博士論文　（要約）

Molecular Detection Methods for Assessing Virus Occurrence and Viability in Water Supply Systems

(分子生物学的手法を用いた水道におけるウイルスの存在及び感染性の評価)

ブ ドゥック　カン
Vu Duc Canh

Drinking water safety can be threatened by the presence of enteric viruses since only few infectious virus particles can cause diseases. Recently, outbreaks associated with the presence of enteric viruses in drinking water have been reported worldwide. Therefore, ensuring viral safety in drinking water is essential for protecting public health. To assess and manage the risk of viral infection via drinking water, it is important to evaluate the effectiveness of virus removal by treatment processes at drinking water treatment plants (DWTPs). Although several studies have investigated the virus removal from laboratory or pilot experiments, virus removal by full-scale treatment processes has not been evaluated. In addition, to guarantee the safety of drinking water, it is necessary to verify the presence of infectious enteric viruses in drinking water. Recently, viability (RT-)qPCR has been developed to rapidly determine the potential infectivity of viruses, wherein water samples are pretreated with viability markers prior to (RT-)qPCR. However, the efficacy of viability (RT-)qPCR was recently shown to depend on the capsid structure of viruses and inactivation mechanism. Also, the applicability of viability (RT-)qPCR for testing actual drinking water is relatively less well-established. Therefore, this dissertation conducted a study on molecular detection methods for assessing virus occurrence and viability in water supply systems. Particularly, the efficiency of virus removal by full-scale drinking water treatment processes was evaluated and the application of viability (RT-)qPCR was evaluated to assess the virus occurrence and viability in drinking water source and tap water.

Chapter 1 introduced the background, limitation and research gaps and objectives. Chapter 2 was the literature review related to the risk of viral infection associated with drinking water, commonly used virus concentration and detection methods and their challenges to determine the infectivity of viruses. Chapter 3 provided the experimental methods and materials that were used in this study.

Chapter 4 investigated the virus removal efficiency of treatment processes at full-scale drinking water treatment plants (DWTPs). According to Water Safety Plan, each unit treatment process DWTP is a critical point for controlling the risks in drinking water. Although viruses can be reduced effectively by disinfection treatments, nevertheless, it is essential to evaluate the performance of other physical treatment processes to ensure robust multi-barrier treatment. Thus, this chapter focused on investigating the efficiency of virus removal by microfiltration (MF) and slow sand filtration (SSF) processes at two full-scale DWTPs in Japan. The pepper mild mottle virus (PMMoV) was used as a surrogate to evaluate the virus removal since it has been proposed as a virus indicator for fecal pollution in water sources and as a useful process indicator that

is readily detectable in water treatment systems. MF was able to remove PMMoV only 0.0 to >0.9 $\log_{10}$. SSF removed PMMoV by up to 2.8 $\log_{10}$; however, the removal efficiency decreased to 0.0–1.0 $\log_{10}$ under cold water temperatures. Thus, it was likely that the provision of drinking water safe from viruses in these DWTPs may rely solely on the performance of the chlorination.

Chapter 5, 6 and 7 focus on the development and application of viability (RT-)qPCR method to determine the potential infectivity of viruses in drinking water. In Chapter 5, viability (RT-)qPCR was improved to more accurately determine the infectivity of viruses. The pretreatment of sodium deoxycholate (SD) surfactant was applied to enhancing the penetration of viability markers including ethidium monoazide (EMA) and propidium monoazide (PMA) and cis-dichlorodiammineplatinum (CDDP), into inactivated Aichi virus. The results indicate that the pretreatment with 0.1% was optimal for enhancing the performance of all three viability markers (EMA, PMA and CDDP) by excluding a majority of false-positive RT-qPCR signals after heat and chlorine treatments. Among the viability markers tested, the use of CDDP with the 0.1% SD pretreatment was most effectively reflected the virus infectivity by RT-qPCR detection (SD-CDDP-RT-qPCR). Although SD-CDDP-RT-qPCR was still not able to completely eliminate the false-positive RT-qPCR signal, this method can be used to provide a more accurate estimate on the virus infectivity than RT-qPCR alone.

