(Fig. 8). In the third set of experiments (described below), the amount of Al_a, the colloid charge density, and both together were also correlated with the efficiency of infectious or total MS2 removal at all coagulant dosages, except for the total MS2 removal ratio at a coagulant dosage of 0.54 mg-Al/L (Table S4). In addition, the removal ratios tended to increase as the amount of Al_a in the PACls decreased (that is, as $Al_b + Al_c$ increased) and as the colloid charge density of the PACls increased. However, the virus removal efficiencies during the coagulation process with aluminum-based coagulants were not solely dependent on either $Al_b + Al_c$ or the colloid charge density. Further investigation is needed to elucidate a completely reliable indicator for the effectiveness of virus removal during the coagulation process.

3.3. *Third set of experiments*

3.3.1. *Effect of coagulant dosage on bacteriophage removal*

Fig. 9a shows the effect of coagulant dosage on the efficiency of infectious MS2 removal from treated water at around pH 8, as evaluated by means of the PFU method after settling. The infectious MS2 removal ratio increased as the coagulant dosage was increased from 0.54 to 2.16 mg-Al/L; although the removal ratios observed with $AlCl_3$ and PACl-1.5ns were unaffected by the increase in coagulant dosage, approximately 1–2-log_{10} improvements were obtained for the other aluminum-based coagulants. For most of the coagulants, the infectious MS2 removal ratio reached a maximum at a dosage of 2.16 mg-Al/L, and retained its virus removal performance when the coagulant dosage was further increased to 5.4 mg-Al/L, except in the case of PACl-1.5s. A similar trend was observed for removal of turbidity and UV260-absorbing NOM (Fig. S3). These results indicate that re-stabilization likely did not occur at this dosage range for any of the coagulants except PAC-1.5s.

The infectious MS2 removal ratios during the coagulation process with high-basicity PACls (i.e., PACl-2.1b, PACl-2.1c, and PACl-2.7) were larger than the ratios with the other aluminum-based coagulants used in the present study at all coagulant dosages. A similar trend was observed for the total MS2 removal ratios, as evaluated by the PCR method (Fig. 9b). Therefore, increasing coagulant basicity tended to lower the coagulant dosage required for effective removal of viruses. In addition, the coagulation process with PACl-2.1c removed MS2 more efficiently than the other aluminum-based coagulants at all coagulant dosages; PACl-2.1c was therefore useful for virus removal over a broader pH range and wider coagulant dosage range compared to commercially available aluminum-based coagulants.

3.3.2. *Overall comparison of coagulation efficiency of the tested coagulants*

As described above, PACl-2.1c, which contains Al_{30} species, removed viruses more efficiently than the other aluminum-based coagulants, especially at weakly alkaline pH. In contrast, at pH 8, the UV260-absorbing NOM removal and the residual aluminum concentration attained with PACl-2.7 were better than those attained with PACl-2.1c (Fig. S3). Because a low residual aluminum concentration is associated with a low content of monomeric aluminum species in the coagulant, our previously reported coagulation process with PACl-2.7 (Kimura et al., 2013), which has a low Al_a content (Fig. 4c), attained a very low residual aluminum concentration. Taken together, our results suggest that the development of novel aluminum-based coagulants for different purposes such as efficient virus removal and low residual aluminum concentration can be achieved. The experimental results obtained in the present study will be useful for the development and investigation of highly effective aluminum-based coagulants.

4. Conclusions

(1) An increase in PACl basicity (from 1.5 to 2.1) and the absence of sulfate in the PACls improved virus removal efficiency.
(2) The efficiency of virus removal at around pH 8 observed with PACl-2.1c, a nonsulfated high-basicity PACl with a high Al_c content, was larger than that with PACl-2.1b, a nonsulfated high-basicity PACl with a high Al_b content.
(3) Although extremely high basicity PACls (PACl-2.7ns and PACl-2.7) effectively removed turbidity and UV260-absorbing NOM and resulted in a very low residual aluminum concentration, the virus removal ratios of these two PACls were smaller than the ratio with PACl-2.1c at around pH 8, possibly as a result of a reduction in the colloid charge density of the PACl due to the increase in basicity from 2.1 to 2.7.
(4) Al_{30} species probably played the major role in virus removal during the coagulation process.
(5) Among the various aluminum-based coagulants used in the present study, PACl-2.1c, which has a high Al_c content (including Al_{30} species) and a high colloid charge density, showed the highest virus removal ratio (>4 log_{10} for infectious viruses) in the pH range from 6 to 8 and a coagulant dosage range from 0.54 mg-Al/L to 5.4 mg-Al/L.
(6) The virus removal ratios tended to increase as the amount of Al_a in the coagulant decreased (that is, as $Al_b + Al_c$ increased) and as the colloid charge density of the coagulant increased.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.watres.2013.09.052.

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Virus removal by an in-line coagulation–ceramic microfiltration process with high-basicity polyaluminum coagulation pretreatment

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ABSTRACT

The ability of in-line coagulation pretreatment with high-basicity polyaluminum chloride (PACl) coagulants to enhance virus removal by ceramic microfiltration (MF) was examined by comparing virus removal efficiencies from water pretreated with PACl-2.2 (basicity 2.2) and PACl-2.5 (basicity 2.5) versus alum, a synthetic aluminum chloride ($AlCl_3$) solution, and two commercially available PACls, PACl-1.5 and PACl-1.8. The virus removal ratios for $AlCl_3$, alum, PACl-1.5, and PACl-1.8 decreased markedly when the pH of the treated water shifted from 6.8 to 7.8, but was high at both pHs for PACl-2.2 and PACl-2.5. PACl-2.5 contains Al_{13} species and possibly Al_{30} species, and has a high colloid charge density. It removed viruses more efficiently than the other aluminum-based coagulants, not only at neutral pH, but also under weakly alkaline conditions. Moreover, the in-line coagulation–ceramic MF process with PACl-2.5 pretreatment removed not only viruses but also dissolved organic carbon and UV260-absorbing natural organic matter more efficiently and resulted in a lower residual aluminum concentration than did commercially available PACls, especially under weakly alkaline conditions. A combination of coagulation pretreatment with a high-basicity PACl and ceramic MF can provide effective treatment of drinking water over a broader pH range than is possible with commercially available aluminum-based coagulants.

Key words | aluminum hydrolyte species, bacteriophage, ceramic microfiltration, colloid charge density, in-line coagulation

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INTRODUCTION

Low-pressure membrane (LPM) filtration, including microfiltration (MF) and ultrafiltration, is widely used for drinking water treatment because of its ability to produce high-quality water, its small footprint, and its relatively low costs (Huang *et al.* 2009). To improve the filtration efficiency of LPM, integration of pretreatment with LPM filtration has been widely employed in actual drinking water treatment plants. Pretreatment methods include adsorption, coagulation, and oxidation. Among these methods, coagulation is the most successful pretreatment for controlling membrane fouling (Huang *et al.* 2009), which reduces membrane permeability and increases the frequency of hydrodynamic or chemical cleaning. In addition, coagulation pretreatment is also useful for improving the quality of treated water. Enhancement of removal of dissolved organic carbon (DOC) and natural organic matter (NOM) can be expected with a combination of pre-coagulation and membrane filtration (Lee *et al.* 2000). Moreover, effective removal of waterborne enteric viruses, those having diameters of 20–100 nm, is possible by this hybrid process. For example, Tanneru *et al.* (2013) have reported that a coagulation–MF process with a coagulant dosage of more than 20 mg-Al/L produced 5.5–6.0 log reduction of viruses when the pH of the treated water was about 6.4, whereas MF alone with a 0.22-μm-pore-size, hydrophilic polyvinylidene difluoride filter produced only a 0.2-log

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removal of viruses. Our research group has also reported the effectiveness of the coagulation–MF process: an approximately 6-log reduction of viruses was achieved at around pH 6.8 with a combination of in-line coagulation and a 0.1 μm-pore-size ceramic membrane filter (Shirasaki *et al.* 2009). This means that the coagulation–MF process has the potential to effectively mitigate the public health risk posed by virus contamination of drinking water.

Many factors affect the virus removal performance of the coagulation–MF process. Matsushita *et al.* (2005) have reported the effects of coagulant dosage, coagulation time, and MF membrane pore size on virus removal. They concluded that coagulant dosage strongly affected virus removal compared with two other factors when the pH was near 7. In addition, Zhu *et al.* (2005) have investigated the effect of pH on virus removal and reported a significant reduction of virus removal as the pH increased from 6.3 to 8.3 when a solution of ferric chloride ($FeCl_3$) was used as the coagulant. Adjustment of the pH during coagulation pretreatment is therefore one of the important steps that must be taken to control virus removal in the coagulation–MF filtration process. However, an increase in the pH of drinking water sources from neutral to alkaline conditions because of excessive algal growth has been reported throughout the world (Hu *et al.* 2006; Matsukawa *et al.* 2006). Under these circumstances, reducing the pH of the drinking water source with acid or adding more coagulant is sometimes required to improve coagulation efficiency when commercially available aluminum-based coagulants such as polyaluminum chloride (PACl) and alum are used (Hu *et al.* 2006; Yan *et al.* 2008). However, both of these methods have some disadvantages, including an increase of the residual aluminum concentration in treated water (Matsukawa *et al.* 2006) and treatment cost (Yan *et al.* 2008).

