Environmental Science & Technology

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Perspective

Waterborne Human Pathogenic Viruses in Complex Microbial Communities: Environmental Implication on Virus Infectivity, Persistence, and Disinfection

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Cite This: https://doi.org/10.1021/acs.est.2c00233

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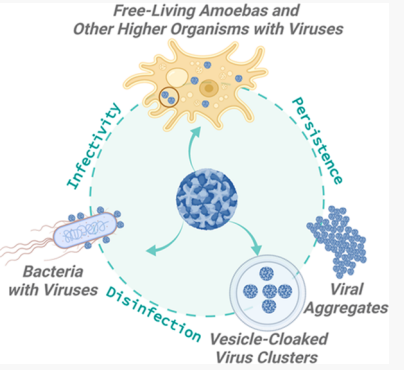
ABSTRACT: Waterborne human pathogenic viruses challenge global health and economy. Viruses were long believed to transmit among hosts as individual, free particles. However, recent evidence indicates that viruses also transmit in populations, so-called en bloc transmission, by either interacting with coexisting bacteria, free-living amoebas, and other higher organisms through endosymbiosis and surface binding, or by being clustered inside membrane-bound vesicles or simply self-aggregating with themselves. En bloc transmission of viruses and virus−microbiome interactions could enable viruses to enhance their infectivity, increase environmental persistence, and resist inactivation from disinfection. Overlooking this type of transmission and virus−microbiome interactions may underestimate the environmental and public health risks of the viruses. We herein provide a critical perspective on waterborne human pathogenic viruses in complex microbial communities to elucidate the environmental implication of virus−microbiome interactions on virus infectivity, persistence, and disinfection. This perspective also provides insights on advancing disinfection and sanitation guidelines and regulations to protect the public health.



KEYWORDS: *waterborne human pathogenic virus, amoeba, bacteria, vesicle-cloaked virus clusters, environmental persistence, disinfection*

INTRODUCTION

Human pathogenic viruses are a global burden for both public health and the economy. People can get viral infection through direct contact, body fluids, insect vectors, and environment-mediated transmission such as contaminated water, food, air, and fomites. Viruses in the stool, saliva, and/or respiratory droplets and aerosols of infected people are normally present with a high load that can easily contaminate the environment. While usually only a low virus dose is needed to infect humans, they can be resistant to environmental stressors and even disinfectants, all of which facilitate virus transmission and infection.[1,2] Particularly, virus survival in different environmental conditions, for example, temperature, humidity, pH, organic loads, salinity, sunlight, and disinfectants, has been extensively investigated,[3−11] leading to the advancement of engineering interventions for controlling the spread of infectious diseases.

While most studies consider viruses as isolated and individual particles during transmission and infection, the interactions between human pathogenic viruses and their microbial neighbors such as higher organisms like free-living amoebas (FLAs), bacteria, and viruses have not been recognized until recent years. Viruses are never alone in the environment; instead, they are an essential part of complex microbial communities. Viruses can interact with other microbial community members through endosymbiosis, surface binding, clustering, and self-aggregation (Figures 1a−c). Water is a complex environment containing many coexisting microbes, and the interactions of waterborne human pathogenic viruses and microbiome have significant environmental and public health impacts. This is because viruses in microbial communities could show enhanced infectivity, environmental persistence, and resistance to disinfection, due to the en bloc transmission of the viruses and unique virus−microbiome interactions.[12−17] Though waterborne human pathogenic viruses do not propagate in their microbial neighbors, virus−microbiome interactions can gather multiple virions for collective infection and potentially provide multiple benefits to the viruses such as increasing the multiplicity of infection (MOI), facilitating genome recombination and reassortment, and evading host immune systems (Figure 1d). Viruses surrounded by their microbial neighbors (e.g.,

