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Traceback and Testing of Food Epidemiologically Linked to a Norovirus Outbreak at a Wedding Reception

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Abstract

We investigated a suspected norovirus outbreak associated with a wedding reception in Wisconsin in May 2015. Fifty-six of 106 (53%) wedding attendees were interviewed, and 23 (41%) reported symptoms consistent with norovirus infection. A retrospective cohort study identified fruit salad as the likely vehicle of infection (risk ratio 3.2, 95% confidence interval 1.1—8.3). Norovirus was detected by real-time reverse transcription polymerase chain reaction (RT-qPCR) in stool specimens collected from four attendees and one food handler and in 12 leftover fruit salad samples from both an opened and a sealed container. Norovirus-positive clinical samples ($n = 4$) were genotyped as GII.4 Sydney and norovirus-positive fruit salad samples ($n = 2$) confirmed the presence of GII.4 norovirus by Sanger sequencing with 98% nucleotide ($n = 236$) similarity in 5' end of ORF2 between fruit salad and clinical specimens. In conclusion, this comprehensive norovirus outbreak investigation combined epidemiologic, virologic, and environmental findings to traceback the contaminated food as the source of the outbreak.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: 'The findings, conclusions, and views expressed in this manuscript are those of the authors and do not necessarily reflect the official policy of the Department of Health and Human Services, the U.S. Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), or the U.S. Government. Reference to any commercial materials, equipment, or process does not in any way constitute approval, endorsement, or recommendation by the FDA or the CDC.'

Keywords

Foodborne outbreak; Norovirus; Outbreak investigation; Produce

Human noroviruses have been identified by the WHO Foodborne Disease Burden Epidemiology Reference Group as the leading causes of foodborne illness and among the top five causes of foodborne disease-associated deaths (Kirk et al., 2015). A recent systematic review highlighted that norovirus was responsible for almost 49% of the produce-associated acute gastroenteritis (AGE) outbreaks reported from 29 different countries between 1974 and 2016 (Nasheri et al., 2019). Identifying a specific food item associated with a foodborne AGE outbreak is challenging as often there is no left-over product available for laboratory testing or no method exists to test the implicated food (Hida et al., 2018).

When a viral etiology is identified during a foodborne outbreak investigation, it is most often conveyed through epidemiological evidence and laboratory testing of stool specimens. In only a handful of published reports has food testing successfully identified a particular food item as the vehicle of illness (Calder et al., 2003; Cook et al., 2021; Rajiuddin et al., 2020; Raymond et al., 2022; Saupe et al., 2021). In the majority of these reports, the virus was detected with one of the standardized protocols available for single-ingredient matrices (ISO, 2019; Williams-Woods et al., 2022). Human noroviruses are small nonenveloped single-stranded RNA viruses that belong to the genus *Norovirus* in the family *Caliciviridae*. They are classified into at least 10 genogroups (G) of which viruses from GI and GII cause the vast majority of AGE (Chhabra et al., 2019). Although significant advances have been made in cell culture methods to detect infectious norovirus in recent years (Ettayebi et al., 2016; Van Dycke et al., 2019), these methods are not yet sensitive enough to detect low levels of contamination typically found in foods as well as a variety of different strains (Wales et al., 2023). As a result, molecular-based methods (e.g., RT-qPCR) are primarily used to detect norovirus in foods and environmental samples (Vinje, 2015).

In the past several years, significant improvements in food virology testing for both norovirus and hepatitis A virus (HAV) have been developed and validated. The International Organization for Standardization has published a set of protocols for the recovery and detection of these viruses from a variety of food matrices (Lowther et al., 2019). Standardized methods have been employed in several surveillance studies internationally on high-risk commodities (Dirks et al., 2021; Li et al., 2018; Pavoni et al., 2022). Similarly, the US Food and Drug Administration has more recently published a chapter in the Bacteriological Analytical Manual with protocols for isolation and detection of the same viruses in certain foods (Williams-Woods et al., 2022). As a result, the capacity of food testing during outbreak investigations has increased considerably, potentially enabling identification of the source of illness and preventing further spread. In addition, recent epidemiological evidence (Somura et al., 2019) has highlighted that more diverse and complex food matrices are often associated with outbreaks indicating that standardized methods for testing viruses in multiingredient foods are required rather than relying on in-house methodologies often used by food testing laboratories. Sanger sequencing has been

traditionally used to confirm contamination in real-time RT-PCR-positive food samples. More recently, techniques based on next-generation sequencing (NGS) have advanced and allowed the sequencing analysis of the whole genome of foodborne pathogens to confirm and type viruses such as norovirus and hepatitis A virus, in naturally contaminated food matrices (Chiapponi et al., 2014; Made et al., 2013; Yang et al., 2024).

In this report, we describe the epidemiologic, virologic, and environmental investigations of a foodborne norovirus outbreak at a wedding reception in Wisconsin, US, in 2015, to identify the likely source of transmission. Due to the COVID-19 pandemic and method optimization, the publication of the findings of this study shifted substantially several years after the actual outbreak.

Materials and methods

Epidemiological investigation.

On May 20, 2015, an outbreak of gastrointestinal illness among attendees of a wedding was reported to a local health department (LHD) in Wisconsin. The wedding had occurred on the evening of May 15 and was held outdoors under a large tent with 106 attendees. Dinner was served as a buffet and was catered by Establishment A. Cakes were purchased from Establishment B and were cut and served by two wedding guests. Based on the symptoms reported (loss of appetite, nausea, vomiting, fatigue, diarrhea, abdominal cramps, muscle aches, chills, headache, fever, and bloody diarrhea) by the wedding guests and report of an ill employee at Establishment A, norovirus was suspected as the causative agent. The LHD initiated an outbreak investigation to identify the etiology of the outbreak, the vehicle of transmission, and the source of contamination.

As part of the public health investigation, a retrospective cohort study was conducted among wedding guests. All wedding guests were asked to complete an online questionnaire created in Survey Gizmo (<https://www.SurveyGizmo.com>). Employees of Establishments A and B were invited by the LHD to complete questionnaires created in Survey Gizmo, either online or orally with Public Health Sanitarians (PHS). Collected data included demographics, clinical symptoms, job duties, and potential secondary transmission to other household members. The wedding guest questionnaire included questions on food consumed at the wedding and clinical symptoms, including any symptoms one week prior to the wedding.

