Marine Pollution Bulletin 112 (2016) 313–317

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Differences in the viability of murine norovirus in different aquatic locations

Hajime Takahashi [a,*], Michiko Takahashi [a], Chihiro Ohshima [a], Yukino Izawa [a], Masashi Uema [b], Takashi Kuda [a], Bon Kimura [a], Mamoru Noda [b]

[a] Department of Food Science and Technology, Faculty of Marine Science, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan
[b] Division of Biomedical Food Research, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

ARTICLE INFO

Article history:
Received 24 December 2015
Received in revised form 27 July 2016
Accepted 2 August 2016
Available online 12 August 2016

Keywords:
Norovirus
MNV-1
Seawater
Salt concentration
Water temperature

ABSTRACT

Norovirus is detected from shellfish and environmental water more frequently in winter than in other seasons. However, there is no report regarding its viability in actual seawater in situ. We investigated the viability of murine norovirus strain 1 (MNV-1), a surrogate for human norovirus, in 2 types of aquatic locations, a seawater pool carrying oceanic water and inner bay carrying brackish water. Sterilized seawater was inoculated with MNV-1 and enclosed in dialysis tubes, which were placed at the 2 locations. MNV-1 exhibited higher level of viability in brackish than in oceanic water. Factors that influenced the viability of MNV-1 included salt concentration as well as temperature of the seawater. Therefore, based on our findings, coastal brackish water that is routinely used for harvesting or cleaning seafood at fishing ports may promote the viability of norovirus.

1. Introduction

Norovirus belongs to the family Caliciviridae and is a major cause of acute gastroenteritis worldwide (Koopmans and Duizer, 2004). In Japan, norovirus accounts for 31% cases of food poisoning in 2014 (Ministry of Health, Labour and Welfare, 2014). Norovirus is transmitted via the oral route by ingestion of contaminated food or water and can also spread via direct contamination of food and surfaces with vomits from infected people (Koopmans and Duizer, 2004). Norovirus outbreaks generally occur in areas where people tend to gather, such as hospitals, schools, and restaurants (Centers for Disease Control and Prevention, 2014; Leuenberger et al., 2007). The infective dose of norovirus is extremely low; <10 viral particles can lead to the onset of symptoms (Teunis et al., 2008).

Norovirus is known to contaminate seawater and bivalve mollusks, such as oysters, clams, and mussels (Metcalf et al., 1995), some of which is often consumed raw in many countries. It has been reported that norovirus was detected in abundance from oysters (Nishida et al., 2003), seawater (Katayama et al., 2002), and sewerage systems (Katayama et al., 2008). Although the viability of norovirus in laboratory scale is available (Flannery et al., 2013; Hewitt et al., 2013; Takahashi et al., 2011), that in actual seawater in situ has not yet been reported. Many factors in natural environment, which may be different between coastal water and oceanic water, must influence the viability of norovirus.

The aim of this study was to investigate the viability of norovirus in actual seawater in different environment. Due to a lack of suitable cultivation method for human norovirus, murine norovirus strain 1 (MNV-1) (as a surrogate for human norovirus) is used to measure the infectivity by cultivation method. MNV-1, also belongs to the family Caliciviridae and genus norovirus (Karst et al., 2003), is more closely related to human norovirus than the other surrogate viruses (Wobus et al., 2006). There are many reports that used MNV-1 as a surrogate for human norovirus (Bae and Schwab, 2008; Seo et al., 2012; Takahashi et al., 2011). Therefore, MNV-1 is preferable over the other surrogate viruses (Cannon et al., 2006), and used in this study. The differences of MNV-1 viability in oceanic water and in brackish water in summer season and winter season were evaluated and the effect of salt concentration in seawater on the viability of MNV-1 was examined.

2. Materials and methods

2.1. Virus and cells

In this study, MNV-1 was used as a surrogate for human norovirus.

Murine macrophage cells (RAW 264.7) cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin, were infected with MNV-1 at 0.1 multiplicity of infection (MOI) and cultured for 3 days at 37 °C in 5% CO_2. After development of the cytopathic effect, the cells were frozen and thawed 4 times and centrifuged at 8000 ×*g* for 20 min to precipitate the virus particles for purification. Infectivity

* Corresponding author.
E-mail address: hajime@kaiyodai.ac.jp (H. Takahashi).

http://dx.doi.org/10.1016/j.marpolbul.2016.08.001
0025-326X/

was measured by plaque assay as described in Section 2.4 and the extracted virus was stored at −80 °C until use.

2.2. Viability of MNV-1 in 2 aquatic locations

MNV-1 was diluted with seawater which was sterilized by filtration (0.20 μm pore size, Advantec, Tokyo, Japan) to obtain 1×10^5 plaque-forming units (PFU)/mL and 5 mL of this solution was enclosed in cellulose ester (CE) dialysis tubes (8–10 kD, 31-mm diameter; Spectra/Por® Dialysis Tubing, Spectrum Laboratories. Inc., CA, USA). The tubes were sealed and affixed within a cage (Fig. 1), which was placed at 2 aquatic locations in Japan during summer and winter: a seawater pool (carrying oceanic water from Tateyama bay in Chiba) and in brackish water (inner bay at Tokyo port) (Fig. 2). The cages were kept in places for 7 days during summer (September 2013 for oceanic water, and August 2013 for brackish water) and winter season (February 2014 for oceanic water, and January 2014 for brackish water). Characteristics of each location were measured using a pH meter (B-712, HORIBA, Ltd., Kyoto, Japan), salt concentration meter (B-721, HORIBA, Ltd., Kyoto, Japan), and Chemical Oxygen Demand (COD) test kit (Kyoritsu Chemical-Check Lab. Corp., Tokyo, Japan). Seawater temperature was measured every 2 h using THMchip (THM10-TH, Wako Pure Chemical Industries, Osaka, Japan) for 7 days.

