

論文の内容の要旨

論文題目 Occurrence and Behavior of Human Enteric Viruses
in Urban Coastal Water
(都市沿岸水中におけるヒト腸管系ウイルスの存在
およびその消長)

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Human enteric viruses have been recognized as one of the major causes of waterborne outbreaks which have been reported worldwide in both developed and developing countries. Enteric viruses related to waterborne diseases include noroviruses, enteroviruses, hepatitis viruses and adenoviruses. Viruses are excreted in feces of infected individuals in high concentration and gathered in sewage. Viruses in sewage can contaminate recreational water body and shellfish harvesting area in coastal water to pose a great risk to the public health. Molecular methods have recently been used as a tool to detect viruses in the environment but most studies provided only qualitative data i.e., presence or absence of the viruses, which lead to the limited information on their behaviors.

The main point sources of viral contamination in urban coastal water are combined sewer overflow and wastewater treatment plant, but actual effect of combined sewer overflow and effluent as well as the degree to which they contribute to viral contamination in coastal water has not been well characterized. Thus, this research aimed to evaluate the occurrence and behavior of human enteric viruses in urban coastal water which contaminated by combined sewer overflow and effluent. The target viruses in this study include norovirus G1, norovirus G2, enterovirus and adenovirus. The occurrence and behavior of microbial indicators was studied in parallel with these viruses. The microbial indicators examined include (i) conventional fecal pollution indicators (total coliform, fecal coliform, *E.coli*), (ii) enterococcus which is recently recommended fecal indicator in marine water, (iii) *Clostridium perfringens* spores, and (iv) two types of bacteriophage viz., FRNA bacteriophage and somatic coliphage, which recently has been proposed as viral indicators. The real-time PCR was used to quantify concentration of viruses. Plate culture assay was used to determine concentration of microbial indicators.

In Chapter 4, the profile of viruses in sewage and their behaviors during passing through wastewater treatment processes was investigated at two wastewater treatment plants (Morigasaki and Ariake wastewater treatment plants) in Tokyo. Combined sewer overflow can be represented by raw sewage in wastewater treatment plant. The raw sewage, effluent before chlorination and final effluent after chlorination were collected on monthly basis for one year. The results showed that human enteric viruses were detected in all sewage samples. The most prevalent virus detected was adenovirus. Seasonal pattern was observed in case of norovirus G1 and norovirus G2 in which their concentrations were high in winter and low in summer. The concentration of enterovirus and adenovirus in sewage was relatively constant throughout the year. An average of overall removal efficiency in both wastewater treatment plants for all types of viruses tested, *C. perfringens* spores, bacteriophages, and bacterial indicators were 2.4 - 3.6 logs, 2.3 logs, 3.4 - 3.6 logs, and 4.5 - 5.5 logs, respectively. Obviously, wastewater treatment plants were more effective in removing bacterial indicators than enteric viruses. The results indicated that the behavior of bacterial indicators does not adequately explain virus removal in wastewater treatment plants. Among viruses, norovirus G1 was the most resistant virus whereas norovirus G2 was the least resistant virus against biological treatment and chlorination. The difference of ratio of concentration of resistant microbes (viruses, bacteriophage or spore) to fecal coliform between sewage and wastewater treatment plant effluent gave useful information for tracking the viral pollution sources in coastal area.

Chapter 5 was focused on distribution and persistence characteristics of viruses following the combined sewer overflow discharge to coastal water under laboratory controlled condition. The vertical distribution of solid-associated viruses through sedimentation process (60 minute settling times) in column experiment was studied. The results showed that the amount of viruses existing in solid-associated form more than free-suspended form, except adenovirus which tended to exist more in free-suspended form. Viruses in solid-associated form were increased in saline water. The percentage of all type of viruses in solid-associated form was in the range of 50 - 76% at salinity 0% condition while it was in the range of 62 - 96% when salinity increased to 1% or 2.5%. The changing in concentration of viruses was governed by their persistence to environmental conditions. Norovirus G1 was the most persistent virus whereas norovirus G2 was the least persistent virus among all type of viruses tested in dark condition. Light irradiation of metal halide lamp accelerated degradation of viruses. The influence of salinity on disappearance of viruses was observed greater in the presence of light than dark condition. Solid particles reduced degradation of viruses under light exposure and also in dark condition.

Chapter 6, field monitoring was carried out to study the occurrence and behavior of viruses in coastal area of Tokyo Bay as well as to identify source of viral contamination. Water and sediment samples were collected in three sampling sites in Tokyo Bay on monthly basis for one year. The most abundant virus found in Tokyo Bay was adenovirus. The prevalence of noroviruses was observed in winter. The results showed that viruses were present even when fecal coliform level was below the Japanese recreational standard. The elevated level of viruses and microbial indicators in Tokyo Bay occurred after heavy rainfall event. The level of virus concentration was not only depended on rainfall quantity and duration of dry days but also persistence of viruses. The persistence of microorganisms in Tokyo Bay had the same tendency in wastewater treatment plant. The order from greatest persistence to the least persistence was *C. perfringens* spore > viruses and bacteriophages > bacterial indicators. The mean concentration of viruses and microbial indicators in sampling sites in Tokyo Bay was higher than mean concentration in effluent from the wastewater treatment plants. The ratio between concentration of resistant microbes (viruses, bacteriophage or spore) to concentration of fecal coliform in Tokyo Bay samples was increased because fecal coliform was degraded more rapidly than resistant microbes. The results of field monitoring demonstrated that viral contamination in coastal zone in Tokyo Bay was governed by combined sewer overflow rather than daily effluent discharge from wastewater treatment plant. The impact of direct combined sewer overflow discharge was observed in both water and sediment in one of the three sampling sites, which is near the outlet of combined sewer overflow.

In Chapter 7, the occurrence of enteric viruses in environmental samples in Indonesia and Vietnam, where epidemiological data on viral prevalence is unavailable, were studied and compared to the occurrence in Japan. The results demonstrated that novovirus G1, norovirus G2, enterovirus and adenovirus were prevalent in Indonesia, Vietnam and Japan but hepatitis A virus was prevalent only in Indonesia. This result revealed that occurrence of enteric viruses varied from country to country. The concentration of viruses in surface water in Indonesia and Vietnam was greatly varied depended on level of contamination. Some surface water samples or floodwater samples contained enteric viruses with concentration similar to raw sewage in Japan. This would pose high risk of viral infection to people through contamination of drinking water or direct contact with waters. The result of Chapter 7 suggested that determining of occurrence of viruses in environmental samples is useful for assessing the prevalence of viruses in the community.