



Role of pepper mild mottle virus as a tracking tool for fecal pollution in aquatic environments

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Abstract

The plant pathogen pepper mild mottle virus (PMMoV) has recently been proposed as a water quality indicator, it is a RNA virus belonging to the genus *Tobamovirus* in the family *Virgoviridae* that causes harm to the pepper crops. After consuming processed food products containing infected peppers, such as hot sauces, PMMoV is excreted in high concentrations in feces; therefore, this is the most common RNA virus, constantly found in the feces of humans. The fecal–oral pathway is emerging as an environmental problem. The presence of high concentrations of pathogens associated with human excreta in environmental waters or water reuse supplies poses a threat to public health. Due to the difficulty in determining the presence of pathogens effectively in water, attempts to monitor microbial water quality often use surrogates or indicator organisms that can be easily detected; therefore, PMMoV is used as a viral surrogate in aquatic environment. This paper describes the incidence and persistence of PMMoV in aquatic environments and in waste treatment plants and its usefulness for quantifying virus reductions by advanced water treatment technologies. In recent research, SARS-CoV-2 was reported to be found in wastewater and utilized for the purpose of monitoring coronavirus illness outbreaks. Since PMMoV is readily identified in the human feces and can also serve as an indicator of human waste, the determined PMMoV concentrations may be utilized to give the normalized report of the SARS-CoV-2 concentration, so that, the amount of human waste found in the wastewater can be taken into consideration.

Keywords Pepper mild mottle virus (PMMoV) · Fecal contamination · Water indicator

Introduction

Water contamination due to associated fecal microorganisms of human excreta is a major concern in the health sector. Viruses are considered as etiological agents, which are the reason for many epidemics. The presence of easy-to-detect surrogates or indicator organisms that reflect pathogen persistence is often used in microbial water quality monitoring as it is difficult to directly measure the waterborne pathogens of concern. A toolbox such as Microbial Source Tracking

(MST) is required to identify fecal matter in environments. In many traditional systems, the fecal indicator bacteria (FIB) have been used as microbial surrogates in environmental surveillance systems to classify fecal pollution. For instance, *Escherichia coli* and *Enterococcus faecalis* are the bacterial species that dwell in the guts of animals and are used as fecal indicators in wastewater treatment plants (Harwood et al. 2014). The use of FIB for water quality assessment has a number of drawbacks as the incidence of FIB does not always indicate the presence of human pathogens or associated health risks, particularly in the case of human enteric viruses (Greaves et al. 2020). Furthermore, FIB can be detected in extra-intestinal reservoirs (soil), so it is not always an indicative of human fecal contamination. Another way to track feces pollution is to look for specific viral pathogens of interest, like norovirus and adenovirus. Many enteric viral pathogens are more resistant to traditional wastewater treatment and chlorination than FIB. The tools for assessing reference enteric viruses are expensive, require a lot of work in the lab, and are often less than 10% effective.

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In addition, they are further diluted when discharged into environmental waters, making detection more difficult (Zhu et al. 2018).

In light of this, a wide range of new fecal indicator organisms have been proposed, including pepper mild mottle virus. Plant viruses were found to be the most abundant viral members of the human gut microbiome (Zhang et al. 2006). Zhang et al. discovered that PMMoV, a plant pathogen, was found to be the most prevalent virus type in human fecal samples, although this virus is a plant pathogen. PMMoV is less tolerant and can survive in harsh conditions and wastewater treatment processes (Otaki et al. 2021). PMMoV is the most common virus which can be used as a surrogate of human enteric virus. It has been proposed as a possible viral indicator for human fecal contamination in water (Ferraro et al. 2021; Colson et al. 2010; Anderson-Coughlin et al. 2021). Zhang et al. (2006), reported high concentrations of up to 10^9 virion particles of PMMoV present in per gram of feces by dry weight. These values indicate that this virus is quite stable and can pass through the gut easily. In many studies, the researchers observed that the presence of pathogens particularly viruses in wastewater, cannot be detected directly as it is infeasible and costly. Methodological approaches for identifying enteric viruses are inefficient, costly and labor-intensive; therefore, their success rates frequently fall below 10%. Enteric viruses are found in very low concentrations after being discharged into environmental waterways; therefore, contaminants are further diluted, which makes their identification more difficult. As a result, indicators are frequently proposed in drinking water (Canh et al. 2021).

To remove viruses in drinking water treatment plants (DWTPs), PMMoV has also been used as a process indicator (Kato et al. 2018). Due to a significantly higher amount in feces, PMMoV's occurrence in the fecal contaminated water has been proposed as a viral marker throughout the world in different water resources, such as potable water and domestic sewage (Symonds et al. 2018). Temporal variables, geographical factors, natural disturbances (e.g., typhoons), and human activities can influence the viral community in freshwater environments. Among them, agriculture adds surplus nutrients and agricultural chemicals to surface waterways, depleting oxygen and creating algal blooms. Surface runoff from urbanization affects water quality. Human pathogenic viruses have been identified more frequently in watersheds with dominating urban and agricultural land cover (Gu et al. 2018). PMMoV causes significant loss of pepper, tobacco and tomato throughout the world, and it was reported to be mechanically transmitted (Filipić et al. 2021). So, the viral marker can be used to detect the presence of feces contamination and is useful in a log reduction of the virus at the time of wastewater treatment (Schmitz et al. 2016). Compared to human enteric viruses and bacteria, PMMoV is the most

common plant virus reported as a viral marker found in sewage water (Stokdyk et al. 2020).

Groundwater is greatly affected by fecal contamination therefore researchers evaluated pathogen–indicator ratios for PMMoV and reported values greater than 1.0. Pathogen–indicator ratio is an estimation of concentration ratio of indicator to pathogen and it is efficiently used to detect the pathogen in wastewater (Stokdyk et al. 2020). It was shown that the tap water had a higher number of gene copies for PMMoV. As a result, the number of infections in both adults and children has increased by 23% after drinking tap water (Stokdyk et al. 2020). Groundwater and treated effluent showed the occurrence of PMMoV at greater concentration whereas the other human pathogenic viruses were absent (except adenovirus). Its prevalence and tenacity in the different environments make it a suitable choice to use it as a viral indicator for log removal in reuse programs of wastewater treatment plants (Morrison et al. 2020). The presence of PMMoV in feces can infect host plants and is viable after passing through the gut (Colson et al. 2010). Colson et al. (2010) reported that the individuals who were positive for PMMoV showed symptoms, such as pain in the stomach and mild fever, plant viruses as a cause of illness were reported for the first time in this study.

Researchers used microbial source tracking (MST) system to identify fecal contamination sources in water canals (Ahmed et al. 2020). To better understand the influence of viruses in water, their risk factors for waterborne illnesses and their potential indicators, should be studied parallelly (Hata et al. 2018). PMMoV use as a fecal contamination indicator in Kenya was investigated by Van Zyl et al. (2019). PMMoV has been suggested as a new human enteric virus surrogate for evaluating the effectiveness of water treatment processes (Shirasaki et al. 2020). Otaki et al. (2021) in their study, used PMMoV as an indicator to assess fecal-associated viral contamination in drinking water wells at on-site sanitation (OSS) system. Various studies on viral metagenomics have revealed that viruses are the potential indicators that can be utilized as markers for fecal contamination of water compared to bacterial fecal indicators (Hamza et al. 2019). Human enteric viruses (HEV) were identified in surface water less often (18–59%) than PMMoV, most likely due to their quicker degradation (Kuroda et al. 2015). For measuring human fecal contamination, use of PMMoV as an indicator in water bodies that were greatly influenced by anthropogenic activities has been proved (Saeidi et al. 2018).

The purpose of this review is to present the research that has been conducted on the detection of PMMoV, its reduction and prevalence in major water ways. These water ways include wastewater treatment plants, water reclamation systems, environmental waters, and potable water treatment systems. This review's objective is to consolidate the present state of knowledge to provide researchers with the tools

they need to accurately evaluate the utility and application of PMMoV as an indicator for water quality assessment. The most notable benefit of PMMoV as an indicator organism is that, it can be detected in measurable and higher quantities more consistently than any other human virus, with no significant seasonal variations in its incidence. RT-PCR and RT-quantitative PCR (also known as RT-qPCR) are the two methods of molecular detection and quantification of PMMoV that are utilized most frequently in the research on fecal contamination. Wherever human enteric viruses are prevalent, PMMoV can be present. Researchers have also shown that PMMoV is more stable than other human enteric viruses in environmental water. This holds true for all forms of water in general, including wastewater, river water, groundwater, seawater, and drinking water among others. Therefore, it is ideally suited for use as a performance indicator in water and wastewater treatment facilities, where it may be used to gauge how well the treatment processes are working.