Case definitions.

The following case definitions were used: a confirmed case was defined as an individual who attended the wedding or who worked at Establishment A or B and had a stool specimen collected in May 2015 that tested positive for norovirus GII by RT-qPCR. A probable case was defined as an individual who attended the wedding or who worked at Establishment A or B and had vomiting or diarrhea and at least one of the following symptoms (nausea, abdominal cramps, fever, chills, muscle aches, headache, or fatigue) with onset between May 16 and 18, 2015, but did not have a stool specimen collected that tested positive for norovirus GII. A negative case was defined as an individual who attended the wedding or

who worked at Establishment A or B and had a stool specimen collected in May 2015 that tested negative for norovirus GII by RT-qPCR.

Data analysis.

To determine if food items were statistically associated with illness, risk ratios (RRs) with corresponding 95% confidence intervals (CIs) were calculated for each food item. Associations between consumption of food items associated with illness were assessed by calculating odds ratios (OR) with corresponding 95% CI. Analyses were conducted using Epi Info version 3.5.3 (Centers for Disease Control & Prevention (CDC)).

Environmental investigation.

PHS conducted onsite visits to Establishments A and B on May 20, performed inspections focusing on areas that could contribute to the spread of norovirus, inquired about ill employees and whether other complaints had been received; they asked for contact information on other large groups served the same weekend as the wedding, and obtained the menus for the wedding. Both establishments were ordered to perform cleaning per the LHD's norovirus cleaning guidelines. Establishment A was ordered to close until PHS could verify that the recommended cleaning had been completed. In addition, an onsite visit was conducted by the Department of Agriculture (DOA) at the plant (plant A) that had packaged the fruit salad served at the wedding.

Laboratory investigation: Clinical sample analysis.

Stool specimens from four wedding attendees and ten employees of Establishment A were collected 4–8 days after symptom onset and viral RNA was extracted from clarified 10% fecal suspensions and tested for norovirus RNA by RT-qPCR at the Wisconsin State Laboratory of Hygiene (WSLH) (Cannon et al., 2017). Genotyping of norovirus-positive stool specimens was approved by CDC (project determination # 0900f3eb81e60989).

Food sample analysis: Sample preparation.

Fruit salad was identified as the most probable source of infection among the various food items served at the dinner. As a result, a bag of leftover fruit salad (containing cantaloupe, pineapple, honeydew, watermelon and grapes) previously thawed, opened, and served during the wedding and a sealed (unopened) container with frozen fruit salad (consisting of the same fruits minus watermelon) were collected and shipped overnight on dry ice to the Center for Food Safety and Applied Nutrition (CFSAN) at the Food and Drug Administration in Maryland, U.S. Immediately upon receipt, all samples were aliquoted in approximately 50 g portions in sterile filtered Whirl-Pak® plastic bag (Nasco; Fort Atkinson, Wisconsin) and four samples (two from the opened and two from the unopened container) were kept at 4 °C overnight to thaw and be tested the next day, while the rest were stored at –80 °C. A total of 4 aliquots from opened and 8 from unopened were tested within a month of receipt following a protocol previously described (Hida et al., 2018; Yang et al., 2017). All four aliquots from the opened container included watermelon as this fruit was added at Establishment A. Briefly, 100 µl of 3×10^5 PFU of murine norovirus (MNV-1) (ATCC PTA-5935) was spiked onto each 50 g food item as a process control. Viruses were eluted

from the food items with 0.1 M Tris–HCl, 0.05 M glycine, 1% beef extract buffer (TGBE) at pH 9.2 containing 500 µl pectinase ($\geq 3,800$ units/ml, Sigma; St. Louis, MO) and 2% w/v polyvinylpyrrolidone (PVP; molecular weight 360 K) (Thermo Fisher Scientific; Waltham, MA). Virus particles were subsequently concentrated by ultracentrifugation (170,000g, at 4 °C for 1 h). RNA was isolated using the RNeasy PowerPlant Kit (Qiagen, Germantown, MD) following the manufacturers' instructions.

Norovirus detection by RT-qPCR.

RT-qPCR was performed as previously described (Hida et al., 2018). An internal amplification control (IAC) was also incorporated in each reaction (Nordstrom et al., 2007). Samples were run in triplicate. Norovirus was quantified (RNA genomic copies) by a standard curve generated by 10-fold serial dilutions of a synthetic norovirus GII RNA transcript (ATCC, Gaithersburg, Maryland). An aliquot was considered positive by RT-qPCR when: (1) the RT-qPCR amplification curve had a typical S curve shape, (2) all three replicates tested positive, (3) no inhibition was observed, and (4) the negative and positive PCR controls performed as expected.

Sanger sequencing of norovirus from stool and food samples.

Norovirus-positive nucleic acid from four fruit salad aliquots (two from the opened and two from the unopened container) was shipped overnight to CDC and tested by hemi-nested RT-PCR (Park et al., 2017). PCR amplicons of the expected size were subsequently sequenced by Sanger sequencing and genotyped using the online human calicivirus typing tool (Tatusov et al., 2021). Norovirus sequences obtained from the contaminated fruit salad and the stool samples of the four quests were aligned with GII reference strains representing all known GII.4 viruses using Clustal W in MEGA X (Kumar et al., 2018). Phylogenetic trees were computed with Maximum-likelihood phylogeny using the Tamura-Nei (nucleotide) models (Tamura & Nei, 1993) with gamma distribution of evolutionary rates among sites and 100 bootstrap replications.

Whole genome sequencing on norovirus-positive fruit salad samples.

Nucleic acid from a norovirus RT-qPCR positive aliquot (from the sealed container where no MNV was added) was sequenced on a MiSeq sequencer (Illumina) (Yang et al., 2016). Raw sequence reads were imported into CLC Genomics workbench v11.0 (CLC bio) and analyzed by either sequence alignment against an in-house custom database or using an abundance estimation algorithm with an in-house k-mer tool (github.com/mmammel8/kmer_id) with default parameters (Yang et al., 2017).

Results

Epidemiological investigation.

