2008; Minbaeva et al., 2013; Wilking et al., 2016); *Cryptosporidium* is a leading cause of diarrheal disease worldwide with young children highly susceptible to infection (Khalil et al., 2018); and *Giardia duodenalis* (one of six morphologically distinct species of *Giardia* to infect humans) results in approximately 4000 laboratory-confirmed giardiasis cases each year in the UK and 2.8×10^8 cases worldwide (Horton et al., 2019). Yet, studies identifying protozoan pathogens on plastic waste in the environment are substantially fewer than for bacteria or even fungi.

Transmission of terrestrial protozoa to humans is entirely dependent on factors that affect their transport and survival and mostly occurs through drinking contaminated water, consuming contaminated foods, or via the faecal-oral route (Tenter et al., 2000). Pathogenic protozoa can survive adverse environmental conditions including nutrient depletion, increased osmotic pressure, temperature changes, low pH, and desiccation (King and Monis, 2007). Their survival is mainly due to the production of thick-walled organelles, i.e., cysts (Giardia) or oocysts (*Cryptosporidium* and *Toxoplasma*), which allow them to remain viable in soil and water for years (Dumètre et al., 2013; Omarova et al., 2018; Pumipuntu and Piratae, 2018). Wastewater treatment strategies vary between countries, with some treating their waste more thoroughly to remove biological contaminants than others (Pauwels and Verstraete, 2006; Carraro et al., 2016; Yan et al., 2020). Developing countries often do not have the infrastructure for pre-treatment of waste and so raw wastewater discharge directly into the environment is common (Laffite et al., 2016; Al Aukidy et al., 2018). This means that the risk of contamination of water courses by *Cryptosporidium* and *Giardia* can be considerable, with cysts and oocysts frequently found in discharge from wastewater treatment works (Cacciò et al., 2003; Robertson et al., 2006); although *T. gondii* oocysts are isolated from wastewater less commonly (Sotiriadou and Karanis, 2008; Gallas-Lindemann et al., 2013). Whether this translates into an enhanced ability to survive in the plastisphere has never before been examined.

Under laboratory conditions, *Cryptosporidium* oocysts and *Giardia* cysts can adhere to, and survive on, abiotic surfaces such as brushed stainless steel, Formica® laminate, ceramics, and fabrics (Alum et al., 2014). The addition of organic matter can increase their survival on surfaces, which may be due to enhanced aggregation of cysts/oocysts, or physio-chemical changes to the properties of the abiotic surface, or to the surface of the bilayered wall of the cyst (Alum et al., 2014). In freshwater, the surface of the oocyst is hydrophilic, negatively charged, and faintly adhesive, which is thought to be important for dispersal in the environment (Shapiro et al., 2009; Dumètre et al., 2012; Dumètre et al., 2013); while in estuarine or marine waters, the oocyst surface becomes neutrally charged, enhancing the interactions between marine biofilms and algae (Shapiro et al., 2014), with evidence that *Cryptosporidium* oocysts readily incorporate into biofilms (Lefebvre et al., 2021) In contrast, it has been suggested that the negatively charged surface of the *T. gondii* oocyst may be an evolutionary strategy to prevent its aggregation with other particles in a bid to aid with dispersal in fresh water (Shapiro et al., 2014).

Cryptosporidium oocysts can readily adhere to polymicrobial biofilms on polyvinylchloride (PVC) coupons submerged in wastewater (Skraber et al., 2007), adhere to and integrate with established river water biofilms (Wolyniak et al., 2009), and attach and detach from biofilms on polycarbonate coupons (Helmi et al., 2008). Under in vitro settings *T. gondii, C. parvum,* and *G. enterica* cysts/oocysts can bind to established biofilms on polyethylene microbeads and polyester microfibers (Zhang et al., 2022). While providing preliminary evidence that these pathogens can associate with plastisphere biofilms, these limited studies only demonstrate a superficial association with the plastisphere due to the experimental parameters used, with oocysts and cysts adhering to the surface of the established biofilm rather than being incorporated within the true biofilm matrix itself. It is likely that cysts and oocysts could attach to the biofilm surface and become embedded within the biofilm matrix, although it remains unknown if this could happen under realistic environmental conditions. A stronger association with biofilm would potentially enhance protozoan survival due to the protective environment provided by the plastisphere community, but could also increase the potential for widescale dissemination and transport throughout the landscape with greater opportunities for exposure to animal or human hosts.

