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Surveillance of norovirus contamination in commercial fresh/frozen berries from Heilongjiang Province, China, using a TaqMan real-time RT-PCR assay

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ABSTRACT

Norovirus (NoV), a major food-borne virus, causes non-bacterial acute gastroenteritis in humans. Berries are generally harvested from low-growing bushes by hand and are minimally processed before being sold to consumers. Therefore, the consumption of berries has been linked to numerous food-borne gastroenteritis outbreaks caused by NoV in many countries. We performed a survey of NoV contamination in commercial fresh/frozen berry fruits collected from 2016 to 2017 in the Heilongjiang Province, the main berry-producing area in China, using a TaqMan-based real-time reverse transcription-PCR assay. Among 900 frozen and 900 fresh domestic retail berry samples, the prevalence of NoV was 9% (81/900) and 12.11% (109/900), including 35.80% (29/81) and 29.36% (32/109) of genotype GI alone, 54.32% (44/81) and 60.55% (66/109) of GII alone, and 9.88% (8/81) and 10.09% (11/109) of both GI and GII, respectively. No NoV was detected among the 677 frozen berry samples for export. Thus, the occurrence of NoV contamination was significantly higher in domestic berries than in exported berries and higher in fresh berries than in frozen berries. This study highlights the need for further risk surveillance for NoV contamination in berries produced in the Heilongjiang Province and recommends region-extended monitoring of retail berries for NoV.

1. Introduction

Noroviruses (NoVs), non-enveloped viruses belonging to the genus *Norovirus* of the family *Caliciviridae* with a positive-sense, single-stranded RNA genome, are the major causative agent of nonbacterial acute gastroenteritis in humans worldwide (Glass et al., 2009; Hall, 2012; Hutson et al., 2004; Lindesmith et al., 2003). Currently, norovirus infections constitute a serious disease burden in both developed and developing countries and are responsible for approximately 20% of all acute gastroenteritis cases (Ng et al., 2017; Patel et al., 2008). In the United States, NoVs are responsible for nearly 60% of all acute gastroenteritis cases each year (Patel et al., 2009). In China, the first case of norovirus infection was reported in 1995 and since then, incidences of norovirus-associated diarrhea outbreaks have gradually increased. In recent years, NoVs are responsible for more than 60% of acute viral gastroenteritis cases with severe symptoms of vomiting, diarrhea, and abdominal cramps (Liao et al., 2016; Shang et al., 2017; Yu et al., 2017). In China, among children with diarrhea and under the age of five years, the detection rate of NoV is more than 15% (Rouhani et al., 2016). Annually, nearly 70,000 norovirus-associated deaths are recorded among children less than 5 years of age worldwide (Lanata et al., 2013).

NoVs cannot be cultivated *in vitro*, which prevents their classification into distinct serotypes. Therefore, NoVs are genetically subdivided into six established genogroups denoted as GI to GVI with more than 30 different genotypes according to the phylogenetic analysis of NoV capsid gene (Vinje, 2015; Zheng et al., 2006), while a tentative genogroup VII has been proposed (Tse et al., 2012). Of these, NoV genogroups GI and GII are the most common strains epidemiologically linked to acute gastroenteritis cases (Cheng et al., 2018; Liao et al., 2016; Vinje, 2015). In particular, strains of NoV genogroup GII has been responsible for causing an increasing number of gastroenteritis

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outbreaks (Kobayashi et al., 2016), such as NoV GII.4 (Lu et al., 2015), NoV GII.17 (Qin et al., 2017), NoV GII.P16–GII.2 (Ao et al., 2017, 2018).

Until now, norovirus-associated diarrhea outbreaks have been mainly associated with person-to-person spread, particularly in long-term care facilities and hospitals (Hall et al., 2013). In addition, many foodborne norovirus outbreaks have been reported, most commonly associated with consumption of contaminated seafood or fresh produce (Bae et al., 2018; Doyle et al., 2004; Jiang et al., 2018; Hall et al., 2013; Park et al., 2015; Patel et al., 2008). In recent years, the consumption of berries, such as raspberries, cranberries, blueberries, and blackcurrants that are recognized as green and healthy food has substantially increased (Evans and Ballen, 2017; Farruggia et al., 2016; Li et al., 2017; Tamada et al., 2009). Generally, berries come from low-growing, ground-based bushes grown under a variety of agricultural systems. Berries are usually harvested better by hand, rather than by mechanization, and are subjected to minimal processing before being sold to consumers. Thus, the minimally processed berries are potential transmission vectors for NoVs (Ryu et al., 2015). Norovirus infections cause many foodborne disease outbreaks via contaminated fresh/frozen berries worldwide (Bae et al., 2018; Bernard et al., 2014; Dietrich et al., 2013) and have become an important food safety concern. Therefore, good agricultural practice (GAPs) systems are needed in the berry-producing industry to minimize the risk of microbial contamination (Li et al., 2018).

The Heilongjiang Province located in Northeast China is the major berry-producing area in China, and includes Daxinganling, Heihe, Qiqihar, Daqing, Mudanjiang, Jiamusi, Yichun, and Harbin (Fig. 1). The main berry varieties produced are blueberries, strawberries, raspberries, cranberries, blackberries, and blackcurrants (Fig. 2), which are sold all over the country and also exported to some countries in Europe and America. To protect consumer health, the NoV contamination in retail and exported fresh/frozen berries sourced from the Heilongjiang Province, China was monitored over the last two years (from 2016 to 2017), using a TaqMan-based real-time reverse transcription (RT)-PCR assay.