An alternative investigated by some researchers has been the effect of adjusting the aluminum hydrolyte ratio (basicity = $[OH^-]/[Al^{3+}]$) in PACl on DOC removal and residual aluminum concentration during the coagulation process. They have reported that high-basicity PACls (basicity 2.1–2.7) yield higher removal of DOC and lower residual aluminum concentrations than commercially available PACls with basicities of 1.5–1.8, especially under weakly alkaline conditions (Yan *et al.* 2008; Kimura *et al.* 2013). Accordingly, effective removal of viruses as well as DOC is possible not only under neutral pH conditions but also under weakly alkaline conditions when the MF process is combined with a coagulation pretreatment with high-basicity PACl instead of commercially available PACl or alum. However, there is no report about the effectiveness of coagulation pretreatment with high-basicity PACl for virus removal during the coagulation–MF process. Our objective in the present study was to investigate the effects of coagulant type and PACl basicity on virus removal during the coagulation–MF process by comparing four PACls with different basicity, a synthetic aluminum chloride ($AlCl_3$) solution, and commercially available alum.

MATERIALS AND METHODS

Source water, coagulants, and MF membrane

On 17 July 2009 and 10 November 2009, river water was sampled from the Toyohira River (Sapporo, Japan), the water quality of which is shown in Table 1. The coagulants used for the coagulation process were six aluminum-based coagulants, the specifications of which are shown in Table 2. Two commercially available PACls with normal basicities of 1.5 (PACl-1.5) and 1.8 (PACl-1.8) were provided by the Taki Chemical Co., Ltd (Kakogawa, Japan). For experimental purposes, the same company also supplied high-basicity PACls with basicities of 2.2 (PACl-2.2, presently available commercially) and 2.5. To provide a comparison of coagulation efficiency with PACls, a synthetic $AlCl_3$ solution, which was prepared by dilution of reagent-grade aluminum (III) chloride hexahydrate ($AlCl_3{\cdot}6H_2O$, Wako Pure Chemical Industries, Ltd, Osaka, Japan) dissolved in Milli-Q water

Table 1 | Water quality of the Toyohira River

	River water 1	River water 2
Sampling date	17-Jul-09	10-Nov-09
pH	7.5	7.7
Turbidity (NTU)	2.0	0.8
DOC (mg/L)	0.9	0.8
UV260 (cm^{-1})	0.031	0.027
Alkalinity (mg-$CaCO_3$/L)	14.5	22.2

Table 2 | Specifications of aluminum-based coagulants used in the present study

Coagulants	Basicity	Aluminum concentration	Sulfate concentration	Relative density at 20 °C	Aluminum species distribution		
					Al_a(%)	Al_b(%)	Al_c(%)
$AlCl_3$	0.0	2.7 g-Al/L	0.0 g/L	1.0	75.8	4.6	19.6
Alum	0.0	8% (w/w) as Al_2O_3	23% (w/w)	1.3	73.3	9.4	17.3
PAC1-1.5	1.5	10% (w/w) as Al_2O_3	3% (w/w)	1.2	46.2	15.5	38.3
PAC1-1.8	1.8	10% (w/w) as Al_2O_3	3% (w/w)	1.2	42.2	11.6	46.3
PAC1-2.1	2.2	10% (w/w) as Al_2O_3	3% (w/w)	1.2	36.4	6.3	57.3
PAC1-2.5	2.5	23% (w/w) as Al_2O_3	0% (w/w)	1.3	24.6	3.8	71.6

(Milli-Q Advantage, Millipore Corp., Billerica, MA, USA), and commercially available alum (Taki Chemical Co., Ltd), were used in the present study. The distributions of aluminum species in the coagulants were analyzed by a Ferron method (Wang *et al.* 2004) and are shown in Table 2. On the basis of the kinetic differences between the reactions of the aluminum species and the Ferron reagent (8-hydroxy-7-iodoquinoline-5-sulfonic acid, Wako Pure Chemical Industries), aluminum hydrolyte species were categorized as monomeric species (Al_a), fast-reacting polymeric species (Al_b), or slow-reacting colloidal species (Al_c) (Wang *et al.* 2004). The details of the Ferron method have been described in our previous study (Kimura *et al.* 2013). A monolithic, modular, ceramic MF membrane (55-channel tubular; nominal pore size 0.1 μm; effective filtration area 0.043 m^2; NGK Insulators, Ltd, Nagoya, Japan) installed in a stainless-steel casing was used for the MF process.

Characterization of coagulants

Electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS)

ESI-TOF-MS was used to analyze the aluminum hydrolyte species in the coagulants. Each coagulant was diluted with Milli-Q water to 2 mg-Al/L, and the diluted coagulant was introduced into an ESI-TOF-MS (model JMS-T100LP; JEOL Ltd, Tokyo, Japan) at a constant flow rate of 30 μL/min by using a syringe pump. Analysis was conducted in a positive-ion mode at a needle voltage of 2,000 V, an orifice 1 voltage of 10–30 V (0–100%), an orifice 2 voltage of 5 V, a ring lens voltage of 10 V, and a mass range *m/z* of 10–500.

Colloid titration analysis

The positive colloid charges of the coagulants were determined by colloid titration with a COM-555 potentiometric titrator (Hiranuma Sangyo Co., Mito, Japan). Each coagulant was diluted with Milli-Q water to 1–2 mg-Al/L (analytical pH condition was approximately 4–5), and then 150 mL of diluted coagulant was transferred to a titration vessel. After addition of 0.3 mL of toluidine blue indicator (Wako Pure Chemical Industries) to the vessel, the solution was titrated by means of a pump with 0.001 mol/L potassium polyvinyl sulfate (a standard negative colloid, Wako Pure Chemical Industries) at a constant rate of 10 mL/min. The vessel contents were homogenized with a magnetic stirrer during the titration, and the absorbance at 630 nm was recorded continuously until there was little change in the absorbance (i.e. subtle change in the color of the indicator from light blue to bluish-purple). The positive colloid charge was determined from the volume of potassium polyvinyl sulfate that corresponded to the half-height of the descending side of the recorded absorbance curve.

Bacteriophage

F-specific RNA bacteriophage Qβ (NITE Biological Research Center (NBRC) 20012) was obtained from the NBRC (Kisarazu, Japan). The bacteriophage Qβ is widely used as a surrogate for waterborne enteric viruses in the membrane filtration process (Matsushita *et al.* 2005; Shirasaki *et al.* 2009) because of its morphological similarities to hepatitis A viruses and polioviruses, which are important to remove during drinking water treatment. The bacteriophage Qβ is the

prototype member of the genus *Allolevivirus* in the virus family Leviviridae. The genome of this bacteriophage contains a single molecule of linear, positive-sense, single-stranded RNA, which is encapsulated in an icosahedral protein capsid with a diameter of 24–26 nm (Fauquet *et al.* 2005). Bacteriophage was propagated and purified as described in our previous report (Shirasaki *et al.* 2010) prior to the preparation of a bacteriophage stock solution.

In-line coagulation–ceramic MF experiments

The river water, placed in a raw water tank, was spiked with Qβ at approximately 10^8 PFU/mL. Throughout the experiments, the raw water was mixed constantly with an impeller stirrer. The raw water was fed into the experimental system at a constant flow rate (83.3 L/(m^2 h) = 2.0 m/d) by a peristaltic pump. To maintain the MF filtrate at pH 6.8 or 7.8, hydrochloric acid or sodium hydroxide was added to the water before it reached the first in-line static mixer (hydraulic retention time (HRT) 1.8 s; 1/4-N40-172-0, Noritake Co., Ltd, Nagoya, Japan). Because about 1 mg-Al/L of PACl is usually dosed for the treatment of Toyohira River water (the source water in the present study) in the actual drinking water treatment plant (Moiwa drinking water treatment plant, Sapporo, Japan), coagulant was injected after the first in-line static mixer and before the second in-line static mixer at a constant dose rate (1.08 or 2.16 mg-Al/L). To obtain a coagulation time of 1 min, a combination of the in-line static mixer (G value 260 s^{-1}, HRT 1.8 s) and a subsequent Tygon tube reactor (inside diameter 1.6 mm, total HRT 1 min) was used as the second in-line static mixer. After the coagulant had been admixed with the water, the water was fed into the ceramic MF module in dead-end mode. Filtration was performed for 4 h without any backwashing. Bacteriophage concentrations in the raw water tank (C_0) and in the MF filtrate (C_f) were measured every hour. In addition, DOC concentrations and UV260-absorbing NOM were quantified with a SIEVERS 900 laboratory TOC analyzer (GE Analytical Instruments, Boulder, CO, USA) and a UV-1700 Pharma spectrophotometer (Shimadzu Corp., Kyoto, Japan), respectively. After adding the nitric acid (1% (v/v), ultrapure, Kanto Chemical Co., Inc., Tokyo, Japan) into the MF filtrate, the aluminum concentration was analyzed with an HP4500 inductively coupled plasma–mass spectrometer (Yokogawa Analytical Systems Inc., Tokyo, Japan).

