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Case Report

A review on mpox (monkeypox) virus shedding in wastewater and its persistence evaluation in environmental samples

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ABSTRACT

Since May 2022, an outbreak of mpox (formerly known as monkeypox) has been spreading in non-endemic countries and this disease is caused by a mpox virus (MPXV) belonging to *Orthopoxvirus* genus of the *Poxviridae* family. Public health surveillance is essential to assess the disease prevalence and spread in the populations. In this purpose, wastewater surveillance has proved to be an effective and affordable tool to detect and monitor disease threats and outbreaks, such as SARS-CoV-2, poliovirus, and other viruses. Prior to the current mpox outbreak, previous studies suggested that MPXV shedding is possible after viral genome and infectious particles were detected in the feces of infected animals. On the other hand, using metagenomic and sequencing approaches to assess the diversity of human viral pathogens in wastewater, contigs assigned to orthopoxviruses and poxviruses were among the most abundant contigs detected in collected samples. Recently, MPXV DNA has been detected in wastewater samples from wastewater treatment plants in cities and airports in the United States, the Netherlands, Italy, France, and Spain. It has long been reported that poxviruses are stable in the environment for long periods and might represent as a source of transmission. However, the stability of MPXV in the environment is unknown and the presence of infectious MPXV in wastewater has not yet been determined. The present work is devoted to reviewing past and recent advances in MPXV detection in wastewater. It also addressed the survival data of orthopoxviruses, which may be of interest to evaluate the persistence of MPXV species in environmental matrices.

1. Introduction

Mpox (formerly known as monkeypox) virus (MPXV) is an enveloped double-stranded DNA virus that belongs to the *Orthopoxvirus* genus of the *Poxviridae* family and the *Chordopoxvirinae* subfamily [1,2]. The genome size of MPXV is approximately 190 kb and contains ≈190 nonoverlapping ORFs >180 nucleotides long [3,4]. In addition to MPXV, the *Orthopoxvirus* genus includes another 8 species responsible for animal and human pox such as variola virus (which causes smallpox), vaccinia virus (VACV) which was used in the smallpox vaccine, buffalopox virus, camelpox virus, and cowpox virus [5,6].

MPXV was first identified in 1958 after the pox-like disease outbreaks occurred in monkeys transported from Africa to Copenhagen, Denmark for research purposes [7], hence the name 'monkeypox'.

The first human case of mpox associated with a 9-month-old child was reported in September 1970 in the Democratic Republic of Congo (former Zaïre) [8] during a period of intensified effort to eliminate smallpox. Smallpox vaccination campaigns have provided cross-immunity against MPXV until the early 1980's [9]. The eradication of smallpox and the cessation of smallpox vaccination changed the picture dramatically, particularly in Kasai Oriental, Democratic Republic of Congo where the mpox disease became endemic [10]. Since then, other countries of Central and West Africa reported mpox cases. From that time, MPXV is considered as an emerging pathogen and the most common cause of human orthopoxvirus infections in the smallpox post-eradication era [11].

Since the identification of the first human mpox, sporadic cases and outbreaks associated with travel from endemic regions have been recognized in non-endemic countries where infection has been primarily linked to zoonotic transmission from small mammals [1].

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In early May 2022, laboratory confirmed cases of mpox, not linked to travel to endemic countries, were diagnosed in multiple European countries and then cases were identified in the United States and in several non-endemic countries around the world. Human-to-human transmission by close contact with infected people (e.g. respiratory droplets, skin-on-skin, or sexual contact) is currently considered the main route of MPXV transmission during this global outbreak [1,12,13]. Recently, airborne transmission has been suggested [14]. In late July 2022, WHO declared mpox a public health emergency of international concern [15].

Historically, two genetically distinct clades of MPXV have been described: the MPXV West African clade and the MPXV Congo Basin clade, also known as the central African clade [16,17]. The West African clade viruses appear to cause less severe disease with lower mortality, and less frequent human-to-human transmission [16].