Fifty-six of 106 (53%) wedding attendees were included in the data analysis, 23 of whom were classified as cases (four confirmed, 19 probable). Sixty-one percent of cases were female and case ages ranged from one to 83 years (mean = 42, median = 49.5). Incubation periods ranged from 15 to 56 h (mean = 38, median = 36) (Fig. 1). Symptoms included loss of appetite (reported by 83% of cases), nausea (83%), vomiting (83%), fatigue (79%),

diarrhea (75%), abdominal cramps (67%), muscle aches (58%), chills (50%), headache (42%), and fever (29%). One individual reported experiencing bloody diarrhea. The duration of illness ranged from 13 to more than 189 h. No one sought medical care. Symptoms were similar in both the confirmed and probable cases with at least half of the individuals in each group reporting abdominal cramps, diarrhea, fatigue, loss of appetite, muscle aches, nausea, and vomiting.

Three food items were significantly associated with illness (Table 1) including fruit salad (RR = 3.2, 95% CI = 1.1, 8.3), carrot cake (RR = 2.8, 95% CI = 1.5, 5.3), and lemon poppy seed cake (RR = 2.3, 95% CI = 1.4, 4.0). The carrot cake and lemon poppy seed cake both contained Establishment B's signature frosting. Twenty (87%) of 23 cases reported eating fruit salad and 15 cases (65%) reported eating cake with signature frosting. It is possible the cakes with signature frosting were associated with illness because wedding guests who ate fruit salad were more likely to eat a cake with signature frosting than individuals who did not eat fruit salad (OR = 3.5, 95% CI = 0.97, 12.59). Guests who ate fruit salad were not more likely to eat any cake or a cake with buttercream frosting (chocolate or vanilla) than people who did not eat fruit salad.

Thirteen Establishment A and 10 Establishment B employees completed illness questionnaires. One employee (employee 1) of Establishment A reported symptoms of vomiting and diarrhea with onset 11 days prior to the wedding and recovery on the same day. This employee's stool was collected six days after the wedding and tested negative for norovirus. No other employees reported symptoms prior to the wedding. One employee of Establishment A who ate fruit salad served at the wedding tested positive for norovirus without reporting having had symptoms.

Environmental investigation.

Inspection of Establishments A and B showed no Food Code violations and both establishments completed the ordered cleaning <https://docs.legis.wisconsin.gov/code/register/2015/712B/insert/atcp75>. Establishment A was able to reopen on May 21. The LHD contacted other large groups who purchased food from Establishments A and B on the same weekend and none reported illness among members of their groups. Neither establishment received any illness complaints from other customers.

At Establishment A, the food for the wedding was prepared by employees 2 and 3. All food items except for the beef tips were products of Company A. Employee 2 heated the hot food items and placed them in containers for transport and serving. Employee 3 transferred the cold food items from their factory containers to containers for transport and serving. In addition, employee 3 cut a watermelon and added it to the fruit salad and transported all food items, plates, and silverware from Establishment A to the wedding site, set up and broke down the buffet, and transported leftovers back to Establishment A. Employee 4 reportedly ate some of the leftover fruit salad with watermelon after it was returned to Establishment A. Upon inspection of Plant A, there were no obvious sources of contamination and no ill employees during the time the fruit salad was packaged.

At Establishment B, the four cakes for the wedding were baked on the Monday, Tuesday, or Wednesday prior to the wedding and frosted on Thursday or Friday. The cakes were picked up on Friday and transported to the wedding site. The cakes were cut and served by two wedding guests using plates and silverware supplied by the bridal couple.

Laboratory investigation.

Stool specimens from four wedding guests and employee 4 from Establishment A tested positive for norovirus GII by RT-qPCR. Nucleic acid from all 12 fruit salad aliquots tested positive for norovirus GII with cycle threshold (Ct) values ranging from 31 to 42 (Table 2) corresponding to approximately 10–1,000 RNA copies/50 g sample. MNV-1, which was included as a process control, could consistently be detected in all food samples (data not shown).

Sanger sequencing of norovirus-positive stool specimens and food samples.

Both the norovirus-positive clinical specimens and fruit salad samples could be typed as GII.4 norovirus in the 5'-ORF2 region. Sequences from the 4 fruit salad samples were 100% identical and likewise, there was 100% sequence identity between the 4 clinical specimens. However, there was only a 98% match (6 nucleotide substitutions between the fruit salad samples and clinical specimens (Fig. 2).

Metagenomic sequencing of norovirus-positive fruit salad samples.

Of the 2.1 M total reads, 208 miSeq reads (0.01%) generated three fragments of which the largest fragment (493 nucleotides) showed the presence of ORF3 fragment of GII.4 noroviruses in NCBI BLAST search. However, since these sequences did not correspond to 5'-end ORF2 Sanger sequences from either fruit salad or clinical samples, the sequences could not be directly compared. When WGS was attempted on the clinical specimens, no quality norovirus sequences could be recovered, most likely due to either low viral load or virus RNA degradation (data not shown).

Discussion

We report a norovirus outbreak among guests attending a wedding reception in Wisconsin in May 2015. Wedding attendees who ate the fruit salad were 3.2 times more likely to report gastrointestinal symptoms than wedding attendees who did not eat the fruit salad. GII norovirus RNA was identified in both opened and unopened fruit salad products and genotyped as GII.4.

This study highlights the importance of conducting a comprehensive outbreak investigation allowing for timely collection of human clinical specimens, left-over food items from the implicated meal, and patient interviews to determine the likely source of infection. The epidemiological investigation showed that wedding guests who had consumed fruit salad and cakes with frosting were significantly associated with AGE. Of the left-over food items, only the fruit salad was available for laboratory testing. Availability of left-over food for testing during AGE outbreak investigations is frequently a challenge. Food may be still available for collection, but may not have been stored at refrigerated temperature or the

left-over food was not the food that was actually consumed during the outbreak under investigation, which can lead to misleading conclusions on which food item was associated with illness (Hida et al., 2018). Reportedly, only 35% of all foodborne norovirus outbreaks reported between 2009 and 2015 could be attributed to a specific food vehicle (Marsh et al., 2018).