Received: January 11, 2022

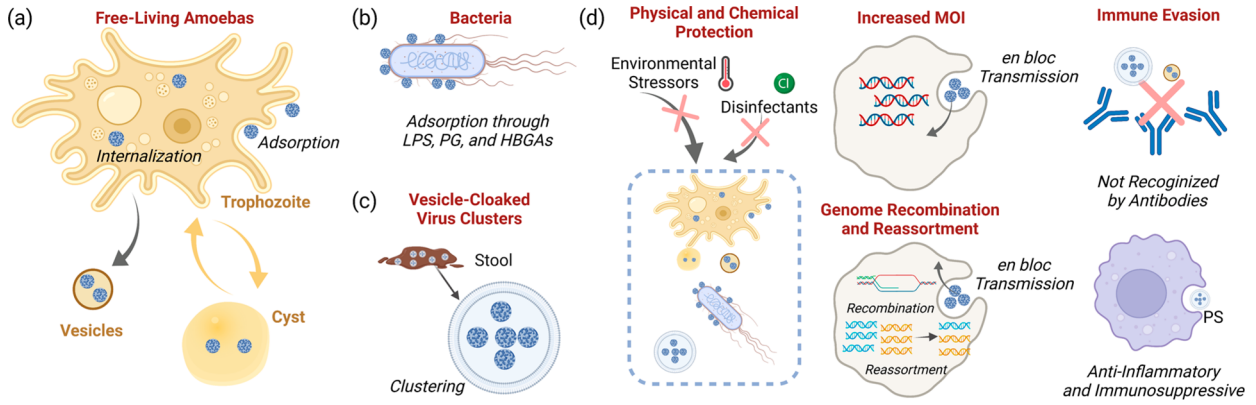


Figure 1. Waterborne human pathogenic viruses interact with (a) free-living amoebas (FLAs), (b) bacteria, and (c) viruses as vesicle-cloaked virus clusters in the environment. FLAs adsorb or internalize viruses, and the viruses could be present in the whole lifecycle of FLAs including the trophozoite and the cyst. FLAs also release vesicles that contain viruses. Viruses bind to bacteria by interacting with bacterial lipopolysaccharides (LPS), peptidoglycan (PG), and histo-blood group antigens (HBGAs). Vesicle-cloaked virus clusters presenting in the human stool of infected hosts are phospholipid-bilayer encapsulated fluid sacs that contain multiple virions or multiple copies of naked viral genomes. (d) Mechanisms of viruses interacting with microbiome to promote their environmental persistence, infectivity, and resistance to disinfection. Physical and chemical protection by the neighboring microbiome, increased multiplicity of infection (MOI), genome recombination and reassortment among the viruses, and immune evasion by the vesicles can all be responsible for the increased persistence, infectivity, and disinfection resistance of the viruses. PS is phosphatidylserine that is enriched on the outer leaflet of the viral vesicle membrane.

internalized within FLAs, bound with bacteria, and cloaked within extracellular vesicles) can also dodge the damage caused by environmental stressors and disinfectants (Figure 1d). Similar to waterborne viruses, respiratory viruses also show enhanced stability and airborne transmission by interacting with their microbial neighbors.[18,19] Without acknowledging viruses as a cohesive component of complex microbial communities, we could easily underestimate the environmental risks of the viruses yet overestimate the efficacy of disinfection and sanitation.[20−23] This perspective aims to highlight the impact of the interactions of waterborne human pathogenic viruses and their microbial neighbors on virus infectivity, environmental persistence, and resistance to disinfection, and guide engineering design and regulations for developing reliable and robust disinfection and sanitation practices to protect the public health.

INTERACTIONS OF VIRUSES AND FLAS AND OTHER HIGHER ORGANISMS

FLAs are primary predators of many microorganisms. They are widely present in vegetables, soils, natural and engineering water systems, air conditioning systems, and other habitats, and they are resistant to many disinfectants.[24−26] Recently, they have been recognized as a reservoir and vector for human pathogenic viruses in water, as well as by providing protection for the viruses, beyond their well-known roles in promoting the survival and dispersion of bacterial pathogens such as *Legionella pneumophila*.[27−30] Virus-like particles were first reported to be present in protozoa in the 1960s,[31−35] but only in the recent decade have researchers revealed the interactions between FLAs and human pathogenic viruses in aqueous environments. Adenovirus,[36−39] coxsackievirus,[21,40,41] norovirus,[42] rotavirus,[41] and reovirus[20] have been found to associate with *Acanthamoeba* spp., *Vermamoeba vermiformis* (*V. vermiformis*), and *Willaertia magna* (*W. magna*), the most common FLAs in water systems. Particularly, *Acanthamoeba* spp. are the most widely studied FLAs to host viruses.[12] FLAs could harbor viruses through adsorption and internalization (e.g., engulfment or ingestion) to promote virus persistence.