More recently, MPXV was classified into three clades: clade 1 (formerly designated as Congo Basin clade), and clade 2 and clade 3 (belong to the formerly designated West African clade) [18,19]. These clades have distinct geographical, clinical, genomic and epidemiological differences and differ mostly in their coding regions, which are primarily related to immunomodulatory and host recognition antigenic determinants like H3L and B21R [19]. Moreover, they correlate with the different epidemiological MPXV outbreaks. For instance, MPXV clades 1 and 2 include most of isolates linked to outbreaks from the Democratic Republic of Congo (MPXV Clade 1) and from West Africa (MPXV Clade 2) [19]; while MPXV clade 3 includes isolates originating from the 2017–2019 [19] and the current outbreaks [18,19].

Mpox disease is generally characterized on infected people by non-specific initial symptoms including fever, and then an eruptive phase, characterized by skin lesions, begins one to three days after the onset of hyperthermia. These lesions, which first affect the face before appearing on other parts of the body including the palms and soles of the feet, mature into crusts and fall off [13,20]. Lesions may also affect genital areas, and the presence of genital lesions is particularly common in infected individuals in the current extra-African episode [20] as well as in the outbreak in Nigeria during 2017–2018 [21].

The most susceptible populations, i.e. those with a higher than average probability of developing symptoms or severe clinical forms of mpox after exposure to MPXV, are immunocompromised individuals, pregnant women and young children [22]. Children are recognized to have a more severe forms of mpox than adults [23,24]. This higher susceptibility of neonates and young people is also reported in animals experimentally infected with MPXV [25]. According to the literature, the probability of transmitting MPXV to people living with an infected person (secondary attack rate) is in the order of 10% [26], without specifying the routes of exposure involved.

Since the first reported cases of mpox in May 2022, the public health system has made efforts to detect the circulating virus disease [27]. However, clinical testing may be limited by the availability of adequate biosafety laboratory equipment, clinician awareness and training levels, and the potential for asymptomatic cases [27,28].

A complementary approach to public health surveillance through wastewater testing provides a potential additional tool for monitoring the presence of MPXV circulation in a community. As for other pathogens agents, it can also capture asymptomatic and/or infraclinical infections and detect infections in a community with poor healthcare accessibility [29].

To monitor MPXV through wastewater-based epidemiology (WBE), the virus must be shed in wastewater. Previous studies have described that MPXV can be excreted in the feces of infected animals [30,31]. During the 2022 outbreak, shedding of MPXV DNA was reported in feces, urine, saliva, semen and skin lesions from infected individuals [32,33]. These excretions can enter the sewage system through toilets, sinks, and shower drains, which has been confirmed by the detection of MPXV genome in wastewater samples [2,27,34–38]. However, no data on the stability of MPXV in environmental samples have been described to date [39]. Published works only described the thermal inactivation and environmental stability of members of the genus *Orthopoxvirus* including VACV and variola virus. Therefore, the aim of this review is to cover the aspect of MPXV shedding from previous and recent evidence and to compile its detection in wastewater. It also addressed the survival data of others orthopoxviruses, used as surrogates, including VACV and variola virus in environmental samples. Because MPXV seems to have diverged from a close common ancestor of cowpox virus [40], such orthopoxviruses survival data may be of interest to evaluate the persistence of MPXV species in environmental matrices.

2. Methodology

The review was prepared based on literature search in the SCOPUS database for publications up to November 10, 2022. The keywords employed for the search were monkeypox virus and wastewater or shedding or feces or urine or environment or survival or persistence as well as orthopoxvirus and environment or survival or persistence, being the results presented in Table S1.

All results of the search were screened manually for relevant information and their references searched for additional publications that may be relevant. There are several manuscripts in the pre-print form with a wealth of information relevant for the review were also selected.

A total of 368 articles were selected, in which the abstract was screened to verify whether they really contained the information described above. At this stage, 311 articles were excluded because they did not contain the required information, resulting in 57 eligible articles that were full text screened and used as references for this review. These articles were 7 for MPXV and wastewater, 11 for MPXV and shedding, 12 for MPXV and feces, 10 for MPXV and urine, 3 for MPXV and survival and 3 for MPXV and persistence. In addition 4 articles were selected for orthopoxvirus and environment, 4 articles for orthopoxvirus and survival and 3 articles for orthopoxvirus and persistence.

