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EVALUATION OF THE SUITABILITY OF PEPPER MILD MOTTLE VIRUS (PMMOV) AS AN INDICATOR VIRUS FOR WATER SAFETY AND QUALITY

Vu Duc Canh[a], Nguyen Thuy Lien[b], Tran Thi Viet Nga[b,*]

[a]*University of Tokyo, Bunkyo-ku 7-3-1, Tokyo 113-0031, Japan*
[b]*Department of Environmental Engineering, Hanoi University of Civil Engineering, 55 Giai Phong road, Hai Ba Trung district, Hanoi, Vietnam*

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Abstract

Wastewater pollution is one of the main causes of waterborne diseases (e.g., diarrheal diseases) because wastewater commonly contains a wide range of pathogenic microorganisms, notably human enteric viruses. Monitoring multiple pathogenic viruses in waters simultaneously is impractical and expensive, so monitoring through virus indicators is essential to ensure water safety and quality. Recently, pepper mild mottle virus (PMMoV) was found as one of the most prevalent viruses in the human gut microbiota and consistently present at high concentrations in human feces and domestic wastewater. This study reviewed the latest information on the presence of PMMoV and human enteric viruses in water environments and in wastewater (water) treatment systems to evaluate the suitability of PMMoV as an indicator virus for water safety and quality. PMMoV was present in all types of waters (e.g., wastewater, surface water, groundwater, coastal water and drinking water) in greater prevalence than human enteric viruses. PMMoV was also removed less or similar to human enteric viruses in various wastewater (water) treatment systems (including disinfection treatment). These results suggest that PMMoV can be used as a suitable indicator virus for 1) assessment of water quality polluted by domestic wastewater; 2) assessment of virus removal efficiency in drinking water treatment plants and 3) assessment of viral safety for drinking water.

Keywords: indicator virus; PMMoV; water quality; virus removal; drinking water.

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1. Introduction

Wastewater pollution is a major public health concern, especially in developing countries where a large proportion of wastewater is not collected and treated adequately before discharging into water environments. According to UNESCO report 2017 [1], about 70% of wastewater (domestic and industrial) is treated in high-income countries. However, this rate is about 38% in middle-income countries and only about 28% in low-income countries. Wastewater pollution is one of the main causes of waterborne diseases (e.g., diarrhea) in the human community since wastewater commonly contains pathogenic microorganisms. Every year, about 2.2 million people die from diarrheal diseases, mainly due to unsafe drinking water and poor sanitation [2].

*Corresponding author. *E-mail address:* ngattvn@huce.edu.vn (Nga, T. T. V.)

Among pathogenic microorganisms, human enteric viruses such as norovirus (NoV), adenovirus (AdV), hepatitis A virus (HAV), hepatitis E virus (HEV), rotaviruses (RV) and enteroviruses (EV) are major causes of diarrhea, with rotavirus alone causing more than half a million deaths each year [3]. These enteric viruses (e.g., AdV, NoV, EV, HAV) are also included in the contaminant candidate list 4 (CCL4) by the United States Environmental Protection Agence (USEPA) as common drinking water microbial contaminants. Currently, there are about 140 types of enteric viruses that are known to be able to infect humans [3]. In addition to causing gastroenteritis, enteric viruses can cause respiratory infections, conjunctivitis and some dangerous diseases such as hepatitis, encephalitis, and myocarditis [3]. Enteric viruses are excreted in extremely high concentrations in the feces of infected people (up to 1011 copies/gram) and can persist for a long time in water environments [3]. In addition, the presence of enteric viruses has been reported in all water environments, including wastewater, surface water (rivers and lakes), groundwater [4] or even drinking water and tap water [4–10]. Most recently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing the ongoing coronavirus disease 2019 (COVID-19) pandemic is also a great concern since this virus has spread over 200 countries and has caused over 5.9 million deaths worldwide (8 March 2022) [11]. This virus has been detected in wastewater in many countries around the world, raising concerns about the potential transmission of SARS-CoV-2 through contaminated waters [12]. Therefore, monitoring the presence of pathogenic viruses in water (wastewater) treatment systems and water environments plays an important role in protecting the public health.