The identification of protozoa within the plastisphere is likely to be hindered by the lack of sensitivity of current methods. As few as 10 oocysts of *Cryptosporidium* or *G. duodenalis* can cause infection in humans (Teunis et al., 2002) (Erickson and Ortega, 2006), while a single oocyst of *T. gondii* can cause infection in rodent models (Dubey, 2016). With current methodologies it is difficult to detect such low concentrations of protozoa in environmental samples. For example, extracting sufficient nucleic acid from cysts/oocysts for PCR or qPCR when the pool of available nucleic acid is heavily diluted with DNA from other eukaryotic species, such as fungi, and where organic inhibitors of PCR, such as humic acid, will be common, the process of identifying and quantifying protozoan pathogens in the plastisphere will be challenging (Hawash, 2014; Sidstedt et al., 2020). More sensitive methods, including immunofluorescence assays (IFA), nested PCR, and loop mediated isothermal amplification (LAMP), have been employed to identify *Giardia* and *Cryptosporidium* in wastewater (Gallas-Lindemann et al., 2016). However, these approaches are likely to lead to overestimations of viable cysts/oocysts in the plastisphere, and measurements of gene transcription would be needed to quantify pathogen viability in this environmental niche (Fradette et al., 2022).

4. Helminths

Soil-transmitted helminths infect >1.5 billion people globally (WHO, 2022), with the most common helminthiases caused by infection with the intestinal helminths, *Ascaris* (roundworm), *Trichuris* (whipworm), *Ancylostoma* and *Necator* (hookworm). Infections are widely distributed in tropical and subtropical areas, and particularly affect those residing in low- and middle-income countries (LMIC) with poor environmental sanitation and high levels of water pollution (Ziegelbauer et al., 2012). Helminth infections arise from contact with faecally-contaminated soils, consumption of unwashed or raw foods, or contact with contaminated water sources (Amoah et al., 2018). Helminth eggs are highly infectious, with a single egg being sufficient to cause infection (Jiménez et al., 2017); and are extremely persistent in the environment, e.g., eggs of *Ascaris* spp. can survive for 20 days at temperatures as low as −27 °C and have been recovered from frozen soils after 10 years (Jiménez et al., 2017). In developing countries, concentrations of helminth eggs in wastewater (16,000 eggs/L) and sludge (up to 23,000 eggs/g) (Amoah et al., 2018) often exceed limits set by the WHO guidelines for wastewater/sludge reuse (≤1 egg/L) (WHO, 2006).

Despite the known ability for helminth eggs to persist in the environment for long periods of time and their well-described ability to survive traditional decontamination processes, no studies have attempted to identify helminths (or their eggs) in the plastisphere (Table 1). There is evidence

