First norovirus outbreaks associated with consumption of green seaweed (*Enteromorpha* spp.) in South Korea

J. H. PARK[1,2], H. S. JEONG[3], J. S. LEE[4], S. W. LEE[3], Y. H. CHOI[2], S. J. CHOI[2], I. S. JOO[4], Y. R. KIM[5], Y. K. PARK[6] AND S. K. YOUN[2]*

[1] *Department of Preventive Medicine, Dongguk University College of Medicine, South Korea*
[2] *Division of Epidemic Intelligence Service, Korea Centers for Disease Control and Prevention, South Korea*
[3] *Division of Vaccine Research, Korea Centers for Disease Control and Prevention, South Korea*
[4] *Food Microbiology Division, Food Safety Evaluation Department, National Institute of Food and Drug Safety Evaluation, South Korea*
[5] *Microbiology and Epidemiology Division, Gyeongsangnam-do Government Institute of Health and Environment, South Korea*
[6] *Epidemiology Division, Busan Institute of Health and Environment, South Korea*

SUMMARY

In February 2012, an outbreak of gastroenteritis was reported in school A; a successive outbreak was reported at school B. A retrospective cohort study conducted in school A showed that seasoned green seaweed with radishes (relative risk 7·9, 95% confidence interval 1·1–56·2) was significantly associated with illness. Similarly, a case-control study of students at school B showed that cases were 5·1 (95% confidence interval 1·1–24·8) times more likely to have eaten seasoned green seaweed with pears. Multiple norovirus genotypes were detected in samples from students in schools A and B. Norovirus GII.6 isolated from schools A and B were phylogenetically indistinguishable. Green seaweed was supplied by company X, and norovirus GII.4 was isolated from samples of green seaweed. Green seaweed was assumed to be linked to these outbreaks. To our knowledge, this is the first reported norovirus outbreak associated with green seaweed.

Keywords: Epidemiological investigation, foodborne illness, green seaweed, norovirus, South Korea.

INTRODUCTION

Norovirus (NoV) has historically been designated 'winter vomiting diseases', later as 'Norwalk virus' and 'Norwalk-like virus', and finally as 'norovirus' [1, 2]. NoV can be classified into five genogroups (GI–GV) by phylogenic analysis of the capsid protein. Human NoV infections are caused by GI, GII, and, to a lesser extent, GIV [3]. Genogroups GI and GII of human NoV are further classified into 14 and 17 sub-genotypes, respectively [4]. Symptoms of NoV include acute-onset vomiting, watery diarrhoea, and nausea. Healthy adults usually recover without complications [5]. NoV can be transmitted efficiently due to the low infectious dose and the high viral load in faeces and vomitus [6].

Historically, NoV outbreaks were under-reported; however, with development of reverse transcription–polymerase chain reaction (RT–PCR)-based diagnostics, NoV has been recognized as a leading cause of gastroenteritis worldwide [7]. Over-reporting of disease is also possible due to a lack of proof that NoV detected by PCR is viable, and able to cause illness

* Author for correspondence: Dr S. K. Youn, Division of Epidemic Intelligence Service, Korea Centers for Disease Control and Prevention, Cheongwon-gun, Chungcheongbuk-do, South Korea (363-700).
(Email: yunki7777@naver.com)

or be transmitted. In the USA, NoV is the most common cause of foodborne illness outbreaks. The primary mode of transmission is person-to-person, followed by foodborne, environmental contamination, and waterborne transmission [8]. As many as 19–21 million NoV cases, including 56 000–71 000 hospitalizations, and 570–800 deaths, are reported annually in the USA [9].

Outbreaks of gastroenteritis (defined as ⩾2 cases of a similar illness) in South Korea are reported to the Korea Centers for Disease Control and Prevention (KCDC), and NoV was designated in South Korea as a national notifiable infectious disease in 2006. During 2007–2009, NoV was the most common pathogen identified in foodborne disease outbreaks, occurring most frequently from November to May [10].