Ecology and genetic background of PMMoV

Natural incidence, virulence and pathogenicity of PMMoV

Pepper (*Capsicum annuum*, family: *Solanaceae*), which originated in the Americas, is currently a major global vegetable crop with many cultivars. However, several significant diseases have a detrimental impact on this crop production and quality. PMMoV has been isolated from resistant pepper in Italy (Wetter et al. 1984). More than 20 distinct viruses can infect pepper plants, and most of them belong to the genus *Tobamovirus*. PMMoV is 6400 nucleotides long positive-sense genomic RNA, encodes four proteins, namely replication proteins (130 K and 180 K), a movement protein 30 kDa, and a coat protein 17.5 kDa (CP). Tsuda et al. (2007) showed that the viral replication and RNA silencing suppression of PMMoV are mediated by replication protein. In capsicum plants, CP of PMMoV is considered to have an essential function as an inducer of tolerance, mediated through the four L genes (L1, L2, L3, and L4), and it is one of the most widely utilized markers for evolutionary studies of viruses in the family, *Virgaviridae* (Adams et al. 2009). Leaves growing at the tip of the plant were affected mainly by PMMoV in pepper plants by producing mosaic symptoms (Tsuda et al. 2007). Tsuda et al. (2007) reported the connection between viral pathogenicity determining domain and the emergence of mosaic symptoms. The results showed that the concentration of viruses in a plant does not always correspond to the development of mosaic symptoms. The PMMoV mutant, which showed no symptoms, had shown reduced activity in suppressing the post-transcriptional gene

silencing (PTGS). Viral accumulation has no role in causing mosaic symptoms; however, viral symptoms are regulated by the host PTGS system. The pepper mild mottle virus (PMMoV) is characterized by its intact capsid which makes them to live in the soil for an extended period of time; this offers significant risks to crops output (Jiao et al. 2020). Upon infection, PMMoV first causes mild foliar mosaic symptoms followed by mottling and malformation of leaves and fruits; veins are also varied in thickness, leading to substantial losses of pepper production (Kim et al. 2012; Rialch et al. 2015). The 5' region of the capsid protein of PMMoV recovered from the stools of thirteen patients, and four Tabasco sauce samples (made up from pepper) had also shown the same result (Colson et al. 2010).

Genetic variants and strains of PMMoV

In Japan, 12 water samples collected from 9 drinking water treatment plants (DWTPs) showed a virulent gene of PMMoV and based on phylogeny, the distinct types of strains reported in this study revealed their high variability in water samples (Haramoto et al. 2013). Antignus et al. (2008) reported that the strain L4 (PMMoV-Is) was shown to cause severe symptoms in crops due to its resistance breaking nature. This strain showed similarity to the Israeli pathotypes (P1, P2 and P3) because of the mutations in the amino acids, leucine to glutamine (at 47th position) and alanine to glycine at 87th position, respectively. Berendsen and Schraevesande (2020) reported two techniques to identify the complete genome sequence of a PMMoV using Oxford Nanopore Technologies and Sanger sequencing methods on pepper seeds cultivated during 2019 in Chile. PMMoV isolates in this study showed 89% and 91% nucleotide sequence identities which were relatively low, and their study further suggested that these isolates belonged to a new genotype. In this study, the entire genome sequence for strain PRO54348 was retrieved using the PMMoV genome (NC 003630) as the reference sequence through the Whole Genome Alignment (beta) toolbox. It encodes (183 kDa protein of 1612 aa and 126 kDa protein of 1117 aa) a replication protein, a movement protein of 257 aa, and a coat protein of 157 aa, which shows similarity of 97% to replication protein, 92% to movement protein, and 96% to coat protein sequence identity to PMMoV. The whole-genome sequence of PRO54348 was assigned with the accession number MT385868 in GenBank, and it belonged to a novel genotype of PMMoV (Berendsen and Schraevesande 2020). According to the study of phylogenetics of PMMoV, analysis of 171 nucleotide sequences from the coat protein gene that were collected between 1980 and 2016 revealed that its evolutionary rate has substantial variance (9.363×10^{-4} substitutions/site/year), and this is comparable to the evolutionary rates predicted for animal RNA viruses, showing that PMMoV has been evolving

rapidly (Guan et al. 2018). Between 2014 and 2015, a nationwide survey conducted in the pepper fields of South Korea reported two new isolates of PMMoV [Sangcheong 47 (S-47, KX399390) and Jeongsong 76 (J-76, KX399389)], which shared 99% nucleotide identity (Han et al. 2017). Synthesis of small interfering RNAs (siRNAs) and microRNAs (miRNAs) are inhibited by replication protein (Han et al. 2017).

PMMoV and environment

Route of entry of PMMoV

PMMoV enters the human host through food items, when they consume processed/tobacco products and spice powders, and subsequently end up in the wastewater through feces (Shrestha et al. 2018). Processed pepper items were

also tested positive for PMMoV, which showed its dietary origins (Zhang et al. 2006). Figure 1 in this review depicts the route of entry of the PMMoV viral particles after consumption of processed foods like spicy sauces, which include contaminated peppers, causing significant levels of PMMoV excretion from people. Since PMMoV is always present in large proportions in wastewater, it is possible to utilize it as a marker to track down untreated wastewater as well as treated wastewater. Through the consumption of polluted drinking water, raw agricultural products, or shellfish reared in contaminated environmental waters, and the accidental ingestion of polluted environmental water, humans are frequently exposed to water and food resources that are contaminated directly or indirectly as a result of wastewater discharges (e.g., lakes, reservoirs, rivers, coastal waters). Water is a crucial source for the quick dissemination of plant viral elements (Mehle et al. 2014); hence, water should be considered as an essential source of contamination in future

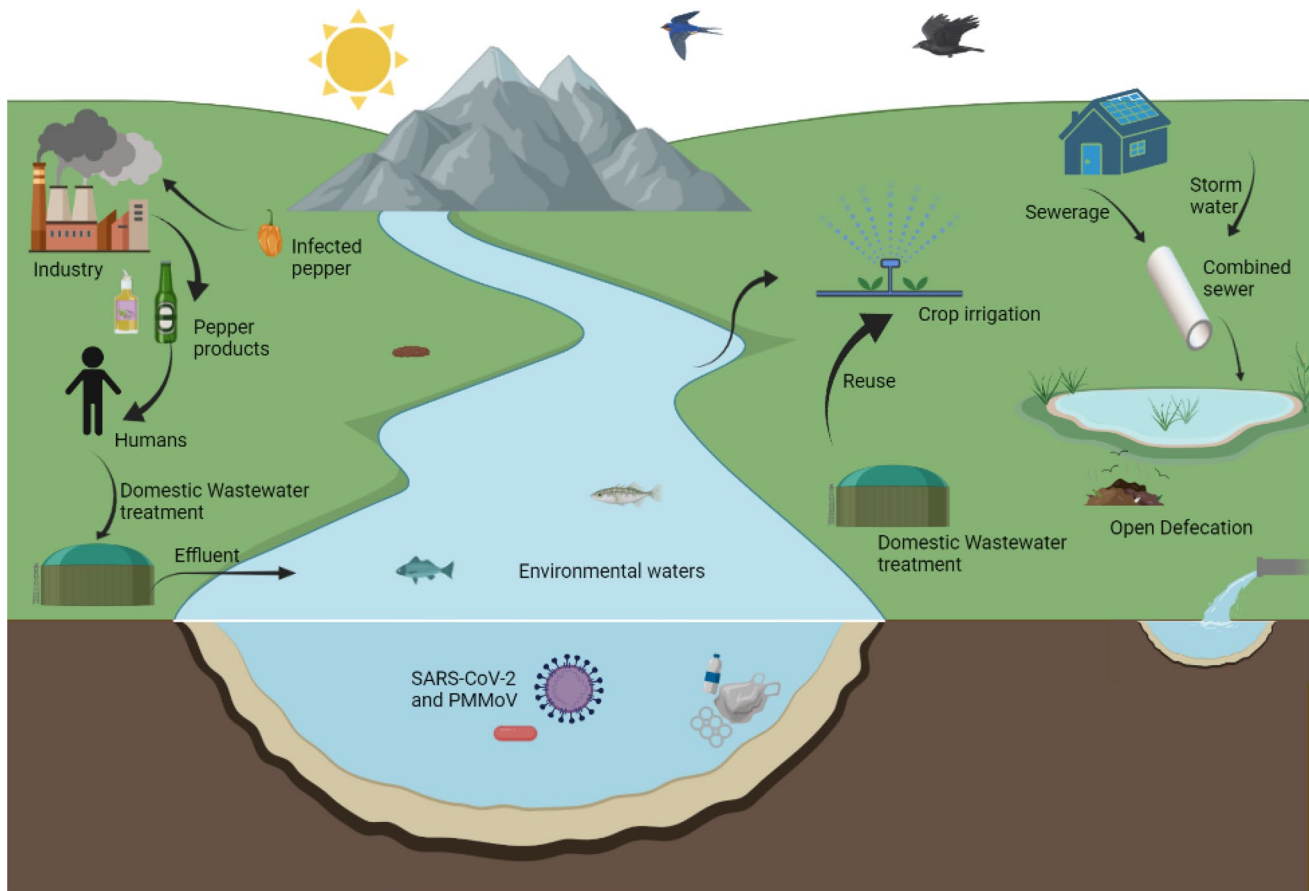


Fig. 1 Route of entry of PMMoV by industries into water sources and different sources of PMMoV released into water. Infected bell peppers were sent to industries for sauce and spice making as they cannot be sold at markets. After consumption viral particles can pass through gut and end up in wastewater. PMMoV is now being used as a control in SARS-CoV-2-contaminated water. Water is contaminated by PMMoV through different ways. Effluents from the WWTs (waste-