This outbreak investigation also provides useful information on the level of norovirus genomic copies present in contaminated food, adding important data to the very limited number of published reports. Though the sampling size and the method of quantification can vary between studies, norovirus Ct values usually range between the mid-30s and low-40s (Li et al., 2018; Pavoni et al., 2022; Stals et al., 2011; Torok et al., 2019). Of 56 viral foodborne outbreaks in France reported between 2012 and 2017, 10 norovirus GII-positive samples were detected with genomic levels ranging from $<10^2$ to 10^5 genome copies per gram (Hennechart-Collette et al., 2020). Another study found that 3 out of 26 raspberry batches were contaminated with an average of 4.3 log genomic copies of norovirus GII per 20 g (De Keuckelaere et al., 2015). Viral RNA copy numbers quantified in our study varied significantly between aliquots suggesting uneven distribution throughout the fruit salad, which has been previously observed for contaminated semidried tomatoes and dates (Boxman et al., 2012; Donnan et al., 2012; Gallot et al., 2011). GII viruses were predominantly associated with foodborne norovirus outbreaks in agreement with other studies looking into the detection rate of different norovirus genotypes from food-associated items (Hennechart-Collette et al., 2020), and this strain was also the most prevalent virus during the time of the wedding outbreak (Vinje, 2015).

Detection of $<100\%$ identical norovirus sequences in the fruit salad compared to the stool specimens from the wedding guests suggests that contamination of the implicated food items possibly occurred inadvertently prior to serving rather than from a single infected food handler. Possible explanations for different strains in clinical specimens and food samples that were tested include a more infectious strain that infected the wedding guests and another strain that contaminated the fruit salad. More specifically, we cannot rule out that employee 1 from Establishment A who was sick eleven days prior to the event might still have been shedding the virus as infected people with NoV have been reported to shed high numbers of virus for prolonged periods of time (Sabrià et al., 2016; Teunis et al., 2015). Although no other employee reported symptoms prior to the wedding, there could have been an asymptomatic one, especially since not all employees submitted stools for testing. Another possible explanation is that contamination may have occurred at the venue where the fruit salad was cut and prepared as aliquots of the salad both with and without watermelon (that was added at the wedding venue) tested positive for norovirus. Lastly, although there is limited evidence from published literature that contamination can directly occur during picking or preharvest conditions (Bozkurt et al., 2021), we cannot completely exclude this route of contamination. Codetection of multiple norovirus strains is not unusual, including outbreaks in oysters, bottled water, strawberries, and raspberries (Blanco et al., 2017; Le Guyader et al., 2004; Rispens et al., 2020).

In the last decade, an increasing number of reports on the use of whole or partial genome sequencing to detect and characterize viral contamination in clinical, food, and

environmental samples have been published (Enkirch et al., 2018; Muller et al., 2015; Rajiuddin et al., 2020; Yang et al., 2024). For example, a low level of HAV contamination was detected in dried dates associated with an outbreak in Denmark with a 100% match with sequences detected from stools (Rajiuddin et al., 2020). Similarly, in a norovirus outbreak associated with consumption of raspberries, GI.6 sequences detected in the frozen fruits were identical to the virus in the stool specimens. In our study, we were unable to obtain whole genome sequencing data by using the described NGS approach. One reason can be that the targeted preamplification sequencing was not used to amplify norovirus sequences (Ford-Siltz et al., 2019). In addition, the food samples preparation for the miseq run did not include any additional steps (such as filtration, concentration, specific capture strategies, etc.) that may have enhanced the number of sequence reads for viruses and improved sequencing coverage (Desdouits et al., 2020). These examples highlight how analytical challenges such as virus type, viral load, viral nucleic acid quality, and lack of standardized protocols frequently limit the applicability of this technology (Aw et al., 2016; Desdouits et al., 2020; Imamura et al., 2016; Yang et al., 2017).

Our study has several limitations. First, the questionnaires administered to employees and wedding attendees relied on individuals to accurately recall and report their symptoms and which foods they had eaten. Second, although epidemiologically two types of cake with “signature frosting” were significantly associated with illness, no left-over cake was available for testing. Third, employees from Establishment B did not submit stool specimens for testing. Finally, stool specimens were collected several days after symptom onset (in some cases 7–10 days after exposure) that may have contributed to the virus being undetected by RT-qPCR in some of them.

Conclusion

This study highlights the importance of a multidisciplinary investigation to successfully investigate a viral foodborne outbreak. It also underscores how a thorough environmental health and laboratory investigation can support the epidemiological findings. Although food testing may take 2–3 days to complete, it can assist in confirming the implicated food item and whether infected food handlers contributed to the contamination of the food. While the results of just the epidemiological study and patient specimen testing would likely have attributed this outbreak to fruit salad contaminated by an infected food worker, laboratory testing of the suspected food item and subsequent phylogenetic analysis strengthened the evidence that the fruit salad was the food vehicle and provided evidence that the fruit salad was likely contaminated at some point prior to its arrival at Establishment A. With standardized methods for the detection of norovirus in foods becoming more widely available, and advances in NGS techniques for viral pathogens, future outbreak investigations should consider testing of left-over food items to help identify appropriate opportunities for prevention, including promoting and adhering to hand hygiene, staying home while sick, and mitigating potential contamination prior to preparation of fresh produce.