In February 2012, a NoV outbreak in elementary school A was reported. In succession, another NoV outbreak occurred in elementary school B, about 20 km from school A. We conducted epidemiological investigations to characterize the extent of the outbreaks, identify the mode of transmission, and determine the relationship between the outbreaks.

METHODS

The outbreaks

On 4 February 2012, a school nurse from school A reported to a local health authority that more than 20 students had diarrhoea or vomiting. On 15 February, a local clinic reported to another local health authority an outbreak of gastroenteritis of more than ten students from school B. These outbreaks were reported to the KCDC; each local health authority first separately initiated an epidemiological investigation. After the common features of these outbreaks became apparent, the KCDC assumed responsibility for the epidemiological investigations.

Case definition and study design

Cases were defined as illness in students with diarrhoea (⩾3 loose stools in any 24-h period) or vomiting (at least one episode). Standardized self-administered questionnaires were used to elicit information on demographic characteristics, clinical history, and potential exposures (food, water, milk, snacks). In school A, all students were enrolled and a retrospective cohort study was undertaken. In school

Table 1. *General characteristics of norovirus outbreaks in the two schools*

Characteristics	School A	School B
No. of exposed students	936	574
No. of cases*	60	31
Attack rates (%)	6·7	5·4
Onset of first case (date)	4 February	14 February
Symptoms, *n* (%)		
Diarrhoea	21 (35·0)	5 (17·9)
Fever	9 (15·0)	9 (29·0)
Chills	3 (5·2)	13 (41·9)
Nausea	25 (41·7)	22 (71·0)
Vomiting	57 (95·0)	31 (100·0)
Abdominal pain	32 (53·3)	24 (77·4)
Incubation period		
Median (h)	38	40
Range (h)	48	34

* Cases were defined as illness with diarrhoea (⩾3 loose stools in any 24-h period) or vomiting (at least one episode) in students.

B, suspected students experiencing symptoms of loose stool or vomiting were detected in cooperation with teachers. A case-control study was conducted using healthy controls that were two randomly selected students in the same class. We calculated the relative risk (RR) or odds ratio (OR) with 95% confidence intervals (CI) to assess associations between illness and potential exposures. These public health investigations undertaken to control outbreaks did not require approval by an institutional review board.

Site inspection

The cafeterias and kitchens in schools A and B were separately inspected for environmental exposures to the pathogens on 2 February and 15 February, respectively. Inspection of suspected company X was undertaken on 17 February to review the green seaweed production process.

Laboratory testing

Students who met the case definition as well as food handlers were encouraged to provide stool samples. Samples of preserved food items served on 2 February in school A and 13 February in school B and environmental samples from kitchen knives, chopping boards, and dishcloths were collected. Drinking and tap water was also tested. From
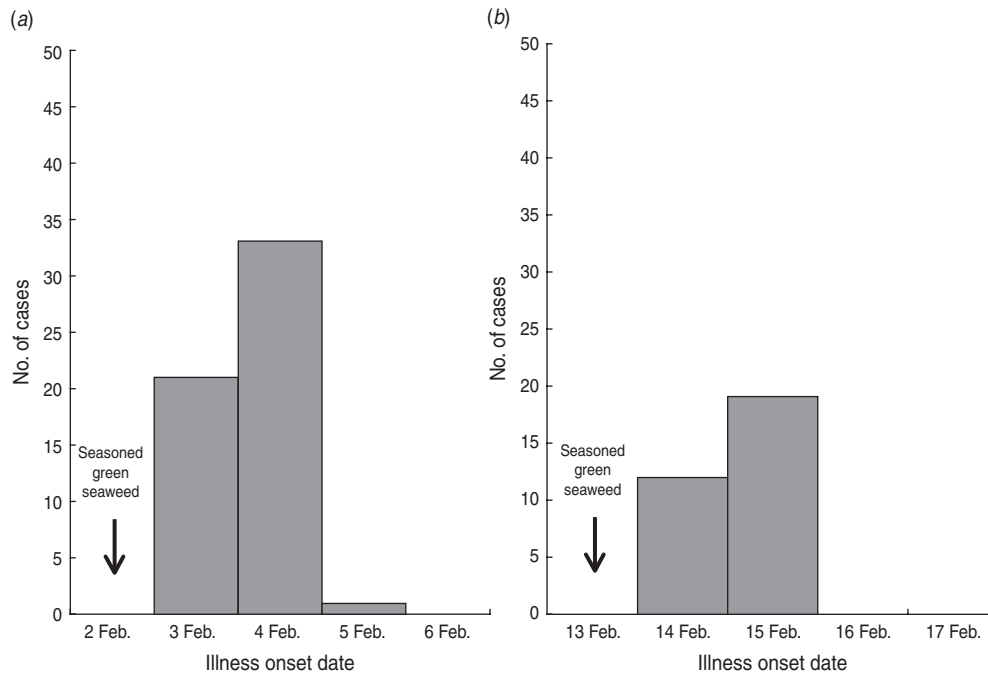
Fig. 1. Epidemic curves of norovirus outbreaks in (*a*) school A and (*b*) school B.