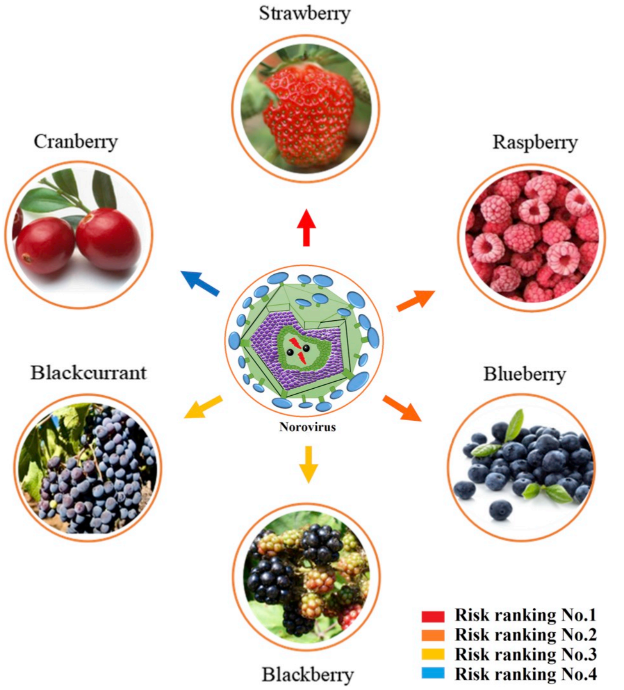

Fig. 2. The major berry varieties, including blueberry, strawberry, raspberry, cranberry, blackberry, and blackcurrant, sourced from the Heilongjiang Province, the major berry-producing area in China and sold all over the country and also exported to some countries in Europe and America, were collected to evaluate the NoV prevalence in this study, and the rankings of NoV contamination in different berries are shown based on the detection results.

2. Materials and methods

2.1. Berry fruits sampling

In this study, a total of 2477 fresh and frozen strawberry, blueberry,

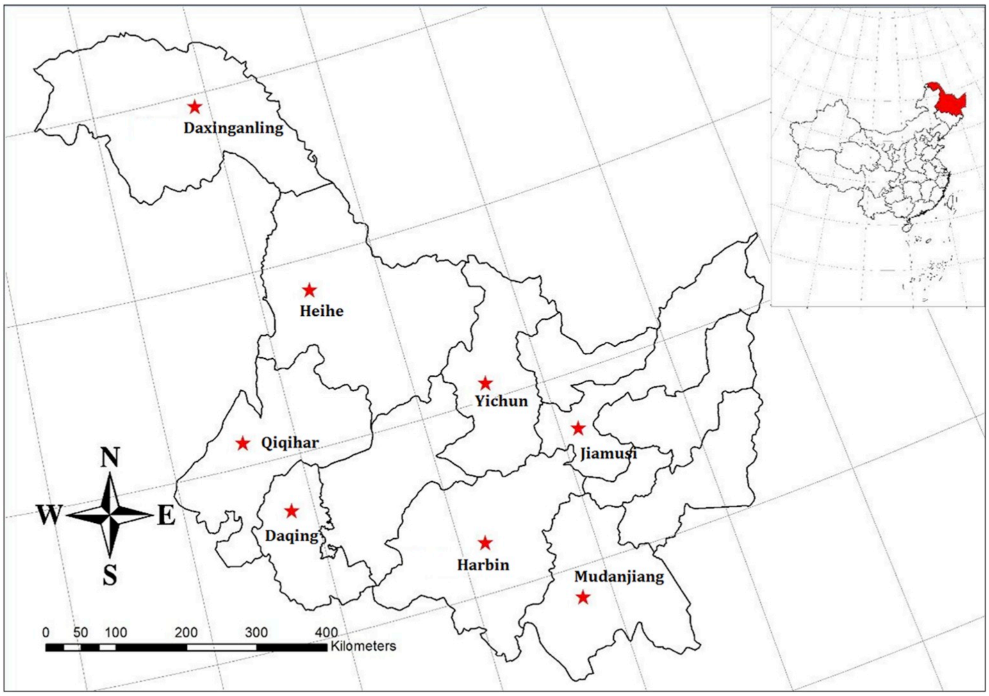

Fig. 1. The major berry-producing areas in the Heilongjiang Province located in Northeast China, including Daxinganling, Heihe, Qiqihar, Daqing, Mudanjiang, Jiamusi, Yichun, and Harbin.

Table 1
Oligonucleotide sequences and probes used for the quantitative reverse transcription PCR assay.

Virus species	ID	Sequence (5′ to 3’)	Positions	Size	Reference
NoV GI	GI-F	CGCTGGATGCGNTTCCAT	5291–5376[a]	86 bp	ISO 15216-1 (2013), Liu et al. (2016)
	GI-R	CCTTAGACGCCATCATCATTTAC			
	GI-Probe	FAM-TGGACAGGAGAYCGCRATCT-TAMRA			
NoV GII	GII-F	ATGTTCAGRTGGATGAGRTTCTCWGA	5012–5100[b]	89 bp	ISO 15216-1 (2013), Liu et al. (2016)
	GII-R	TCGACGCCATCTTCATTCACA			
	GII-Probe	FAM-AGCACGTGGGAGGGCGATCG-TAMRA			

[a] Positions correspond to Norwalk virus (GenBank accession no. m87661).
[b] Positions correspond to Lordsdale virus (GenBank accession no. x86557).

raspberry, cranberry, blackberry, and blackcurrant samples listed in Table 2 were used to investigate the level of NoV contamination in berries (Fig. 2), which were exposed to the potential risk of being contaminated by NoVs, due to their planting, harvesting, and processing methods. Of these, 1800 batches of domestic retail berry samples (900 frozen berry samples and 900 fresh berry samples) were mainly collected from retail markets (supermarkets) and morning markets, and 677 batches of frozen export berry samples (mainly exported to Russia, Germany, Canada, Poland, Holland, Thailand, and Belgium, etc.) were collected from export food enterprises located in Daxinganling, Heihe, Qiqihar, Daqing, Mudanjiang, Jiamusi, Yichun, and Harbin of the Heilongjiang Province, China from January 2016 to December 2017 (covering a period of 24 months: 25–30 batches of export berry samples, 35–40 batches of domestic fresh berry samples, and 35–40 batches of domestic frozen berry samples were collected per month, respectively, of which sampling batches of strawberry were more than those of the other berries, based on its high yield and consumption). During the sampling period, the fresh and frozen berry samples were directly transported to the laboratory using refrigerated vehicles at 4 °C and −20 °C, respectively, and NoV extraction was carried out within 24 h.