The presence of human adenovirus in *Acanthamoeba* spp. isolates from natural water was first reported in 2007.[36] Besides natural water, human adenovirus was identified from *Acanthamoeba* spp. isolates in recreational waters like swimming pools, with a concentration of up to 5.14×10^5 gene copies per mL of the FLA isolate.[37] There is always a debate on how viruses are associated with FLAs, either through surface adsorption or internalization. Surface adsorption is a prerequisite for virus internalization into FLAs, and early studies suggested poliovirus and echovirus only adsorbed on but were not internalized into *Acanthamoeba castellanii* (*A. castellanii*).[40,43] Other studies also did not observe the internalization of freely suspended human adenovirus, coxsackievirus, and human rotavirus into *A. castellanii* and *Acanthamoeba polyphaga* (*A. polyphaga*), but virus-infected mammalian cells could be ingested by FLAs.[39,41] This is probably because FLAs usually have a preferred prey size of ca. 0.8−1.2 μm, not allowing the internalization of small free viruses.[41] In contrast, freely suspended human reovirus was internalized into FLAs with virions accumulated within the nucleus, and the FLAs did not actively seek out free virions as food.[20] Human adenovirus was internalized into the food vacuole or the cytoplasm of *Acanthamoeba* spp., most probably via phagocytosis, while whether viruses were cell-associated or freely suspended remains an open question.[38] Other studies also confirmed that freely suspended coxsackievirus and murine norovirus (MNV, a human norovirus surrogate) could be internalized into *A. castellanii* and *V. vermiformis*.[21,42] Whether human pathogenic viruses only adsorb on FLAs or they can also be internalized into the FLAs could depend on the specific species or strains of the viruses and the FLAs. It might also be possible that viruses are phagocytized into FLAs but then released from the FLAs shortly after. The discrepancy shown in the studies of virus−FLA interactions could be the result from artifacts in the experimental design and observation. Future studies should explore whether virus adsorption on organic compounds or particles can facilitate their internalization into FLAs and how viruses retain in or pass through FLAs in complex aquatic environments.

https://doi.org/10.1021/acs.est.2c00233
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Advanced imaging tools including immunofluorescence microscopy, transmission electron microscopy, and immunoelectron microscopy should be used to clearly demonstrate the spatial relationship between waterborne human pathogenic viruses and FLAs and understand the mechanism of their interactions.

Once internalized, infectious viruses can persist along the whole lifecycle of FLAs, from the trophozoite to the cyst (Figure 1a). Infectious coxsackievirus persisted in all life stages of *V. vermiformis*, without causing any injury to the FLA.[21] The internalized human reovirus remained infectious for at least 4 days in *V. vermiformis*, *A. polyphaga*, and *W. magna*.[20] MNV survived and stayed infectious within the whole lifecycle of *A. castellanii* for 28 days, while no infectious MNV was detected after 4 days in *A. polyphaga*.[42] These results indicate that the persistence and viability of viruses in FLAs could also be specific to the species and strains of both microorganisms. Bacteriophage Phi6, a surrogate of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was also internalized in *V. vermiformis* and remained infectious after 2 months within FLA cysts.[19]

FLAs could also protect waterborne human pathogenic viruses from inactivation caused by environmental stressors and disinfectants, possibly through shielding, limiting biocide diffusion, and providing a favorable physiological environment, as how they protect the intra-amoebic bacteria (Figure 1d).[29,44,45] Therefore, FLAs can be excellent vectors to promote virus transmission in aquatic environments. Human adenovirus survived within the cytoplasm of *A. polyphaga* after exposure to 5 mg L^{-1} of sodium hypochlorite for 24 h, while free viruses were completely inactivated under the same treatment (3 $\log_{10}$ reduction).[39] *V. vermiformis*, *A. polyphaga*, and *W. magna* also protected their intracellular human reoviruses from ultraviolet (UV) disinfection, which provided 0.5−1.0 $\log_{10}$ reduction in disinfection efficacy when compared to the virus-only control under the same UV irridation.[20] Researchers have found that internalized bacteria were well-protected by FLA cysts against physical and chemical stressors.[46] The fact that Phi6 was still infectious after 2 months within *V. vermiformis* cysts in distilled water implies FLA cysts might contribute to the resistance of human viruses to disinfection.[19] Moreover, inactivated FLAs can still protect their intracellular bacteria from disinfection by Cl_2, ClO_2, and UV_{254},[29] which could also be applicable for intracellular viruses.