In Chapter 6, the effects of genome features (genome types and amplicon lengths) on the performance of viability (RT-)qPCR using EMA, PMA and CDDP markers were investigated. The different types of naked viral genomes including RNA viral genomes extracted from PMMoV, NoV II, AiV, HAV, PV-1 and DNA viral genomes extracted from AdV-5, AdV-40, PhiX174. Among the viability markers tested, CDDP was found the most effective to reduce the signal of (RT-)qPCR detection for all naked viral genomes (including RNA and DNA genomes). All viability (RT-)qPCR methods were more effective to eliminate RNA viral genomes than DNA viral genomes. The viability (RT-)qPCR methods seemed to work more efficiently to remove viral genomes with longer amplicon lengths than those with shorter ones. In addition, the performance of viability (RT-)qPCR methods was evaluated under the effects of environmental matrices from raw and treated water in three full-scale DWTPs. A total of 36 water samples (18 raw water and 18 treated water samples) were collected and concentrated by a virus concentration method using a negatively charged filter cartridge. It was found that all viability RT-qPCR methods could be influenced by environmental matrices from raw and treated water concentrates, but their influences on RT-qPCR were greater than

those on viability treatments. When compared among the viability RT-qPCR methods, SD-CDDP-RT-qPCR was still more effective than SD-EMA/PMA-RT-qPCR methods to eliminate the signal of the inactivated virus under the effects of environmental matrices. Therefore, it is important to evaluate the efficiency of RT-qPCR and the effectiveness of viability treatments when using viability (RT-)qPCR methods to evaluate environmental water samples.

Chapter 7 investigated the application of SD-CDDP-(RT-)qPCR (the most effective viability (RT-)qPCR method) to assessing the virus occurrence and viability in drinking water sources and tap water. A total of 63 water samples (20 source water and 43 tap water) were collected in the Kanto region (covering 7 prefectures) in Japan between August 2018 and March 2019. The source water samples (n=20) with an average volume of 14 L were collected from four rivers and one lake. The tap water samples (n=43) with an average volume of 365 L were collected from 11 sports centers that received drinking water from 11 different drinking water treatment plants (DWTPs) taking raw water from surface waters (8 DWTPs) and groundwaters (3 DWTPs). All water samples were concentrated using a negatively charged filter cartridge and then quantified by conventional (RT-)qPCR and SD-CDDP-(RT-)qPCR for various types of viruses including PMMoV, AiV, EV, NoV-I and NoV-II, AdV40-41, BK and JC PyV.

For source waters, PMMoV was detected more prevalently (100%) and at higher concentration (3.0–7.4 $\log_{10}$ copies/L) than other enteric viruses (46-69%, 1.7–6.1 $\log_{10}$ copies/L) when determined by conventional (RT-)qPCR. The similar tendencies were also observed when determined by SD-CDDP-(RT-)qPCR but at lower detection rate and concentration, particularly PMMoV (95%, 3.1-6.2 log1$_0$ copies/L) and other enteric viruses (1.7-6.1 $\log_{10}$ copies/L). It suggests that most of the viruses in source waters had a partially damaged capsid and so the SD-CDDP-(RT-)qPCR generally provided a better estimate on the occurrence and viability of viruses in source waters than conventional (RT-)qPCR. For tap water, most of the target viruses were not detected in the tap water samples by conventional (RT-)qPCR (LoD=0.3 $\log_{10}$ copies/L) with an exception of PMMoV (9%, 2.20–2.90 $\log_{10}$ copies/L) and AiV (5%, 0.62–1.18 $\log_{10}$ copies/L). The presence of PMMoV and AiV in tap water might be due to their high abundance in the source waters and their high persistence against treatment processes in DWTPs. In addition, only PMMoV was detected in tap water by SD-CDDP-(RT-)qPCR. It might be that the capsid of PMMoV was also more stable than that of other enteric viruses against drinking water treatment processes. Interestingly, although PMMoV seemed to be abundant and persistent in tap water, it was not detected in the samples positive for

AiV, which were all originated from groundwaters. It appears that PMMoV did not correlate with AiV in tap water produced from groundwater. This evidence suggests that PMMoV might not be used as a good indicator to estimate the risk of viral contamination in tap water that was produced from groundwater. However, for the tap water produced from surface waters, the absence of PMMoV can be a useful indicator to ensure the absence of other enteric viruses and so it can be used to ensure the safety of drinking water from the viral contamination.

Chapter 8 summarized the overall conclusion of this thesis, proposed the consideration for the application of SD-CDDP-(RT-)qPCR to assessing the potential infectivity of viruses in aquatic environments and recommendations for the future study based on SD-CDDP-(RT-)qPCR method.