water treatment plants) are directly discharged into water bodies. In urban areas, heavy raining causes the mixing of sanitary wastewater and storm water, which result in overflow of sewer water directly into the water. In rural areas, Oss (Onsite sanitation system) is the source of PMMoV. If this water is used for irrigation purpose, it will cause enormous loss of crops. Illustration created using trial version of Biorender

epidemiological and risk assessment research. According to Yarwood (1960), the roots of infected plants that are dead or wounded also play a significant role in releasing plant viral particles into drain water. Van Dorst et al. (1988) discovered that when a waste dump is nearby, water canals can become a source of infection, and virus particles can persist there for up to 6 months. Tomlinson et al. (1983) suggested that sewage is also a significant contributor to plant viruses. Irrigation is also reported as a transmission route of the viruses to water (Bačnik et al. 2020).

Colson et al. (2010) reported that 21 pepper or spice-containing food products showed the presence of twelve (57%) PMMoV RNA sequences. Healthy individuals can excrete up to 10^9 gene copies in 1 g of feces (Colson et al. 2010). A person's age and food choices may influence the amount of PMMoV in an individual's stools, this virus has been found in untreated wastewater from Africa, the Americas, Asia and Europe suggesting that this virus is prevalent and internationally dispersed. The maximum viral load was found in tabasco sauce compared to the other food products, and it was reported to be almost 10^7 PMMoV RNA copies/mL on a cycle threshold of 22 by real-time PCR (Colson et al. 2010). PMMoV is highly infectious and can spread through contaminated seeds and soil (Kumari et al. 2020). Recreational activities, such as swimming, boating, surfing and consumption of shellfish collected from fecally polluted water, are the routes of a pathogenic virus entry into humans (Ahmed et al. 2020). The study by Aguado-García et al. (2020) reported that tobamovirus could easily pass from mother to child via breast milk. This may be because of improper treatment of the mother or airborne transmission of the virus. The water used to produce baby formula or the infusions in infants is due to tobamovirus contamination. After two weeks of delivery, the samples collected from the oropharynx and feces of the newborns showed 65% and 100% tobamovirus, respectively. This shows that food and water are the main sources of tobamovirus in fecal contamination (Aguado-García et al. 2020).

Resistance to environment and survival rate

Plant viruses also adsorb to various materials, such as clay, organic and soil particles, which protect them from many factors (physical and chemical) in the soil and water (Tošić and Tošić (1984). It also depends on environmental factors, such as pH, salts, and other materials (Piazzolla et al. 1986). Many viruses can travel great distances in water because of their high stability (Bačnik et al. 2020). PMMoV can easily pass through the human gastrointestinal system and spread in water (Filipić et al. 2021). It is more resistant than human enteric virus and bacteria when undergoing different wastewater plant treatments (Schmitz et al. 2016). Evaluation of conventional wastewater

treatment effectiveness on plant virus inactivation is not yet investigated (Thebo et al. 2017). But recently, inactivation of PMMoV is carried out by the CAP (cold atmospheric plasma) treatment. Unlike other water-transmitting tobamoviruses, PMMoV is able to survive the human digestive tract. Transmission electron microscopy, molecular techniques, and test plants' infectivity tests were used to assess PMMoV inactivation effectiveness in terms of infectivity and virion integrity. (Filipić et al. 2021). Anderson-Coughlin et al. (2021) examined the different atmospheric conditions to see if there were any links between EVs (enteric virus) and PMMoV in other parameters like air, the temperature of water and cloud cover, to observe the precipitation at different durations (24 h, 7 days, and 14 days). The presence of toxicants, for example, heavy metals in natural matrices, especially in water, reduces the effectiveness of capsid integrity testing. As a result, an efficient analysis like (RT)-qPCR is required to determine the inhibitory influence of natural matrices in drinking water samples (Canh et al. 2021). In DWTPs, PMMoVs are highly resistant to the treatments, namely coagulation, sedimentation, filtration and disinfection treatments by heat and chlorine, compared to the human pathogenic viruses (Kato et al. 2018; Shirasaki et al. 2020). Kaupinen et al. (2018) in their research, noticed that viruses could survive in the subterranean ecosystem for months to years. It has been observed that tobamovirus is extremely resistant to physical and chemical agents, intense heat as well as dehydration (Wetter et al. 1984). In the wastewater treatment process, PMMoV, due to its intact capsid, was more resistant than other enteric viruses (human adenovirus and norovirus) and MST indicators (Hughes et al. 2017). The most significant factors of viral community in the freshwater ecosystem depend upon territorial elements (such as storage tanks and land usage), environmental elements (such as pH and precipitation), physicochemical properties, and land management practices (Gu et al. 2018). Despite the prevailing weather or location in Japan, PMMoV was the most common virus, suggesting its use to indicate viral contamination of surface water (Hata et al. 2018). Few viral characteristics such as the resistance of viral particles to wastewater treatments, their survival rate in different environmental conditions, changes in season, a quantity of viral load from an infected person during defecation and cause of outbreak condition are the reasons for the viral incidence in the aquatic environment (Hata et al. 2018). Shirasaki et al. (2020) in their findings showed that PMMoV, due to its different capsid morphology, was highly resistant to heat-treatment in comparison to other human enteric viruses like AdV (adenovirus) and CV (cytomegalovirus) and even after 90 °C treatment, PMMoV was not completely inactivated.

Different sources that harbor PMMoV

Many researchers have shown the occurrence of the virus in different water resources. Sinigalliano et al. (2016), reported that the primary source of fecal contamination to the coastal marine ecosystem was due to the discharges of municipal and industrial wastewater into the marine ecosystem. It had many zoonotic viruses, which were the cause of outbreaks. To avert such outbreaks of sickness, there is an urgent need to identify the sources of fecal contamination, which leads to a surge of pathogenic microorganisms in water (Malla et al. 2019). Due to a lack of water supply, it has been a common practice to recycle wastewater from a variety of sources. Despite this, wastewater that was recycled for agriculture purpose was found to contain plant viruses (Anderson-Coughlin et al. 2021; Thebo et al. 2017). Whether the capsid is intact or damaged, the virulent nature of the viruses was not addressed in drinking water and tap water by conventional (RT)-qPCR (Canh et al. 2021). A public water system supplying groundwater (wells) to many communities (municipalities, agriculture) and non-community areas (schools, factories, restaurants, resorts, and churches) in the USA was greatly affected by HF183 (human-associated bacterial cluster) and PMMoV (Stokdyk et al. 2020). Influent and effluent of wastewater treatment plants had reported high quantities of PMMoV (up to 10^9 GC/L) from various regions across the world (Hamza et al. 2011; Kitajima et al. 2014; Rosario et al. 2009; Schmitz et al. 2016). In Japan, surface water is the greatest source of PMMoV with a concentration $> 1.7 \log_{10}$, which is quite high compared to human enteric viruses GII-Nov (the second most prevalent) reported in the research by Hata et al. (2018). Irrigation with river water and wastewater is common in the Kathmandu Valley, the primary source of crop yield degradation (Shrestha et al. 2018). Wet weather overflows (WWOs) were caused when rainwater entered into the sewerage system due to heavy rain; this resulted in the release of sewage water that led to the mixing of raw sewage to other water bodies. These were also considered the channel for plant virus contamination into the water (Ahmed et al. 2020). Shallow dug wells are highly vulnerable to aquatic virus infection because of OSSs, especially PMMoV (Otaki et al. 2021). In Hanoi, wastewater tracer (PPCP) is compared with the concentration ratio of PMMoV in wastewater (Kuroda et al. 2015). PPCP (pharmaceuticals and personal care products) is used by the individual for health or cosmetics purposes (caffeine and carbamazepine). PMMoV was found in all agricultural, river, sewer and pond samples.