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References

- Aw TG, Wengert S, & Rose JB (2016). Metagenomic analysis of viruses associated with field-grown and retail lettuce identifies human and animal viruses. *International Journal of Food Microbiology*, 223, 50–56. 10.1016/j.ijfoodmicro.2016.02.008. [PubMed: 26894328]
- Blanco A, Guix S, Fuster N, Fuentes C, Bartolome R, Cornejo T, Pinto RM, & Bosch A (2017). Norovirus in bottled water associated with gastroenteritis outbreak, Spain, 2016. *Emerging Infectious Diseases*, 23(9), 1531–1534. 10.3201/eid2309.161489. [PubMed: 28820131]
- Boxman IL, te Loeke NA, Klunder K, Hagele G, & Jansen CC (2012). Surveillance study of hepatitis A virus RNA on fig and date samples. *Applied and Environmental Microbiology*, 78(3), 878–879. 10.1128/AEM.06574-11. [PubMed: 22138987]
- Bozkurt H, Phan-Thien KY, van Ogtrop F, Bell T, & McConchie R (2021). Outbreaks, occurrence, and control of norovirus and hepatitis A virus contamination in berries: a review. *Critical Reviews in Food Science and Nutrition*, 61(1), 116–138. 10.1080/10408398.2020.1719383. [PubMed: 32008374]
- Calder L, Simmons G, Thornley C, Taylor P, Pritchard K, Greening G, & Bishop J (2003). An outbreak of hepatitis A associated with consumption of raw blueberries. *Epidemiology and Infection*, 131(1), 745–751. 10.1017/s0950268803008586. [PubMed: 12948375]
- Cannon JL, Barclay L, Collins NR, Wikswo ME, Castro CJ, Magana LC, Gregoricus N, Marine RL, Chhabra P, & Vinje J (2017). Genetic and epidemiologic trends of norovirus outbreaks in the United States from 2013 to 2016 demonstrated emergence of novel GII.4 recombinant viruses. *Journal of Clinical Microbiology*, 55(7), 2208–2221. 10.1128/JCM.00455-17. [PubMed: 28490488]
- Chhabra P, de Graaf M, Parra GI, Chan MC, Green K, Martella V, Wang Q, White PA, Katayama K, Vennema H, Koopmans MPG, & Vinje J (2019). Updated classification of norovirus genogroups and genotypes. *The Journal of General Virology*, 100(10), 1393–1406. 10.1099/jgv.0.001318. [PubMed: 31483239]
- Chiapponi C, Pavoni E, Bertasi B, Baioni L, Scaltriti E, Chiesa E, Cianti L, Losio MN, & Pongolini S (2014). Isolation and genomic sequence of hepatitis A virus from mixed frozen berries in Italy. *Food and Environmental Virology*, 6(3), 202–206. 10.1007/s12560-014-9149-1. [PubMed: 24859055]
- Cook N, Vickers-Smith L, & D'Agostino M (2021). Detection of hepatitis A virus in strawberries implicated in an outbreak in the USA in 1997. *Food and Environmental Virology*, 13(3), 421–422. 10.1007/s12560-021-09480-2. [PubMed: 34106432]
- De Keuckelaere A, Li D, Deliens B, Stals A, & Uyttendaele M (2015). Batch testing for noroviruses in frozen raspberries. *International Journal of Food Microbiology*, 192, 43–50. 10.1016/j.ijfoodmicro.2014.09.024. [PubMed: 25306298]
- Desdoutis M, de Graaf M, Strubbia S, Oude Munnink BB, Kroneman A, Le Guyader FS, & Koopmans MPG (2020). Novel opportunities for NGS-based one health surveillance of foodborne viruses. *One Health Outlook*, 2, 14. 10.1186/s42522-020-00015-6. [PubMed: 33829135]
- Dirks RAM, Jansen CCC, Hagele G, Zwartkruis-Nahuis AJT, Tijsma ASL, & Boxman ILA (2021). Quantitative levels of norovirus and hepatitis A virus in bivalve molluscs collected along the food chain in the Netherlands, 2013–2017. *International Journal of Food Microbiology*, 344, 109089. 10.1016/j.ijfoodmicro.2021.109089. [PubMed: 33662900]
- Donnan EJ, Fielding JE, Gregory JE, Lalor K, Rowe S, Goldsmith P, Antoniou M, Fullerton KE, Knope K, Copland JG, Bowden DS, Tracy SL, Hogg GG, Tan A, Adamopoulos J, Gaston J, & Vally H (2012). A multistate outbreak of hepatitis A associated with semidried tomatoes in Australia, 2009. *Clinical Infectious Diseases*, 54(6), 775–781. 10.1093/cid/cir949. [PubMed: 22238166]
- Enkirch T, Eriksson R, Persson S, Schmid D, Aberle SW, Lof E, Wittesjo B, Holmgren B, Johnzon C, Gustafsson EX, Svensson LM, Sandelin LL, Richter L, Lindblad M, Brytting M, Maritschnik S, Tallo T, Malm T, Sundqvist L, & Ederth JL (2018). Hepatitis A outbreak linked to imported frozen

strawberries by sequencing, Sweden and Austria, June to September 2018. *Eurosurveillance*, 23(41). 10.2807/1560-7917.ES.2018.23.41.1800528.