3. Evidence of MPXV shedding

WBE can be is an effective tool for the public health surveillance to detect and monitor infectious disease trends because wastewater sample represents a collection of biological samples from a population served by wastewater treatment plant. Many infectious agents are excreted into the sewerage by feces, urine, but also through respiratory secretions and skin abrasions [29]. WBE has been shown to be an effective tool for monitoring poliovirus circulation [36] and the epidemiological trends of SARS-CoV-2 [27,29] as well as for the detection of the respiratory virus outbreaks such as influenza A virus and respiratory syncytial virus [27]. Many studies of SARS-CoV-2 have reported a positive correlation between the incidence of daily new cases of infections and the wastewater viral titers at the WWTP [41,42]. People infected with MPXV excrete the virus DNA via skin lesions, saliva, feces, and urine and these can enter the wastewater via toilets, sinks, and shower drains [27,32,33].

A few studies investigated the detection of MPXV in experimental animals and human feces. However, it is not confirmed whether feces either may contain excreted MPXV, or become contaminated when defecating [27] as a high proportion of positive rectal swabs among asymptomatic [43,44] and symptomatic individuals was reported [32, 45].

Surveillance of this virus in wastewater could therefore represent a potentially interesting tool for monitoring the level of circulation in the population. However, precedent work comparing trends of wastewater data with epidemiological data (virological and clinical) in the population would be necessary to provide proof of concept before considering routine wastewater surveillance to track a mpox outbreak. Furthermore, such comparison would only be possible in countries with population-based clinical or virological surveillance systems.

The MPXV genome was recently detected in the urine [32,46] and feces of infected patients [32,33]. Such excretion was already observed

in chimpanzees where Patrono et al. [31] analyzed 492 fecal and 55 urine samples from wild chimpanzees in Ivory Coast MPXV outbreaks. Viral DNA was detected in 12.6% of fecal and 20% of urine samples collected respectively from 19 (7 symptomatic, 12 asymptomatic) and 3 (2 symptomatic, 1 asymptomatic) individuals (Table 1). They also identified the circulation of at least two distinct MPXV lineages and documented the shedding of infectious particles in feces as infectious MPXV was found in one out 10 analyzed positive PCR samples. Similar observation was also reported by Hutson et al. [30] who experimentally infected prairie dogs with MPXV and showed that viral DNA was detected in fecal samples, and notably, tissue culture results showed that DNA fecal samples were associated with infectious viral particles [30] (Table 1). Further, using sequencing and metagenomic approaches to examine viral diversity and abundance in wastewater samples, a high abundance of contigs assigned to *Orthopoxvirus* and *Poxviridae* was also observed [47,48] (Table 1) suggesting that genomes of poxviruses and orthopoxviruses members, including MPXV, are likely to be detected in wastewater.

Interestingly, MPXV excretion was modeled to explore the probability of detection of mpox in wastewater under different settings and detection limit ranges [49]. This theoretical work, based on animal studies, suggests that monitoring of MPXV in sewage is feasible. However, it also advocates for the acquisition of data on infection kinetics and shedding in different human body fluids to relate sewage monitoring data to mpox clinical cases.