2.2. Virus concentration

NoV concentration in the berry fruits was evaluated according to the method previously described (ISO 15216-1, 2013; Liu et al., 2016). Briefly, 25 g of berries were placed into a sample bag with a mesh filter and 50 mL of Tris Glycine Beef Extract buffer (TGBE; Sigma-Aldrich, USA) solution supplemented with 30 U of *Aspergillus niger* (*A. niger*) pectinase (Amresco, USA) was added. The samples were vibrated at 60 times/min for 20 min at 25 °C (pH was kept at 9.0–9.5, adjusted with 1 mol/L NaOH every 10 min). Then, the filtered liquid was transferred into a centrifuge tube and centrifuged at $10000 \times g$ for 30 min at 4 °C. The supernatant was transferred into a clean tube, the pH was adjusted to 7.0, and PEG8000-NaCl was added to the supernatant until a final concentration of 100 g/L PEG8000 (Sigma, USA) and 0.3 mol/L NaCl was attained. The samples were vibrated at 60 times/min for 60 min at 4 °C, followed by centrifugation at $12000 \times g$ for 30 min, and the pellet was used for RNA extraction. In this study, the virus concentration from each batch of berry sample was performed three times in parallel.

2.3. Viral RNA extraction and purification

A QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) was used to extract and purify viral RNA according to the manufacturer's instructions. Briefly, the whole pellet obtained from each batch (25 g) of berry sample after the virus concentration performed in Section 2.2 was used for viral RNA extraction, and following the processes of viral lysis and RNA extraction and purification, 35 μL of RNase-free dH$_2$O was used for the final elution of viral RNA. Moreover, losses of viral RNA can occur during RNA extraction and purification. Therefore, in this work, Mengo virus strain MC$_0$, a genetically modified organism, was used as a control for viral RNA extraction according to the methods previously described (ISO 15216-1, 2013; Jiang et al., 2018; Liu et al., 2016).

2.4. Reverse transcription and TaqMan-based real-time PCR

After viral RNA extraction, approximately 1.0 μg of RNA sample was reverse transcribed into single-stranded cDNA in a single 40 μL reaction system using the High Capacity cDNA Reverse Transcription Kit with Oligo(dT)18 primer (Thermo Fisher Scientific, USA) according to the manufacturer's instructions and stored at −80 °C for not longer than

Table 2
Berry samples collected in this study and results of the norovirus detected by the TaqMan-based real-time reverse transcription PCR assay.

Berries	Year	Export berries			Domestic retailed berries					
		Frozen			Frozen			Fresh		
		Total No.	+[a]	-[b]	Total No.	+	–	Total No.	+	–
Strawberry	2016	108	0	108	150	24	126	150	29	121
	2017	121	0	121	150	23	127	150	35	115
Raspberry	2016	55	0	55	60	6	54	60	3	57
	2017	69	0	69	60	7	53	60	8	52
Blackcurrant	2016	37	0	37	60	0	60	60	4	56
	2017	40	0	40	60	4	56	60	4	56
Blueberry	2016	52	0	52	60	5	55	60	7	53
	2017	56	0	56	60	3	57	60	5	55
Cranberry	2016	35	0	35	60	1	59	60	2	58
	2017	30	0	30	60	0	60	60	3	57
Blackberry	2016	33	0	33	60	3	57	60	3	57
	2017	41	0	41	60	5	55	60	6	54
Total		677	0	677	900	81	819	900	109	791

[a] "+" represents NoV positive.
[b] "-" represents NoV negative.

one week, until used as the template for real-time polymerase chain reaction (PCR). Oligonucleotide primers and probes used for the detection of the NoV genotypes GI and GII are listed in Table 1. To determine the limit of detection (LOD) of the TaqMan-based quantitative reverse transcription PCR method, 2-fold series of dilutions from 16 viral genome copies per microliter quantified by a Norovirus GI Q and Norovirus GII Q Standard kit (CeeramTooLs, France) according to the manufacturer's instructions were used as the template. The optimized TaqMan-based real-time PCR reaction was set up in a total volume of 25 μL as follows: 2.5 μL of 10 × PCR buffer (500 mmol/L KCl, 15 mmol/L MgCl2, 1% Triton X-100, and 100 mmol/L Tris-HCl, pH 8.8) (TaKaRa, China), 2 μL of dNTPs (2.5 mmol/L for each) (TaKaRa, China), 5 μL of cDNA template, 0.5 μL of HiFi Taq DNA polymerase (5 U/μL) (TaKaRa, China), 1 μL of the primer pair (10 mmol/L for each), 0.2 μL of the probe (10 μmol/L), and 13.8 μL of deionized water. The real-time PCR reaction conditions were as follows: 95 °C for 5 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min (fluorescence signals were collected during this step). The TaqMan-based real-time PCR was performed in a 7900HT Fast Real-time PCR System (ABI, USA). All samples extracted in this study were screened for the presence of NoV GI and GII. In parallel, RNase-free ultrapure water was used as blank control, and cDNA reverse transcribed from RNA of Norovirus-free berries was used as a negative control, and recombinant plasmids pMD-GI and pMD-GII containing NoV genotype GI and GII amplification target sequences, respectively (prepared in our lab), were used as positive controls. Each sample was tested three times in parallel.

2.5. The criteria for determination of NoV contamination

In this study, the detection results were judged in accordance with the following rules (i) when the cycle threshold (Ct) value of samples detected by the real-time PCR assay was less than or equal to 35, those samples were considered NoV positive; (ii) when the Ct value was more than 38, the sample was considered NoV negative; (iii) when the Ct value was between 35 and 38, the sample should be evaluated repeatedly, and if the Ct value was above 38, the result was judged NoV negative; otherwise, it was positive.

2.6. Statistical analysis

In this work, Tukey's multiple comparison tests were used to analyze differences between groups by SigmaPlot v10.0 software (Systat Software Inc., USA). A $p < 0.05$ (*) was considered significant, and a $p < 0.01$ (**) was considered highly significant.