Bacteriophage assay

PFU method

The infectious bacteriophages were enumerated according to the double-layer method (Adams 1959) by using the bacterial host *Escherichia coli* (NBRC 13965). Serially diluted raw water or MF filtrate (1 mL) was poured onto a solid-bottom agar plate followed by 0.3 mL of host *E. coli* culture mixed with 3 mL of molten top agar. The plates were incubated for 16–24 h at 37 °C. To measure the concentrations of infectious bacteriophage in the water samples, we calculated the average plaque counts of triplicate plates prepared from one sample on plates with 30 to 300 PFU, which we considered a countable number of plaques, and determined the number of plaque forming units per millilitre.

For quantification of low infectious bacteriophage concentrations (i.e. <30 PFU/mL) in the MF filtrate, 50 mL of MF filtrate was mixed with 5 mL of bacterial host *E. coli* culture and 50 mL molten agar, and the mixture was then poured into 10 plates (without bottom agar). The plates were incubated for 16–24 h at 37 °C. We calculated the number of plaque forming units per millilitre by dividing the total plaque counts for the 10 plates by the sample volume (50 mL).

Real-time reverse-transcription polymerase chain reaction method (RT-PCR)

The viral RNA of bacteriophages was quantified by the real-time RT-PCR method. This method detects all bacteriophages, regardless of their infectivity and the existence of aggregates. The detailed procedure for the real-time RT-PCR method has been described in our previous study (Shirasaki *et al.* 2010).

RESULTS AND DISCUSSION

Effect of coagulant type on bacteriophage removal

Figure 1(a) shows the effect of coagulant type on the removal of infectious Qβ, assessed by the PFU method, in the in-line coagulation–ceramic MF process (source water was river water 1). Because the diameter of Qβ

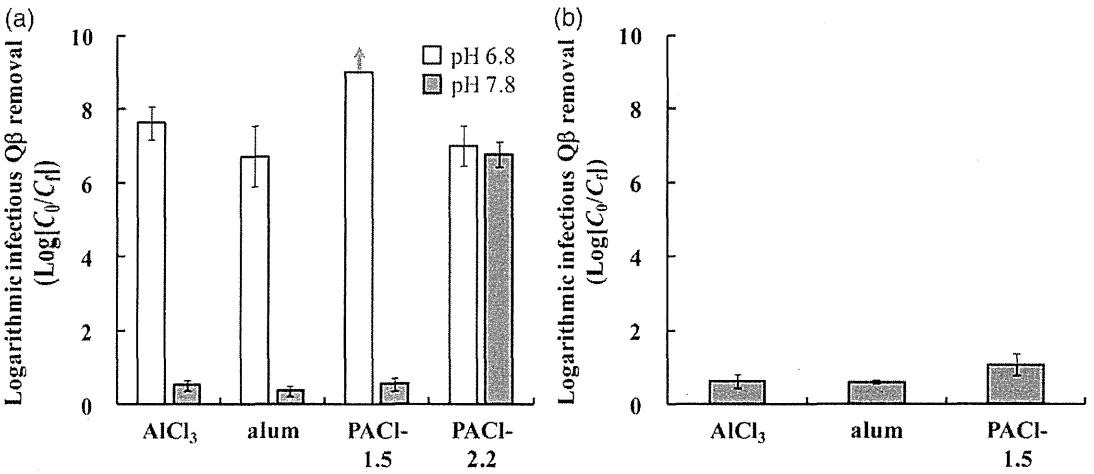

Figure 1 | Effect of coagulant type on removal of infectious Qβ by the in-line coagulation–ceramic MF process as evaluated by the PFU method. Source water was river water 1. Coagulant dosage was 1.08 (a) or 2.16 (b) mg-Al/L. Values are filtration time-averaged removals, and the error bars indicate standard deviations. The absence of a bar indicates that the Qβ concentrations were below the quantification limit in the treated water.

(approximately 25 nm) was smaller than the nominal pore size of the ceramic MF membrane (0.1 μm), no removal of infectious Qβ was observed in the absence of coagulation pretreatment (data not shown). In contrast, in-line coagulation pretreatment dramatically enhanced the removal ratios ($\log[C_0/C_f]$) of infectious Qβ compared with ceramic MF alone at around pH 6.8 of treated water. Greater than 6-log removals were achieved by a combination of in-line coagulation pretreatment and ceramic MF filtration, regardless of the type of coagulant, because during the coagulation pretreatment, Qβ was effectively incorporated into the aluminum flocs, which were larger than the nominal pore size of the ceramic MF membrane, and the flocs with incorporated Qβ were removed by ceramic MF filtration.

The infectious Qβ removal performances of $AlCl_3$, alum, and PACl-1.5 were markedly decreased when the pH of the treated water shifted from 6.8 to 7.8. Even when the coagulant dosage was increased from 1.08 to 2.16 mg-Al/L, <1-log removals were observed for those coagulants (Figure 1(b)). In contrast, PACl-2.2 continued to remove viruses with high efficiency (>6-log removal of infectious Qβ) even under weakly alkaline pH conditions (Figure 1(a)). This result indicates that the type of coagulant affects virus removal performance in the in-line coagulation–ceramic MF process, especially under weakly alkaline pH conditions, and that a high-basicity PACl (e.g. PACl-2.2) can remove viruses more effectively than the other commercially available aluminum-based coagulants used in the present study.

Effect of PACl basicity on bacteriophage removal

As described above, PACl-2.2 removed infectious Qβ more efficiently than did other aluminum-based coagulants, including PACl-1.5, especially under weakly alkaline pH conditions. To precisely quantify the effect of PACl basicity on virus removal, we compared the removal of Qβ by in-line coagulation pretreatment with four PACls with different basicities followed by ceramic MF filtration within the pH range 6.8–7.8 (Figure 2, source water was river water 2). More than 8-log removals of infectious Qβ, assessed by the PFU method, were attained by the in-line coagulation–ceramic MF process with all the PACls when the pH of the treated water was about 6.8, regardless of their basicity (Figure 2(a)). In contrast, the removal efficiencies of infectious Qβ depended on the basicity of the PACls when the pH of the treated water was about 7.8. The in-line coagulation–ceramic MF process with PACl-1.5 and PACl-1.8 achieved approximately a 1-log removal of infectious Qβ, but approximately 6-log and >8-log removals were achieved with PACl-2.2 and PACl-2.5, respectively. A similar trend was observed for removal ratios of total Qβ, assessed by the RT-PCR method. Although high removal efficiencies of total Qβ, at least

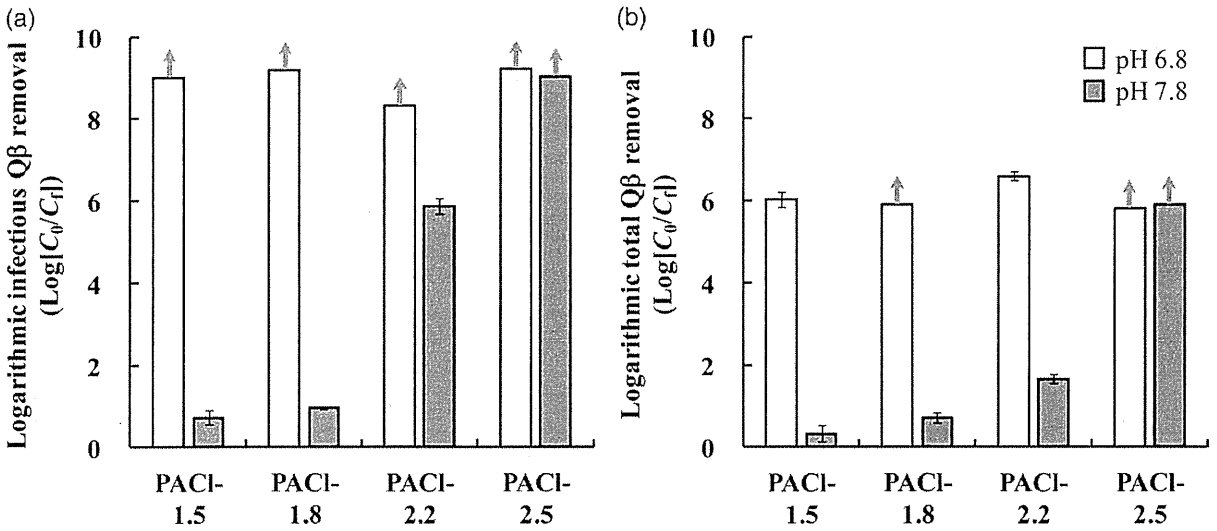

Figure 2 | Effect of PACl basicity on infectious Qβ removal as evaluated by the PFU method (a) and on total Qβ removal as evaluated by the RT-PCR method (b) by the in-line coagulation–ceramic MF process. Source water was river water 2. Coagulant dosage was 1.08 mg-Al/L. Values are filtration time-averaged removals, and the error bars indicate standard deviations. The absence of a bar indicates that the Qβ concentrations were below the quantification limit in the treated water.