The concentration ratio of PMMoV to caffeine in surface water remained unchanged compared to that in WWTP influents, implying that the persistence of PMMoV in surface water was comparable to that of caffeine (Kuroda et al. 2015). In stagnant waters, PMMoVs are commonly found

and can be used as an indicator for predicting their presence. Because of its high concentrations as well as 100% sensitivity and specificity, PMMoV was used as an essential indicator in the Costa Rican MST arsenal for monitoring household wastewater pollution (Symonds et al. 2017). Gene sequences having nucleotide similarities of 99–100% were found among the 85% of samples collected from different sources that were positive for PMMoV. In the Karst aquifer system, PMMoV was used as a potential indicator of fecal pollution because of its presence at different concentrations, representing its durability in environmental water (Rosiles-González et al. 2017). A diversified array of tobamoviruses, such as pepper mild mottle virus, tropical soda apple mosaic virus and opuntiatobamovirus, had spread among the population, these findings imply that a wider range of tobamoviruses were the cause of illness in children (Aguado-García et al. 2020). Wang et al. (2018) reported the detection of nine distinct plant virus families in the oropharynx samples, among them they found the tobamoviruses being the most common once again. They found more than 20 plant viruses in the nasal wash specimens at Beijing Children's Hospital. Aguado-García et al. (2020), compared infection rates in breastfed infants versus formula-fed infants and estimated risk for infection of tobamovirus. They addressed that breastfeeding timing and maternal infection are the source of tobamovirus in breast milk. During heavy rain in Japan, combined sanitary waste and stormwater were mixed in the same tunnel causing overflow through the weir wall which was released into combined sewer systems without any treatment [combined sewer overflows (CSOs)] (Inoue et al. 2020). Figure 1 depicted in this review article shows that PMMoV contamination in urban areas occurs, when the heavy rain causing the mixing of sanitary wastewater and stormwater results in the overflow of sewer water directly into the water sources, whereas in rural areas, OSS is the source of PMMoV. HF183 and PMMoV were present at 100% concentration in river samples and ocean samples, so based on this, they can be used as indicators to check chronic sewage pollution in the aquatic environment. In river water, PMMoV was found along with other members, such as *C. perfringens* and somatic coliphages, and in ocean water samples, PMMoV and enterococci were mostly found (González-Fernández et al. 2021).

Persistence of PMMoV in human and animal feces

From two different continents (San Diego in North America and Singapore in Southeast Asia), 18 samples of feces obtained from healthy people showed 66.7% occurrence of PMMoV. The occurrence of PMMoV in feces is not limited by geography. Among six of the nine Singaporeans who had recently consumed peppers in meals, five of them defecated PMMoV in their feces. The detection rate

of PMMoV is incredibly varied from person to person throughout time (Zhang et al. 2006). The potential indicator is suggested based on its prevalence in fecally polluted water and its less severe pathogenicity as compared to NoVs (nova virus), SaVs (Salmonid alphavirus); therefore, PMMoV is suggested as a potential fecal indicator (Hata et al. 2018). PMMoV has been found in human feces at high concentrations (10^5 – 10^9 genome copies (GC)/g) in the study conducted in the United States, Singapore, Germany, and France (Colson et al. 2010; Zhang et al. 2006; Hamza et al. 2011). Rosario et al. (2009) reported that PMMoV is the most common plant pathogen, which is ubiquitously found in wastewater of the United States, implying that this virus can be used as an indication of human fecal contamination. The prevalence of PMMoV in animal fecal samples showed a positive result for chicken and seagull samples (Rosario et al. 2009). According to Ferraro et al. (2020), PMMoV is highly abundant in the feces of a healthy individual and is suggested as the viral biomarker for fecal contamination in aquatic habitats. Plant virus shows higher stability in the gut of vertebrates, because of which it remains inactivated (nonviable due to its intact capsid) and can spread over long distances. PMMoV shows an extremely resistant nature which makes it survive through the human digestive tract and can be easily moved to the aquatic environment (Filipić et al. 2021). According to Stokdyk et al. (2020), a reported marker that is associated with feces is found in wells, among them, HF183 and PMMoV were frequently found. The feces samples of 22 out of 304 adult samples (7.2%) confirmed the presence of PMMoV by Real-time PCR (Colson et al. 2010). In the patients who were positive for PMMoV in their feces samples, symptoms were compared to those of 31 PMMoV-negative patients, based on univariate analysis, it was revealed that stomach discomfort, such as fever and inflammation in colon wall, was substantially noticed. There was no major biological indication linked to the presence of PMMoV RNA. The discovery of PMMoV RNA in stools, food products and antibodies (Anti-PMMoV IgM) in serum suggests that this virus can cause mild symptoms (Colson et al. 2010). In comparison to the control group, Anti-PMMoV IgM were found in serum of all PMMoV-positive patients who had symptoms after consumption of contaminated products and viral load was also found to be higher in feces (Colson et al. 2010). PMMoV detected in human feces is in viable form with an intact capsid. Gyawali et al. (2019) reported for the very first time the incidence of PMMoV in black swan feces, thereby stating the occurrence of PMMoV in the aquatic environment and shellfish in New Zealand. In this study, they investigated the cultivating waterways that were used for oysters, mussels, cockles, and shellfish which were affected by the contaminants of human feces. Consequently, PMMoV may be used as an indication of human feces contamination in New Zealand. In influent

wastewater, PMMoV occurrence is at higher concentration, therefore can be used as a potential indication of fecal contamination in wastewater. PMMoV occurrence in the feces of Black swan, Canada goose, seagull and dog were reported by Gyawali et al. (2019). PMMoV use as a fecal contamination indicator in Kenya was investigated by Van Zyl et al. (2019). Shrestha et al. (2018), mentioned that affected individuals defecate more enteric viruses (10^{12} viruses per gram feces) than enteric bacteria. Enteric viruses have an extraordinarily high survival rate in freshwater and high infectivity. PMMoV prevalence in feces was stable after passing through the gut compared to human enteric viruses (Hamza et al. 2019). Based on dry weight, PMMoV viral load is lesser in meals in comparison to feces, which means during defecation, more RNA particles were released, this may be due to replication, but currently, there are no supportive data for a copy (Zhang et al. 2006) of this virus in the gut. In a study of gut virome, breastfed children were detected with plant viruses in their stomach and oropharynx. Using next-generation sequencing, fecal and oropharynx samples were examined from three healthy infants in a semi-rural neighborhood. At least one plant virus was found in the oropharynx (65%) and feces (100%) samples (Aguado-García et al. 2020). Figure 2 shows that the occurrence of PMMoV is geographically distributed. It is found in varying percentage at different areas from various countries, therefore PMMoV can be used as indicator for wastewater contamination of the aquatic environment.