Besides, the practice of wastewater reuse for potable purposes is presently increasing worldwide due to the pressure of population growth, urbanization, and impacts of climate change [13, 14]. Since wastewater commonly contains a wide range of viruses with high concentrations, virus removal is a critical factor to be regulated in potable reuse facilities. Indeed, according to California regulations, the level of viruses from raw wastewater to finished water must be reduced 12 log10 and 20 log10 for the indirect and direct potable reuse, respectively, to ensure a safe level of potable reuse [15]. However, directly monitoring the removal of pathogenic viruses through water treatment facilities is impractical since pathogenic viruses are commonly present at low concentrations in treated water (especially after drinking water treatment processes [e.g., disinfection treatments]). Therefore, indirectly monitoring the viral indicator is essential to estimate the presence of pathogenic viruses or to evaluate the virus removal efficiency in water treatment processes.

To date, indicator bacteria (such as *E.coli* and total coliforms) are used to identify the fecal contamination in water environments or regularly monitored to ensure the microbial safety of drinking water worldwide. However, the structure or morphology of these indicator bacteria is very different from those of pathogenic viruses. Viruses are more resistant to the water (wastewater) treatment processes and are more likely to persist in water environments than indicator bacteria [3]. In fact, pathogenic viruses have been detected in drinking water in the absence of indicator bacteria [5]. Thus, indicator bacteria cannot be used to indicate the presence or absence of pathogenic viruses or to ensure the viral safety of drinking water.

Recently, pepper mild mottle virus (PMMoV), a plant virus belonging to the genus Tobamovirus in the family *Virgaviridae*, was identified as one of the viruses that have the highest concentrations in human feces (up to 10^9 viruses/gram dry stool) [16]. In metagenomic studies, the abundance of PMMoV genomes (accounting for 75.7–99.4% of total virus genomes) was also reported in healthy human fecal samples [17]. Besides, the presence of PMMoV was also higher abundant than that of human enteric viruses in untreated wastewater, treated wastewater, drinking water source and treated drinking water in the literature (Table 1). This evidence supports for the use of PMMoV as a useful

indicator virus to indicate the potential fecal pollution.

In this review, we describe the most up-to-date information on the presence of PMMoV and human enteric viruses in water environments and in drinking water treatment facilities. Besides, we also evaluate the use of PMMoV as 1) an indicator virus to indicate wastewater pollution; 2) a process indicator virus to evaluate the efficiency of virus removal in drinking water treatment facilities and 3) an indicator virus to investigate the viral safety of drinking water.

2. PMMoV detection methods

PMMoV and other RNA viruses in water are generally detected with the same methodologies, i.e., virus concentration processes followed by virus detection. Virus concentration methods are used to enrich the number of viruses before the virus detection methods owing to the small number of viruses in environmental waters (especially drinking water). Various virus concentration methods have been used to concentrate PMMoV and enteric viruses in water, such as virus adsorption-elution (VIRADEL) using a negatively or positively charged filters [18–22], hollow-fiber ultrafilters [23–25], tangential-flow ultrafilter |cite 26 and glass-wool filters [26].

Cell culture detection methods and molecular detection methods can be applied to quantify the number of PMMoV in water samples. Although cell culture is a gold standard method to detect infectious viruses, it is time-consuming, laborious and expensive. Indeed, infectious PMMoV can be detected based on observing the infection results of PMMoV on leaves of *Nicotiana tabacum cv. Xanthi-nc*. However, it takes approximately 1 month to grow and culture *Nicotiana tabacum cv. Xanthi-nc* from theirs seeds and seedlings [27]. After inoculation, it also takes 4-5 days for incubating the plants in the growth chamber [27]. Therefore, the cell culture method might not be a suitable tool for monitoring the presence of PMMoV in water. Molecular detection methods such as reverse transcriptase-polymerase chain reaction (RT-PCR) or RT-quantitative PCR (RT-qPCR) are more commonly used to detect PMMoV in water due to its rapidness, specificity and sensitivity [19–21, 28]. However, molecular detection methods are not able to discriminate between infectious and inactivated viruses and so generally overestimate the actual number of infectious viruses in water.