6-log removals, were achieved for all PACls at around pH 6.8, marked differences in removal ratios were observed among the four PACls when the pH of the treated water was about 7.8 (Figure 2(b)). These results suggest that the basicity of PACls affects virus removal performance in the in-line coagulation–ceramic MF process, especially under weakly alkaline conditions.

As shown Figure 2, the in-line coagulation–ceramic MF process with PACl-2.5 effectively removed viruses compared to the performance of the other PACls used in the present study. In addition, this process removed DOC and UV260-absorbing NOM more efficiently and resulted in a lower residual aluminum concentration than did the processes with PACl-1.5, PACl-1.8, and PACl-2.2, especially under weakly alkaline conditions (whereas approximately 20–30% and 40–50% removals for DOC and UV260-absorbing NOM, respectively, and 0.08–0.15 mg-Al/L residual aluminum concentrations were observed for PACl-1.5, PACl-1.8, and PACl-2.2 at a pH of about 7.8, approximately 50% and 70% removals for DOC and UV260-absorbing NOM, respectively, and <0.01 mg-Al/L residual aluminum concentrations were attained for PACl-2.5). Accordingly, the combination of coagulation pretreatment with high-basicity PACl and ceramic MF is effective in treating drinking water, including virus removal, over a broader pH range compared to commercially available aluminum-based coagulants.

The Qβ removal ratios determined by the PFU and RT-PCR methods differed markedly, depending on the coagulation conditions. The infectious Qβ removal ratios (Figure 2(a)) were larger than the total Qβ removal ratios (Figure 2(b)). Because our previous study demonstrated that Qβ loses its infectivity after being mixed with aluminum hydrolyte species during coagulation with PACl-1.5 (Matsushita *et al.* 2011), the virucidal activity of the PACls contributed to the removal efficiency of infectious Qβ during the in-line coagulation–ceramic MF process.

Characterization of coagulants

To elucidate why PACl-2.5, which has a higher basicity, was more effective in removing viruses, we used ESI-TOF-MS to analyze the aluminum hydrolyte speciation in PACl-2.5. In the ESI-TOF-MS spectra of all the coagulants (Figure 3), we found the most intensive peak at *m/z* 97, which is assigned to the monomeric aluminum species $[Al(OH)_2(H_2O)_2]^+$ (Urabe *et al.* 2007). In addition, fragment ions of tridecameric species – i.e. Al_{13} species such as $[(Al_{13}O_4(OH)_{24}(H_2O)_{12})]^{7+}$ with peaks at *m/z* 303 $[Al_{13}O_4(OH)_{28}(H_2O)]^{3+}$ and 454 $[Al_{13}O_4(OH)_{29}]^{2+}$ (Stewart *et al.* 2009) – were observed for

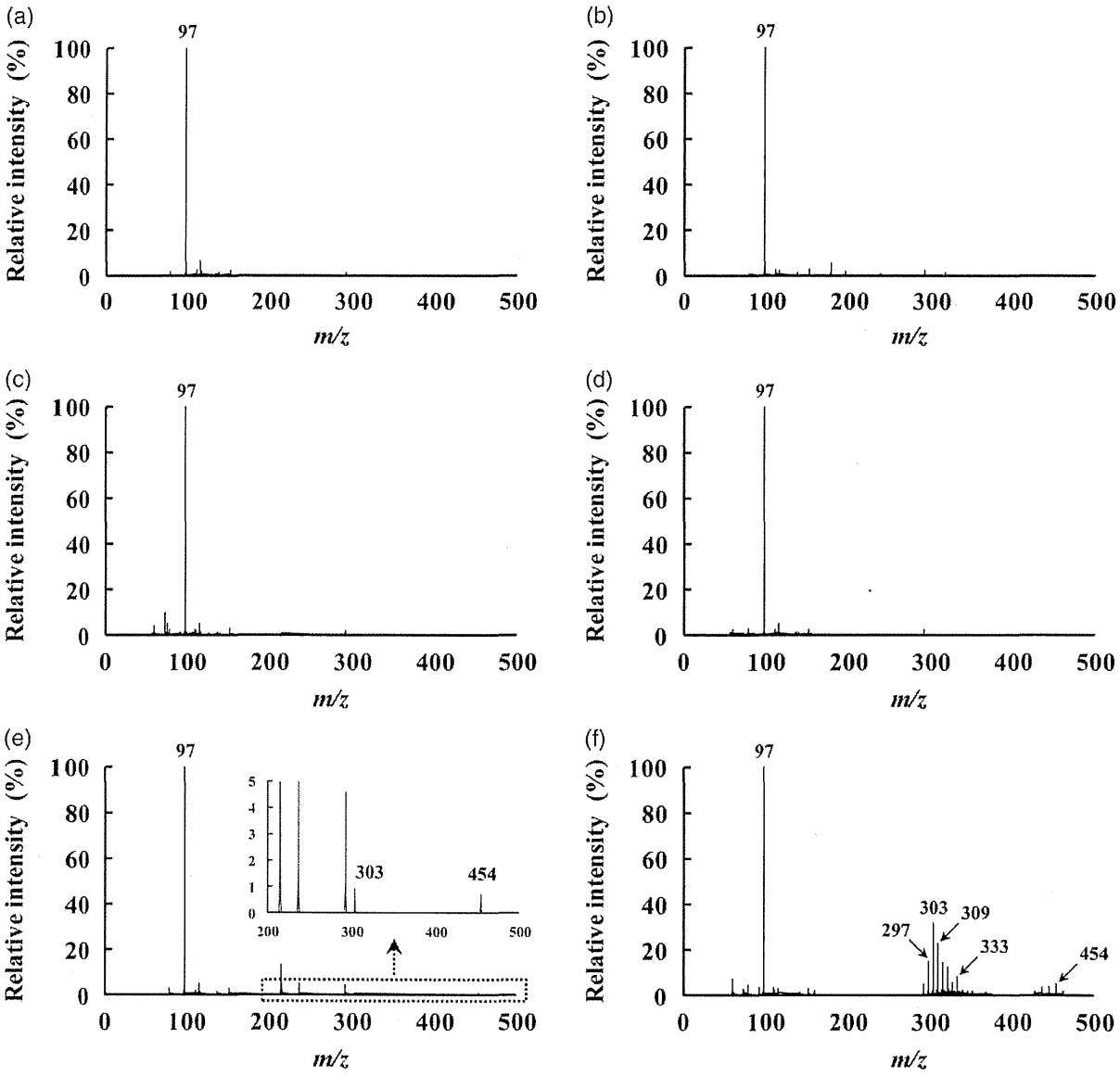

Figure 3 | ESI-TOF-MS spectra of coagulants: $AlCl_3$ (a), alum (b), PACl-1.5 (c), PACl-1.8 (d), PACl-2.2 (e), and PACl-2.5 (f).

PACl-2.2 (the relative intensities of these two peaks were 0.7–0.9%) and PACl-2.5. In contrast, no peak for Al_{13} species was confirmed in the spectra of $AlCl_3$, alum, PACl-1.5, and PACl-1.8. Moreover, other strong peaks of Al_{13} species at m/z 297 $[Al_{13}O_4(OH)_{28}]^{3+}$, 309, 315, 321, 327, and 333 $[Al_{13}O_4(OH)_{28}(H_2O)_{2\text{-}6}]^{3+}$ (Stewart *et al.* 2009) were observed in the spectrum of PACl-2.5. These results suggest that high-basicity PACls such as PACl-2.2 and PACl-2.5 contain Al_{13} species, but these species are not present in $AlCl_3$, alum, PACl-1.5, and PACl-1.8.

Some researchers have demonstrated that the content of Al_{13} species in a coagulant is approximately equal to that of Al_b species categorized by the Ferron method (Chen *et al.* 2006). In the present study, whereas strong peaks related to Al_{13} species were observed for PACl-2.5, the content of Al_b in PACl-2.5 was very similar to those in other

aluminum-based coagulants used in the present study (Table 2). In contrast, the content of Al_c varied as a function of the coagulant type and was highest in PACl-2.5 (Table 2). Because the Al_{30} species $[(Al_{30}O_4(OH)_{56}(H_2O)_{24})]^{18+}$ does not react with Ferron reagents within 120 min, it is categorized as Al_c by the Ferron method (Chen *et al.* 2007). In addition, the Al_{30} species is known to consist of two Al_{13} species connected by four monomeric aluminum species (Chen *et al.* 2007), the implication being that the peaks of monomeric aluminum species and fragment ions of Al_{13} species will also be observed when the Al_{30} species in a coagulant is decomposed by fragmentation in the ESI-TOF-MS analysis. Actually, these peaks were clearly confirmed in the ESI-TOF-MS spectrum of PACl-2.5, as shown in Figure 3. Therefore, PACl-2.5 possibly contained not only Al_{13} species but also Al_{30} species.