MNV-1 solution in the CE dialysis tubes was sampled at 0, 1, 3, 5, and 7 days post-placement, the virus was filtered using a CA filter (0.20-μm

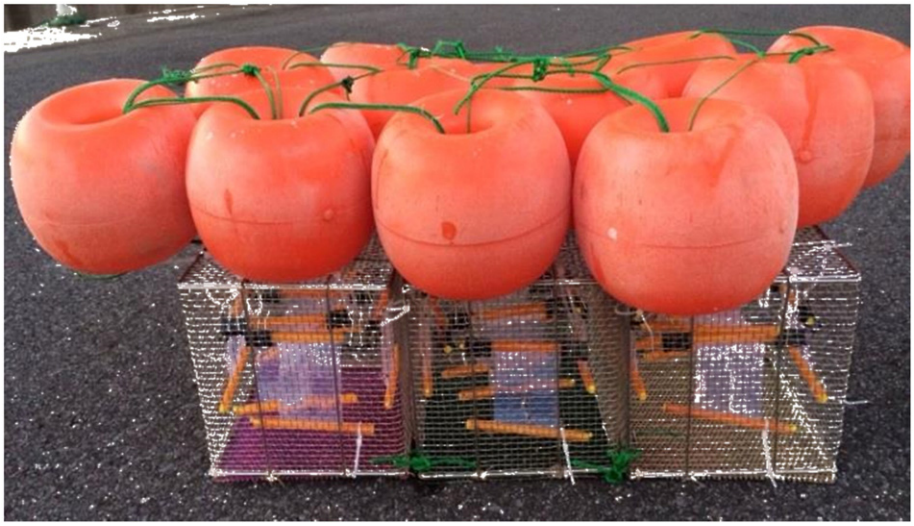

Fig. 1. The cages used in this study. Cellulose ester (CE) dialysis tubes (8–10 kD, 31-mm diameter) containing MNV-1 were fixed using cable ties to prevent collision. The cages were gently placed at 2 aquatic locations during summer and winter season.

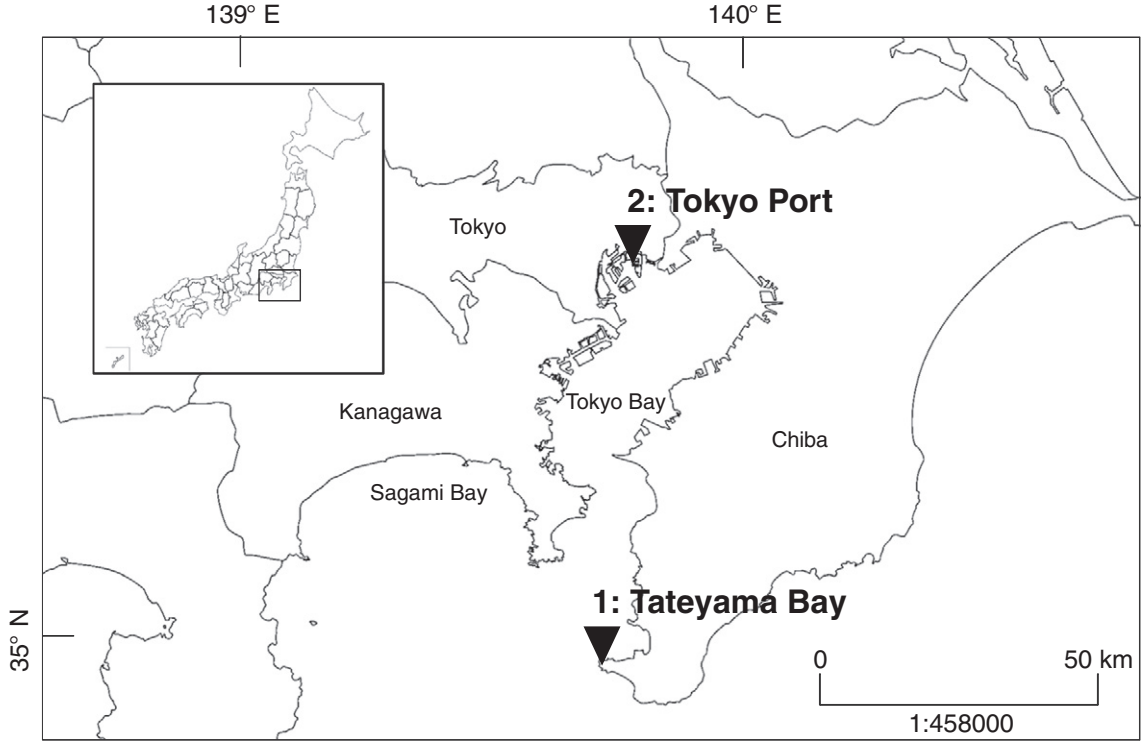

Fig. 2. Sampling locations. MNV-1 aliquots were placed at 2 aquatic locations in Japan: in an oceanic water pool from Tateyama bay in Chiba and in brackish water at Tokyo port, Japan.