During the current human outbreak, Wolfe et al. [27] investigated the presence of MPXV in settled solids and liquid influent samples from nine wastewater plants in Greater Bay Area of California, USA over the period of approximately 4 weeks. MPXV DNA was detected, by droplet digital PCR (ddPCR) targeting the G2R_G and the G2R_WA assays, in the settled solids samples and ranged from non-detect to 24,114 genome copies (GC)/g dry weight of wastewater solids (Table 1). All liquid influent samples (15/15) were positive for the presence of MPXV genome and DNA concentrations were near the detection limit. The viral DNA concentrations measured in the solid fraction was 3 $\log_{10}$ unit higher than those detected in the liquid fraction of wastewater on a mass-equivalent basis. In a retrospective study, positive detection of MPXV by qPCR was reported in wastewater samples originating from WWTPs in Amsterdam and Schiphol airport in Netherlands based on the MPXV generic (G2R_G) and MPXV West African specific (G2R_WA) assays [35]. No concentrations were reported in the analyzed samples and the study was based on the cycle threshold (Ct) detection. Ct values of the positive samples ranged between 38 and 45, indicating the presence of relatively low DNA concentrations (Table 1). Samples with Ct values below 40 were confirmed for the presence of MPXV using an additional conventional PCR specific for the West African clade. In another study, La Rosa et al. [36] investigated the presence of MPXV in 20 wastewater samples from Rome airport using qPCR targeting the G2R region (TFN gene), F3L, and N3R genes (Table 1). They detected the presence of MPXV in two samples with Ct of 38.37 and 40.18, indicating low DNA concentrations in the tested samples (estimated concentrations $\approx$ 0.5 and 1.7 GC/µl of nucleic acid, equivalent to approximately 277 and 944 GC/L of wastewater). In France, concentrations of MPXV DNA were estimated between 1000 and 10,000 GC/L after analyzing 264 wastewater samples from 16 sewersheds located in Paris [34] (Table 1). In Spain, Giron Guzman et al. [37] analyzed 312 samples from 24 different WWTPs and showed that 63 positive samples were detected with concentration range from 2.2×10^3 to 8.7×10^4 GC/L of wastewater. These studies seem promising although low genome copies were reported [27] or estimated in some studies [34–36]. Regardless of the level of circulation of the virus among the population, the viral load in wastewater is affected by several factors. First, feces that reach the sewerage undergo a large dilution [50] and this dilution causes a decrease in the viral load in wastewater in comparison to the viral concentration in the feces. Second, not the entire population served by WWTP shed the virus because of the limited percentage of mpox positive cases. Third, as others enveloped viruses, MPXV in the sewerage system could be adsorbed onto solid particles and finally, the viral load is influenced by microbial activities and diverse pollutants present in wastewater.

The detection of MPXV genome in wastewater samples is a matter of concern for the virus transmission routes and the release of infectious virions in the environment. However, concerning the possible presence of infectious MPXV in the detected samples, Paran et al. [51] correlated MPXV Ct values to virus infectivity and defined a PCR threshold value Ct $\geq$ 35 (viral DNA $\leq$4300 GC/mL) that predicts no or low infectivity in the viral cultures. According to their prediction, we can estimate that positive MPXV wastewater samples were no or poorly infectious in laboratory conditions.

4. Environmental resistance of poxviruses

Detection of MPXV genome in wastewater samples does not imply that the virus is viable and infectious as many factors, such as temperature, pH, solids, micropollutants, may affect the persistence of the infectious virus in environmental samples [52]. Based on a literature search, no study to date has reported the persistence of MPXV in environmental samples [39]. However, the survival of other viruses of the *Poxviridae* family, such as VACV and variola virus, has been documented. Therefore, in the absence of MPXV data, results obtained with members of *Poxviridae* family may be used as surrogates to describe the survival of MPXV in environmental samples.

The virion of a poxvirus is distinguished from other enveloped viruses by the presence of a low lipid content in their envelope, although there are considerable differences among the various subfamilies and genera of poxviruses [53]. Poxviruses are also characterized by the presence of small amounts of carbohydrates (about 3%) [53]. Due to their low lipid content, they are less sensitive to organic solvents/disinfectants compared to other enveloped viruses. Despite all these aspects, poxviruses are extremely sensitive to all generally approved disinfection regimens [53].

Viruses of the *Orthopoxvirus* genus are also known to be stable in the environment for long periods of time [54]. Materials from infected patients (e.g., skin crusts) or fomites (e.g., bed linen) can remain infectious for months to years [53]. Besides, nosocomial transmission of MPXV to a health worker through mpox patient clothing and bedding was reported in the UK in 2018 [55]. Compared to other enveloped viruses, poxviruses exhibit exceptional resistance to drying, increased temperature and pH tolerance [54].