company X, samples of green seaweed (1 kg), seawater (40 litres) used for washing raw green seaweed, and seawater (40 litres) collected near company X were obtained to test for NoV.

Enteric pathogens were examined as described previously [11]. NoV was identified using conventional nested RT–PCR [4]. All PCR products were sequenced using an ABI Prism 3100 automatic sequencer and Big Dye Terminator Cycle Sequencing Mix (Invitrogen, USA). The 185 bp of NoV capsid genes were aligned with ClustalW and compared to those of reference strains obtained from GenBank. Phylogenetic analysis was conducted using the neighbour-joining method with 1000 bootstrap replicates in MEGA, version 4.0 [4].

RESULTS

Epidemiological investigation

Of 936 students in school A, 893 (response rate 95·4%) participated in the retrospective cohort study. Sixty pupils (attack rate 6·7%) met the case definition. The primary symptoms were vomiting (95·0%), abdominal pain (53·3%), and nausea (41·7%) (Table 1). The outbreak onset occurred in the early morning of 3 February, with the number of cases peaking the following morning (Fig. 1*a*).

Of 574 pupils in school B, 31 (attack rate 5·4%) of 39 suspected student cases met the case definition. The most common symptom was vomiting (100·0%), followed by abdominal pain (77·4%) and nausea (71·0%) (Table 1). The first case displayed symptoms early in the morning of 14 February, and the peak in the number of cases occurred the next morning (Fig. 1*b*). The median incubation period was 38 h (range 14–62 h) from lunch on 2 February and 40 h (range 14–48 h) from lunch on 13 February in schools A and B, respectively (Table 1). The attack rates in different grades showed no difference in schools A and B.

On 1 February, school A opened after the winter vacation and there were no major events or visits to other places from 1 January to 5 February. Lunch had been served on weekdays since 2 February. Among lunch food served on 2 February, uncooked green seaweed, and seasoned vinegar served with sliced radishes, was significantly associated with illness (RR 7·9, 95% CI 1·1–56·2) (Table 2). There was no common exposure of snacks or drinking water (brought from home) in cases.

In school B, lunch was served on weekdays and there were no major events or visits to other places from 1 January to 15 February. Among lunch food served on 13 February (Monday), cases were 5·1 times more likely to have eaten uncooked green seaweed served with vinegar seasoning and sliced pears

Table 2. *Association of illness with lunch food served on 2 February in school A*

	Exposed			Unexposed			
Food items	Illness	Total	AR (%)	Illness	Total	AR (%)	RR (95% CI)
Cooked germinated brown rice	59	852	6·9	1	12	8·3	0·8 (0·1–5·5)
Bean sprout soup with beef	57	837	6·8	1	27	3·7	1·8 (0·3–12·8)
Pan-fried tofu with soy sauce	59	852	6·9	1	19	5·3	1·3 (0·2–9·0)
Seasoned green seaweed with radishes	59	772	7·6	1	103	1·0	7·9 (1·1–56·2)
Kimchi	59	834	7·1	1	42	2·4	3·0 (0·4–20·9)

AR, Attack rate; RR, relative risk; CI, Confidence interval.