3. Results and discussion

3.1. Limit of detection and standard curves of the TaqMan quantitative reverse transcription PCR for NoV GI and GII

The real-time fluorescence quantitative PCR method with high sensitivity and specificity was considered a useful tool for detecting NoVs in contaminated foods (Jiang et al., 2018). In this study, the standard curves and LOD of the TaqMan-based quantitative reverse transcription (RT) PCR for the detection of NoV genotypes GI and GII were determined using the Norovirus GI and GII Standard (CeeramTooLs, France). The results showed that the standard curve of the method for NoV GI/GII (as shown in Fig. 3: A) was generated using 10-fold series of dilutions from 10^5 to 10^1 viral genome copies per microliter as the template. The LOD of the TaqMan real-time RT-PCR method was 10 viral genome copies per reaction for NoV GI/GII in buffer (Fig. 3: B) with a Ct value of less than 38, using 2-fold series of dilutions from 16 viral genome copies per microliter as the template. Because NoV was difficult to culture *in vitro*, the LOD of the method for detecting NoV in spiked berry samples was not determined. However, we used a similar method for LOD determination for NoV in the spiked berries as we did for the LOD for NoV in buffer, and the results showed that the LOD of the TaqMan real-time RT-PCR method was 20 viral genome copies per reaction for NoV GI/GII in the spiked berries (Fig. 3: C) with a Ct value of less than 38.

3.2. NoV prevalence in commercial berries

To investigate NoV prevalence in berries, we collected 2477 batches of commercial fresh and frozen berry fruit samples from the Heilongjiang Province, China from 2016 to 2017. Our results demonstrated that in the 900 domestic retail frozen berry samples collected, the prevalence of NoV was 9% (81/900) (Table 2), including 35.80% (29/81) of genotype GI alone, 54.32% (44/81) of genotype GII alone, and 9.88% (8/81) of both GI and GII. Further, in the 900 domestic retail fresh berry samples collected, the prevalence of NoV was 12.11% (109/900) (Table 2), including 29.36% (32/109) of genotype GI alone, 60.55% (66/109) of genotype GII alone, and 10.09% (11/109) of both genotypes GI and GII. In addition, there were no NoVs detected in the 677 frozen export berry samples collected (Table 2). Moreover, in all the 87 NoV-positive samples collected from 2016, there were 48 batches of fresh berry samples positive for NoV, including 14 positive for GI strain alone, 27 positive for GII strain alone, and 7 positive for both GI and GII strains, and there were 39 frozen berry samples positive for NoV, including 16 batches positive for the GI strain alone, 20 batches positive for the GII strain alone, and 3 batches positive for both GI and GII strains. Out of the 103 batches of NoV-positive samples collected from 2017, there were 61 batches of NoV-positive fresh berry samples, including 18 positive for the GI strain alone, 39 positive for the GII strain alone, and 4 positive for both GI and GII strains, and there were 42 batches of NoV-positive frozen berry samples, including 13 positive for the GI strain alone, 24 positive for the GII strain alone, and 5 positive for both GI and GII strains. The prevalence rate of NoV contamination in fresh/frozen berries collected in 2016 and 2017 was analyzed with the results of real-time RT-PCR given in terms of Ct value (as shown in Fig. 4), and it was clear that the quantities of viruses detected by the real-time PCR assay in different NoV-positive berry samples varied greatly, of which 13.16%, 47.37%, 36.31%, and 3.16% of NoV-positive samples had Ct values of < 25, 25–30, 30–35, and 35–38, respectively, and the lowest Ct value of 20.3 and the highest Ct value of 36.2 was recorded. Moreover, based on the detection results of NoV contamination in berries, we found that in addition to fresh berry fruits, frozen berries are also an important source of NoV transmission, which is consistent with previous reports (Dietrich et al., 2013; Maunula et al., 2009; Sarvikivi et al., 2012). In this study, the Ct cut-off value was used as a criterion for the determination of NoV contamination in berry samples. Although the method has been widely used (Jiang et al., 2018; Liu et al., 2016), the use of the Ct cut-off leads to underestimation of positive samples in practice. Therefore, the positive rate of NoV contamination in berries collected in this work may be higher.

3.3. Risk of NoV transmission via berries in China

In order to assess the risk of NoV transmission via berries in China, an investigation was conducted on fresh/frozen berries produced in Heilongjiang province, one of the major berry-producing areas in China, from 2016 to 2017. From the results of investigation, significant levels of NoV contamination in fresh strawberries ($^{**}p < 0.01$) and frozen strawberries ($^{*}p < 0.05$) were detected compared to those in fresh/frozen blueberries, raspberries, cranberries, blackberries, and blackcurrants (Fig. 5: A). The comparison of domestic and export berries showed that levels of NoV contamination in domestic berries were significantly higher than those in export berries ($^{*}p < 0.05$) (Fig. 5: B). Moreover, the levels of NoV contamination in GI vs. GII (Fig. 5: C), different years (Fig. 5: D), different regions (Fig. 5: E), and different times of year (Fig. 5: F) were also subjected to statistical analysis. The

