ABSTRACT
論文の内容の要旨

TITLE (**論文題目**) Molecular Epidemiological Analysis of Pathogenic Viruses in Water Environments and Risk Assessment

(水環境中における病原ウイルスの分子疫学的解析および感染リスク評価)

NAME (**氏名**) **北島　正章**

Enteric viruses, such as noroviruses (NoVs), enteroviruses, and adenoviruses, have been a major research target of environmental virology. Development and improvement of methods to detect enteric viruses in environmental water, especially virus concentration methods and molecular detection techniques, have enabled us to accumulate qualitative and quantitative data on occurrence and behavior of enteric viruses in the environments. NoVs are considered to be an important agent in causing waterborne gastroenteritis. Although no routine in vitro infectivity assay system for human NoVs is available, discovery of murine noroviruses (MNVs) that can be cultivated in routine cell culture system have facilitated our understanding on environmental stability of NoVs. That is, recent progress in environmental virology has been elucidating the behavior of enteric viruses including human NoVs in water environments and water treatment systems. On the other hand, emerging and re-emerging viral diseases for humans have been reported. In 1997, highly pathogenic avian influenza A (HPAI) of the H5N1 subtype viruses transmitted from birds to humans and caused the deaths of 6 out of 18 infected persons in Hong Kong. This was the first incidence of transmission of avian influenza virus to humans with fatal outcome. Further outbreaks of HPAI H5N1 viruses had started since 2003 in Hong Kong, Vietnam, Indonesia, and Thailand. The HPAI H5N1 viruses have then spread to other Asian countries (including Japan), the Middle East, Southeast Europe, Central Europe, and Africa. Therefore, human infection with HPAI H5N1 virus is of a great public health concern in the world, and the H5N1 avian influenza is one of the important emerging diseases for humans. As represented by the H5N1 avian influenza, a novel virus with unknown biological characteristics from non-human source can emerge, and thus, environmental virologists should target broad range of pathogenic viruses to understand their behavior in the environments and control risks.

On the basis of the above background, the present study was performed to obtain

systematic and comprehensive knowledge to manage infection risks caused by waterborne viruses. The major objectives of the present study were (1) to investigate the prevalence, seasonality, and genetic diversity of enteric viruses, namely NoVs, sapoviruses (SaVs), and Aichi viruses (AiVs), in water environments and (2) to characterize infection risks of highly pathogenic avian influenza viruses to humans through water.

Chapters 3 and 4 focus on gastroenteritis viruses, namely NoVs, SaVs, and AiVs. Circulation of the viruses between contaminated environmental water and human populations is a key issue in understanding their epidemiology and health risks for humans. Since wastewater contains viruses shed from all populations regardless of their health status, monitoring of viruses in wastewater and urban river water receiving effluents from multiple wastewater treatment plants (WWTPs) could be an appropriate approach for determining the actual prevalence and molecular epidemiology of gastroenteritis viruses in catchment areas rather than clinical studies. In Chapter 3, prevalence and genetic diversity of NoVs, SaVs, and AiVs in wastewater and river water was investigated during a 1-year period. Presence of viral genomes in the water samples were examined with RT-PCR assays, and the strains were further characterized based on the nucleotide sequences of the PCR amplicons. GI NoV strains were more frequently detected than GII NoV strains in both wastewater and river water samples. This result disagrees with the epidemiological data obtained from the Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Japan, where GII strains were much more frequently detected than GI in feces of hospitalized patients, suggesting that GI strains are more widely spread among humans than previously appreciated. Moreover, GIV NoV strains were successfully identified in both wastewater and river water samples by utilizing the newly developed semi-nested RT-PCR assay specific for GIV. Interestingly, it was demonstrated that genetically diverse GIV NoV strains, including those of a newly identified genetic cluster, were circulating in Japan. The newly developed assay can be a powerful tool to detect and characterize GIV strains in water environments. SaV strains were also successfully identified in both wastewater and river water. A nested RT-PCR assay utilizing a novel primer (1245Rfwd), rather than any previously reported assays, more efficiently amplified SaV genomes in the samples. The newly developed RT-PCR assay is useful for identifying SaV strains in environmental samples. Another highly important results obtained in this chapter is that Aichi viruses showed the highest prevalence in both wastewater (23/24; 96%) and river water (36/60; 60%) among the viruses tested, suggesting that AiV are prevalent in aquatic environments of Japan. The results

described in this chapter demonstrates that genetically diverse NoV, SaV, and AiV strains are circulating between human populations and water environments. These results provide novel findings contributing our understanding of the prevalence and genetic diversity of the viruses in water environments. Gastroenteritis viruses in water environments may reflect, more accurately, the actual prevalence and molecular epidemiology in the human population rather than reported cases which represent a small portion of total cases.