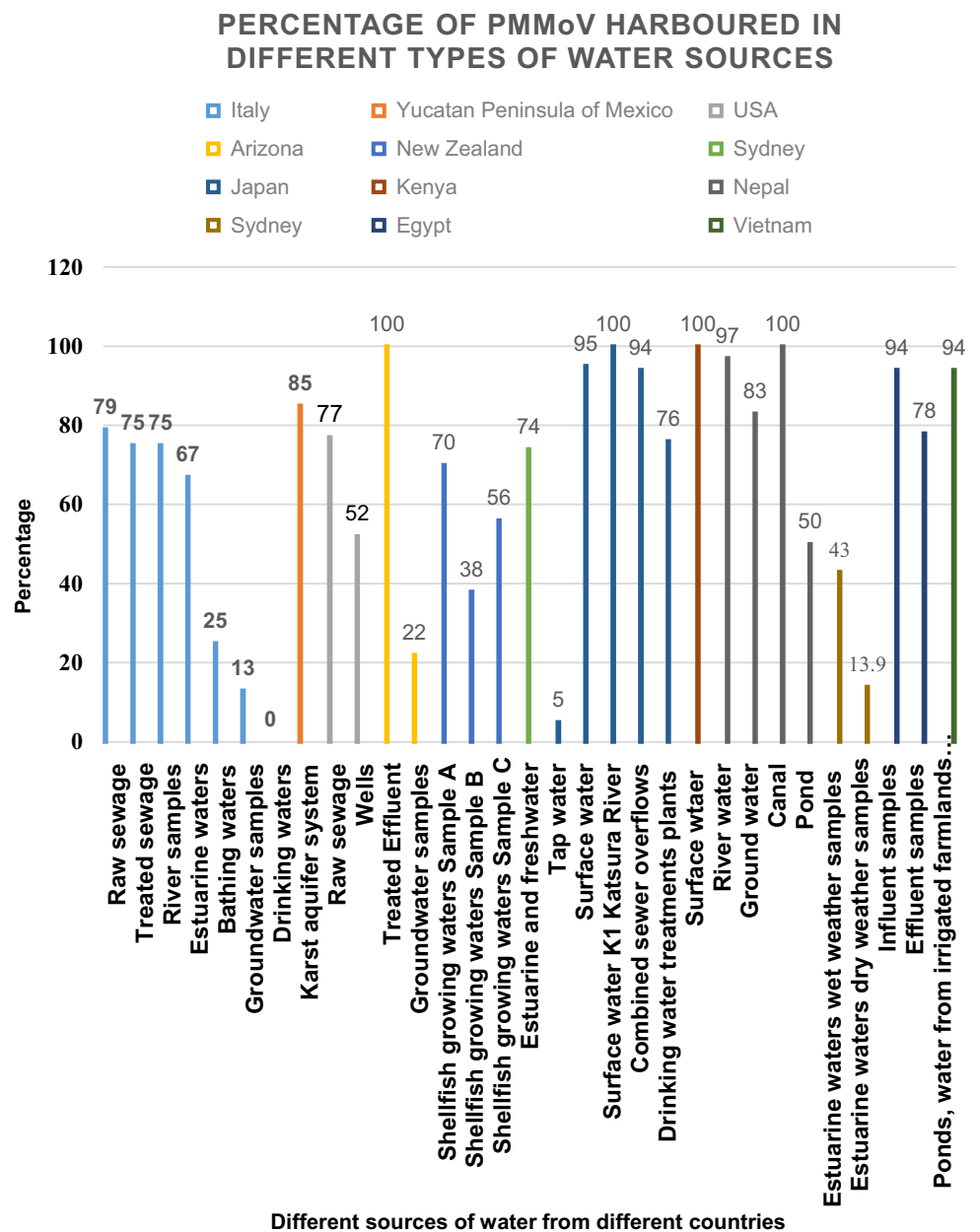
PMMoV is found in the Karst aquifer system throughout the dry and wet seasons, which may be used as a potential fecal indicator (Rosiles-González et al. 2017). Tobamovirus sequences were found in samples of children with respiratory tract illnesses in the research conducted in Mexico (8% in lower and 35% in the upper respiratory tract) (Taboada et al. 2014). PMMoVs were numerous in coastal areas of Tokyo (1.4×10^4 – 6.8×10^6 genome copies/L), but the quantities of the other enteric viruses were modest (Avian influenza virus (AiV), 1.3×10^2 – 2.9×10^4 ; norovirus GI, 2.9×10 – 5.6×10^3 ; norovirus GII, 2.5×10 – 1.2×10^4 genome copies/L) (Inoue et al. 2020). Viral integrity (capsid integrity (RT) qPCR) of PMMoV is used to know the presence of a viable and nonviable forms of the virus in tap water (Canh et al. 2021).

PMMoV and its management

Water conditions and sanitation management

Inoue et al. (2020) reported that even though FIB significantly decreased in saline conditions, PMMoV had no effect. According to Rosario et al. (2009), PMMoV was not found in clean seawater, but it was observed after one week in the marine environment, indicating fresh contamination.

Fig. 2 This figure depicts the percentage of PMMoV in various water samples at different concentration. X-axis denotes different sources of water samples from different countries; Y-axis denotes percentage of PMMoV in water. Figure created using MS excel



Logistic regression analysis revealed that water salinity had a significant effect on the detection of both HEVs and PMMoV ($P < 0.05$), but to test the presence of viruses in brackish groundwater, sources used for irrigation purposes turned out to be more difficult (Anderson-Coughlin et al. 2021). These findings shed light on the extent and nature of the relationship between PMMoV, human enteric (pathogenic) viruses and water quality data in mid-Atlantic surface and reclaimed waters as potential agricultural irrigation sources. The physical and chemical properties of water were monitored by Canh et al. (2021). Table 1 summarizes all the aquatic habitats in which PMMoV can survive in different water conditions. In the study, HI 98129 water tester was used to measure the temperature and pH of the water, and a

2100Q portable turbidimeter was used to measure turbidity. Using N, N-diethyl-p-phenylenediamine (DPD) colorimetric technique, total amount of chlorine values were determined in a DR890 colorimeter. Clay minerals and metal oxides in the subsurface of water have a high adsorption capability for viruses such as PMMoV (Kuroda et al. 2015). The low effectiveness of the viral recovery can be used as a significant viral indicator; Inoue et al. 2020, showed that, because of the low effectiveness of viral recovery, the virus concentration at the salinity area showed a P value of $P < 0.1$, which means that, it is statistically significant. So, the low effectiveness of PMMoV in saline water, therefore, can be used as an indicator for saline water habitat. Multiple factors influence viral persistence in water sources (Banerjee 2011). Plant viruses

Table 1 Geographical distribution of PMMoV in different water sources

Sample collection area	Source	Positive samples (%)	Concentration (genome copies/L)	Remarks, Presented argument	Detection method	References
Italy	92 raw sewage	79	2.2×10^6	In various Italian water environments PMMoV reported its first occurrence	RT-qPCR	Ferraro et al. (2021)
	32 treated sewage	75	2.9×10^5			
	16 river samples	75	6.1×10^2			
	9 estuarine waters	67	4.8×10^2			
	20 bathing waters	25	8.5×10^1			
	67 groundwater samples	13	5.9×10^1			
Yucatan Peninsula of Mexico	18 drinking waters	Nil	2.2×10^6	The quantity and prevalence of PMMoV in the karst aquifer system may represent its environmental durability and potential as a faecal indicator	RT-qPCR	González Fernandez et al. (2021)
	Karst aquifer system	85	1.7×10^1 to 1.0×10^4 GC/L			
Japan	Surface water, $n=20$	95	$3.1\text{--}6.2 \log_{10}$ copies/L	PMMoV was found in its intact form in tap water derived from surface waters but shows its absence which derived from the ground water	Capsid integrity RT-qPCR SD-CDDP-RTqPCR	Canh et al. (2021)
	Tap water, $n=43$	5	$0.62\text{--}1.18 \log_{10}$ copies/L			
USA	Wells ($n=52$)	5.4	920 (GC/L)	Sporadic fecal contamination	qPCR	Stokdyk et al. (2020)
Arizona	Treated effluent ($n=6$)	100	NA	This is the first study to look at how wetting/drying cycles affect viral elimination by SAT	RT-qPCR	Morrison et al. (2020)
	Groundwater ($n=18$)	22				
New Zealand	Shellfish growing waters	14 (70%) No of positive samples based on DNQ, 3 (15%) No. of positive samples based on LLOQ		This is the first research in New Zealand to report the presence of PMMoV in black swan feces, as well as the first study to describe the presence of PMMoV in the aquatic environment and shellfish	RT-qPCR assay	Gyawali et al. (2019)
	Sample A	6 (38%)	$3.9 \pm 0.1 \log_{10}$ GC/L			
	Sample B	1 (6%)	NA			
	Sample C	9 (56%)	3.9 ± 0.1			

Table 1 (continued)

Sample collection area	Source	Positive samples (%)	Concentration (genome copies/L)	Remarks, Presented argument	Detection method	References
Sydney	Estuarine and freshwater			qPCR systems, PCR inhibitors, nucleic acid extraction efficiency, and low target levels might all have contributed to the reported differences	qPCR marker assays-based gene	Ahmed et al. (2020)
	CSIRO land and water (CLW) Sydney water (SW) laboratory	74	4.42 ± 0.65 log ₁₀ copies/L 3.89 ± 0.41 log ₁₀ copies/L			
Japan	Surface water K1 Katsura River	100(52/52)	5.0 log ₁₀ copies/L	Regardless of season or location, PMMoV was the most common virus, suggesting its use as an indication of viral contamination of water	RT-qPCR	Hata et al. (2018)
Kenya	Surface water BMFS (bag-mediated filtration system) n = 59	100	6.1 × 10 ⁵ GC/L	Polluted sites, PMMoV might be utilized as a BMFS process control for enteric virus environmental surveillance	qRT-PCR	Van Zyl et al. (2019)
Nepal	Irrigation water sources River water—Sep 14 Apr 15 Aug 15 Ground water—Aug 15 Canal—Apr 15 Aug 15 Pond—Sep 14 Apr 15 Aug 15	100(8/8) 100(9/9) 91(10/11) 83(5/6) 100(1/1) 100(1/1) 50(1/2) 0(0/1) 100(2/2)	8.7 log ₁₀ copies/L	This is the first research to suggest and investigate TMV's potential as an index virus. PMMoV and TMV were prevalent in all irrigation water sources and seasons	TaqMan (MGB)-based qPCR assays	Shrestha et al. (2018)
Sydney	Estuarine waters	43	3.89 ± 0.28 log ₁₀ GC/L	These findings indicate that estuarine water	qPCR assays	Ahmed et al. (2020)
	Wet weather overflows	13.9	3.97 log ₁₀ GC/L	greatly affected by WWOs (wet weather overflows)		
Egypt	Influent samples	~94	3.9 × 10 ⁴ to 3.3 × 10 ⁸ genome copies/L (GC/L)	Their finding suggests that in wastewater treatment process, PMMoV can be used as fecal indicator	qRT-PCR	Hamza et al. (2019)
	Effluent samples	78	3.9 × 10 ² to 1.2 × 10 ⁷ GC/L			