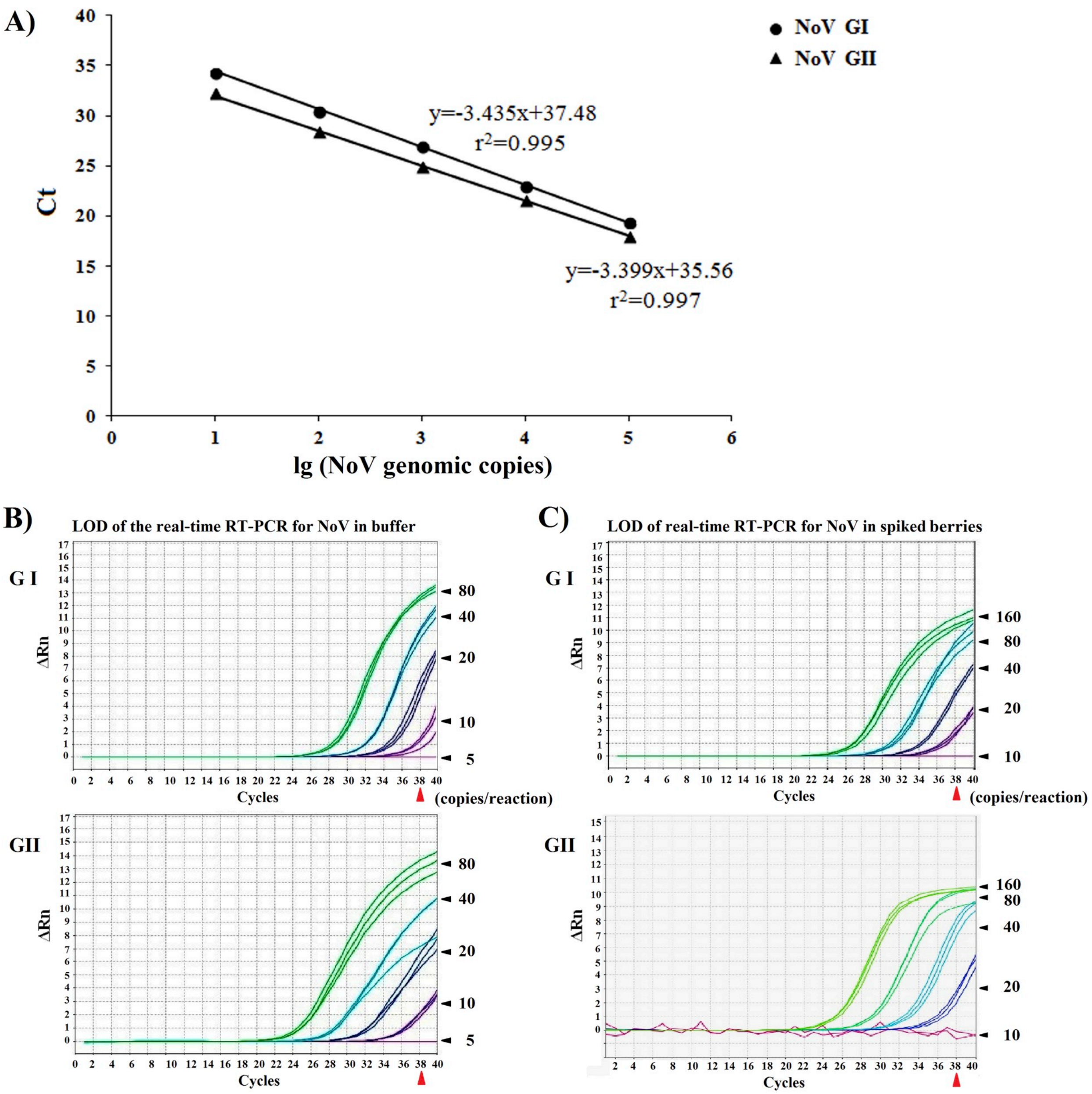

Fig. 3. Standard curves for TaqMan quantitative reverse transcription PCR detection of NoV genotypes GI and GII (A). The limit of detection (LOD) of the real-time RT-PCR assay for NoV genotypes GI and GII in buffer (B). The LOD of the real-time RT-PCR assay for NoV genotypes GI and GII in the spiked berries (C).

results indicated that the risk of NoV transmission via strawberries was highest among these berry varieties.

Currently, NoV with strong resistance to environmental conditions and low-dose virus infections have become one of the most commonly reported causes of non-bacterial acute gastroenteritis outbreaks and sporadic incidences worldwide (Lanata et al., 2013; Park et al., 2015; Patel et al., 2008; Schmid et al., 2004). Usually, NoV is transmitted by the fecal-oral route, which may be via contaminated food (foodborne) or water (waterborne) or human-to-human contact, and may also spread via contaminated surfaces or through the air (Hyun et al., 2018; Solano et al., 2014). For the surveillance of NoV prevalence in berries, we investigated the risk of NoV contamination at each stage in the berry production chain, particularly the harvesting and processing procedures. Generally, the places for cultivating berries are located in rural areas in China. Sometimes, there are open-air toilets or livestock farms close to the water in rural areas; thus, the water used for irrigation during the planting process could be easily contaminated by human and livestock excrement. Moreover, farmyard manure could further increase the risk of norovirus contamination because berries, particularly strawberries, generally come from low-growing bushes. Therefore, NoV colonization can occur by direct contact with contaminated water used for irrigation or due to the use of human manure and poor worker hygiene. Furthermore, because berries have high juice content and are fragile, they are sensitive to handling and damage. Therefore, since artificial picking by hand is still the main way of harvesting berries in China and there is a lack of personal hygienic awareness of workers, the chances of norovirus contamination are greatly increased. In addition, in order to avoid damaging the berries, the producers rarely wash the harvested berries. Thus, post-harvest, the berries generally go straight into final packaging as fresh ready-to-eat produce or are frozen, resulting in the persistence of NoV in fresh and frozen berry products. On the other hand, in order to export the berries to countries in Europe and America, GAPs and hazard analysis and critical control point (HACCP) systems of food production have to be carried out to protect food safety. However, these GAPs and HACCP systems have not been widely applied to berry production in China. This explains the significantly higher