In Chapter 4, TaqMan-based real-time RT-PCR assays for rapid detection, quantification, and genotyping of AiVs have been established. The assays were subsequently applied for quantitative analysis of occurrence and behavior of AiVs at two WWTPs (A- and B-WWTP) during a 1-year period. AiVs were detected in all influent as well as effluent samples of both WWTPs, demonstrating that they are continuously circulating between human populations and water environments. A trend observed in both WWTPs was that the concentrations of AiVs in influent increased in winter season, and were comparably lower in summer–autumn season. The removal ratios of AiVs at A-WWTP and B-WWTP were 2.41±0.42 log10 (n=12) and 2.96±0.40 log10 (n=12), respectively. It should be noted here that AiVs can potentially be an appropriate indicator of viral contamination in the environments because of their high prevalence in the environments and structural as well as genetic similarity with some of other important enteric viruses. AiVs are small round viruses possessing a single-stranded RNA genome and are members of the family Picornaviridae that includes enteroviruses and hepatitis A virus. These latter two viruses are listed in the latest U.S. Environmental Protect Agency's Contaminant Candidate List (CCL3), which identifies emerging contaminants of aquatic environments that may pose a public health risk. This is the first study providing protocols for quantification and genotyping of AiVs by real-time RT-PCR assays and describing quantitative data on the occurrence of AiVs in wastewater over a 1-year period. Behavior of AiVs in water environments had been unknown, since there have been only a few studies reporting detection of AiVs in environmental samples. This study provides useful knowledge toward understanding their occurrence and fate in water environments.

Chapters 5, 6, and 7 involve experimental and analytical studies on influenza A viruses. It is likely that avian influenza viruses with feces or other secretions from both symptomatic and asymptomatic waterfowl will be released into water environments where the birds gather. Although avian influenza viruses can persist for extended periods of time in water, the occurrence of the viruses in environmental water remains unknown. Since virus concentration is an essential step to detect viruses at low levels in

water, Chapter 5 was designed to determine recovery yields of influenza A viruses in water by conventional virus concentration methods with both plaque assay and real-time RT-PCR assay. The results demonstrated that infectious influenza viruses were not efficiently recovered by Mg- or Al-methods because of pH sensitivity of influenza viruses. A novel method using an HA electronegative filter and surfactant-based eluting solution (pH 7.9) was judged to be an appropriate concentration method that can efficiently recover viable influenza A virus particles from water to obtain a recovery rate of more than 30%.

Open bodies of water, including drinking water sources, can be contaminated by infected waterfowl. The oral ingestion or aspiration of water containing influenza A virus could be a possible mode of transmission to humans, although no evidence has been reported. Despite growing concerns about the public health threat posed by influenza A viruses, there has been limited knowledge on efficacy of disinfectants in inactivating influenza A viruses in water. In Chapter 6, inactivation kinetics of influenza A viruses by disinfectants, namely chlorination, chloramination, and UV irradiation, were investigated. For the purpose of drinking water production, the U.S. Environmental Protection Agency requires free chlorine CT values of 6 and 8 mg-min/L to achieve enteric virus inactivation of 3 and 4 log10, respectively. According to the results obtained in this study, these CT values would be more than sufficient to inactivate influenza A viruses in water. Inactivation ratio of influenza A viruses by each disinfectant was compared with those of enteric viruses, which demonstrated that influenza A viruses are less resistant to the disinfectants than most of enteric viruses. These results demonstrated that water disinfection process could achieve appreciable inactivation of influenza A viruses when a proper pre-treatment process (e.g. coagulation, sedimentation and rapid sand filtration) is performed to remove the chlorine demand and suspended solid. The information on inactivation of influenza A viruses described in this study is useful for developing risk management procedures regarding the role of water in the transmission of the viruses to humans and poultry.

Recently, highly pathogenic avian influenza A (HPAI) of the H5N1 subtype viruses have infected humans and caused severe diseases in many countries. Quantitative microbial risk assessment (QMRA) framework can be a powerful tool to understand how to control pandemics mediated by environmental reservoirs or human-to-human transmission (e.g. calculating risk of infection due to a low dose). The objectives of Chapter 7 were to estimate risks of waterborne and airborne infections, and to develop a time-dependent dose-response model, based on the QMRA framework. Waterborne infection risks of the HPAI H5N1 virus quantitatively evaluated assuming that a single

HPAI H5N1 virus-infected duck shed the virus in the river water used for drinking water production and recreational purposes. The results of the Monte Carlo simulation demonstrated that the median probability of infection associated with swimming in the contaminated river was 9.4×10? 11 [infection/person/swim]. The median probability of infection associated with consumption of tap water was less than 10? 13 [infection/person/year] when the virus reduction efficiency at the drinking water treatment plant was 4 log10. Furthermore, a basic model to evaluate a household, airborne, secondary transmission risk was constructed. An essential step in the QMRA process is dose-response assessment; however, to date, none of the prior studies investigated the dose-response relationship to describe HPAI H5N1 virus infection in humans. In this study, the time-dose-response model to describe mortality of mice exposed to an HPAI H5N1 virus, describing the mortality over time and represents experimental responses accurately, has been successfully developed and validated. This is the first study describing a time-dependent dose-response model for HPAI H5N1 virus. These models developed in the present study will be useful to evaluate the risks of HPAI H5N1 virus infection under various exposure scenarios and to estimate the mortality of HPAI H5N1 virus, which may depends on time post exposure, for preparation of a future influenza pandemic caused by this lethal virus.

Environmental virology consists of four major elements: (1) development of concentration and detection methods of viruses in water, (2) investigation of the occurrence and behavior of viruses in water environments, (3) characterization of inactivation of viruses by disinfectants used for water treatment and water distribution processes, and (4) risk assessment. A lot of knowledge on environmental and medical virology has been accumulated since several decades ago. The present study provides novel knowledge on the prevalence, seasonality, and genetic diversity of enteric viruses, namely NoVs, sapoviruses (SaVs), and Aichi viruses (AiVs), in water environments, and on infection risks of highly pathogenic avian influenza viruses to humans through water. This dissertation summarizes previously known and newly obtained knowledge that greatly contributes to evaluate and reduce infection risks of pathogenic viruses in water.