Interestingly, internalized viruses, like internalized bacteria,[47] could be released as clusters within vesicles from FLAs (Figure 1a). High concentrations of infectious coxsackievirus[21] and human respiratory syncytial virus[48] have been reported presenting in extracellular amoebal-vesicles (*V. vermiformis* and *W. magna*, respectively), which suggests new pathways of virus transmission. In addition, viral vesicles released by FLAs might share similar features with vesicle-cloaked virus clusters released from human hosts, which enhance virus infectivity, evade host antibodies, suppress host immune defenses, protect viruses from inactivation by disinfectants, and thus call for urgent health concerns.[17,23,49]

Besides FLAs, waterborne human pathogenic viruses can also interact with other higher organisms including nonamoeba protozoa, nematodes, and zooplankton in both natural and engineering water systems. Ciliates play a dual role in virus−ciliate interactions, activating[50] or protecting internalized viruses,[51] or facilitating virus removal and inactivation in water treatment,[52−56] possibly depending on both ciliate and virus species. Nematodes, which are well-known vectors of human pathogenic bacteria and protect ingested microorganisms from removal and inactivation in water treatment,[57] were also reported transporting and protecting human coxsackievirus and echovirus.[58,59] Filter-feeding zooplankton can uptake waterborne viruses like echovirus through prey−predator interactions, which may either facilitate virus removal and inactivation or act as a vector by transferring infectious viruses to higher trophic levels through the food chain.[53] Future research on the interactions of waterborne human pathogenic viruses and microbiome should broaden the scope from FLAs to other higher organisms, focusing on not only the risks of the microbes facilitating virus persistence and transport but also the benefits of higher organisms promoting virus removal and inactivation for water purification.

INTERACTIONS OF VIRUSES AND BACTERIA

Bacteria could collaborate with waterborne human pathogenic viruses and facilitate virus persistence and infection both in vitro and in vivo. Particularly, bacteria and their cellular components can promote the virus binding, increase the MOI and virus coinfection rate, and protect viruses from environmental stressors and disinfectants (Figure 1d).[14−16,60] Most studies have explored the interaction of bacteria and human enteric viruses and their surrogates, for example, poliovirus,[22,61−63] rotavirus,[64] reovirus,[65] coxsackievirus,[22,66] echovirus,[22] Aichi virus,[66] and MNV and Tulane virus.[67−70] A recent study has also highlighted that respiratory bacteria promoted the stability and airborne transmission of influenza viruses.[18]

Virus binding to bacteria plays a key role in enhancing virus infectivity (Figure 1b). Enteric bacteria enhanced poliovirus infectivity both in vitro and in vivo through the interaction of bacterial lipopolysaccharide (LPS) and peptidoglycan (PG) with polioviruses.[61] LPS stabilized viruses by preventing premature RNA release and enhancing virus binding to host cells to increase virus infectivity.[62] Histo-blood group antigens (HBGAs) have been suggested as receptors or coreceptors for human norovirus,[71] and the presence of enteric bacteria that express HBGAs was a prerequisite for successful human norovirus infection in vitro.[67] Human norovirus-like particles were observed to bind to *Enterobacter* spp. through HBGA-like substances in extracellular polymeric substances,[68] while their binding to Caco-2 and HT-29 cells was inhibited by a non-HBGA-expressing bacteria *Bifidobacterium adolescentis*.[72] However, discrepancy was observed for Tulane virus and Turnip crinkle virus, two human norovirus surrogates, interacting with bacteria, and HBGA-expressing bacteria were found to bind the Tulane virus but not the Turnip crinkle virus, which suggested virus−bacteria interactions might be microbe-type and virus-strain dependent.[70] Enteric bacteria can also act as the scaffold for the en bloc transmission of pathogenic viruses in vitro by binding multiple virions to each bacterium, which enhances the MOI, genetic recombination and reassortment between different virions, and fitness of the viruses (Figure 1d).[63] A variety of enteric bacteria, for example, *Lactobacillus johnsonii*, *Staphylococcus* spp., *Bacteroides acidifaciens*, and *Clostridium symbiosum*, were capable of binding multiple poliovirus particles, adhering to host cells, and thus increased the MOI for virus coinfection.[63] As an RNA virus, poliovirus is prone to mutate because of the lack of proofreading mechanism of the RNA-dependent RNA polymerase during

https://doi.org/10.1021/acs.est.2c00233
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genome replication, and the mutation is usually deleterious for the viruses.[73] Virus coinfection introduces multiple viral genomes into one host cell, which allows genetic recombination or reassortment at the initial stage of infection. Hence, virus variants complement each other's defects, resulting limited abortive infections and enhanced viral fitness.[63] Bacteria-mediated virus coinfection could also promote virus evolution and zoonotic transmission.[16,74]