Table 3. *Association of illness with lunch food served on 13 February in school B*

	Cases ($n = 31$)		Controls ($n = 51$)		
Food items	No.	%	No.	%	OR (95% CI)
Cooked rice with beans	30/1	96·8	50/1	98·0	0·6 (0·0–10·0)
Blue crab stew	30/1	96·8	49/2	96·1	1·2 (0·1–14·1)
Spicy hot chicken stew	30/1	96·8	44/2	95·7	1·4 (0·1–15·7)
Seasoned green seaweed with pears	29/2	93·5	34/12	73·9	5·1 (1·1–24·8)
Kimchi	25/2	92·6	42/7	85·7	2·1 (0·4–10·8)

OR, Odds ratio; CI, Confidence interval.

(95% CI 1·1–24·8) (Table 3). Other food items, cafeteria drinking water, and snacks were not significantly associated with illness. Tap water was used for cooking and none of food handlers in schools A or B reported symptoms. During the investigations, we observed proper food handling practices and hygiene practices in schools A and B.

The food items served in schools A and B were seasoned green seaweed and kimchi. Kimchi, a traditional fermented food in South Korea, consists of vegetables and a seasoning mixture that includes spices and minor additives. The green seaweed used by both schools came from company X, located on the coastal area of Busan. The kimchi, however, was sourced from different food companies. Company X obtained raw green seaweed from more than two farms. The seaweed was washed with seawater at company X to remove foreign matter. Company X then provided green seaweed to school A (2 February) and school B (13 February).

Laboratory testing

In school A, stool samples were available from 19 cases and seven food handlers. Fifteen stool samples (78·9%) from cases and four stool samples (57·1%) from food handlers were positive for NoV. In school B, stool samples were obtained from 30 cases and eight food handlers. Five stool samples (16·7%) from cases were positive for NoV. Stool samples from food handlers were negative for NoV.

Samples of drinking and tap water from schools A and B were negative for routine tests for general bacteria, total coliforms, and *Escherichia coli*. Samples of preserved food items and environmental samples from kitchen knives, chopping boards, and dishcloths from schools A and B were negative for bacterial pathogens, but were not tested for viral pathogens including NoV. Samples of green seaweed, seawater used for washing, and seawater collected near company X were positive for NoV.

Phylogenetic analysis (Fig. 2)