Recently, to overcome the limitation of conventional molecular detection methods, an advanced technique has been developed to discriminate between infectious and inactivated viruses, in which water samples are treated by capsid integrity reagents prior to (RT-)qPCR (capsid integrity [RT-]qPCR). Capsid integrity reagents (e.g., propidium monoazide [PMA], ethidium monoazide [EMA] and dichlorodiammineplatinum [CDDP]) can bind with genomes of compromised viruses with damaged capsid and subsequently block (RT-)qPCR amplification while these reagents cannot bind to genomes of infectious viruses with an intact capsid. Therefore, Capsid integrity RT-qPCR can detect only infectious viruses. This method has been successfully applied to discriminate between infectious and inactivated PMMoV after disinfection treatments (e.g., heat and chlorine treatments) [27]. Besides, successful applications of capsid integrity RT-qPCR were also reported to determine potential infectivity of various enteric viruses in environmental waters [27, 29, 30]. Therefore, this method can be a promising tool to routinely monitor the presence of intact PMMoV and enteric viruses in environmental waters. However, the efficiency of capsid integrity RT-qPCR was found to depend on types of viruses and inactivation modes. Because capsid integrity RT-qPCR relies on the integrity of viral capsid to determine the infectivity of viruses, this method can overestimate the detection of infectious viruses if viruses are inactivated while their capsid structure remains intact (e.g., viruses are inactivated by UV irradiation) [31–33].

3. PMMoV as an indicator virus for wastewater pollution

Monitoring pathogenic viruses play an important role in managing and controlling the virus infection through water environments. However, wastewater commonly contains a wide variety of viruses, thus monitoring multiple viruses simultaneously is expensive and not feasible. Some human enteric viruses (e.g., AdV, NoV, EV or AiV) or bacteriophages (e.g., F-specific and somatic coliphages) have been proposed as potential virus indicators for the presence of human enteric viruses in environmental waters because these viruses were commonly present in human feces and wastewater at high concentrations [34–36]. However, the concentration of human enteric viruses was found to fluctuate seasonally and depend on their infection level in the human community [37]. For bacteriophages, several studies have reported the presence of human enteric viruses in water environments while bacteriophages are absent [38, 39]. Therefore, using these enteric viruses or bacteriophages as indicator viruses can face critical limitations.

Recently, the presence of PMMoV has been reported in domestic wastewater and environmental waters in many countries around the world. PMMoV was consistently present at a higher concentration than human enteric viruses (Table 1). Indeed, the concentration of PMMoV in wastewater was often high above 10^5 copies/L, while the concentration of human enteric viruses (NoV, EV and AdV) was commonly less than 10^5 copies/L (Table 1). In addition, PMMoV (6.0×10^5 copies/L) was also detected in most of the treated wastewater samples with concentrations at least 10 times higher than that of human enteric viruses (including NoV, EV, AdV, RV, JC polyomaviruses [JC PyV] and BK polyomaviruses [BK PyV]) [40]. In environmental waters (such as rivers and lakes, coastal seawater) that receive treated wastewater, PMMoV was also more commonly detected than human enteric viruses (Table 1). Indeed, in Germany, when collecting river water samples at the distance of 1.5–9 km downstream from the wastewater treatment plant, PMMoV was detected in 100% (108/108) of water samples with a concentration of 3.0×10^3–1.1×10^6 copies/L while human enteric viruses (e.g., AdV) were present in 20–97% of samples at lower concentrations (5.0×10^1–5.6×10^4 copies/L) [41]. A similar tendency was also reported in a study conducted in Viet Nam indicating a higher prevalence of PMMoV than human enteric viruses in river waters [22]. For coastal water, PMMoV (1.4×10^4–6.8×10^6 copies/L) was present more commonly than human enteric viruses (e.g., NoV and AiV) (2.9×10^1–5.6×10^3 copies/L) in a study conducted at Odaiba Bay in Tokyo, Japan [20]. More notably, the presence of PMMoV in water sources (rivers, lakes) used for drinking water treatment plants has also been reported more abundant than that of human enteric viruses. Particularly, Canh et al. [5] found that PMMoV presents in 100% (20/20) of the river and lake water samples with high concentrations (1.0×10^3–2.5×10^7 copies/L) whereas human enteric viruses (AdV, EV, NoV, JC and BK PyV) were detected in 30–65% (20/20) of the collected samples at lower concentrations (6.3×10^1-1.3×10^6 copies/L). In another study, Haramoto et al. [28] investigated the presence of PMMoV in 184 source water samples from 30 drinking water treatment plants across 7 geographical regions of Japan (including Hokkaido, Tohoku, Kanto, Chubu, Kinki, Chugoku-Shikoku, and Kyushu-Okinawa). PMMoV was positive in 76% (140/184) of the collected samples with relatively high concentrations (2.0×10^3–2.9×10^6 copies/L). A similar result was also reported in other studies in Thailand indicating the high prevalence of PMMoV in water sources (Table 1).