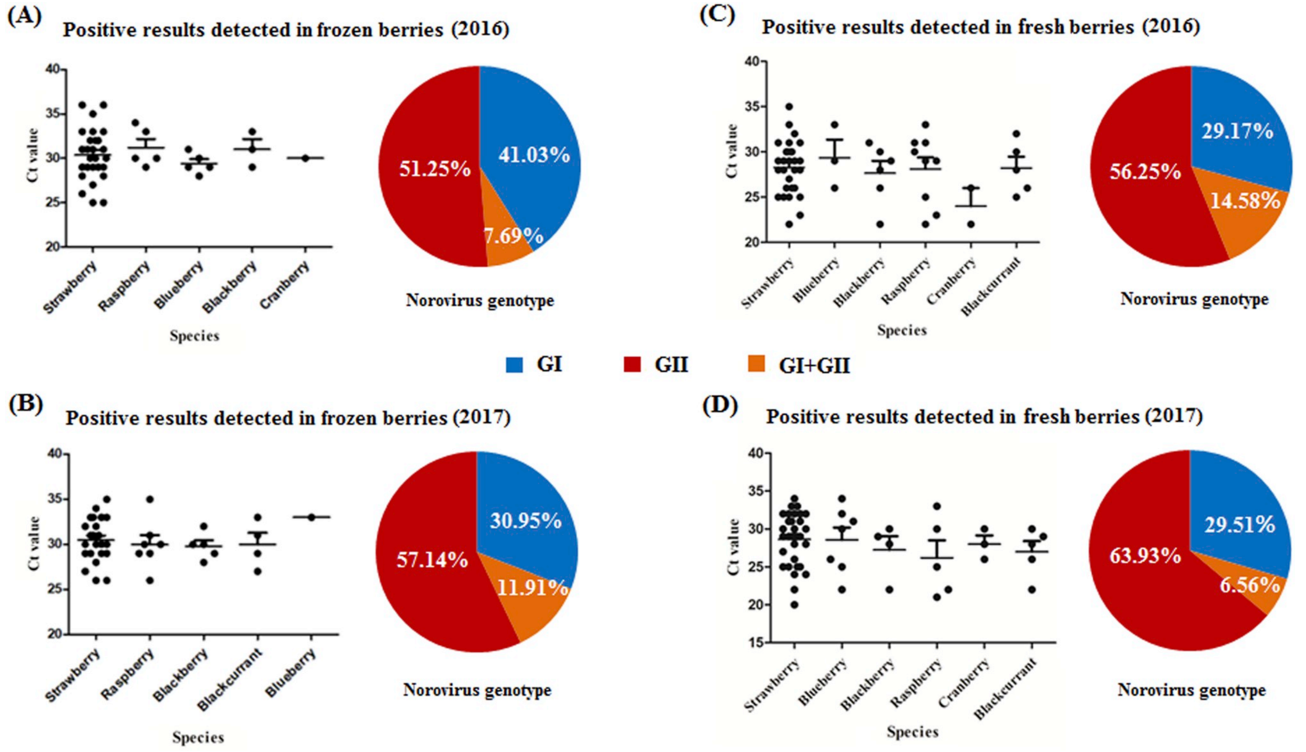

Fig. 4. NoV positive results and the rate of the genotypes GI and GII detected in fresh and frozen berry samples collected in 2016 and 2017.

occurrence of NoV contamination in domestic retail berries than in exported berries, where no NoV-positive samples were detected. Furthermore, the temporal and geographical distribution of berry-related NoV is being subjected to further investigation in order to extend our knowledge of NoV contamination in China.

3.4. Suggestions of minimizing NoV contamination in berries

The results from our surveillance of NoV prevalence in berries clearly indicate that effective measures are needed to minimize NoV contamination in berries and protect food safety and human health. For the specific risks associated with fresh and frozen berries, our recommendations include attention to appropriate food safety measures

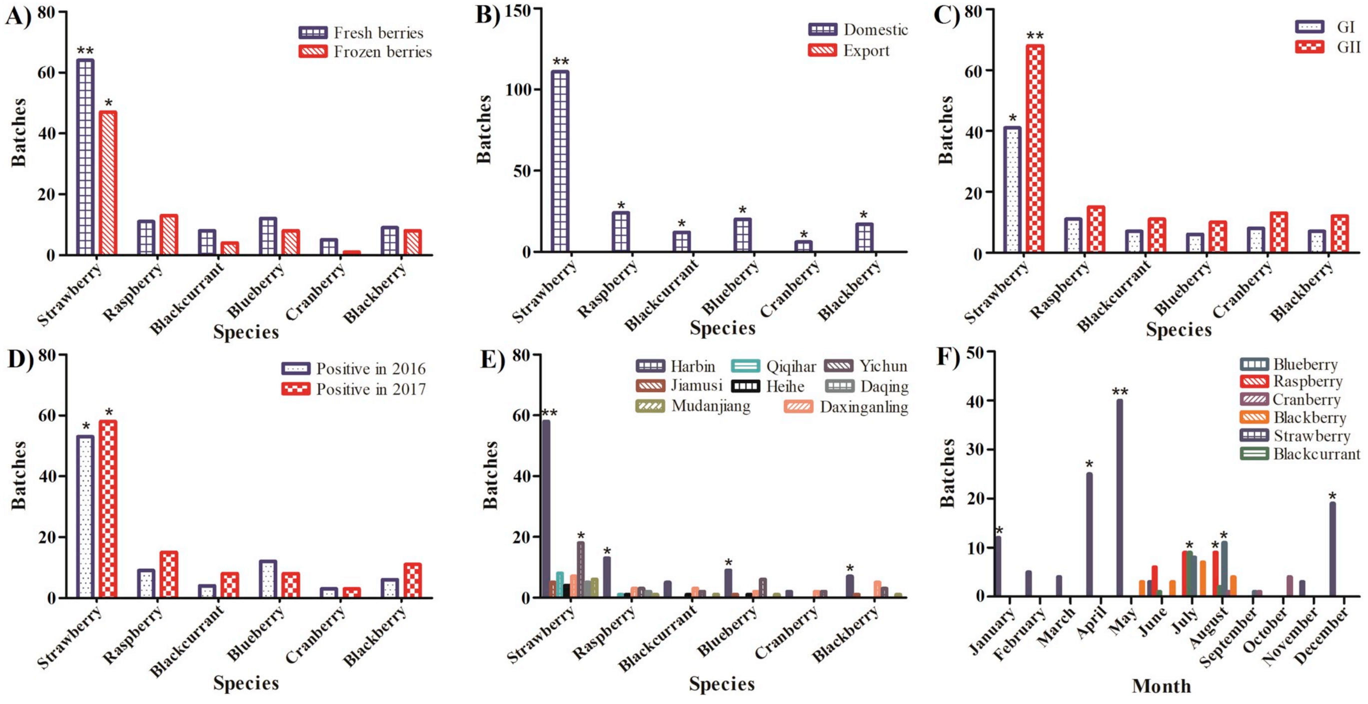

Fig. 5. The statistical analysis of the detection results. Statistical analyses were carried out on the investigation results of NoV contamination in berries from fresh vs. frozen (A), domestic vs. export (B), GI vs. GII (C), different years (D), different regions (E), and different times of year (F). **$P < 0.01$, *$P < 0.05$.

that are applicable to berry production, such as GAPs, HACCP, good manufacturing practices (GMPs), and good hygiene practices (GHPs). In China, routine testing is not carried out for NoVs in domestic retail berries and there are no available process hygiene criteria or procedures for managing NoVs or other microbial surveillance in berries. Therefore, we strongly advise the implementation of GAPs, HACCP, GMPs, and GHPs to avoid the contamination of fresh and frozen berries with NoVs and other pathogens. Moreover, certain practices should be implemented at each stage in the berry production chain in order to avoid NoV contamination, namely improving detection standards for NoV contamination in berries, developing effective detection assays for NoVs, investigating the efficacy of decontamination procedures for berries, frequently performing surveillance on norovirus prevalence in berries, carrying out risk assessment for the development of criteria designed to control NoVs contamination in fresh and frozen berries, and strengthening personal hygienic awareness.