Phylogenetic analysis was used to evaluate the relatedness of NoV strains detected in the two outbreaks and company X. The genotypic distribution of the 18 NoV strains isolated from the two outbreaks was GII.6, 38·9% ($n = 7$); GI.12, 16·7% ($n = 3$); GII.5, 5·6% ($n = 1$); GII.7, 5·6% ($n = 1$); GII.14, 5·6% ($n = 1$); GII.17, 5·6% ($n = 1$); GI.1, 5·6% ($n = 1$); GI.2, 5·6% ($n = 1$); GI.9, 5·6% ($n = 1$); GI.14, 5·6% ($n = 1$).
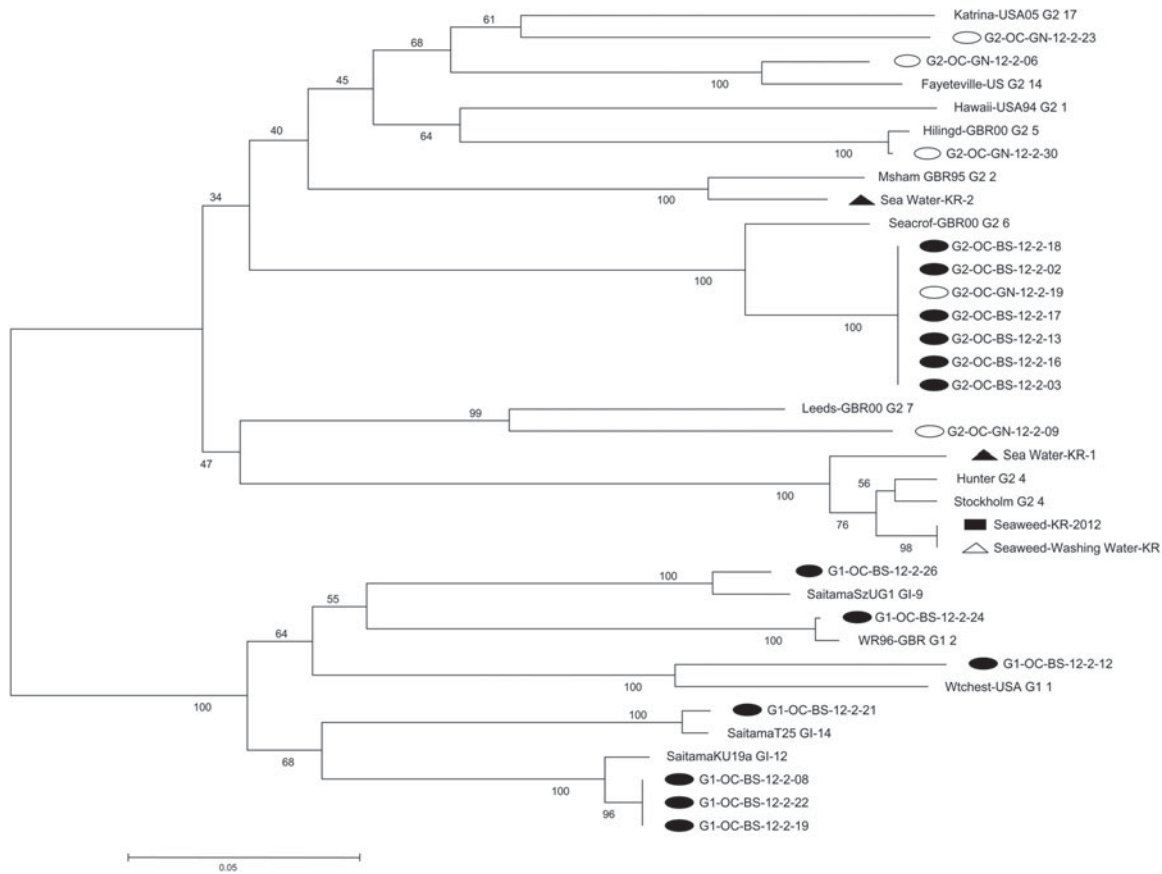
Fig. 2. Phylogenetic analysis of outbreaks based on partial sequence of the norovirus capsid region (open reading frame 2). Nucleotide sequences were analysed by the neighbour-joining method. The numbers at the branches indicate bootstrap values for 1000 replicates. Closed circles, 13 strains detected in school A; open circles, five strains detected in school B; closed rectangle, one strain detected in green seaweed from company X; closed triangles, two strains detected in seawater near company X; open triangle, one strain detected in seawater used for washing raw green seaweed from company X.

School A yielded 13 NoV strains belonging to six genotypes including GI.1, GI.2, GI.9, GI.12, GI.14, and GII.6. School B yielded five NoV strains belonging to five genotypes including GII.5, GII.6, GII.7, GII.14, and GII.17. NoV GII.6 samples from the two outbreaks were indistinguishable.

Company X yielded four NoV strains of two genotypes: GII.4 ($n = 3$; green seaweed, seawater used for washing, and seawater near company X) and GII.2 ($n = 1$; seawater near company X). NoV GII.4 from green seaweed and seawater used for washing was clustered and showed 98% sequence homology.

DISCUSSION

The most common symptom associated with the outbreaks in these elementary schools was vomiting. Vomiting is more frequently reported than diarrhoea in children diagnosed with NoV [12]. The median

incubation periods were 38 h and 40 h in these two outbreaks, consistent with the reported average incubation period of 24–48 h [13]. Seasoned green seaweed was significantly associated with illness in both NoV outbreaks, and company X provided green seaweed to both schools. Phylogenetic analysis revealed that NoV GII.6 was indistinguishable between the two outbreaks. Samples obtained from company X of green seaweed and seawater used for washing were positive for NoV GII.4. They showed 98% sequence homology in phylogenetic analysis. No additional NoV outbreaks were identified in prospective surveillance of adjacent schools, clinics, and hospitals.