that helminth eggs can readily adhere to soil particles, resulting in their persistence in these environments (Landa-Cansigno et al., 2013) but in order to further advance our understanding of the environmental dissemination pathways of these pathogens, more information is needed on the association between helminths and environmental plastic pollution. Structurally, helminth eggs have many similar characteristics to protozoan cysts and oocysts (multi-layered, protein and sugar coated, and chitinous), and such adhesive properties could facilitate the association of helminth eggs with plastics in the environment. Helminth eggs adhere more readily to soil when there is an abundance of siliceous surfaces such as those found in sandy and clay-filled soils, although the hydrophobic surface of the helminth egg may result from its association with the organic component of the soil (Capizzi and Schwartzbrod, 2001). There is evidence that Ascaris eggs can adhere to polystyrene microspheres through hydrophobic interactions (Capizzi-Banas et al., 2002). Although conducted under controlled in vitro settings, this study does highlight the potential for helminths to associate with the plastisphere (Jiménez et al., 2017). Nothing is known about the interaction between helminths and prokaryotes in the plastisphere; however, some helminths have bactericidal properties, with components of the pseudocoelomatic fluid of the porcine intestinal nematode *Ascaris suum*, inhibiting the growth of *Bacillus megaterium* and *Staphylococcus aureus* (Wardlaw et al., 1994); and compounds secreted by *A. suum* (lectins, cystatin, and members of the antibacterial factor and cecropin AMP families) can inhibit biofilm formation of *Escherichia coli* (Midha et al., 2018). Therefore, if helminths (or eggs) did become associated with plastisphere biofilms, they have the potential to alter the community composition, including the presence and persistence of bacterial pathogens. There remain many unanswered questions about the potential for helminths to associate with plastic debris in the environment, and whether this could increase their survival and transfer.

5. Future perspectives

While several studies have identified eukaryotic pathogens in the plastisphere, this has often relied on metagenomic approaches. Future work must include a more in-depth characterisation of the plastisphere and the ability of fungal, protozoan, and helminth pathogens to traverse environmental matrices and the points at which they pose a human health risk. Central to this, is establishing the pathogenic potential of eukaryotic pathogens in the plastisphere, which is particularly significant as simply identifying whether a pathogen is present in the plastisphere is not enough to infer a human health risk (Beloe et al., 2022). While there has been some success in identifying the presence of prokaryotic virulence genes in the plastisphere (Kirstein et al., 2016; Silva et al., 2019) the resolution of amplicon sequencing data is often insufficient to determine pathogenesis as virulence factors are commonly found on mobile genetic elements (Sakib et al., 2018). Furthermore, gene presence does not always translate into a transcribed and functional protein (e.g., DNA from VBNC or eDNA released from dead cells can also give positive identification of virulence genes that do not pose a direct threat to public health) (Wolffs et al., 2005; Li et al., 2013). This should be taken into consideration when examining the presence of eukaryotic virulence genes in future plastisphere studies. Similar difficulties in identification of virulence potential will likely be apparent in the study of eukaryotic pathogens in the plastisphere. To overcome this, following recovery of eukaryotic pathogens from the plastisphere, future studies could employ models such as in vivo challenge or challenge of in vitro cell lines to establish if eukaryotic pathogens adhering to environmental abiotic debris present a risk to human health. Recent work has utilised a *Galleria mellonella* model of infection to indicate retention of pathogenicity of prokaryotic pathogens following their recovery from the plastisphere (Ormsby et al., in press). Furthermore, the use of RNA sequencing could allow for the identification of virulence-protein encoding RNA transcripts.

With many pathogens known to survive primary, secondary, and in some instances, tertiary wastewater treatment (Mbanga et al., 2020), there is increasing risk of microbial pathogens entering the environment. With increasing reports of plastic pollution and contaminated plastic waste being found in locations where human exposure can be high, there is a heightened potential for the spread of disease to the human population. Recent plastisphere research has focused on identifying which genera are present in the plastisphere, but few identify and confirm the virulence potential of these organisms (Beloe et al., 2022). Quantifying whether eukaryotic pathogens can persist in the plastisphere, and crucially, understanding their ability to retain virulence is a neglected environmental and public health issue, which requires urgent attention.

CRediT authorship contribution statement

MJO, **RSQ**: Conceptualisation; **MJO**: Writing - original draft preparation; **RSQ**: Supervision; **MJO**, **AA**, **RSQ**: Writing, reviewing, and editing; **RSQ**: Funding acquisition.

Data availability

No data was used for the research described in the article.

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.

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