Table 1 (continued)

Sample collection area	Source	Positive samples (%)	Concentration (genome copies/L)	Remarks, Presented argument	Detection method	References
Japan	Drinking water treatment plants (DWTPs)	76	2.03×10^3 copies/liter to 2.90×10^6 copies/liter	PMMoV was commonly found in samples that tested negative for human enteric virus or <i>E. coli</i>	qRT-PCR	Haramoto et al. (2013)
USA	Raw sewage Treated sewage	77	8.04×10^5 to 1.9×10^6 copies/mL 2.02×10^4 to 1.01×10^6 copies/mL	In marine environment, PMMoV is potential biomarker	qPCR	Rosario et al. (2009)
Vietnam	Ponds, water from irrigated farmlands and rivers WWTP influents WWTP effluents	94	5.5×10^5 – 7.2×10^6 copies/L 6.5×10^5 – 8.5×10^5 copies/L	For the first time, PMMoV was assessed as a fecal indicator and tracer in aquatic settings by comparing it to wastewater tracer PPCPs (pharmaceuticals and personal care products)	qPCR	Kuroda et al. (2015)
Japan	Combined sewer overflows	94	1.4×10^4 – 6.8×10^6 GC/L	PMMoV found at high concentration in saline water	qRT-PCR	Inoue et al. (2020)

NA not available; DNQ Detected but not quantifiable; LLOQ Lower limit of quantification

adsorb to various matter like clay organic. Soil particles protect them from many influencing factors (physical and chemical) in the soil as well as in water, and this depends on the environmental factors, such as pH, salts, and the presence of different materials; therefore, these properties make the virus less resistant toward wastewater treatment process and readily available for its use as an indicator (Piazzolla et al. 1986).

The availability of clean water, proper sanitation, waste management and hygienic conditions, is important for the prevention and protection of human health during the outbreaks of infectious illness (WHO 2018). PMMoV has evolved as a novel surrogate for enteric viral infections with established applications for monitoring microbiological water quality, wastewater treatment efficiency, and food safety (WHO 2018). This comes from the unexpected finding that plant viruses prevail in human feces. PMMoV has the potential to serve as a substitute for enteric viruses in the evaluation of food safety in addition to being a viral indicator (Filipić et al. 2021). Quantitative microbiological risk analysis based on PMMoV concentrations in irrigation water permitted the evaluation of optimal practices and practical suggestions on safe water reuse for users. The design, building, operation, and use of toilets should all be carried out in such a way that people are kept safe from waste products (Otaki et al. 2021). The construction of the toilet needs to be designed in such a way that it prevents animals and insects from entering, as well as precipitation and runoff from stormwater. Permeable containers, soak pits, and leach fields should be kept at least 15 m away from the local groundwater table and 1.5 m away from drinking water sources to prevent contamination of the drinking water supply (Otaki et al. 2021). It is essential that the treatment facility be developed and run in accordance with the precise end-use/disposal purpose.

PMMoV as an alternative to FIB

Water samples necessitate the development of appropriate indicators for viral pathogen contamination, which leads to epidemics (Anderson-Coughlin et al. 2021). *Escherichia coli* concentration is greatly decreased in saline conditions; these findings suggest that this cannot be used as an indicator in saline water environments (Inoue et al. 2020). Table 2 provided in this review gives the information about the use of PMMoV as a better indicator than *E. coli*. Indicator bacteria (*E. coli* and total coliforms) are used to detect the presence of microorganisms in drinking water and different environmental conditions. To detect a rate of pathogenic viruses during treatment processes, the surrogate is required, but these indicator bacteria could not be used (Canh et al. 2021). Fecal indicator bacteria do not always represent contamination due to viruses in different environmental conditions (Greaves et al. 2020). The presence of fecal indicator bacteria cannot be reliably predicted or related to human enteric viruses as there is a lack of human host specificity (Jang et al. 2017). Some requirements are needed to assign them as viral indicators. They must possess high persistence in the environment and be detected throughout the year; in such cases, PMMoV is the most abundant (Shrestha et al. 2018). Novel indicators of sewage contamination such as PMMoV were compared to the known indicators (*E. coli* and Enterococci) to assess the extent of sewage contaminants in wastewater (Ahmed et al. 2020). Otaki et al. (2021), showed that PMMoV was a suitable indicator for fecal contamination because of its capsid structure, it is more environmentally tolerant than *E. coli*, and in shallow dug, *E. coli* was not a great indicator. Other indicator bacteria were not always found in water, but PMMoV shows its presence, making it a good viral indicator. A bacterial marker may not be able to assess viral risk (Rosario et al. 2009).

Researchers showed that if the distance between OSS and well water was more than 10–20 m, there was a gradual

Table 2 Comparative studies between PMMoV and fecal indicator bacteria properties

PMMoV	Fecal indicator factor	References
It is the RNA biomarker able to offer an evaluation of viral risk in comparison to other enteric viruses	A bacterial marker may not be able to offer an evaluation of viral risk	Rosario et al. (2009)
PMMoV found at high concentration in saline water	As the salt concentration increases, the <i>E. coli</i> concentration decreases	Inoue et al. (2020)
SARS-CoV-2 RNA deterioration status was similar to PMMoV	Enteric viruses and fecal indicator bacteria shown large differences	Ai et al. (2021)
PMMoV is a suitable indicator for fecal contamination due to its capsid structure; it is more environmentally tolerant than <i>E. coli</i> , and in shallow dug	<i>E. coli</i> was not a great indicator	Otaki et al. (2021)
Same samples that are tested positive for presence of PMMoV 68%	Same samples that are tested negative for presence of human enteric virus or <i>E. coli</i>	Haramoto et al. (2013)
It shows higher concentration in feces 10 ⁹ virions per gram	It is present in less concentration	Zhang et al. (2006)

decrease in PMMoV concentration. Therefore, toilets should be more than 20 m away from water resources (Otaki et al. 2021). This means that contamination due to OSSs can be reduced by increasing the distance between water sources and OSSs. Researchers reported that *E. coli* was found in shallow dug wells; therefore, it may be from feces or any other contaminants. To know the source of *E. coli*, wells should be completely covered to minimize the contamination from outside sources. Otaki et al. (2021) reported that, while both PMMoV and *E. coli* were present in well water, the concentrations of PMMoV was found to be higher. Its presence in the well water shows that it is essential to ensure hygienic safety, as the OSS sludge near the water resources may be the source of water contamination. To detect the viral community in aquatic environment or wastewater treatment process, traditional fecal indicator bacteria are not advisable because of their characteristics like more susceptibility to wastewater treatment, disinfectants, circumstances of poor environmental resistance, and their co-occurrence with animal species (Hamza et al. 2019). In many countries, virus outbreak studies have revealed a lack of connection between bacterial indicators and viral epidemics in recreational and drinking waters (Hamza et al. 2019). Samples that were tested negative for human enteric virus or *E. coli* showed the presence of PMMoV up to 68% (65/95) and 64% (27/42) (Haramoto et al. 2013). Plant viral particles have been shown in the past to be assembled in *E. coli* cells (Hwang et al. 1994). *Escherichia coli* and Enterococcus have shown good associations with PMMoV, suggesting that the virus can be used as a biomarker in the investigation (Saeidi et al. 2018). In tropical surface waters, PMMoV can be used as a biomarker in fecal contamination through humans by comparing FIB and viral indicator results. PMMoV exhibited a strong significant correlation with land use weightage ($\rho = 0.728$; $P < 0.01$) compared to traditional FIB ($\rho = 0.583$; $P < 0.01$) (Saeidi et al. 2018).