4. Conclusion

We evaluated the NoV contamination in commercial fresh/frozen berry fruits collected from 2016 to 2017 in the Heilongjiang Province, which is the main berry-producing area in China, using a TaqMan-based real-time reverse transcription-PCR assay. Our findings show that the occurrence of NoV contamination was significantly higher in domestic berries than in exported berries, and that NoV was more often detected in fresh berries than in frozen berries. Thus, this study highlights the need to perform further risk surveillances for NoV contamination in berries produced in the Heilongjiang Province. Moreover, based on our findings, we recommend region-extended monitoring of retail berries for NoV contamination. In summary, the harvesting, packaging, and distribution of berries should be rigorously and independently assessed for suitable contamination management practices throughout the whole berry production chain. Further, research into developing official food safety procedures for the control of NoV contamination in domestic retail berries should be a priority.

Declarations of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.01.017.

References

Ao, Y., Wang, J., Ling, H., He, Y., Dong, X., Wang, X., Peng, J., Zhang, H., Jin, M., Duan, Z., 2017. Norovirus GII.P16/GII.2-associated gastroenteritis, China, 2016. Emerg. Infect. Dis. 23, 1172–1175.

Ao, Y., Cong, X., Jin, M., Sun, X., Wei, X., Wang, J., Zhang, Q., Song, J., Yu, J., Cui, J., 2018. Genetic analysis of re-emerging GII. P16–GII. 2 noroviruses in 2016-2017 in China. J. Infect. Dis. 218, 133–143.

Bae, G., Kim, J., Kim, H., Seok, J.H., Lee, D.B., Kim, K.H., Chung, M.S., 2018. Inactivation of norovirus surrogates by kimchi fermentation in the presence of black raspberry. Food Control 91, 390–396.

Bernard, H., Faber, M., Wilking, H., Haller, S., Höhle, M., Schielke, A., Ducomble, T., Siffczyk, C., Merbecks, S., Fricke, G., 2014. Large multistate outbreak of norovirus gastroenteritis associated with frozen strawberries, Germany, 2012. Euro Surveill. 19, 20719.

Cheng, D., Zou, S., Liao, N., Shi, X., Chen, J., Zhang, Y., Sun, L., Zhang, R., 2018. Evaluation of an extraction method for the detection of GI and GII noroviruses in fruit and vegetable salads. J. Food Sci. 83, 393–400.

Dietrich, M., Katja, T., Eckehard, N., Marina, H., Reimar, J., 2013. Detection and typing of norovirus from frozen strawberries involved in a large-scale gastroenteritis outbreak in Germany. Food. Environ. Virol. 5, 162–168.

Doyle, A., Barataud, D., Gallay, A., Thiolet, J.M., Le Guyaguer, S., Kohli, E., Vaillant, V., 2004. Norovirus foodborne outbreaks associated with the consumption of oysters from the Etang de Thau, France, December 2002. Euro Surveill. 9, 24–26.

Evans, E.A., Ballen, F.H., 2017. An overview of US blueberry production, trade, and consumption, with special reference to Florida. Food. Resour. Econ. FE952, 1–6.

Farruggia, D., Crescimanno, M., Galati, A., Tinervia, S., 2016. The quality perception of fresh berries: an empirical survey in the German market. Agr. Agr. Sci. Proc. 8, 566–575.

Glass, R.I., Parashar, U.D., Estes, M.K., 2009. Norovirus gastroenteritis. N. Engl. J. Med. 361, 1776–1785.

Hall, A.J., 2012. Noroviruses: the perfect human pathogens? J. Infect. Dis. 205, 1622–1624.

Hall, A.J., Wikswo, M.E., Manikonda, K., Roberts, V.A., Yoder, J.S., Gould, L.H., 2013. Acute gastroenteritis surveillance through the national outbreak reporting system, United States. Emerg. Infect. Dis. 19, 1305–1309.

Hutson, A.M., Atmar, R.L., Estes, M.K., 2004. Norovirus disease: changing epidemiology and host susceptibility factors. Trends Microbiol. 12, 279–287.

Hyun, J., Ko, D.H., Lee, S.K., Kim, H.S., Kim, J.S., Song, W., Kim, H.S., 2018. Evaluation of a new multiplex real-Time PCR assay for detecting gastroenteritis-causing viruses in stool samples. Ann. Lab. Med. 38, 220–225.

ISO 15216-1, 2013. Microbiology of the Food Chain–Horizontal Method for Determination of Hepatitis A Virus and Norovirus Using Real-Time RT-PCR–Part 1: Method for Quantification.

Jiang, T., Han, C., Seamus, F., Li, N., Wang, J., Zhang, H., Zhang, J., Li, F., 2018. Norovirus contamination in retail oysters from Beijing and Qingdao, China. Food Control 86, 415–419.

Kobayashi, M., Matsushima, Y., Motoya, T., Sakon, N., Shigemoto, N., Okamoto-Nakagawa, R., Nishimura, K., Yamashita, Y., Kuroda, M., Saruki, N., Ryo, A., Saraya, T., Morita, Y., Shirabe, K., Ishikawa, M., Takahashi, T., Shinomiya, H., Okabe, N., Nagasawa, K., Suzuki, Y., Katayama, K., Kimura, H., 2016. Molecular evolution of the capsid gene in human norovirus genogroup II. Sci. Rep. 6, 29400.

Lanata, C.F., Fischer-Walker, C.L., Olascoaga, A.C., Torres, C.X., Aryee, M.J., Black, R.E., 2013. Global causes of diarrheal disease mortality in children < 5 years of age: a systematic review. PLoS One 8, e72788.

Li, D., Butot, S., Zuber, S., PROFEL, Uyttendaele, M., 2018. Monitoring of foodborne viruses in berries and considerations on the use of RT-PCR methods in surveillance. Food Control 89, 235–240.