Although the environmental resistance of poxviruses is high at ambient temperatures, it is even greater at lower temperatures. Dried VACV can be stored at 4 °C for more than 35 weeks without decrease in infectivity. Frozen in buffer at −20 °C, a 3 $\log_{10}$ reduction in VACV titer was observed after 15 years [53], which can strongly reflect their potential stability. Mahnel et al. [56] studied the persistence of VACV strain Elstree in drinking water (pH 7.52), lake water, and river water (pH 8.05) spiked with VACV strain Elstree at 10^4 $TCID_{50}$/ml and stored at 10 °C (Table 2). They observed that the viral titer had decreased to 10^{2-3} $TCID_{50}$/ml after 200 days. Essbauer et al. [57] examined the viability of VACV strain Munich 1 (VACV M1) in slightly acidic (pH 5.4–5.7), nonsterile storm water stored at 4 °C for 2 weeks (Table 2). The virus remained viable throughout the experiment without significant reduction in viral titer. Interestingly, prolonged experiments showed that VACV M1 stored at 4.5 °C remained infectious for up to 56 days in storm water and for up to 166 days in storm water supplemented with 10% fecal calf serum. At room temperature, the virus remained infectious in storm water until the third day, and in storm water supplemented with 10% fecal calf serum for two weeks. However, in storm water supplemented with potting soil, the viruses were infectious for 5 days at 4.5 °C and for 2 days at room temperature.

Hahon and Kozikowski [58] studied the thermal inactivation kinetics of variola virus (Yamada strain) in phosphate buffered saline, pH 4.5 at different temperatures (40 °C, 45 °C, 50 °C and 55 °C). The decay rate of

Table 1
Data on the detection of MPXV DNA in feces and wastewater samples.

Reference	Location	Type of sample, treatment or collection conditions	Number of samples	MPXV concentration method	MPXV detection assay	Results
Wolfe et al. [27]	Greater Bay Area of California, USA	Settled solids and liquid influent samples from nine wastewater plants over the period of approximately 4 weeks (from 19 June 2022 to 20 July 2022)	287 settled solids and 15 influent samples	For settled solid samples: without concentration step For influent samples: affinity-based capture method with magnetic hydrogel Nanotrap Particles	Droplet digital PCR using MPXV generic (G2R_G) and MPXV West African specific (G2R_WA) assay	In wastewater solids: •Concentrations of MPXV DNA ranged from non-detect to 24,114 GC/g dry weight of solids •No significant difference between results from the G2R_G assay and the G2R_WA assay In liquid influent: • 15/15 were positive for the G2R_G assay and G2R_WA assay • Concentrations in all samples were near the detection limit Concentrations of MPXV DNA is 10^3 times higher in the solid fraction compared to the liquid fraction
de Jonge et al. [35]	Amsterdam and Schiphol airport, Netherlands	Wastewater from Amsterdam and Schiphol airport (since 23 May 2022)	Not indicated	Without concentration step	qPCR using MPXV generic (G2R_G) and MPXV West African specific (G2R_WA) assay	Frequency of MPXV DNA detection in wastewater samples according to periods: • 64% (58/90) during 23 May – 3 July 2022 • 25% in week 21 • More than 50% of the samples from week 22 onwards Low DNA concentrations: Ct values ranged between 38 and 45 Positive results with additional conventional PCR was performed on samples with Ct values below 40 using the G2R_G_WA assay
La Rosa et al. [36]	Rome airport, Italy	Wastewater samples since 30 May 2022	20	PEG/NaCl precipitation	qPCR targeting the G2R region (TFN gene), F3L, and N3R genes Nested PCR assays were also used for confirmation by sequencing	2 samples tested positive: •Ct values obtained were 38.37 and 40.18 •Estimated concentrations ≈ 0.5 and 1.7 GC/µl of nucleic acid, equivalent to approximately 277 and 944 GC/L of wastewater Sequencing results confirmed the occurrence of MPXV in the airport's wastewater
Wurtzer et al. [34]	Paris, France	Wastewater samples from 16 sewersheds located in the city of Paris from 11 April to 11 July 2022	264	Ultracentrifugation	qPCR using the MPXV TaqMan assay (#Vi07922155_s1, ThermoFisher scientific) targeting the gene J1L	Frequency of MPXV DNA detection increased from 18% (May 23rd) to 57% on July 11th, 2022 MPXV DNA concentrations were estimated between 1000 and 10,000 GC/L
Giron Guzman et al. [37]	Spain	Wastewater samples from 24 different wastewater treatment plants between 9 May and 4 August 2022	312	Aluminum-based adsorption precipitation	qPCR using MPXV West Africa (G2R_WA) assay and for some sample, an additional assay of MPXV generic (G2R_G) was used	63 samples tested positive: • Ct values ranged between 34.5 and 44.3 • Concentrations range from 2.2×10^3 to 8.7×10^4 GC/L of wastewater
Antinori et al. [33]	Italy	Feces	10 samples from 4 mpox patients	NA	qPCR using Orthopoxvirus PCR Kit (Altona Diagnostics GmbH) and the MPXV G2R_G assay targeting the tumour necrosis factor (TNF) receptor gene	Viral DNA was detected in 2 samples from 2 different patients: •Ct values obtained were 22.6 and 26.1
	Spain	Feces		NA	Samples were analyzed by PCR	