PMMoV was consistently more abundant than human enteric viruses in feces, domestic wastewater, and environmental waters (including coastal water, surface water) (as mentioned above). This evidence suggests that PMMoV can be used as an indicator virus to indicate the presence of viral pathogens in environmental waters. However, the use of PMMoV as an indicator virus might also have limitations. Indeed, PMMoV was highly more stable than human enteric viruses in environmen-

Table 1. Occurrence of PMMoV and human enteric viruses in environmental waters

Type of water	Number of samples	PMMoV		Enteric viruses[a]		Countries
		Positive detection rate	Concentration[b] (copies/L)	Positive detection rate	Concentration[b] (copies/L)	
Domestic wastewater	2	100%	5.5×10^{6}–7.2×10^{6}	80%	3.0×10^{5}–4.2×10^{6}	Vietnam [22]
	24	100%	4.3×10^{5}–1.3×10^{9}	42–100%	3.0×10^{2}–3.0×10^{5}	USA[40]
	12	100%	1.9×10^{8}–9.6×10^{8}	25%	1.0×10^{7}–1.7×108	Germany [41]
	n/a	n/a	5.7×10^{7} (mean value)	n/a	1.4×10^{4}–2.6×10^{5}	Australia [42]
	10	100%	$10^{7.1\pm0.5}$	n/a	n/a	New Zealand [43]
	34	94.1%	9.5×10^{6}	88.2%	$\sim 2\times10^{6}$	Egypt [44]
	12	100%	$10^{7.9\pm10.35}$	58–100%	$10^{3.9\pm8.30}$	Japan [45]
	13	100%	$1.0\times10^{7}\pm3.0\times10^{0}$	69–92%	1.0×10^{5}–3.0×10^{7}	USA [46]
River/ lake	108	100%	3.0×10^{3}–1.1×10^{6}	20–97%	5.0×10^{1}–5.6×10^{4}	Germany [41]
	17	94%	3.0×10^{4}–1.8×10^{6}	18–59%	2.3×10^{5}–2.3×10^{6}	Vietnam [22]
	4	100%	1.8×10^{5}–3.4×10^{5}	100%	2.7×10^{1}–9.6×10^{4}	USA [18]
	184	76%	2.0×10^{3}–2.9×10^{6}	n/a	n/a	Japan [28]
	20	100%	1.0×10^{3}–2.5×10^{7}	30–65%	6.3×10^{1}–1.3×10^{6}	Japan [5]
	13	100%	$10^{5.4\pm0.48}$	n/a	n/a	Japan [19]
	11	100%	$10^{5.3\pm0.34}$	n/a	n/a	Japan [19]
	8	100%	$10^{6\pm0.9}$	n/a	n/a	Nepal [47]
	36	100%	1.0–3.8×10^{5}	n/a	n/a	Costa Rica [48]
	11	100%	$10^{2.9\pm0.35}$	0–82%	$< 10^{2.4\pm0.5}$	Thailand [21]
Coastal water	23	100%	1.4×10^{4}–6.8×10^{6}	97%	2.9×10^{1}–5.6×10^{3}	Japan [20]
	30	60%	8.7×10^{5} (highest)	40%	$< 5.0\times10^{3}$	USA [49]
Ground water	1	0%	n.d	0%	n.d	Vietnam [50]
	12	67%	1.4×10^{1}–4.0×10^{3}	8-17%	1.2×10^{0}–5.0×10^{1}	USA [18]
	20	85%	1.8×10^{1}–1.0×10^{4}	n/a	n/a	Mexico [24]
Drinking water	4	50%	7.6×10^{5}–9.1×10^{5}	0%	n.d	Vietnam [50]
	6	0%	n.d	0%	n.d	Vietnam [22]
	43	9%	1.6×10^{2}–7.9×10^{2}	5%	4.2×10^{0}–1.5×10^{1}	Japan [5]