NoV can be transmitted from person to person [8]; however, the epidemic curves had one peak in each outbreak and we found no evidence of person-to-person transmission among pupils, teachers, and food handlers between school A and school B, which are

located in different provinces. Additionally, multiple NoV genotypes were identified in the two outbreaks, a finding consistent with sewage contamination or foodborne contamination earlier in the food-handling chain. Besides person-to-person transmission, the possibility of waterborne transmission was low because there was no common drinking water or cooking water between schools A and B.

Green seaweed belongs to the genus *Enteromorpha*, which occurs commonly along the North European and Mediterranean shores, and is distributed broadly throughout the coasts of South Korea, China, and Japan. Green seaweed resides in the upper region of the intertidal zone or in eutrophicated coastal water. It has been used for millennia in Asia as a pharmaceutical product and healthcare food, and have previously been utilized in the USA, France, and Chile [14–16].

Among food items served in schools A and B, only seasoned green seaweed and kimchi were uncooked; NoV could survive in both these food items [17]. Green seaweed was supplied from company X, while kimchi was supplied from different companies. The green seaweed in this study was eaten unheated along with vinegar seasoning. While the vinegar could eliminate some microbes, including *E. coli*, NoV remains stable in these acidic conditions [18–20].

A study of freshwater and seawater in Europe using nested RT–PCR reported NoV-positive rates of 6·3% and 16·4% in freshwater and seawater, respectively [21]. In South Korea, the NoV-positive rate using nested RT–PCR was higher (84·4%) in freshwater from four rivers [22], implying a marked potential for contaminated seafood. Shellfish play an important role in the transmission of NoV due to the accumulation by filtration of large volumes of contaminated seawater [23], and a correlation between NoV concentration in wastewater and in oysters has been reported [24].

Company X and at least one green seaweed farm are located on the southern coast of Korea near the Nakdong River, where wastewater flows into the sea. The NoV GII.4 genotypes detected in samples of green seaweed and seawater used for washing were not identical. NoV GII.4 was not isolated from samples obtained from these two outbreaks. We were unable to determine if the NoV contamination occurred at the farms or during washing at company X. However, the green seaweed sample from company X was positive for NoV, and could be the source of infection in these two NoV outbreaks.

To our knowledge, this is the first report of norovirus outbreaks associated with consumption of green seaweed; however, the findings in this study have several limitations. First, the preserved seasoned green seaweed in the two schools could not be tested for NoV owing to small volumes. Second, we could not conduct a retrospective cohort study of the outbreak in school B due to limited time and resources. Third, asymptomatic infections might dilute the association between illness and the consumption of food items. We reviewed the possibility of other contaminated food items as a cause of these outbreaks; however, only consumption of uncooked green seaweed could explain these NoV outbreaks. Fourth, although the food handlers in school A reported no symptoms, four food handlers were positive for NoV. However, these food handlers had eaten the same seasoned green seaweed provided to the NoV-positive cases.

After the outbreaks, company X was forbidden from selling green seaweed. We reported a summary of these outbreaks in the KCDC newsletter to inform the public of the risks of consuming unheated seasoned green seaweed [25]. A seafood monitoring system that includes green seaweed, oysters, freshwater, and seawater might be considered to decrease NoV infections.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Adler JL, Zickl R.** Winter vomiting disease. *Journal of Infectious Diseases* 1969; **119**: 668–673.
2. **Lopman B, Zambon M, Brown DW.** The evolution of norovirus, the 'gastric flu'. *PLoS Medicine* 2008; **5**: e42.
3. **Wang QH, et al.** Porcine noroviruses related to human noroviruses. *Emerging Infectious Diseases* 2005; **11**: 1874–1881.
4. **Cheon DS, et al.** Seasonal prevalence of asymptomatic norovirus infection in Korean children. *Foodborne Pathogens and Disease* 2010; **7**: 1427–1430.
5. **Mattner F, et al.** Risk groups for clinical complications of norovirus infections: an outbreak investigation. *Clinical Microbiology and Infection* 2006; **12**: 69–74.