- Ettayebi K, Crawford SE, Murakami K, Broughman JR, Karandikar U, Tenge VR, Neill FH, Blutt SE, Zeng XL, Qu L, Kou B, Opekun AR, Burrin D, Graham DY, Ramani S, Atmar RL, & Estes MK (2016). Replication of human Journal of Food Protection xxx (2024) 100395 noroviruses in stem cell-derived human enteroids. *Science*, 353(6306), 1387–1393. 10.1126/science.aaf5211. [PubMed: 27562956]
- Ford-Siltz LA, Mullis L, Sanad YM, Tohma K, Lepore CJ, Azevedo M, & Parra GI (2019). Genomics analyses of GIV and GVI noroviruses reveal the distinct clustering of human and animal viruses. *Viruses*, 11(3). 10.3390/v11030204.
- Gallot C, Grout L, Roque-Afonso AM, Couturier E, Carrillo-Santistevé P, Pouey J, Letort MJ, Hoppe S, Capdepon P, Saint-Martin S, De Valk H, & Vaillant V (2011). Hepatitis A associated with semidried tomatoes, France, 2010. *Emerging Infectious Diseases*, 17(3), 566–567. 10.3201/eid1703.101479. [PubMed: 21392466]
- Hennechart-Collette C, Martin-Latil S, Fraisse A, Niveau F, & Perelle S (2020). Virological analyses in collective catering outbreaks in France between 2012 and 2017. *Food Microbiology*, 91, 103546. 10.1016/j.fm.2020.103546. [PubMed: 32539952]
- Hida K, Papafragkou E, & Kulka M (2018). Testing for human norovirus and recovery of process control in outbreak-associated produce items. *Journal of Food Protection*, 81(1), 105–114. 10.4315/0362-028X.JFP-17-134. [PubMed: 29280676]
- Imamura S, Haruna M, Goshima T, Kanezashi H, Okada T, & Akimoto K (2016). Application of next-generation sequencing to investigation of norovirus diversity in shellfish collected from two coastal sites in Japan from 2013 to 2014. *The Japanese Journal of Veterinary Research*, 64(2), 113–122. [PubMed: 27506085]
- ISO (2019). *Microbiology of the Food Chain: Horizontal Method for Determination of Hepatitis A Virus and Norovirus Using Real-Time RT-PCR. Part 2: Method for Detection*. ISO 15216–2:2019. Geneva, Switzerland.
- Kirk MD, Pires SM, Black RE, Caipo M, Crump JA, Devleeschauwer B, Dopfer D, Fazil A, Fischer-Walker CL, Hald T, Hall AJ, Keddy KH, Lake RJ, Lanata CF, Torgerson PR, Havelaar AH, & Angulo FJ (2015). Correction: World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases, 2010: a data synthesis. *PLoS Medicine*, 12(12), e1001940. [PubMed: 26701262]
- Kumar S, Stecher G, Li M, Knyaz C, & Tamura K (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–1549. 10.1093/molbev/msy096. [PubMed: 29722887]
- Le Guyader FS, Mittelholzer C, Haugarreau L, Hedlund KO, Alsterlund R, Pommepuy M, & Svensson L (2004). Detection of noroviruses in raspberries associated with a gastroenteritis outbreak. *International Journal of Food Microbiology*, 97(2), 179–186. 10.1016/j.ijfoodmicro.2004.04.018. [PubMed: 15541804]
- Li D, Butot S, Zuber S, & 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. 10.1016/j.foodcont.2018.02.024.
- Lowther JA, Bosch A, Butot S, Ollivier J, Made D, Rutjes SA, Hardouin G, Lombard B, In't Veld P, & Leclercq A (2019). Validation of EN ISO method 15216 – Part 1 – Quantification of hepatitis A virus and norovirus in food matrices. *International Journal of Food Microbiology*, 288, 82–90. 10.1016/j.ijfoodmicro.2017.11.014. [PubMed: 29229293]
- Made D, Trubner K, Neubert E, Hohne M, & John R (2013). Detection and typing of norovirus from frozen strawberries involved in a large-scale gastroenteritis outbreak in Germany. *Food and Environmental Virology*, 5(3), 162–168. 10.1007/s12560-013-9118-0. [PubMed: 23888384]
- Marsh Z, Shah MP, Wikswo ME, Barclay L, Kisselburgh H, Kambhampati A, Cannon JL, Parashar UD, Vinje J, & Hall AJ (2018). Epidemiology of foodborne norovirus outbreaks – United States, 2009–2015. *Food Safety (Tokyo)*, 6 (2), 58–66. 10.14252/foodsafetyfscj.2017028.
- Muller L, Schultz AC, Fonager J, Jensen T, Lisby M, Hindsdal K, Krusell L, Eshoj A, Moller LT, Porsbo LJ, Bottiger BE, Kuhn K, Engberg J, & Ethelberg S (2015). Separate norovirus outbreaks linked to one source of imported frozen raspberries by molecular analysis, Denmark, 2010–

2011. *Epidemiology and Infection*, 143(11), 2299–2307. 10.1017/S0950268814003409. [PubMed: 25530074]
- Nasheri N, Vester A, & Petronella N (2019). Foodborne viral outbreaks associated with frozen produce. *Epidemiology and Infection*, 147, e291. [PubMed: 31625499]
- Nordstrom JL, Vickery MC, Blackstone GM, Murray SL, & DePaola A (2007). Development of a multiplex real-time PCR assay with an internal amplification control for the detection of total and pathogenic *Vibrio parahaemolyticus* bacteria in oysters. *Applied and Environmental Microbiology*, 73(18), 5840–5847. 10.1128/AEM.00460-07. [PubMed: 17644647]
- Park GW, Chhabra P, & Vinje J (2017). Swab sampling method for the detection of human norovirus on surfaces. *Journal of Visualized Experiments*, 120. 10.3791/55205.
- Pavoni E, Bertasi B, Galuppini E, Mangeri L, Meletti F, Tilola M, Carta V, Todeschi S, & Losio MN (2022). Detection of hepatitis A virus and norovirus in different food categories: a 6-year survey in Italy. *Food and Environmental Virology*, 14(1), 69–76. 10.1007/s12560-021-09503-y. [PubMed: 34698989]
- Rajiuddin SM, Midgley SE, Jensen T, Muller L, & Schultz AC (2020). Application of an optimized direct lysis method for viral RNA extraction linking contaminated dates to infection with hepatitis A virus. *Frontiers in Microbiology*, 11, 516445. 10.3389/fmicb.2020.516445. [PubMed: 33042044]
- Raymond P, Paul S, Perron A, Bellehumeur C, Larocque E, & Charest H (2022). Detection and sequencing of multiple human norovirus genotypes from imported frozen raspberries linked to outbreaks in the province of Quebec, Canada, in 2017. *Food and Environmental Virology*, 14(1), 40–58. 10.1007/s12560021-09507-8. [PubMed: 35066807]
- Rispens JR, Freeland A, Wittry B, Kramer A, Barclay L, Vinje J, Treffiletti A, & Houston K (2020). Notes from the field: multiple cruise ship outbreaks of norovirus associated with frozen fruits and berries – United States, 2019. *MMWR. Morbidity and Mortality Weekly Report*, 69(16), 501–502. 10.15585/mmwr.mm6916a3. [PubMed: 32324722]
- Sabrià A, Pintó RM, Bosch A, Bartolomé R, Cornejo T, Torner N, Martínez A, Simón M, Domínguez A, & Guix S (2016). Norovirus shedding among food and healthcare workers exposed to the virus in outbreak settings. *Journal of Clinical Virology*, 82, 119–125. 10.1016/j.jcv.2016.07.012. [PubMed: 27479175]
- Saupe AA, Rounds J, Sorenson A, Hedeén N, Bagstad E, Reinberg R, Wagley AG, Cebelinski E, & Smith K (2021). Outbreak of norovirus gastroenteritis associated with ice cream contaminated by frozen raspberries from ChinaMinnesota, United States, 2016. *Clinical Infectious Diseases*, 73(11), e3701–e3707. 10.1093/cid/ciaa821. [PubMed: 32564069]
- Somura Y, Nagano M, Kimoto K, Oda M, Mori K, Shinkai T, & Sadamasu K (2019). Detection of norovirus in food samples collected during suspected food-handler-involved foodborne outbreaks in Tokyo. *Letters in Applied Microbiology*, 69 (3), 175–180. 10.1111/lam.13189. [PubMed: 31220345]
- Stals A, Baert L, De Keuckelaere A, Van Coillie E, & Uyttendaele M (2011). Evaluation of a norovirus detection methodology for ready-to-eat foods. *International Journal of Food Microbiology*, 145(2–3), 420–425. 10.1016/j.ijfoodmicro.2011.01.013. [PubMed: 21333370]
- Tamura K, & Nei M (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10(3), 512–526. 10.1093/oxfordjournals.molbev.a040023. [PubMed: 8336541]
- Tatusov RL, Chhabra P, Diez-Valcarce M, Barclay L, Cannon JL, & Vinje J (2021). Human calicivirus typing tool: a web-based tool for genotyping human norovirus and sapovirus sequences. *Journal of Clinical Virology*, 134, 104718. 10.1016/j.jcv.2020.104718. [PubMed: 33360859]
- Teunis PF, Sukhrie FH, Vennema H, Bogerman J, Beersma MF, & Koopmans MP (2015). Shedding of norovirus in symptomatic and asymptomatic infections. *Epidemiology and Infection*, 143(8), 1710–1717. 10.1017/S095026881400274x. [PubMed: 25336060]
- Torok VA, Hodgson KR, Jolley J, Turnbull A, & McLeod C (2019). Estimating risk associated with human norovirus and hepatitis A virus in fresh Australian leafy greens and berries at retail. *International Journal of Food Microbiology*, 309, 108327. 10.1016/j.ijfoodmicro.2019.108327. [PubMed: 31493567]