[a]Type and the number of enteric viruses might be different among studies;
[b]Virus concentration was determined based on positive samples only;
n/a: not available;
n.d: not detected.

tal waters [51]. The presence of PMMoV was found in some cases where all human enteric viruses were absent [5]. Therefore, the presence of PMMoV might not always correlated with the presence of pathogenic viruses in environmental waters or the use of PMMoV as a virus indicator might overestimate the risk of enteric virus infection. Due to the high environmental stability, a previous study also suggested that PMMoV might not be a suitable indicator virus to indicate the fresh fecal pollution in water bodies or the presence of pathogens [41]. However, in a recent study in Japan, Canh et al. [5] found that at concentrations of PMMoV higher than 8×10^{4} copies/L, PMMoV always coexisted with human enteric viruses in environmental waters, indicating that a threshold concentration of PMMoV can be used to assess the presence of enteric viruses in environmental waters. The threshold concentration of PMMoV might be different depending on geopraphic regions. Thus, more studies are needed to identify the universal threshold concentration of PMMoV for indicating the presence of human enteric viruses in environmental waters. Besides, most previous studies applied (RT-)qPCR to assess the presence of PMMoV and human enteric viruses in environmental water. Nevertheless,

this method is not able to assess viral infectivity. Thus, additional studies are needed to investigate the link between the presence PMMoV and the risk of viral infections.

Besides, although PMMoV was not detected in water uncontaminated by human feces/domestic wastewater, the presence of PMMoV was found in the feces of some animals (especially from seagulls, swans, geese) [4, 41, 43]. Therefore, the presence of PMMoV in environmental waters might not always indicate human fecal pollutions in environmental waters. However, due to the consistent occurence and high abundance of PMMoV in domestic wastewater (including raw and treated wastewater), this virus can be used as a potential viral indicator to indicate the human fecal pollutions, especially in areas with minimal farming activity of avian [43].

4. PMMoV as a process indicator virus to evaluate the efficiency of virus removal in drinking water treatment systems

PMMoV was commonly present in domestic wastewater at high concentrations (up to 10^9 copies/L) (as mentioned above). PMMoV was also found to be more resistant to wastewater treatment processes (such as conventional activated sludge process, trickling filter process and upflow anaerobic sludge blanket reactor [UASB]) than human enteric viruses (e.g., EV, NoV, AdV, JC and BK). PyV) [46, 52, 53]. Therefore, PMMoV has been proposed as a useful indicator virus for evaluating the virus removal efficiency of wastewater treatment systems or full-scale wastewater treatment plants. Furthermore, the abundance of PMMoV is consistently reported in water environments with high concentrations (up to 10^7 copies/L, as mentioned above) and PMMoV can also survive in water environments (e.g., river water) longer than pathogenic viruses (e.g., AdV and PyV) [41]. Therefore, PMMoV has great potential to be used as a process indicator virus to evaluate the virus removal efficiency of drinking water treatment systems.

Currently, there have been several studies evaluating the efficiency of PMMoV removal in drinking water treatment systems at both laboratory scale and full-scale. In a laboratory-scale experiment, Kato et al. [19] found that PMMoV was removed 1.96 ± 0.30 $\log_{10}$ by the coagulation-sedimentation process and 0.26 ± 0.38 $\log_{10}$ by the rapid sand filtration, which was comparable to the removal of tested bacteriophages (e.g., Qb and MS2) and human enteric viruses (e.g., 1.86 ± 0.61 $\log_{10}$ and 0.28 ± 0.46 $\log_{10}$ for NoV II, respectively). Similar results were also observed in other studies indicating that the removal efficiency of PMMoV by the coagulation combined with the rapid sand filtration was similar to that of human enteric viruses (e.g., AdV, coxsackievirus (CV), HAV and MNV) (0.8–2.5 $\log_{10}$) [27]. Furthermore, similar removal efficiency between PMMoV and human enteric viruses (e.g., AdV, CV, HAV and MNV) was also obtained when investigating other water purification systems such as MF and UF membranes [54].