Bacteria and their cellular compounds could promote the environmental persistence, particularly thermostability, of waterborne human pathogenic viruses. Reovirus showed enhanced thermostability at 23−37 °C for hours by interacting with bacterial PG and LPS in phosphate-buffered saline (PBS).[65] Echovirus, coxsackievirus, and poliovirus also survived at 50 °C for 1 h in PBS buffer after PG and LPS binding.[22] The protection was PG and LPS concentration dependent and virus serotype specific, suggesting that the unique capsid protein sequence determined virus−bacteria interactions.[22] Human norovirus like-particles maintained higher antigen integrity when bound to HBGA-expressing bacteria, in contrast to non-HBGA-expressing bacteria, during heat treatment at 90 °C in PBS buffer.[69] However, Tulane virus was not protected against heat inactivation at 56 °C, even when HBGA-expressing bacteria were present in the experiment solution of PBS buffer.[75]

The virus−bacteria interactions could also protect waterborne human pathogenic viruses from disinfection, which raises public health concerns of infectious disease spreading and outbreaks. Poliovirus, coxsackievirus, Aichi virus, and mengovirus, under the protection of bacteria and extracted bacterial LPS in PBS buffer, maintained their infectivity after exposure to 0.0001% bleach for 1 min.[66] Interestingly, bare viruses without any protection or incubated with bovine serum albumin and cellulose lost at least 1-$\log_{10}$ of their infectivity after the same bleach exposure,[66] indicating that specific bacteria−virus binding rather than bleach consumption by organics might contribute to virus survival. After binding to LPS and PG, enterovirus did not show noticeable infectivity loss upon chlorine disinfection at 3 mg·min·L^{-1}; however, 5.3-$\log_{10}$ inactivation was achieved without the presence of bacterial compounds after the same chlorination.[22] Interestingly, UV_{254} disinfection was equally effective for inactivating enterovirus, regardless of the presence or absence of the bacterial compounds.[22] The results highlighted that viruses interacting with bacteria played a critical role in protecting the viruses from disinfection when the disinfectant targeted the virus capsid but not the genome.[22] Bacterial compounds had a direct interaction with the virus capsid to stabilize the capsid, but they did not bind to the virus genome or shield UV_{254} for protecting the virus from UV_{254} disinfection.

INTERACTIONS BETWEEN VIRUSES

Beyond interacting with FLAs and other higher organisms and bacteria through endosymbiosis and surface binding, waterborne human pathogenic viruses themselves can also form clusters and aggregates that are more infectious, and probably more environmentally persistent and resistant to disinfectants.[17,23,76] In contrast to individual viral particles, vesicle-cloaked virus clusters (here referred to as viral vesicles) are phospholipid-bilayer-encapsulated extracellular vesicles comprising multiple virions (from 1 to 5 to >25) or multiple copies of naked viral genomes (Figure 1c).[17,49] In infected host cells, viral vesicles can be derived from a variety of cellular organelles including plasma membranes, autophagosomes, and multivesicular bodies, and vesicle sizes range from 50 to 100 nm to several hundreds of nanometers, depending on the membrane origin. More interestingly, viral vesicles enable nonenveloped viruses to be released from infected cells nonlytically.[77] A broad spectrum of nonenveloped and enveloped viruses can form viral vesicles, including environmentally transmissible viruses, like enteric viruses (e.g., poliovirus, coxsackievirus, rotavirus, norovirus, hepatitis A and E viruses, enterovirus 71, and JC and BK polyomaviruses), respiratory viruses (e.g., rhinovirus), and bloodborne and arthropod-borne viruses (e.g., hepatitis C virus, Langat virus, West Nile virus, thrombocytopenia syndrome virus, Dengue virus, bluetongue virus, and Zika virus).[17,49] This emerging pathogenic unit of viral vesicles may also be abundant in the environment, for example, rotavirus vesicles contributed to up to 45% of the total rotavirus population in stool[78] that could easily contaminate different environments such as water, food, and contact surfaces.