- Van Dycke J, Ny A, Conceicao-Neto N, Maes J, Hosmillo M, Cuvry A, Goodfellow I, Nogueira TC, Verbeken E, Matthijssens J, de Witte P, Neyts J, & Rocha-Pereira J (2019). A robust human norovirus replication model in zebrafish larvae. *PLoS Pathogens*, 15(9), e1008009. [PubMed: 31536612]
- Vinje J (2015). Advances in laboratory methods for detection and typing of norovirus. *Journal of Clinical Microbiology*, 53(2), 373–381. 10.1128/JCM.01535-14. [PubMed: 24989606]
- Wales SQ, Kulka M, Keinard B, Ngo D, & Papafragkou E (2023). Use of Human Intestinal Enteroids for Recovery of Infectious Human Norovirus from Berries and Lettuce. *Foods*, 12(23), 4286. 10.3390/foods12234286. [PubMed: 38231763]
- Williams-Woods J, Rodriguez R, Marchant J, Swinford AG, & Burkhardt W (2022). Concentration, extraction and detection of enteric viruses from food. *Bacteriological Analytical Manual*. Food and Drug Administration.
- Yang Z, Kulka M, Yang Q, Papafragkou E, Yu C, Wales SQ, Ngo D, & Chen H (2024). Whole genome sequencing-based confirmatory methods on RT-qPCR results for detection of foodborne viruses in frozen berries. *Food and Environmental Virology*, 16(2), 225–240. 10.1007/s12560-024-09591-6. [PubMed: 38687458]
- Yang Z, Leonard SR, Mammel MK, Elkins CA, & Kulka M (2016). Towards next-generation sequencing analytics for foodborne RNA viruses: examining the effect of RNA input quantity and viral RNA purity. *Journal of Virological Methods*, 236, 221–230. 10.1016/j.jviromet.2016.07.013. [PubMed: 27435336]
- Yang Z, Mammel M, Papafragkou E, Hida K, Elkins CA, & Kulka M (2017). Application of next generation sequencing toward sensitive detection of enteric viruses isolated from celery samples as an example of produce. *International Journal of Food Microbiology*, 261, 73–81. 10.1016/j.ijfoodmicro.2017.07.021. [PubMed: 28992517]