The positive colloid charges of the coagulants were also determined by using a colloid titration technique (Figure 4). The colloid charge densities of the $AlCl_3$ and alum were very small and almost constant, regardless of the aluminum concentration. In contrast, the colloid charge densities of the PACls increased with increasing concentrations of aluminum. In addition, the colloid charge density of PACl-2.5 was the highest among the aluminum-based coagulants used in the present study. Because Al_{13} species and Al_{30} species are known to be effective coagulants because of their strong charge neutralization capability and high structural stability (Chen *et al.* 2006; Zhang *et al.* 2008), the presence of Al_{13} species, Al_{30} species, or both in PACl-2.5 is possibly associated with the large amount of positive colloid charge. In addition, the absence of sulfate in PACl-2.5 probably also contributed to its high colloid charge density compared with that of other sulfated PACls such as PACl-1.5, PACl-1.8, and PACl-2.2, because the presence of sulfate during the coagulation process reduces the charge neutralization capability of coagulants (Wang *et al.* 2002). These characteristics of PACl-2.5 account for its high capability to neutralize the negative charges on viruses during coagulation pretreatment. Actually, the in-line coagulation–ceramic MF process with PACl-2.5 could effectively remove viruses not only under neutral pH conditions but also under weakly alkaline conditions, as described above.

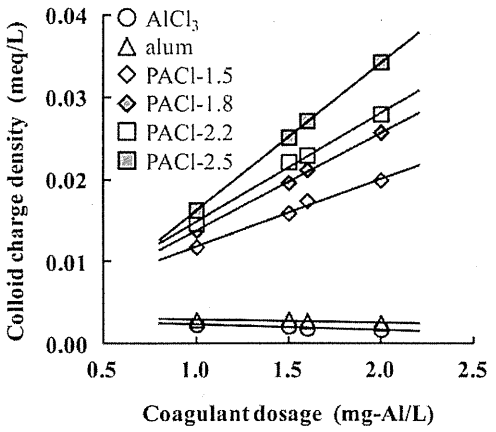

Figure 4 | Colloid charge densities of the coagulants as evaluated by the colloid titration technique.

We have shown through this study that coagulation pretreatment with PACl-2.5 followed by ceramic MF has a variety of advantages, including effective removal of viruses, DOC, and UV260-absorbing NOM, and the process results in very low residual aluminum concentrations compared with other aluminum-based coagulants, even under weakly alkaline conditions. To elucidate whether the in-line coagulation–ceramic MF process with high-basicity PACl is actually useful for drinking water treatment, further investigations using a wide variety of water sources are needed, because the concentration of viruses in the treated water may be affected by the characteristics of the source water.

CONCLUSIONS

1. The basicity of PACls affects the virus removal performance of the in-line coagulation–ceramic MF process. The high-basicity PACls, PACl-2.2 and PACl-2.5, could effectively remove viruses not only under neutral pH conditions but also under weakly alkaline conditions.
2. Among the various aluminum-based coagulants used in the present study, PACl-2.5, which has a high Al_c content (including Al_{13} species and possibly Al_{30} species) and a high colloid charge density, removed viruses more efficiently (>8-log for infectious viruses; >6-log for total viruses) than the other aluminum-based coagulants in the pH range 6.8 to 7.8.
3. The in-line coagulation–ceramic MF process with PACl-2.5 removed not only viruses but also DOC and

UV260-absorbing NOM more efficiently and resulted in a lower residual aluminum concentration than did commercially available PACls, especially under weakly alkaline conditions.

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Quantitative detection of human enteric adenoviruses in river water by microfluidic digital polymerase chain reaction

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ABSTRACT

We describe an assay for simple and accurate quantification of human enteric adenoviruses (EAdVs) in water samples using a recently developed quantification method named microfluidic digital polymerase chain reaction (dPCR). The assay is based on automatic distribution of reaction mixture into a large number of nanolitre-volume reaction chambers and absolute copy number quantification from the number of chambers containing amplification products on the basis of Poisson statistics. This assay allows absolute quantification of target genes without the use of standard DNA. Concentrations of EAdVs in Japanese river water samples were successfully quantified by the developed dPCR assay. The EAdVs were detected in seven of the 10 samples (1 L each), and the concentration ranged from 420 to 2,700 copies/L. The quantified values closely resemble those by most probable number (MPN)-PCR and real-time PCR when standard DNA was validated by dPCR whereas they varied substantially when the standard was not validated. Accuracy and sensitivity of the dPCR was higher than those of real-time PCR and MPN-PCR. To our knowledge, this is the first study that has successfully quantified enteric viruses in river water using dPCR. This method will contribute to better understanding of existence of viruses in water.

Key words | absolute quantification, digital PCR, gastroenteritis, real-time PCR, waterborne infectious diseases

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INTRODUCTION

The accurate quantification of pathogenic microorganisms in water is crucial for estimating and controlling health risk of waterborne infectious diseases. Real-time polymerase chain reaction (PCR) is widely used for quantifying microorganisms in water (Rajal *et al.* 2007; Wyn-Jones *et al.* 2011; Kishida *et al.* 2012a). However, the quantified value by real-time PCR is unstable if the copy number of target genes in the reaction mixture is small, because the effect of annealing error at the beginning of the PCR cycle (PCR drift) on the amplification efficiency is high (Wagner *et al.* 1994). Although it is possible to increase copy number by concentrating microorganisms in water by filtering or centrifugation, excess concentration increases inhibitory substances for PCR, such as humic acids, fulvic acids and polysaccharides, which are typically co-extracted with target DNA from natural water samples (Hata *et al.* 2011). Due to the inhibitory effect, the concentration of microorganisms in water is often underestimated (Hamza *et al.* 2009; Hata *et al.* 2011). If copy number is small, and inhibitory effect is high, estimated quantified value can be less than one copy. This phenomenon sometimes occurred in our previous experiments (data not shown). In addition, accurate preparation of copy number of standard DNA is generally difficult, which increases quantification error.

In this study, we focused our attention on a recently reported novel molecular method for the quantification of nucleic acid sequences named microfluidic digital PCR (dPCR) (Dube *et al.* 2008; Spurgeon *et al.* 2008) as an improved method for the quantification of microorganisms in water. The microfluidic dPCR is a new technology that allows absolute quantification of target genes without the use of standard DNA. As shown in Figure 1, the reaction

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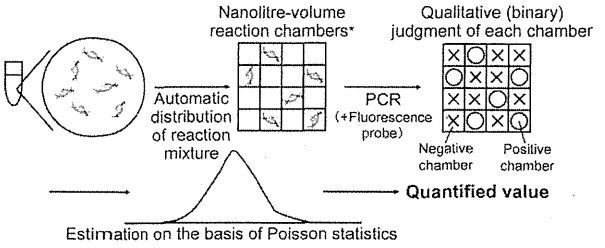

Figure 1 | Schematic presentation of microfluidic dPCR for estimating the target DNA without the use of standard DNA. (*The number of reaction chambers is much larger in an actual device.)

mixture for PCR is automatically distributed and partitioned into a large number of nanolitre-volume reaction chambers. After conducting PCR in the chambers, the amount of target DNA is estimated from the number of chambers containing amplification products on the basis of Poisson statistics, which can predict how input DNA is distributed into all small chambers (Dube *et al.* 2008). This method provides binary output because the PCR reaction in each nanolitre-volume chamber is either positive or negative, and the quantified copy number is independent of the PCR amplification efficiency (Hoshino & Inagaki 2012). Therefore, this method can accurately and simply quantify microorganisms even if inhibitory substances exist in the reaction mixture (Hoshino & Inagaki 2012). However, this method has rarely been applied to quantification of pathogenic microorganisms in water.

In the present study, we developed a microfluidic dPCR assay for accurate quantification of human enteric adenoviruses (EAdVs) as representative microorganisms, and applied the assay to the detection in water samples collected from a Japanese river. To investigate validity of the microfluidic dPCR, conventional real-time PCR assay and most probable number (MPN)-PCR assay were also performed, and the quantification data for the three techniques were compared.

MATERIALS AND METHODS

Collection of river water samples, virus concentration and DNA extraction

In total, 10 river water samples were collected from one sampling site in the Tone River in Japan, from October 2011 to March 2012. The Tone River has a total length of 322 km and a catchment area of 16,840 km^2, with approximately 800 tributaries. The sampling site was located on the right bank at the Tone Diversion Weir, where the surface water is utilized for the production of drinking water for the Tokyo Metropolitan Area. Over 2 million people live in the upper river basin of the sampling site, and there are many wastewater treatment plants and private septic tanks in the area. Enteric AdVs have been frequently detected at this sampling site in a previous study by us (Kishida *et al.* 2012b).