Viral vesicles are persistent both in vivo and under different environmental stressors, including enzyme digestion, low pH, temperature variation, and detergent treatment. Rotavirus vesicles were resistant to digestive enzymes and low stomach pH, because they remained intact when passing through the gastrointestinal tract of mice.[78] Rotavirus vesicles could also keep their integrity in freshwater and wastewater for months (unpublished data). MNV vesicles did not show noticeable decomposition after 20 cycles of freeze−thaw, and the viral vesicles only partially decomposed after treatment by sodium dodecyl sulfate and nonyl phenoxypolyethoxylethanol.[23] This is in sharp contrast to enveloped viruses, which lose fusion capability with host membranes and become inactivated, after viral envelopes are treated with detergents. The enrichment of cholesterol and sphingomyelin on vesicle membranes, known modulators for membrane fluidity and integrity, may be responsible for the stability of viral vesicles in water, freeze−thaw, low pH, and in the presence of detergents.[78−82] Regardless, the viral vesicle is an emerging pathogenic unit of infection that appears to blur the distinction between nonenveloped viruses and enveloped viruses but behaves like neither.

Viral vesicles facilitate the en bloc transmission of viruses, which enables multiple viral genomes being simultaneously transported to the same host cell and enhances virus infectivity, even after disinfection. Poliovirus,[77] coxsackievirus,[83] rhinovirus,[77] marseillevirus,[84] polyomavirus,[85] rotavirus,[78] and norovirus[23] have all been reported to show enhanced infectivity by transmitting en bloc virions in vesicles rather than as individual viral particles, in buffers and cell culture media through both in vitro and in vivo studies. Importantly, this enhanced infectivity over free viral particles was preserved even with UV_{254}[23] and free chlorine disinfection (unpublished data). Viruses can benefit from en bloc transmission through increased MOI, genome recombination, and reassortment between different virions when multiple virions are delivered to the same host cell, and shielding from immune defenses, all of which enhance the infectivity (Figure 1d).[17,49] Replication barriers are more difficult to overcome when viruses enter cells in low numbers as host defenses are triggered before sufficient numbers of viral proteins and genomes can be made.[49] However, when multiple viruses enter simultaneously, high levels of viral proteins and viral genomes can quickly be achieved to shut down host defenses.[77,78,86] Moreover, the en bloc transmission of viruses

https://doi.org/10.1021/acs.est.2c00233
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within vesicles can allow for genetic recombination and reassortment, and multiple mutated or damaged viral genomes in one host cell cooperate and complement each other's deficiencies, resulting in successful infection.[17,49,63] In addition, virus clusters in the vesicles can evade antibody neutralization by being shielded inside the host membrane.[49,87] The vesicle membrane is enriched with phosphatidylserine,[17,49,77,78] a conserved anti-inflammatory, and immunosuppressive signal.[88] The nonlytical release of viral vesicles from host cells could also prevent the spreading of damage- and pathogen-associated molecular patterns and avoid triggering the inflammatory immune response against viral epitopes.[89] Beyond the en bloc transmission of virions, viral vesicles also enable the codelivery of viral components, viral receptors, and induced or altered host factors, which may hamper antiviral responses, broaden viral tropism, and trigger pro-viral effects.[90−92]

Free viral particles can also form viral aggregates that might be persistent in the environment and resistant to disinfection. Viral aggregates are different from viral vesicles, because they are induced under desired water chemistries and aggregation/disaggregation is a reversible process, their sizes range from a couple virions to thousands of virions, and their complex compositions include cell debris, polyelectrolytes, and solid particles in aquatic environments.[76] The formation and persistence of viral aggregates are well-studied, and readers are welcome to refer to a comprehensive review by Gerba et al.[76]

OUTLOOK

The interactions of waterborne human pathogenic viruses in complex microbial communities have been found able to increase virus infectivity, environmental persistence, and resistance to disinfection. Nevertheless, current disinfection and sanitation guidelines are designed based on the assumption that all viruses are free particles, instead of associated with FLAs and other higher organisms or bacteria, or in the form of viral vesicles or aggregates. It is important to scrutinize whether disinfection and sanitation practices are still effective and robust for inactivating the emerging forms of viruses, preventing the spread of infectious diseases, and protecting the public health. Innovations in disinfection and sanitation also call for urgent attention.