(*continued on next page*)

Table 1 (*continued*)

Reference	Location	Type of sample, treatment or collection conditions	Number of samples	MPXV concentration method	MPXV detection assay	Results
Peiró-Mestres et al. [32]			22 samples from 12 mpox patients			MPXV DNA was detected in 14 samples from 8 patients: • Ct values ranged between 17.8 and 31.4 • MPXV DNA was detected in several samples taken between 4 and 16 days post-symptom onset
Patrono et al. [31]	Tai National Park, Ivory Coast	Fecal and urine samples from wild living chimpanzees in Tai National Park were collected during MPXV outbreaks	492 fecal and 55 urine samples	NA	Samples were analyzed by PCR Viability of MPXV was investigated in ten fecal samples showing positive PCR results	Viral DNA was detected in 19 (7 symptomatic, 12 asymptomatic) fecal samples (12.6%)20% of urine samples were positives Infectious MPXV was found in one out of ten positive PCR sample Detection of MPXV DNA in feces spanned a period of time of 12 days before and 2 months after the first clinical signs
McCall et al. [48]	Detroit, Michigan	Untreated wastewater samples collected from Water Resource Recovery Facility	18	NA	Viral diversity and abundance using next-generation sequencing and metagenomic analyses	*Poxviridae*, *Herpesviridae* and *Picornaviridae* displayed the greatest number of contigs
O'Brien et al. [47]	Bugolobi, Uganda	Influent wastewater samples from WWTP	6	NA	Viral diversity and abundance using next-generation sequencing and metagenomic analyses	*Poxviridae* displayed high number of hits with other human and vertebrate viruses
Hutson et al. [30]		Prairie dogs were infected (at a level of $10^{4.5}$ PFU in a total volume of 10 mL) with West African and Congo Basin strains by either an intranasal or intradermal via scarification route	Not indicated	NA	Specimens were first tested for the presence of DNA by PCR Positive sample were subsequently evaluated for viable virus by tissue culture propagation	Fecal shedding begun on day 9 and viral continued until day 15 Tissue culture results showed that the DNA in fecal samples were associated with infectious viral particles

NA: Not applicable.

the virus (K) was 5.7×10^{-2} min^{-1}, 9.6×10^{-2} min^{-1}, 1.8×10^{-1} min^{-1} and 3.5×10^{-1} min^{-1} at 40 °C, 45 °C, 50 °C and 55 °C respectively (Table 2) which corresponded to T_{90} values (time for 90% reduction, or 1-$\log_{10}$ reduction) of 40.39, 23.98, 12.79 and 6.57 min, respectively; after applying the conversion formula $T_{90} = -Ln(0.1)/K$. Comparing with SARS-CoV-2, Batéjat et al. [59] reported that in cell culture medium spiked with 6 $\log_{10}$ $TCID_{50}$/mL of SARS-CoV-2, complete destruction of the virus (6 $\log_{10}$ reduction) was achieved after 30 min at 56 °C and the time for 1-$\log_{10}$ reduction (T_{90}) was 5 min. Because heat treatment at 56 °C is commonly used for inactivation of enveloped viruses and given the fact that poxviruses was shown to be stable over a wide range of pH (4.5–10) [53], we can consider that the orthopoxviruses exhibiting similar sensitivity to elevated temperature as SARS-CoV-2 in laboratory medium. However, it is still unknown the susceptibility to heat in wastewater and other environmental samples.