Several studies have been conducted to evaluate PMMoV removal efficiency in full-scale drinking water treatment systems (Table 2). The removal efficiency of PMMoV by the coagulation + sedimentation process ranged from 2.38 $\log_{10}$ to 2.63 $\log_{10}$ while this value was less than 0.9 $\log_{10}$ by filtration processes (e.g., rapid sand filtration and microfiltration) [19, 55]. Due to the small size, viruses might not be removed effectively by the filtration processes like rapid sand filtration and microfiltration. However, PMMoV was removed more effectively by slow sand filtration (up to 2.8 $\log_{10}$) [55]. In slow sand filtration, viruses can be removed by the upper layers of sand bed (biofilm), which contains many adsorption sites [55]. For the entire treatment processes in the drinking water treatment plant (including the disinfection treatment), the efficiency of PMMoV removal was found in a range from 3.5 $\log_{10}$ to over 6.8 $\log_{10}$ depending on the treatment technology and the condition of treatment processes (e.g., types and dosages of coagulants in coagulation processes or CT values in chlorination

treatment) (Table 2). In addition to PMMoV, the removal efficiency of human enteric viruses was also evaluated in these studies. However, human enteric viruses were commonly present at low concentrations in source water (1.2–6.1 $\log_{10}$) and were completely under the detection limit in treated water when determined by molecular detection methods (e.g., (RT-)qPCR). Therefore, comparing the removal efficiency between human enteric viruses and PMMoV in full-scale drinking water treatment plants is difficult. Previous studies consistently found that PMMoV was frequently detected in source water and treated water whenever pathogenic viruses were detected or not. Thus, PMMoV can be a suitable process indicator virus to evaluate the virus removal efficiency for full-scale drinking water treatment plants.

Table 2. Removal of PMMoV and human enteric viruses in full-scale drinking water treatment systems

	PMMoV ($\log_{10}$)	Enteric viruses ($\log_{10}$)[a]	
Drinking water treatment processes			
Coagulation + Sedimentation	2.38±0.74	-	[19]
	2.63±0.76	-	[19]
Rapid sand filtration	0.26±0.38	-	[19]
Slow sand filtration	< 2.8	-	[55]
Microfiltration	< 0.9	-	[55]
Coagulation + Microfiltration	0.7–1.5	-	[56]
Ozonation	1.91±1.18	-	[19]
Drinking water treatment plant			
Coagulation + sedimentation, rapid sand filtration, chlorine disinfection	> 5.0 – > 6.8	> 2.0 – > 4.6	[5]
Coagulation + sedimentation, rapid sand filtration, chlorine disinfection	4.6 – > 6.7	> 2.3 – > 6.1	[5]
Slow sand filtration, chlorine disinfection	3.5 – > 6.8	> 2.2 – > 5.2	[5]
Microfiltration, chlorine disinfection	4.7 – > 6.4	> 1.2 – > 4.8	[5]
Coagulation + sedimentation, rapid sand filtration, Biological activated carbon (BAC), chlorine disinfection	> 3.0 – > 5.7	> 3.1 – > 4.2	[5]

[a]Type and the number of enteric viruses might be different among studies;
-: not applicable.

5. PMMoV as an indicator virus for the viral safety of drinking water

The presence of human enteric viruses in drinking water is a major public health concern because human enteric viruses are able to cause diseases at low infection doses (only a few virus particles)

[57]. Furthermore, the risk of infection caused by viruses in drinking water is 10 to 10,000 times higher than that of bacteria [58]. Recently, the presence of human enteric viruses in drinking water has been detected in many countries around the world, such as China, Brazil, Ghana, France, Japan and Sweden [4–8, 29, 59]. In addition, several waterborne outbreaks associated with human enteric viruses in drinking water have been reported, particularly in the US, Finland and Australia [10, 60, 61]. Indicator bacteria (such as total coliform and *E.coli*) are commonly used to assess the microbiological quality of drinking water. However, these indicator bacteria were not detected while human enteric viruses were detected in the above studies [5, 8]. Besides, human enteric viruses were also detected in drinking water which has relatively high free chlorine residuals (0.65–0.84 mg/L) [8]. Therefore, the use of indicator bacteria or the free chlorine value cannot sufficiently assess the presence of pathogenic viruses in drinking water. Since regular monitoring of pathogenic viruses in drinking water is not feasible and expensive, indirect monitoring through an indicator virus is essential to ensure the viral safety of drinking water [5].