Detection and removal of PMMoV

The high incidence of PMMoV in treated wastewaters has prompted a large number of studies to investigate the presence of this virus in natural waterways. PMMoV has been detected in water samples using the same techniques (RT-PCR or RT-quantitative) which are used to detect enteric RNA viruses Canh et al. (2018). For the purpose of concentrating PMMoV particles from a variety of water sources, including potable water, groundwater, wastewater, and water reclamation, numerous virus concentration methods, such as electronegative disk or cartridge filters, electropositive filters, and tangential flow ultrafiltration, have been utilized. During the cultivation of crops in fields, methyl bromide is

used for fumigation, and it prohibits viral entry into the soil (Yoneyama 1988). Table 1 summarizes all the geographical areas where PMMoV was found and harbored in different water samples at different concentrations and its detection by RT-qPCR (Reverse transcriptase quantitative PCR).

Table 3 represents various processes/treatments used to reduce (PMMoV) in water. The number in the Table 3 reports the decreased efficiency of PMMoV in wastewater treatment plants. In 2011, it was revealed for the first time that PMMoV reduction efficiencies may be achieved by wastewater treatment (Hamza et al. 2011). According to the findings of this research, a wastewater treatment facility in Germany that applied a typical activated sludge method had shown reductions in PMMoV ranging from 1.7 to 3.7 log₁₀ ($n = 12$) (Hamza et al. 2011). According to the findings of Kitajima et al. 2014, the reduction efficiencies of PMMoV by activated sludge and trickling filter were, 0.76 ± 0.53 log₁₀ ($n = 12$) and 0.99 ± 0.64 log₁₀ ($n = 12$) respectively. Waterborne virus poses a great threat to plants, animals and humans; therefore, virus inactivation is required. In the in vitro cell model system, CAP (cold atmospheric plasma) treatment for 5 min was a perfectly acceptable method to inhibit PMMoV without inducing any cytotoxic or genotoxic effects (Filipić et al. 2021). CAP treatment for 5 min results in complete inactivation of PMMoV; subsequently this treated virus produced no symptoms when tested on plants. Reactive oxygen, nitrogen species, and UV radiation are all components of CAP and showed high antimicrobial properties. RT-qPCR was used to investigate the virus integrity after CAP treatment, and RT-ddPCR (reverse transcriptase digital droplet PCR) was used to determine the concentration of viral RNA in treated PMMoV (Filipić et al. 2021). There was no significant reduction in PMMoV and enteric viruses (NoV genogroup I and rotavirus) in either of two wastewater treatment pond systems studied by Symonds et al. (2014) in Bolivia (three-pond and UASB-pond systems).

Surface water and treated effluents showed the 100% occurrence of PMMoV, whereas drinking water showed no presence of the viral particles. Various water samples such as raw sewage directly released from industry treated wastewater that comes in contact with fecal polluted water and fecal samples of animals showed a high concentration of PMMoV when determined using quantitative PCR (Rosario et al. 2009). Using two different techniques, namely nested RT-PCR (Reverse transcription-polymerase chain reaction) and TaqMan-based quantitative PCR, detection of the presence of a variety of PMMoVs in different water bodies was studied by Ferraro et al. (2020). Many inactivation methods of bacteria have been studied, but few were targeted for the virus. Sometimes inactivation by traditional water treatment

Table 3 Various processes/treatments used for the reduction of Pepper Mild Mottle Virus (PMMoV)

Process or treatment	Country	Log ₁₀ reduction	Initial viral concentration	Viral concentration after treatment	References
CAP (Novel technology for inactivation of PMMoV in water)	Slovenia	ND	3 min– 4.90×10^5	1.26×10^5	Filipić et al. (2021)
			5.29×10^5	5.92×10^4	
			5.29×10^5	3.96×10^4	
			5 min– 4.90×10^5	1.08×10^5	
			5.29×10^5	2.02×10^4	
Gas	Slovenia	ND	5 min– 5.05×10^5	5.07×10^5	Filipić et al. (2021)
Bardenpho	US	$> 2.7 \pm 1.6$	ND	ND	Schmitz et al. (2016)
Soil aquifer treatment (SAT)	Tucson, Arizona	EW-008A–5.8	ND	ND	Morrison et al. (2020)
		WR-398A–5.8			
		WR-069B > 6.2			
Thermal treatment	Japan	60 °C– $1.1 \cdot \text{Log}_{10}$	ND	ND	Shirasaki et al. (2020)
Coagulation sedimentation, Ozonation	Japan	2.38 ± 0.74	ND	ND	Kato et al. (2018)
		1.91 ± 1.18			
Coagulation sedimentation	Bangkok	Rainy season– $0.40 \pm 0.17 \log_{10}$	ND	ND	Asami et al. (2016)
		Dry season– $1.61 \pm \log_{10}$			
Bench-scale coagulation sedimentation and rapid sand filtration processes	Japan		ND	ND	Kato et al. (2018)
	Before typhoon	$1.96 \pm 0.30 \log_{10}$			
	During typhoon	$0.26 \pm 0.38 \log_{10}$			
Activated sludge	Germany	1.7–3.7	ND	ND	Hamza et al. (2011)
Sequential batch reactor (SBR)	Vietnam	0.92	ND	ND	Kuroda et al. (2015)

ND not done; EW production well; WR monitoring well

is ineffective (Bačnik et al. 2020), so inactivation of virus depends on the type of source that needs to be treated. For example, chlorination is effective in potable water (Filipić et al. 2021) and pools. WWTPs purify domestic water for drinking purposes (Malla et al. 2019; Filipić et al. 2021). Plant infectivity assay, TEM (Transmission electron microscopy), PCR methods were used to study the inactivation of the virus. To distinguish infectivity and non-infectivity forms of viruses in a water environment, capsid integrity RT-qPCR was considered the best method. Researchers studied the capsid integrity of the virus in surface water and tap water, and they pretreated with SD (sodium deoxycholate) and CDDP (cis-dichlorodiammineplatinum) followed by RT-qPCR. In traditional RT-qPCR, human pathogenic viruses (30–60%) are less commonly found in comparison to PMMoV (100%), but SD-CDDP-(RT-) qPCR (sodium deoxycholate cis-dichlorodiammineplatinum reverse transcriptase quantitative PCR) reported different results for PMMoV (95%) and other human pathogenic viruses (20–45%) which were intact. PMMoV showed (5%) positive rates after SD-CDDP treatment followed by (RT)qPCR,

but no AiV (Avian influenza virus) was identified, which means PMMoV has an intact capsid (Canh et al. 2021). In water, viral load analysis was measured by the molecular tests, RT-qPCR and reverse transcription-quantitative real-time polymerase chain reaction (RT-qPCR), but these tests could not trace intact viral form. Some studies showed that viruses could lose their infectivity due to capsid modifications and genome damage that prevents the penetration of capsid integrity test reagents (Leifels et al. 2015). Capsid integrity test reagent, a platinum compound (CDDP), was recently proposed to denote the infectivity nature of capsid of bacteria and viruses. It is significantly less expensive and more effective than other monoazide reagents. After several disinfection treatments using heat and chlorination, the nature of capsid can be easily determined by capsid integrity (RT-)qPCR (Canh et al. 2021; Leifels et al. 2015).

For the secondary treatment, the Bardenpho process (a five-stage process that removes biological nutrients nitrogen and phosphorous from wastewater) was more effective at reducing the presence of viruses in effluent 296 wastewaters when compared to other activated sludge and trickling filter bio-towers.