Regarding persistence on surfaces, Wood et al. [60] studied the persistence of VACV inoculated with 10^7 PFU on glass, galvanized steel, painted cinder block and industrial carpet. The study was conducted under different environmental conditions combining low relative humidity (1–10%), high relative humidity (89–100%), low temperature (6–7 °C) and room temperature (21–23 °C) during 56 days. They found that VACV was most persistent at low temperature and low relative humidity, with more than 10^4 PFU recovered from glass, galvanized steel, and painted cinder block at 56 days (representing only about 2 $\log_{10}$ reduction). Therefore, the time for 1-$\log_{10}$ reduction (T_{90}) was equivalent to about 28 days. At room temperature and high relative humidity, a 3.15 $\log_{10}$ reduction was achieved after 3 days in glass and a 5.77 $\log_{10}$ reduction after 1 day in galvanized steel. The time for 1-$\log_{10}$ reduction was 0.95 days (22.8 hours) and 0.17 day (4 hours) in galvanized steel.

High persistence of *Poxviridae* members was also observed in food matrices. Essbauer et al. [57] studied the persistence of VACV M1 in bread, sausage, and salad at 4 °C for 14 days. They found that the virus remained viable throughout the experimental period, with a 0.2–0.3 $\log_{10}$ reduction after 14 days. In milk, samples contaminated with 10^3 plaque-forming units (PFU)/mL and 10^5 PFU/mL VACV strain Guarani P2 (GP2V) remained viable after 48 hours of storage at 4 °C and −20 °C [61]. Reduction in GP2V titer of 14.49% and 25.86% was observed in milk with 10^3 PFU/mL titer at −20 °C and 4 °C, respectively. On the other hand, in 10^5 PFU/mL spiked milk samples, 61.88% and 75.98% reduction in GP2V titer were observed at −20 °C and 4 °C, respectively [61]. In another study, cheese made from contaminated milk with 10^4 PFU/mL of GP2V showed the presence of infectious viral particles in both the whey and curd fractions [62]. Approximately 2 $\log_{10}$ reduction of the viral titer was observed after 60 days of the ripening process at 25 °C and infectious viruses (1.7×10^2 PFU/mL) have been found in cheeses even after 60 days after the ripening process [62]. Using thermal treatment, heating at 65 °C for 30 min of milk samples contaminated with 10^3 PFU/mL and 10^5 PFU/mL of GP2V resulted in a 94.83% and 99.99% reduction in virus titer, respectively, but viable virus particles were still present [61].

5. Conclusion and research needs

The present study reviews the recent evidence on the occurrence of MPXV in wastewater. It highlights the lack of studies on the persistence of MPXV and its transmission risk to the environment. The available data show that members of poxvirus exhibit high persistence at ambient and

Table 2
Environmental persistence of orthopoxvirus infectious particles and DNA in different matrices.