PMMoV was consistently found at higher concentrations than human enteric viruses in environmental waters (Table 1) and removed less or equal to human enteric viruses by various water treatment processes (such as sedimentation, rapid sand filtration, ultrafiltration and microfiltration) [5, 19, 21, 55] (discussed above). Notably, PMMoV was also found to be more resistant to the chlorine treatment than coxsackievirus B5 (CV-B5) [5, 62], which was recognized as the most chlorine resistant among enteric viruses [63, 64]. Indeed, at a free chlorine concentration of 0.5 mg/L, CV-B5 were completely inactivated after a contact time of 3 min (> 4.0 $\log_{10}$inactivation) while PMMoV required longer than 240 min to achieve the similar level of inactivation (4.0 $\log_{10}$). Furthermore, when assessing the presence of viruses in tap water, PMMoV (9%, 4/43) was more frequently detected than human enteric viruses (including NoV [0%], EV [0%], AdV [0%], AiV [5%], JC PyV [0%] and BK PyV [0%]) [5]. PMMoV with an intact capsid (potentially infectious) was also detected in tap water while none of human enteric viruses was detected [5]. This evidence suggests that the absence of PMMoV can be used to ensure the absence of human enteric viruses in drinking water or the safety of drinking water.

However, it should be noted that the presence of PMMoV in various water sources was consistently greater abundant than that of human enteric viruses. In addition, PMMoV showed higher resistance to water treatment processes (including physical treatment processes and disinfection treatments) than human enteric viruses [5, 62]. Therefore, the presence of PMMoV in drinking water might not always indicate the presence of enteric viruses. Besides, a previous study found the inconsistent occurrence between PMMoV and human enteric viruses (e.g., HEV) in groundwater and tap water [22]. It was also reported that PMMoV did not co-exist with human enteric viruses (e.g., AiV) in tap water produced from groundwater although this tendency was not found in tap water produced from surface water [5]. This evidence suggests the limitation of PMMoV as a viral indicator of enteric viruses in tap water produced from groundwater [5]. Since the evaluation of the relationship between PMMoV and human enteric viruses in drinking water remains limited [5, 22], more studies are needed to confirm the use of PMMoV as a useful indicator virus to control the viral safety of drinking water. In addition, it is important to note that most previous studies used quantitative reverse transcription-polymerase chain reaction (RT-qPCR) or quantitative polymerase chain reaction (qPCR) methods to evaluate the presence of viruses in waters. These methods can only detect viral genomes, but cannot distinguish between infectious and non-infectious viruses. Therefore, further studies are recommended to evaluate the relationship between infectious PMMoV and human enteric viruses in drinking water (especially after the disinfection treatment) and the link of the presence of PMMoV

with the infection risk of enteric viruses in drinking water. Recently, capsid integrity (RT-)qPCR has been developed to distinguish between intact viruses (potentially infectious) and inactivated viruses in waters [5, 65–67] (as discussed above). This method can be used to assess more accurately the relationship between PMMoV and human enteric viruses in drinking water and is a useful tool to monitor potentially infectious pathogenic viruses in drinking water.

6. Conclusions

This review revealed that PMMoV was detected in all types of waters (e.g., wastewater, surface water, coastal water and drinking water) more frequently than human enteric viruses. Thus, PMMoV can be considered to use as an indicator virus to assess water quality polluted by wastewater. Besides, PMMoV was consistently removed less or equal to human enteric viruses throughout various wastewater (water) treatment processes. Furthermore, PMMoV was also resistant to disinfection treatment (e.g., chlorination) and was present in drinking water whereas all human enteric viruses were absent. Therefore, PMMoV can also be used as an appropriate indicator virus to investigate the virus removal efficiency in drinking water treatment plants or assess viral safety of drinking water.

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