One litre of sampled river water was concentrated to approximately 0.6 mL using electronegative and ultrafiltration membranes as described previously (Haramoto *et al.* 2005). For detection of EAdVs, 200 μL of the concentrated sample was subjected to DNA extraction using a QIAamp DNA mini kit (Qiagen, Tokyo, Japan) to obtain 200 μL of the resulting DNA.

Quantification of EAdVs by dPCR, real-time PCR and MPN-PCR

The primer pairs and the TaqMan probe used for dPCR, real-time PCR and MPN-PCR were designed to amplify adenovirus stereotypes 40 and 41 (Ko *et al.* 2005). The dPCR was performed using the BioMark Real-time System and 12.765 Digital Array, which consisted of 12 panels containing 765 reaction chambers each (Fluidigm Corporation, San Francisco, USA). Aliquots of 1.15 μL of extracted DNA solution were mixed with 3.45 μL of reaction buffer containing 2.3 μL of TaqMan Gene Expression Master Mix (Life Technologies Japan, Tokyo, Japan), 900 nM of each primer, and 250 nM of TaqMan probe. The reaction mixtures were incubated at 50 °C for 2 min and 95 °C for 10 min followed by 45 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. After amplification, Digital PCR Analysis Software (Fluidigm Corporation) was used to count the number of positive chambers. Finally, the software statistically estimated the absolute copy number of target DNA in panels using Poisson statistics. The theory and equations used in this study have been described elsewhere (Dube *et al.* 2008; Bhat *et al.* 2009). Briefly, the copy number of template DNA (M) is given by

$$M = -C \times \ln\left(1 - \frac{H}{C}\right)$$

where C is the number of all chambers in a panel (=765) and H is the number of positive chambers (Hoshino & Inagaki 2012). Because the molecules in the reaction mix are distributed in small chambers, dPCR quantifies the number of molecules instead of the copy number of genes estimated by real-time PCR. For example, a genome fragment

possessing three copies of the target gene in a reaction chamber should be quantified as one by digital PCR, whereas real-time PCR quantifies it as three (Hoshino & Inagaki 2012).

Real-time PCR and MPN-PCR were performed using LightCycler 480 System II (Roche Diagnostics, Tokyo, Japan). For real-time PCR, aliquots of 1.15 μL of extracted DNA solution were mixed with 18.85 μL of reaction buffer containing 10 μL of 2 × LightCycler 480 Probe Master (Roche Diagnostics), 400 nM of each primer, and 200 nM of TaqMan probe. The reaction mixtures were incubated at 95 °C for 10 min followed by 50 cycles of 95 °C for 10 s and 55 °C for 30 s. When fluorescence intensity is over the threshold value within 45 cycles, the sample was considered to be positive. Tenfold serial dilutions of chemically synthesized oligo-DNAs of adenovirus serotype 40 (Accession No.: X16583; position: 614-723) were used to make a standard curve. The concentration of the standard was calculated from the optical density measurement. Validation of concentration of standard DNA was also performed using dPCR. Namely, standard DNA with the concentration of 500 copies/reaction calculated from the optical density measurement was quantified using dPCR.

For MPN-PCR, DNA sample was manually diluted as described previously (Katayama *et al.* 2008). The reaction mixture composition and incubation condition of MPN-PCR were the same of those of real-time PCR except for template volume. To compare detection sensitivity of each PCR, the total volume of template DNA solution before dilution for MPN-PCR was set as 1.15 μL. Each PCR test was performed in triplicate.

RESULTS AND DISCUSSION

Validation of concentration of standard DNA using dPCR

Figure 2 shows binary results of each reaction chamber of dPCR. Positive and negative chambers were clearly divided. Therefore, the number of positive chambers was successfully counted, and copy number was quantified on the basis of Poisson statistics. The quantified values for standard DNA tested in triplicate resemble each other (78 ± 10 copies/reaction). However, these values were much less than the input copy number (500 copies/reaction). This result indicates that the copy number of standard DNA prepared by the optical density measurement and dilution was not accurate.

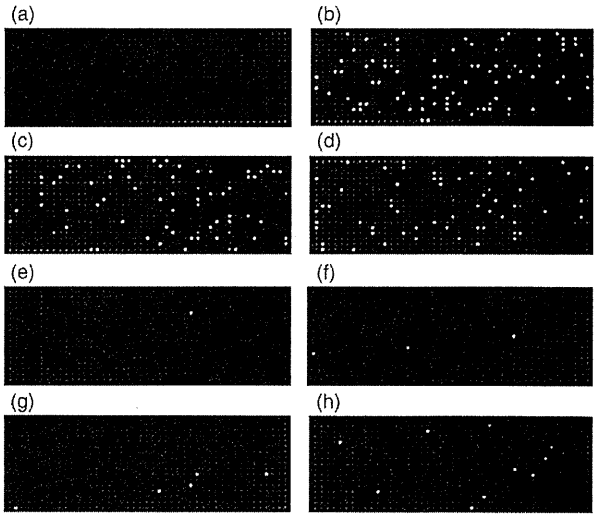

Figure 2 | Images of the digital PCR array after 45 cycles of PCR. Each panel contains 765 chambers, and white chambers are positive. (a) Negative control, (b)–(d) standard DNA ($n = 3$), (e)–(h) river water samples (examples).

Quantification of EAdV concentration in river water sample using dPCR

The EAdVs were successfully detected from river water samples using dPCR. As shown in Table 1, EAdVs were detected in seven of the 10 samples (70%). In this study, the water sampling was conducted only in the winter season when detection frequency of EAdVs is high. In fact, the detection efficiency is higher compared to a previous study conducted year-round which covered all seasons at the same sampling site (Kishida *et al.* 2012b).

Table 1 | Concentration of EAdVs in river water samples from the Tone River in Japan determined by microfluidic dPCR

	Concentration (copies/L) Test no. ($n = 3$)				
Sample no.	1	2	3	Mean	Standard deviation
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	420	420	420	420	0
5	1,000	1,500	1,500	1,300	290
6	460	920	1,400	920	460
7	1,300	2,500	4,000	2,700	1,500
8	440	880	1,800	1,000	670
9	440	1,300	1,300	1,000	510
10	1,600	1,600	2,000	1,700	230

Although the reason why detection efficiency is high in the winter season is not clear, a seasonal variation in the number of infected patients may be one of the reasons. According to limited clinical data reported to the National Institute of Infectious Diseases, Japan, the number of confirmed EAdVs (patients/surveillance site) was larger in the winter season in 2011/2012 compared with other seasons (National Institute of Infectious Diseases 2014). Hence, it is suggested that EAdVs were shed from humans and discharged into the water environment via wastewater treatment plants in this season, which may cause an increase in the concentration of EAdVs in river water. Another reason may be a seasonal variation in rainfall. Near the sampling site, the amount of rainfall was small in the winter season in 2011/2012 (Japan Meteorological Agency 2014), which decreases the water level of the river and the dilution effect by rainfall, and may cause an increase in the concentration of EAdVs in river water. Meanwhile, the first three samples (No. 1–3 in Table 1) were negative for EAdVs. This may be because the amount of rainfall near the sampling period was different. The amount of rainfall in the early winter season that the first three samples were obtained was larger than that in the late winter season when the other samples were obtained (Japan Meteorological Agency 2014). Dilution effect by rainfall would be higher in the early winter season, which decreased the concentration of EAdVs in river water.

Although river water samples were concentrated using electronegative and ultrafiltration membranes, the quantified copy number of each reaction was less than 10, as shown in Figure 2, because the EAdV concentration in river water sample is normally lower compared to those in sewage samples (Haramoto *et al.* 2007). Nevertheless, relatively stable quantified values were obtained. The mean concentration of each sample tested in triplicate ranged from 420 to 2,700 copies/L. This contaminant level is similar to that of the previous study conducted at another Japanese river (Haramoto *et al.* 2007).

Comparison of quantified data among dPCR, real-time PCR and MPN-PCR

Table 2 shows quantified data of EAdVs by dPCR, real-time PCR and MPN-PCR. Standard deviations of positive samples quantified by dPCR are lower than those by real-time PCR. This indicates that stable quantification data can be obtained using dPCR even when the target copy number in the reaction mixture is small. This is because dPCR provides binary output since the PCR reaction in

Table 2 | Concentration of EAdVs in river water samples using three different PCR methods (unit: copies/reaction; $n = 3$)

	dPCR		MPN-PCR		Real-time PCR			
					Adjustment[a]		Non-adjustment[b]	
Sample no.	Mean	S.D.[c]	Mean	S.D.[c]	Mean	S.D.[c]	Mean	S.D.[c]
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	1.0	0	1.9	1.6	0	0	0	0
5	2.7	0.58	4.0	3.4	3.7	3.0	23	19
6	2.0	1.0	0	0	1.2	0.42	7.7	2.7
7	6.3	3.5	6.2	2.7	3.7	3.9	24	25
8	2.3	1.5	4.7	2.7	2.2	2.0	14	13
9	2.3	1.2	2.1	1.8	1.5	0.66	9.7	4.2
10	4.3	0.58	3.7	0.94	1.7	1.2	11	7.6
Mean[d]	3.0	1.4	3.8	2.2	2.3	1.9	15	12
Detection frequency	70%		60%		60%		60%	

[a]Quantified values when the concentration of standard DNA was adjusted (validated) by dPCR.
[b]Quantified values when the concentration of standard DNA was not adjusted (validated) by dPCR.
[c]Standard deviation.
[d]Mean values of the positive samples.

each nanolitre-volume chamber is either positive or negative, and the quantified copy number is independent of the PCR amplification efficiency. In addition, the standard deviations quantified by dPCR are lower than those by MPN-PCR. This is because the handling error does not exist in the dPCR measurement. The reaction mixture is automatically distributed and partitioned into a large number of nanolitre-volume reaction chambers in dPCR whereas it is manually diluted and distributed in MPN-PCR.