Bardenpho process has been designed to improve nutrient removal (nitrogen and phosphorous) rather than microbial contaminants. TaqMan-based qPCR assays were used for the quantification of the viral genome, an electronegative filter was used to concentrate the virus in wastewater (Schmitz et al. 2016). In a study by Stokdyk et al. 2020, samples were tested for pathogens and fecal markers in wells using 23 qPCR assays. qPCR/RT-PCR was used to detect adenovirus, enterovirus, PMMoV and crAssphage, for which the LRVs (log₁₀ reduction values) were calculated. Virus elimination is of special significance during the SAT (soil aquifer treatment) process because viruses, due to their tiny size and colloidal characteristics, can travel greater distances than other bacterial species. During SAT, two viral markers PMMoV and crAssphage, assess the effectiveness of viral elimination (Morrison et al. 2020). A real-time reverse transcription (RT)-PCR technique was used to detect PMMoV RNA (Colson et al. 2010). Ahmed et al. (2020) demonstrated the similarities and differences in laboratory equipment, reagents, and procedures that may impact the findings of qPCR-based marker gene analysis in water samples for the parameters, namely membrane type, sample acidity, sample volume range, filtering type, and nucleic acid extraction kit. PMMoV and other human enteric viruses were removed from wastewater by the new method BMFS (bag-mediated filtration system). It is designed in such a way that wastewater can be reused (Van Zyl et al. 2019). Without substantial seasonal fluctuation, a range between 3.7 log₁₀ decreases and no removal of PMMoV was found during wastewater treatment (Hamza et al. 2019). A virus is concentrated using an electronegative membrane followed by RNA extraction, reverse transcription and was concentrated by tangential flow filtration (Zhang et al. 2006; Haramoto et al. 2013). TaqMan assay was used to study PMMoV in fecal and food samples (Zhang et al. 2006). PMMoV may be utilized as a marker in source water because of its large and constant copy numbers. To evaluate the log₁₀ reduction in the treatment process, indicator of enteric viruses is required. Enteric virus and PMMoV show similar LRVs (Log reduction value). The PMMoV log₁₀ reduction was observed in CS (2.38 ± 0.74 , $n 14 \pm 13$ and 2.63 ± 0.76 , $n 14 \pm 10$ for Plant A and B, respectively) and ozonation, it is a process which destroys all microorganisms by adding ozone gas into water (1.91 ± 1.18 , $n 14 \pm 5$, Plant A). CS and ozone are the most offered treatment processes for virus reduction at DWTPs, based on the result provided in PMMoV reduction for plant A and plant B, a reduction value of 2.38 ± 0.74 log₁₀ and 2.63 ± 0.76 log₁₀ for CS and 1.91 ± 1.18 log₁₀ for ozone treatment of plant A has been observed (Kato et al. 2018). Although PMMoV and enteric viruses differing in morphological characteristics and origins had weak correlations, they had similar detection methods of qPCR and viral replication mechanism fates, such as

adsorption to clay particles, sedimentation, resuspension and inactivation (Saeidi et al. 2018). During soil aquifer treatment (SAT), a viral elimination process, two viral indicators were suggested, namely PMMoV and crAssphage, because of their 100% occurrence in the wastewater samples while the other viruses present were less in concentration (Morrison et al. 2020). Studies on PMMoV's surface charge characteristics and morphology show that it is distinct from other enteric viruses in terms of both size and form, therefore suggesting as a good viral indicator to measure reduction efficiencies.

PMMoV and other viruses

Sustainability of PMMoV in agriculture runoff, compared to other plant viruses

According to Mehle et al. (2014), PepMV (Pepino mosaic virus), PVY (Potato virus y) (NTN strain), PSTVd (Potato Spindle Tuber Viroid) can survive in water for three weeks, one week and seven weeks, respectively. Water is a crucial bridge for rapid virus/viroid dissemination Dombrovsky et al. (2017). Their findings showed that viral dissemination is much faster than other vectors such as insect and infected gardening tools (mechanical transmission). As a result, water should be considered in future epidemiological and risk assessment research. Arid regions are greatly affected by water used for irrigation, as it has been reported that infective tobamoviruses have been released into environmental waters (Filipić et al. 2021). Infected decomposing plant debris and the associated soil top surface, when washed by surface, could introduce plant viruses into water (Thebo et al. 2017). During dry weather, contaminants of a pathogen in water were indicated by PMMoV and the newly suggested TMV (Shrestha et al. 2018). Before using a different source of water (canal, pond, groundwater) for irrigation, contaminants need to be checked using the indicators PMMoV and TMV (Shrestha et al. 2018). As a result of ineffective law enforcement, the water quality in irrigation systems has dramatically damaged crops throughout the world. WWTPs (wastewater treatment plants) release raw or poorly treated wastewater effluent directly into the water bodies (river and its tributaries), which become the source of the pathogenic virus and lead to crop losses (Shrestha et al. 2018). The occurrence of plant viruses outside their host plant or insect vectors has rarely been studied and most of them obtained from aquatic environment due to inefficient detection method (Salem et al. 2016). The other plant viruses found in aquatic environment are ToMV, TMV, CRSV, TBSV, CaRMV and PVX, when tested for longevity in vitro (LIV), their sap remained infectious for several days (Brunt 1996). Tobacco mosaic virus (TMV) was found in river sample of Germany (Koenig 1988). Tomato mosaic

virus (ToMV) was observed in an open pit mine area as well as in rivers (Drava, Vipava, and Krka) in Slovenia (Boben et al. 2007). Tomato mosaic virus (ToMV) was observed in clouds and food in the USA (Castello et al. 1995).

PMMoV as a potentially helpful indicator for monitoring SARS-CoV-2

When clinical trial options are limited, waste-water monitoring may depict a supplementary approach to evaluating viral agents. Based on diagnostic cases reported in Massachusetts on March 25, 2020, viral titers of SARS-CoV-2 were remarkably high. They used PMMoV to know if there were any alterations in initial sample concentration levels and RNA losses during these experiments (Wu et al. 2020). Detection of microorganisms in waste-water or sewerage is the best method for early detection of water-borne outbreaks. During defecation, viral particles are found in the feces of two types of people (asymptomatic or symptomatic); the national wastewater surveillance system (NWSS) and government agencies can detect both types easily. The study of Medema et al. (2020) shows that the concentration of PMMoV in rectal swabs is higher than nasal swabs. Human fecal normalization evaluates the amount of human feces in water by measuring certain microbes or other compounds, such as PMMoV, which is human fecal control. They were used to compare any viral losses due to noise (wastewater dilution, sample collection, and storage or input of feces in treatment plants, laboratory analysis). PMMoV variability is lower due to their capsid structure, but it has shown similar results for SARS-CoV-2. PMMoV RNA is constantly found in the PCS (primary clarified sludge); therefore, it was used in a study of SARS-CoV-2 infection (SARS-CoV-2 N1 and N2 RNA marker) (D'Aoust et al. 2021). Irrespective of climatic circumstances over a three-month period for two WRRFs (Water resource recovery facilities) in PCS (primary clarified sludge), the RNA signal of the PMMoV was less variable (D'Aoust et al. 2021). PMMoV RNA normalization had the highest association with epidemiological measures, minimized the noise, and improved accuracy (D'Aoust et al. 2021). Two human-specific fecal biomarkers (PMMoV and crAssphage) were measured to normalize the SARS-CoV-2 gene (N1, N2, E) concentrations in comparison to other fecal bacteria in wastewater samples to clearly evaluate human-specific fecal strength in wastewater samples from Columbus wastewaters (Ai et al. 2021). Mean RNA concentrations of $\sim 1 \times 10^6$ gene copies/L of PMMoV were found in sewage when SARS-CoV-2 RNA concentrations were normalized (Ai et al. 2021). SARS-CoV-2 RNA was compared to the pepper mild mottle virus for their degradation percentages and

viability percentage. SARS-CoV-2 RNA deterioration status was similar to PMMoV compared to other enteric viruses and fecal indicator bacteria. In each of these cases for 20 days of the study, viral RNA remained stable in river water at two different temperatures, 4 and 20 °C, so PMMoV was considered as surrogates for enteric viruses (Sala-Comorera et al. 2021). In the surveillance study of SARS-CoV-2, researchers aimed to assess the incidence and prevalence of SARS-CoV-2 in health care facilities (affected and recovered patients), found low levels of SARS-CoV-2 RNA as compared to PMMoV (Acosta et al. 2021).

Future prospective and conclusion

Plant and vertebrate viruses belong to separate biological niches and there were no known shared hosts. Researchers showed that PMMoV is distributed all over the world and reported its highest occurrence in feces of humans and few animals too. Its effectiveness is highly maintained in different water conditions and wastewater treatment processes due to its intact capsid structure, therefore it is not restricted to geographical conditions. This virus is mostly found along with other human enteric bacteria. The large percentage of PMMoVs found in water was extremely stable and occurs at greater concentration and can survive for extended period of time outside the host cells. Water from sewage systems is routinely discharged into rivers and ponds, where it is utilized for irrigation. So before releasing the sewerage water into environments, indicators like PMMoVs can be used to detect the contaminants.

PMMoV is a plant virus, so its occurrence in the aquatic environment may differ from that of human enteric viruses. Therefore, there is a possibility that PMMoV in the aquatic environment came from sources other than human feces. Thus, future research should investigate the origin and destiny of PMMoV to validate it as a good enteric viral indicator and also to confirm the constituents of this as MST toolbox. It should extensively explore the health concerns in environmental waters connected with its quantifying and detecting nature and its pathogenicity toward humans. More studies are required to show the existence and survival of PMMoV in the gut, if PMMoV is proved to be not replicating in the gut, then it can be used as a plant-based vaccine to treat some chronic infections. Bacterial and viral interaction in the human gastrointestinal system is required to gain more knowledge on their interactions. In future, studies on the coexistence of PMMoV and those pathogens should be carried out, and more strategies are needed in the ecosystem for determining the risk of infectious agents in sanitation.

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