Reference	Virus (strain)	Method of measurement	Assay matrix	Temperature	Survival results	
Hahon and Kozikowski [58]	Variola virus (Yamada strain)	Titration assay	Phosphate buffered saline, pH 4.5 spiked at 5×10^2 I u/ml		Half life time, $t_{1/2}$	K[a]
				40 °C	12.1 min	5.7×10^{-2} min^{-1}
				45 °C	7.1 min	9.6×10^{-2} min^{-1}
				50 °C	3.8 min	1.8×10^{-1} min^{-1}
				55 °C	2 min	3.5×10^{-1} min^{-1}
Mahnel et al. [56]	Vaccinia virus (strain Elstree)	Titration assay	Drinking water (pH 7.52), lake and river water (pH 8.05) spiked at 10^4 $TCID_{50}$/ml titer	10 °C	Titer reduced to 10^{2-3} $TCID_{50}$/ml after 200 days	
Essbauer et al. [57]	Vaccinia virus (Munich 1)	Titration assay	Storm water spiked with Vaccinia virus (Munich 1) spiked at $5 \times 10^{5.5}$ $TCID_{50}$/ml titer	4.5 °C	Virus remained viable at the initial titer value during the entire experiment (2 weeks). Samples remained infectious for up to 56 days	
		PCR			Positive PCR during the entire experiment	
		Titration assay		Room temperature	Virus remained viable until day 3	
		PCR			Positive PCR during the entire experiment	
		Titration assay	Storm water with 10% FCS spiked with Vaccinia virus (Munich 1) at $5 \times 10^{5.5}$ $TCID_{50}$/ml titer	4.5 °C	Virus remained viable the initial titer value during the entire experiment (2 weeks). Samples remained infectious for up to 166 days	
		PCR			Positive PCR during the entire experiment	
		Titration assay		Room temperature	Virus remained viable the initial titer value during the entire experiment (2 weeks)	
		PCR			Positive PCR during the entire experiment	
		Titration assays	Storm water with 10 g poting soil spiked with Vaccinia virus (Munich 1) at $5 \times 10^{5.5}$ $TCID_{50}$/ml titer	4.5 °C	Virus remained viable until day 5	
		PCR			Positive PCR during the entire experiment	
		Titration assay		Room temperature	Virus remained viable until day 2	
		PCR			Positive PCR until day 12	
Abrahão et al. [63]	Vaccinia virus	Titration assay PCR	Murine feces	Environmental temperature	VACV infectious particles were still detected at 20 days post-environmental-exposure (d.p.e.), while viral DNA was detected until 60 d.p.e	

[a] K: The decay rate constant of virus inactivation.

low temperatures in environmental matrices. Moreover, they can remain stable in surfaces and food matrices for extended periods at low temperature and low humidity.

Since MPXV can be shed in the feces of infected individuals and discharged into the sewerage to WWTP, it could lead to environmental contamination by several routes. For instance, treated wastewater effluent may release viruses into the aquatic environment and, in particular surface water. Moreover, surface water may be contaminated through leakage from sewage network systems. In fact, more studies are required to evaluate the environmental transmission risk.

In general, limited data are available concerning the possible circulation and the contamination level of MPXV viral particles in environmental water matrices. These data are important to acquire for the risk assessment process. The use of quantitative molecular tools (qPCR) associated with adapted and validated concentration and genomic extraction procedures, may allow to quickly acquire this type of data. Additionally, the isolation of the detected genomes would allow the characterization of the viral strains in circulation via the use of a sequencing tool and then the comparison between them.

However, the key question is whether the detected MPXV is infectious or not? It is also of particular interest to study the presence of the infectious state as well as the persistence and inactivation of MPXV in wastewater and related treatments. Moreover, it is necessary to investigate the impact of environmental factors, mainly temperature, on MPXV survival in environmental matrices.

The treatment of wastewater in WWTPs resulted in the production of sludge solids. As others enveloped viruses, MPXV could be adsorbed onto solid particles but its adsorption and desorption behavior could be affected by virus envelope structure, capsid proteins, and virus particle size. Different treatments can be applied to sewage sludge before disposal or recycling. In this regards, studies are needed to understand the fate of MPXV through the stage of wastewater and sludge treatment lines.

Finally, there are lack of information on the infectious dose of MPXV in humans and the level of MPXV excretion by an infected individual, which are needed to evaluate the potential environmental transmission

risk of MPXV.

Beyond the environmental risk, monitoring of MPXV in the environment (wastewater) would also provide additional information on the level of virus circulation in the population. For this purpose, the acquisition of data in wastewater and their comparison with epidemiological data in the population (clinical, virological), when available, would be necessary to assess the correlation between these data sources.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cscee.2023.100315.

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