The detection frequency of dPCR is a little higher than those of real-time PCR and MPN-PCR. This can be expected because amplification efficiency of dPCR is higher. Since the reaction chamber is very small, heat transfer of reaction mixture during PCR cycles would be efficiently completed. In a further study, the detection sensitivity could be thoroughly investigated by applying dPCR to quantification of microorganisms in a larger number of water samples.

The mean values of positive samples determined by real-time PCR are similar to those determined by dPCR and MPN-PCR when the standard DNA was adjusted (validated) by dPCR. However, they varied substantially when the standard was not adjusted as shown in Table 2. This strongly indicates that the standard DNA concentration calculated from the optical density measurement is not accurate. Generally, accurate preparation of standard DNA for real-time PCR is difficult and troublesome. Although the reaction time of dPCR and real-time PCR is almost the same, handling time considerably decreases in dPCR because accurate data can be obtained without the use of standard DNA.

CONCLUSIONS

Concentrations of EAdVs in Japanese river water samples were quantified by dPCR and other current detection techniques. The accuracy and sensitivity of the dPCR were higher than those of current detection techniques. Therefore, the dPCR can be applicable to quantify pathogenic microorganisms in aquatic environments in the cases where accuracy and sensitivity are more important than analytical costs. Accurate analytical data are strongly required when the data are used for regulation. In addition, dPCR measuring may be suitable for quantitative microbial risk assessment because accurate and sensitive data are required to increase the precision of the assessment.

ACKNOWLEDGEMENTS

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本誌に掲載された統計資料は，1)「感染症の予防及び感染症の患者に対する医療に関する法律」に基づく感染症発生動向調査によって報告された，患者発生および病原体検出に関するデータ，2）感染症に関する前記以外のデータに由来する。データは次の諸機関の協力により提供された：保健所，地方衛生研究所，厚生労働省食品安全部，検疫所。

＜特集＞ クリプトスポリジウム症およびジアルジア症　2014年7月現在

クリプトスポリジウム症およびジアルジア症は，消化管寄生性原虫感染による疾患で，非血性の水様下痢等の症状を示し，糞便中に排出されるオーシストやシストを経口摂取することで伝播する（糞口感染）。感染症法においては全数把握の5類感染症で，医師には診断後7日以内の届出が義務付けられている（届出基準はhttp://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou11/01-05-04.html, http://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou11/01-05-08.html)。届出には検査診断（従来の鏡検による病原体検出に加え，2011年からは抗原検出あるいは遺伝子検出が追加された）が必要である（本号13ページ)。また，クリプトスポリジウムは特定病原体の四種病原体としての管理を要する。

クリプトスポリジウム症

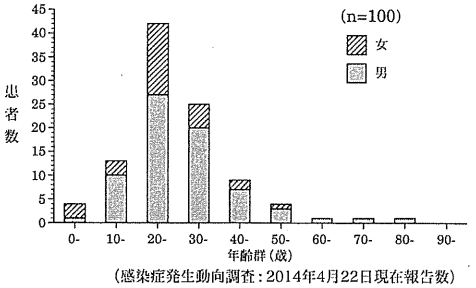
クリプトスポリジウム症は，胞子虫類に属する*Cryptosporidium*の感染に起因する。人には主に*C. hominis*（従来の*C. parvum* genotype Iあるいはヒト型）が，哺乳類には主に*C. parvum*（従来の*C. parvum* genotype IIあるいはウシ型）が感染するが，稀な*C. meleagridis*（トリ型）が集団感染事例から検出されたこともある（IASR 29: 22-23, 2008)。糞便中に排出されるオーシスト（直径5μmの球形）（本号14ページ図1）は，塩素等の消毒薬に抵抗性である。水道，水泳プール，噴水等の水を介した水系集団感染は大規模になりやすく，厚生労働省では「水道におけるクリプトスポリジウム等対策指針」（健水発第0330005号，平成19年3月30日; http://www.mhlw.go.jp/topics/bukyoku/kenkou/suido/kikikanri/dl/ks-0330005.pdf）を定め，水道施設の整備〔水のろ過（急速ろ過，緩速ろ過，膜ろ過等)，紫外線照射〕，原水（河川水など）の検査，浄水処理の運転管理（ろ過池出口の水の濁度0.1度以下の徹底)，水源対策を講じることとされている（本号3ページ)。水や食品を介した感染経路以外にも，患者や動物との接触感染があり，性感染症，人獣共通感染症，日和見感染症としても報告されている。潜伏期間は6日（4～8日）で（本号6ページ)，水様下痢等の症状が10日間程度持続するが，効果的な治療薬はなく，健常者では脱水を防ぐ対症療法が基本である。免疫不全状態では，慢性，難治性，消耗性の下痢を引き起こし，免疫機能を回復させる治療が施されなければ時に致死的となる。日本では1996年の埼玉県越生町の水道水を介した

表1. クリプトスポリジウム症・ジアルジア症報告数，1999年4月～2014年7月

診断年	クリプトスポリジウム症 報告数	ジアルジア症 報告数
1999 (4～12月)	4	42
2000	3	98
2001	11	137
2002	109	113
2003	8	103
2004	92	94
2005	12	86
2006	18	86
2007	6	53
2008	10	73
2009	17	70
2010	16	77
2011	8	65
2012	6	72
2013	19	82
2014 (1～7月)	80	37

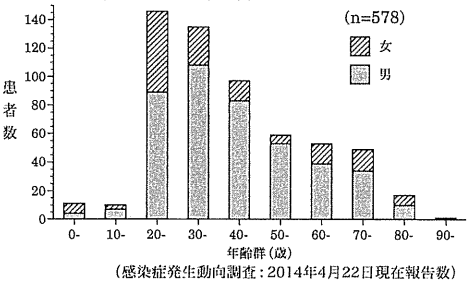
（感染症発生動向調査：2014年7月30日現在報告数）

図1. クリプトスポリジウム症患者の性別年齢分布，2006年1月～2013年12月



（感染症発生動向調査：2014年4月22日現在報告数）

図2. ジアルジア症患者の性別年齢分布，2006年1月～2013年12月



（感染症発生動向調査：2014年4月22日現在報告数）

（2ページにつづく）

(特集つづき)

表2. クリプトスポリジウム症感染要因, 2006～2013年

(n=100)

感染経路・要因	報告数
ウシとの接触	32
海外渡航	27
性的接触 (すべて男性間)	11
食品	4
その他*	2
不明 (うち7例基礎疾患・免疫不全あり)	24

*肥料(有用微生物)の飲用、動物糞便扱い
(感染症発生動向調査:2014年4月22日現在報告数)

表3. ジアルジア症感染要因, 2006～2013年

(n=578)

感染経路・要因	報告数
海外渡航*	250
性的接触*	71
(うち男性間性的接触	42)
下水や糞便等への曝露	6
集団感染(ビルの貯水槽)	4
不明	251

*要因の重複4例を含む
(感染症発生動向調査:2014年4月22日現在報告数)

集団感染がもっとも大規模な事例として知られており, 地域住民の約7割の8,800人が発症した (IASR 17: 217-218, 1996; 埼玉県衛生部報告書, 平成9年3月)。次いで雑居ビル (汚染された貯水槽) の蛇口水 (IASR 15: 248-249, 1994) や水泳プールでの集団感染事例 (IASR 26: 167-168, 168-169, 169-170&170-171, 2005), 牧場実習での感染事例 (IASR 30: 319-321, 2009) 等の報告がある。

感染症発生動向調査: クリプトスポリジウム症の患者届出は, 前回特集 (IASR 26: 165-166, 2005) 以降, 2006～2013年まで年10例程度であった (前ページ表1)。2014年6月には多数の小学生と同行者が体験学習で集団感染した事例が発生しており, 原因の調査中である。