To elucidate the interactions between waterborne human pathogenic viruses and microbial neighbors and assess their environmental, biological, and public health significance, researchers are urged to answer the following open research questions in the future:

(i) What is the presence of waterborne human pathogenic viruses interacting with microbiome in the environment? Past environmental surveillance studies rarely differentiate free viruses from the viruses in other forms. However, more and more evidence has indicated that viruses interact with their microbial neighbors and present together in both natural and engineering water systems. Overlooked virus−microbiome interactions may result in underestimated virus infectivity and overestimated virus removal and disinfection efficacy in water and wastewater treatment. Therefore, it is imperative to understand the contribution of viruses associated with microbial communities to the total viral population in real environments. Future environmental surveillance should fractionate free viruses from viruses associated with FLAs and higher organisms or bacteria, or in vesicles or aggregates. Next-generation sequencing for fractionated virus populations will illustrate the species and strains of microbes in interactions and the intensity of the interactions.

(ii) What is the mechanism of waterborne human pathogenic viruses interacting with microbial communities and how do interactions tailor the environmental and biological behavior of the viruses? Pioneering studies have provided initial insights, but many mechanisms remain elusive. For example, in which cases and why viruses only adsorb to FLAs rather than being internalized? What renders a FLA specie able to internalize viruses and what makes a virus susceptible to FLA predation? What are the conditions for virus storage versus viral vesicles released by FLAs? Beyond LPS, PG, and HBGAs, are there any other bacterial components determining virus binding to bacteria? Advanced microscopic, spectroscopic, and omics tools are required to answer these questions. Moreover, environmental chemistry plays a critical role in determining interactions between viruses and microbiome, for example, virus adsorption to FLAs and other higher organisms and bacteria and virus aggregation could potentially be reversed to release free viruses under a certain environmental condition. The clear definition of environmental matrices and experimental conditions is a prerequisite for understanding virus association with microbiome. Different environmental matrices and experimental conditions, as well as the choice of human pathogenic virus surrogates and experimental models (in vitro versus in vivo), might impact how viruses interacting with their microbial neighbors, result in discrepancies in conclusions, and lead to artifacts in evaluating virus infectivity and virus removal and inactivation efficacies.

(iii) Are current disinfection and sanitation practices safe and robust for inactivating waterborne human pathogenic viruses in diverse forms beyond free viruses? The resistance of FLAs and other higher organisms- and bacteria-associated viruses, viral vesicles, and viral aggregates to disinfection raises serious public health concerns. Disinfection resistance needs to be systematically evaluated for different disinfectants and viruses and prioritized for the viruses that are discovered to be commonly associated with microbial communities in environmental surveillance. Quantitative microbial risk assessment should be adopted for evaluating disinfection performance to improve regulations, where questions like how many virions could be internalized per higher organism like FLA, adsorbed to per bacterium, or cloaked in per vesicle, may be valuable to address. Increasing the dosage of disinfectants could more effectively inactivate the viruses in persistent forms, but it could also produce undesired disinfection byproducts (DBPs). Combing multiple disinfectants or developing new disinfectants could sufficiently inactivate the viruses and limit DBP formation.

(iv) Can we leverage the interactions of waterborne human pathogenic viruses and microbiome to facilitate virus removal and inactivation and improve water quality? Indeed, we have known that viruses can benefit from their interactions in complex microbial communities to

https://doi.org/10.1021/acs.est.2c00233
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enhance their survival and transport in the environment and resistance to disinfection. However, virus control by interacting with microbiome has emerged and attracted attention, for example, higher organisms like ciliates and zooplankton can uptake and remove viruses,[53] bacteria can produce proteolytic enzymes and degrade the viral capsid for inactivating the viruses,[93] and viruses can form aggregates to facilitate physical removal in water treatment like sand and membrane filtration.[94] Future research should elucidate under what environmental conditions virus−microbiome interactions promote virus removal and inactivation.

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Notes

The authors declare no competing financial interest.

Biography

Dr. Danmeng Shuai is an Associate Professor in the Department of Civil and Environmental Engineering at The George Washington University (GW). His research focuses on the development of novel materials and processes for sustainable water purification, renewable energy production, and public health protection. In recent years, he expands his research interests into identifying and understanding emerging environmental pathogens. He is a recipient of the 2018 GW School of Engineering and Applied Sciences Outstanding Junior Researcher Award and the 2019 CAPEES/Nanova Young Investigator Award.

ACKNOWLEDGMENTS

Research reported in this publication was supported by the National Science Foundation under Award Nos. CBET-2028464 and CBET-2028504 and National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award No. R21AI154049. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Science Foundation and National Institutes of Health. We thank National Heart, Lung, and Blood Institute Intramural Research Programs for supporting our work. The table of contents and the figure were created by BioRender.com.

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