6. **Repp KK, Keene WE.** A point-source norovirus outbreak caused by exposure to formites. *Journal of Infectious Diseases* 2012; **205**: 1639–1641.

7. **Kirkwood C.** Viral gastroenteritis in Europe: a new norovirus variant? *Lancet* 2004; **363**: 671–672.

8. **Hall AJ, et al.** Acute gastroenteritis surveillance through the national outbreak reporting system, United States. *Emerging Infectious Diseases* 2013; **19**: 1305–1309.

9. **Hall AJ, et al.** Norovirus disease in the United States. *Emerging Infectious Diseases* 2013; **19**: 1198–1205.

10. **Gwack J, et al.** Trends in water- and foodborne disease outbreaks in Korea, 2007–2009. *Osong Public Health and Research Perspectives* 2010; **1**: 50–54.

11. **DuPont HL, et al.** A randomized, double-blind, placebo-controlled trial of rifaximin to prevent travelers' diarrhea. *Annals of Internal Medicine* 2005; **142**: 805–812.

12. **Sala MR, et al.** Cases of acute gastroenteritis due to calicivirus in outbreaks: clinical differences by age and etiologic agent. *Clinical Microbiology and Infection.* Published online: 30 December 2013. doi: 10.1111/1469-0691. 12522.

13. **Patel MM, et al.** Noroviruses: a comprehensive review. *Journal of Clinical Virology* 2009; **44**: 1–8.

14. **Cho HB, et al.** Clinical and microbial evaluation of the effects on gingivitis of a mouth rinse containing an *Enteromorpha linza* extract. *Journal of Medicinal Food* 2011; **14**: 1670–1676.

15. **Zhang Z, et al.** Synthesized oversulfated and acetylated derivatives of polysaccharide extracted from *Enteromorpha linza* and their potential antioxidant activity. *International Journal of Biological Macromolecules* 2011; **49**: 1012–1015.

16. **Li Y, et al.** Production of enzymes by *Alteromonas* sp. A321 to degrade polysaccharides from *Enteromorpha prolifera*. *Carbohydrate Polymers* 2013; **98**: 988–994.

17. **Wang D, Tian P.** Inactivation conditions for human norovirus measured by an in situ capture-qRT-PCR method. *International Journal of Food Microbiology* 2014; **172**: 76–82.

18. **Chang JM, Fang TJ.** Survival of *Escherichia coli* O157: H7 and *Salmonella enterica* serovars Typhimurium in iceberg lettuce and the antimicrobial effect of rice vinegar against *E. coli* O157:H7. *Food Microbiology* 2007; **24**: 745–751.

19. **Donaldson EF, et al.** Norovirus pathogenesis: mechanisms of persistence and immune evasion in human populations. *Immunological Reviews* 2008; **225**: 190–211.

20. **Lopman B, et al.** Environmental transmission of norovirus gastroenteritis. *Current Opinion in Virology* 2012; **2**: 96–102.

21. **Wyn-Jones AP, et al.** Surveillance of adenoviruses and noroviruses in European recreational waters. *Water Research* 2011; **45**: 1025–1038.

22. **Lee C, Kim SJ.** The genetic diversity of human noroviruses detected in river water in Korea. *Water Research* 2008; **42**: 4477–4484.

23. **Suffredini E, et al.** Norovirus contamination in different shellfish species harvested in the same production areas. *Journal of Applied Microbiology* 2012; **113**: 686–692.

24. **Flannery J, et al.** Concentration of norovirus during wastewater treatment and its impact on oyster contamination. *Applied and Environmental Microbiology* 2012; **78**: 3400–3406.

25. **Korea Centers for Disease Control and Prevention.** Norovirus outbreaks by seaweed in Korea, 2012. *Public Health and Weekly Report* 2012; **5**: 372–373.