2006～2013年に診断された患者の感染要因は, ウシとの接触, 海外渡航関連 (直近の渡航歴があり, 海外での飲食物摂取が疑われる場合), 男性間性的接触, 食品由来であった (表2)。ウシとの接触では, 学生の農場実習において子ウシとの接触が原因と示唆された事例 (本号4ページ), 自然体験学習 (本号5ページ) などの集団感染が報告されていた。食品由来事例としては, 2006年にウシの生肉 (ユッケあるいは生レバー) に関連の集団感染があった (IASR 28: 88-89, 2007)。

海外感染例の渡航先は, 開発途上国が主であった。その中には, ジアルジアや赤痢アメーバなど他の病原体との重複感染も報告されていた (IASR 28: 298-299, 2007)。男性間の性的接触例では, HIVとの重複感染も報告されていた (本号8ページ)。2006～2013年に診断されたクリプトスポリジウム症患者は20代に多く, 男性が多かった (前ページ図1)。

国内では2006年以降, 大規模な水系集団感染はなかった。しかし海外では, 飲料水, プール, 噴水等による水系集団感染が2004～2010年の間に120件が報告され (本号10ページ), 特に2010年には欧州で最大規模の水系集団感染が報告されている (本号11ページ)。

ジアルジア症

ジアルジア症は, 鞭毛虫類に属する消化管寄生性原虫 *Giardia*の感染による。ヒトに感染する *G. lamblia* (あるいは *G. duodenalis*, *G. intestinalis*, 別名ランブル鞭毛虫) は, 8つの遺伝子型 (Assemblage A～H) に分類され, ヒトからはA, Bが検出される。ジアルジアのシスト (短径5～8×長径8～12μmのラグビーボール型) (本号15ページ図2) は, 塩素消毒に抵抗性があるが, クリプトスポリジウムに比べて弱く, サイズが大きいことからろ過等で除去されやすく, ジアルジア対策としてクリプトスポリジウム対策が有効である (前述の対策指針参照)。治療にはメトロニダゾール (公知申請により2012年からジアルジア症への健康保険適用がなされた) が使われる。

感染症発生動向調査: ジアルジア症の届出は, 2006～2013年の間に578例あった (前ページ表1)。2010年には近年の本邦で初めてジアルジアによる集団感染が報告された (本号7ページ)。感染要因は, 海外渡航関連, 性的接触, 下水や糞便等への曝露であった (表3)。海外感染例の渡航先は, 開発途上国が主であった。性的接触は, 男性間が多かった (71例のうち42例)。ジアルジア症の届出は, クリプトスポリジウム症と同様に20代に多く, 男性が多かった (前ページ図2)。他の病原体との重複感染は26例 (578例中の4.5%) 報告されており, 赤痢アメーバ, クリプトスポリジウム, チフス菌, パラチフス菌, 赤痢菌などであった。HIVとの重複感染も報告されていた (本号8ページ)。ジアルジア症が疑われる場合, 他の病原体との重複感染を疑って検査することが重要と考えられた。

症状の多くは下痢であるが, 腹部不快感などのみで下痢, 軟便, 粘液便等の症状の報告のなかった例が98例 (578例中の17%), 無症状が13例 (578例中の2.2%) あり, 無症状病原体保有者 (シストキャリア) の存在が指摘される。感染症法におけるジアルジア症の届出基準には無症状病原体保有者は含まれていないが, 感染源としての注意が必要である。精査目的と思われる内視鏡検査が行われていた63例 (578例中の11%) において, 十二指腸液, 胆汁, 膵液等からのジアルジア検出が報告されていた。胆嚢炎様症状を呈する患者において, ジアルジアが稀に検出されることに注意を要すると考えられた (本号10ページ)。

クリプトスポリジウム, ジアルジア等の原虫類による感染症は, 世界的には多くの患者が発生している。消化管寄生性原虫には, 他にサイクロスポラ (本号12ページおよび15ページ図3), イソスポラ, 赤痢アメーバ等もあり, 水源や動物などの感染源対策, 手洗い, 加熱などの感染予防対策はクリプトスポリジウム, ジアルジア対策と共通である。原因不明の下痢症に対しては, クリプトスポリジウム症やジアルジア症も鑑別診断に挙げての, 糞便の検査が重要である。

＜特集関連情報＞

水道におけるクリプトスポリジウム等検出状況と対応の事例（給水停止等の対応を行ったもの）

2014(平成26)年2月末現在
厚生労働省健康局水道課

年度	件数	都道府県	種別	浄水処理	長期的対応	備考
1996(平成8)	1	埼玉県	上水道	急速ろ過処理	膜ろ過施設設置	浄水からクリプトスポリジウムを検出 住民14,000人のうち8,800人が感染
1997(平成9)	2	鳥取県	簡易水道	塩素処理のみ	上水道事業に併合	原水からクリプトスポリジウムを検出 感染症患者なし
		兵庫県	簡易水道	塩素処理のみ	膜ろ過施設設置	原水からクリプトスポリジウムを検出 感染症患者なし
1998(平成10)	2	福井県	簡易水道	急速ろ過処理	浄水処理管理強化	原水及び浄水からジアルジアを検出 感染症患者なし
		兵庫県	簡易水道	塩素処理のみ	膜ろ過施設設置	原水からクリプトスポリジウムを検出 感染症患者なし
1999(平成11)	1	山形県	上水道	塩素処理のみ	広域用水供給事業から受水	原水からクリプトスポリジウム及びジアルジアを検出 感染症患者なし
2000(平成12)	3	青森県	簡易水道	塩素処理のみ	膜ろ過施設設置	浄水からジアルジアを検出 感染症患者なし
		沖縄県	小規模水道	簡易ろ過及び塩素処理	上水道事業に併合	浄水からクリプトスポリジウムを検出 感染症患者なし
		岩手県	簡易水道	塩素処理のみ	水源変更、急速ろ過施設設置	浄水からジアルジアを検出 感染症患者なし
2001(平成13)	5	愛媛県	上水道	塩素処理のみ	当該水源は使用中止	浄水からクリプトスポリジウムを検出 感染症患者なし
		岩手県	簡易水道	緩速ろ過処理	浄水処理管理強化	原水及び浄水からジアルジアを検出 感染症患者なし
		兵庫県	簡易水道	塩素処理のみ	膜ろ過施設設置	原水からクリプトスポリジウムを検出 感染症患者なし
		鹿児島県	上水道	塩素処理のみ	膜ろ過施設設置予定	原水からクリプトスポリジウムを検出 感染症患者なし
		愛媛県	上水道	急速ろ過、活性炭処理	ろ材入替、浄水処理管理強化を予定	浄水からクリプトスポリジウムを検出 感染症患者なし
2002(平成14)	1	山形県	簡易水道	塩素処理のみ	応急対策として膜処理装置設置、長期的には上水道事業と統合予定	原水からジアルジアを検出 感染症患者なし
2003(平成15)	2	大分県	上水道	塩素処理のみ	当該水源は使用中止	原水からジアルジアを検出 感染症患者なし
		山形県	小規模水道	塩素処理のみ	応急対策として膜ろ過施設設置、長期的には水源変更	浄水からジアルジアを検出 感染症患者なし
2004(平成16)	1	兵庫県	上水道	急速ろ過処理	安全確認迄の間飲用制限、浄水処理管理強化を実施	原水及び浄水からジアルジアを検出 感染症患者なし
2005(平成17)	0	該当なし				
2006(平成18)	1	大阪府	簡易水道	急速ろ過	濁度計を設置し常時濁度管理を徹底	原水及び浄水からクリプトスポリジウムを検出 感染症患者なし
2007(平成19)	2	富山県	簡易水道	塩素処理のみ	上水道事業に併合	原水からジアルジアを検出 感染症患者なし
		富山県	簡易水道	急速ろ過 (濁度管理不可)	紫外線処理施設設置予定	原水からジアルジアを検出 感染症患者なし
2008(平成20)	1	山形県	簡易水道	塩素処理のみ	膜ろ過施設設置	原水からジアルジアを検出 感染症患者なし
2009(平成21)	0	該当なし				
2010(平成22)	2	富山県	専用水道	塩素処理のみ	紫外線処理施設の設置あるいは隣接簡易水道への切り替え	原水からジアルジアを検出 感染症患者なし
		千葉県	小規模貯水槽水道	—	貯水槽を更新	給水栓水からクリプトスポリジウム及びジアルジアを検出。小規模貯水槽水道の利用者43人のうち28人が体調不良。4人がジアルジアに感染
2011(平成23)	1	長野県	簡易水道	急速ろ過		原水及び浄水からクリプトスポリジウムを検出 感染症患者なし
2012(平成24)	1	群馬県	用水供給	急速ろ過		浄水からジアルジアを検出 感染症患者なし
2013(平成25)	2	北海道	飲料水供給施設	塩素処理のみ	膜ろ過施設設置	原水及び浄水からクリプトスポリジウムを検出 感染症患者なし
		東京都	専用水道	除鉄・除マンガン処理		原水からジアルジアを検出 感染症患者なし
計	28					

※ 原水からクリプトスポリジウム等が検出された場合で「対策指針」に基づく対策が講じられていない施設の事例を含む。