Li, Y., Sun, H., Chen, L., 2017. The blueberry industry of China: the past 10 years and the future. Acta Hortic. (Wagening.) 1180, 531–536.

Liao, Q., Ran, L., Jin, M., Cui, S., Yuan, J., Ma, H., Ban, H., Sun, L., Luo, L., Liu, N., Duan, Z., Yu, H., 2016. Guidelines on outbreak investigation, prevention and control of norovirus infection (2015). Zhonghua Yufang Yixue Zazhi 50, 7–16 [Article in Chinese].

Lindesmith, L., Moe, C., Marionneau, S., Ruvoen, N., Jiang, X., Lindblad, L., Stewart, P., LePendu, J., Baric, R., 2003. Human susceptibility and resistance to Norwalk virus infection. Nat. Med. 9, 548–553.

Liu, J.Y., Pan, L.W., Zhang, S.Y., Li, X.H., Wei, M.L., Luo, Z.F., 2016. The National Food Safety Standards of china: Food Microbiology Detection for Norovirus (GB 4789.42-2016). China Standard Publishing House, Beijing.

Lu, Q.B., Huang, D.D., Zhao, J., Wang, H.Y., Zhang, X.A., Xu, H.M., Qu, F., Liu, W., Cao, W.C., 2015. An increasing prevalence of recombinant GII norovirus in pediatric patients with diarrhea during 2010-2013 in China. Infect. Genet. Evol. 31, 48–52.

Maunula, L., Roivainen, M., Keränen, M., Mäkela, S., Söderberg, K., Summa, M., von Bonsdorff, C.H., Lappalainen, M., Korhonen, T., Kuusi, M., Niskanen, T., 2009. Detection of human norovirus from frozen raspberries in a cluster of gastroenteritis outbreaks. Euro Surveill. 14, 1424–1427.

Ng, Y.C., Kim, Y.W., Ryu, S., Lee, A., Lee, J.S., Song, M.J., 2017. Suppression of norovirus by natural phytochemicals from *Aloe vera* and Eriobotryae Folium. Food Control 73, 1362–1370.

Park, J.H., Jung, S., Shin, J., Lee, J.S., Joo, I.S., Lee, D.Y., 2015. Three gastroenteritis outbreaks in South Korea caused by the consumption of kimchi tainted by norovirus GI.4. Foodborne. Pathog. Dis. 12, 221–227.

Patel, M.M., Widdowson, M.A., Glass, R.I., Akazawa, K., Vinje, J., Parashar, U.D., 2008. Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerg. Infect. Dis. 14, 1224–1231.

Patel, M.M., Hall, A.J., Vinje, J., Parashara, U.D., 2009. Noroviruses: a comprehensive review. J. Clin. Virol. 44, 1–8.

Qin, S.W., Chan, T.C., Cai, J., Zhao, N., Miao, Z.P., Chen, Y.J., Liu, S.L., 2017. Genotypic and epidemiological trends of acute gGastroenteritis associated with noroviruses in China from 2006 to 2016. Int. J. Environ. Res. Publ. Health 14, 1341.

Rouhani, S., Yori, P.P., Olortegui, P.M., Salas, S.M., Trigoso, R.D., Mondal, D., Bodhidatta, L., Platts-Mills, J., Samie, A., Kabir, F., Lima, A., Babji, S., Mason, C.J., Kalam, A., Bessong, P., Ahmed, T., Mduma, E., Bhutta, Z.A., Lima, I., Ramdass, R., Lang, D., George, A., Zaidi, A.K.M., Kang, G., Houpt, E., Kosek, M.N., 2016. Norovirus infection and acquired immunity in 8 countries: results from the MAL-ED study. Clin. Infect. Dis. 62, 1210–1217.

Ryu, S., You, H.J., Kim, Y.W., Lee, A., Ko, G.P., Lee, S.J., Song, M.J., 2015. Inactivation of norovirus and surrogates by natural phytochemicals and bioactive substances. Mol. Nutr. Food Res. 59, 65–74.

Sarvikivi, E., Roivainen, M., Maunula, L., Niskanen, T., Korhonen, T., Lappalainen, M., Kuusi, M., 2012. Multiple norovirus outbreaks linked to imported frozen raspberries. Epidemiol. Infect. 140, 260–267.

Schmid, M., Oehme, R., Schalasta, G., Brockmann, S., Kimmig, P., Enders, G., 2004. Fast detection of noroviruses using a real-time PCR assay and automated sample preparation. BMC Infect. Dis. 4, 15.

Shang, X., Fu, X., Zhang, P., Sheng, M., Song, J., He, F., Qiu, Y., Wu, H., Lu, Q., Feng, Y., 2017. An outbreak of norovirus-associated acute gastroenteritis associated with contaminated barrelled water in many schools in Zhejiang, China. PLoS One 12, e0171307.

Solano, R., Alseda, M., Godoy, P., Sanz, M., Bartolome, R., Manzanares-Laya, S., Dominguez, A., Cayla, J.A., 2014. Person-to-person transmission of norovirus resulting in an outbreak of acute gastroenteritis at a summer camp. Eur. J. Gastroenterol. Hepatol. 26, 1160–1166.

Tamada, T., Hummer, K.E., Strik, B.C., Finn, C.E., 2009. Current trends of blueberry culture in Japan. Int. Vaccinium. Symp. 810, 109–116.

Tse, H., Lau, S.K., Chan, W.M., Choi, G.K., Woo, P.C., Yuen, K.Y., 2012. Complete genome sequences of novel canine noroviruses in Hong Kong. J. Virol. 86, 9531–9532.

Vinje, J., 2015. Advances in laboratory methods for detection and typing of norovirus. J. Clin. Microbiol. 53, 373–381.

Yu, J., Ye, C., Lai, S., Zhu, W., Zhang, Z., Geng, Q., Xue, C., Yang, W., Wu, S., Hall, A.J., Sun, Q., Li, Z., 2017. Incidence of norovirus-associated diarrhea, Shanghai, China, 2012-2013. Emerg. Infect. Dis. 23, 312–315.

Zheng, D.P., Ando, T., Fankhauser, R.L., Beard, R.S., Glass, R.I., Monroe, S.S., 2006. Norovirus classification and proposed strain nomenclature. Virology 346, 312–323.