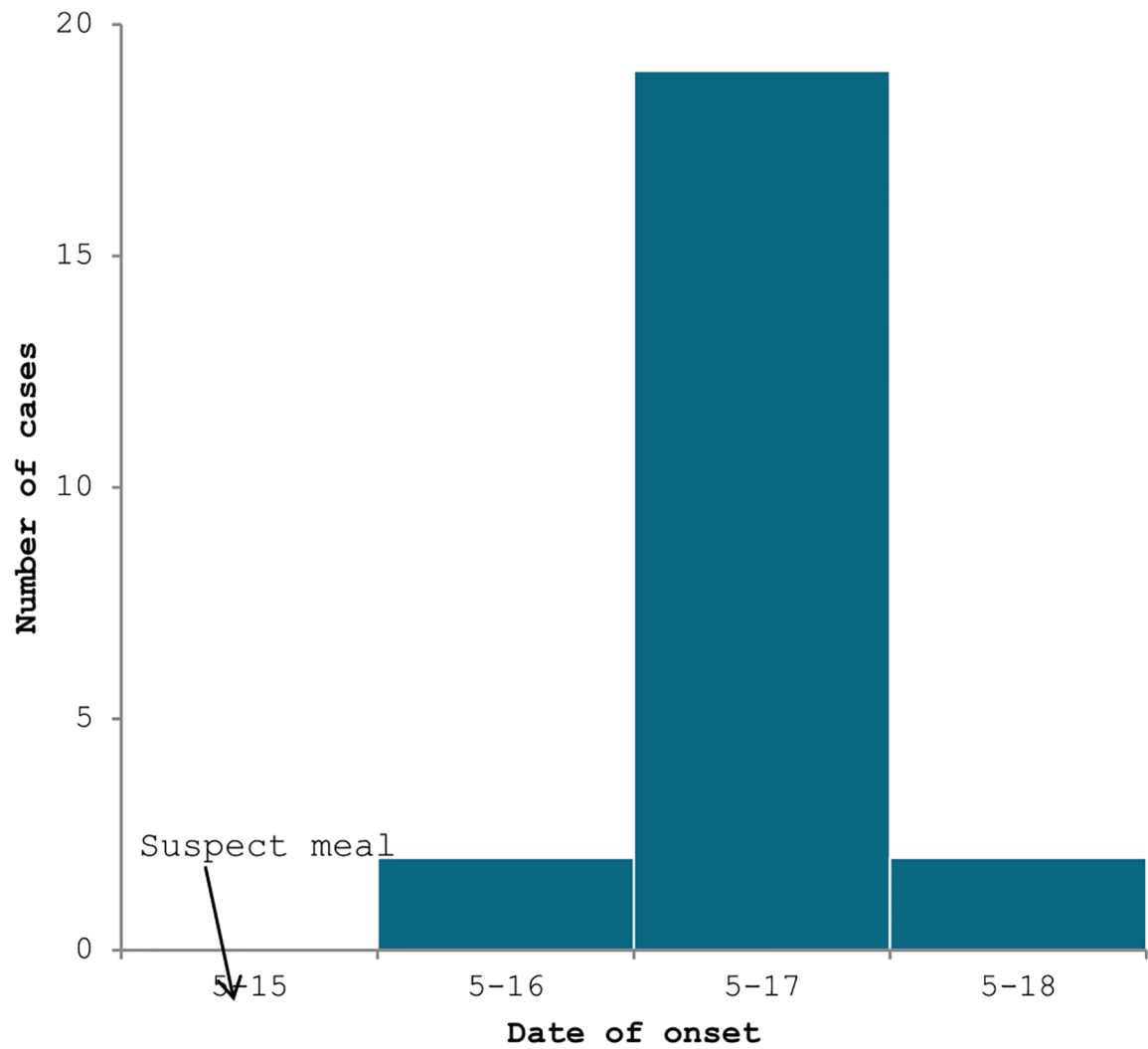


Figure 1. Epidemic curve of the acute gastroenteritis outbreak by date of onset in a wedding reception in Wisconsin in May 2015. The bar chart shows the numbers of acute gastroenteritis cases (y -axis) by date (x -axis).

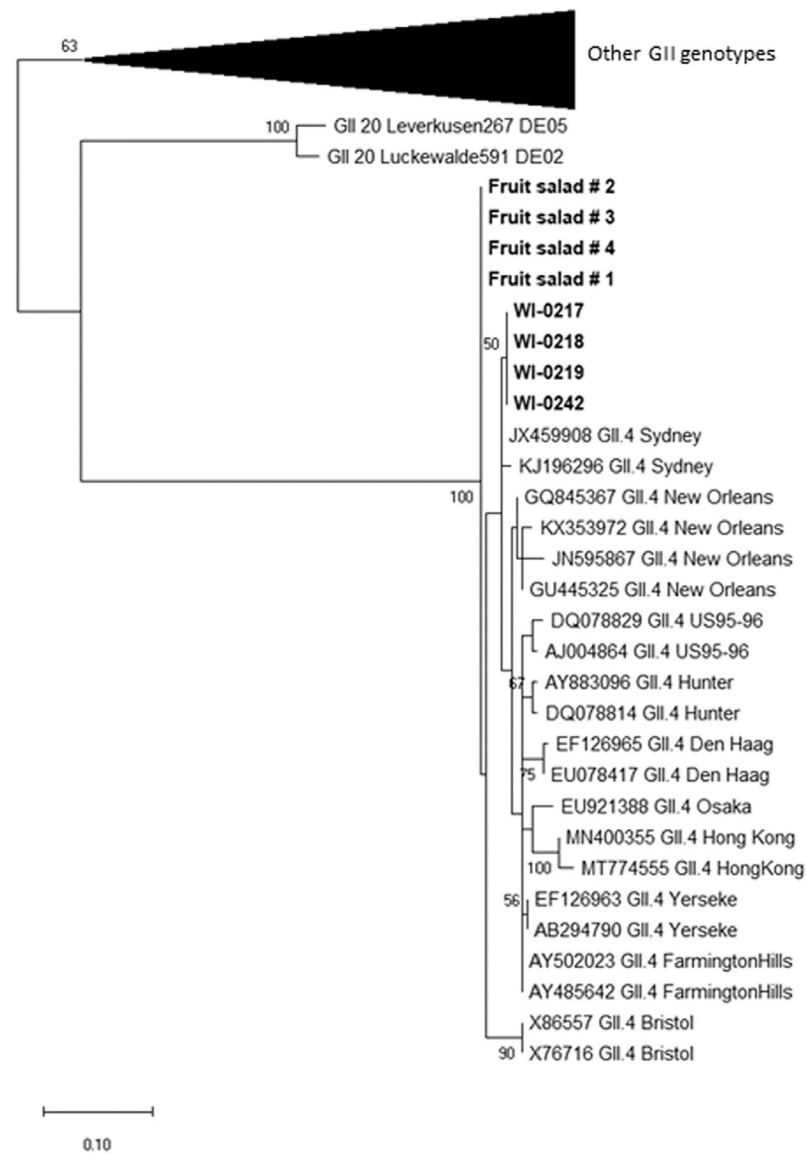


Figure 2.

Phylogenetic tree with partial norovirus sequences from food and clinical samples collected at an acute gastroenteritis outbreak at a wedding. The Phylogenetic trees of the genotyping region C (236 nt from 5' end of ORF2). The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.2640)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 236 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [2]. Scale bar represents nucleotide substitutions per site. Bootstrap (100) values are indicated at the nodes. Sequences from this study are in **bold**.

Table 1
Attack rates and risk ratios for food items consumed by guests attending a wedding in Wisconsin

Food item	Number who ate specified food			Number who did not eat specified food			Attack rate	Risk ratio	95% confidence interval
	Cases	Total	Attack rate	Cases	Total	Attack rate			
Beef tips	23	54	43%	0	2	0%	undefined	undefined	
Coleslaw	9	19	47%	12	35	34%	1.38	0.71	2.67
Corn	16	35	46%	7	20	35%	1.31	0.65	2.62
Dinner rolls	17	37	46%	2	13	15%	2.99	0.80	11.20
Fruit salad	20	38	53%	3	18	17%	3.16	1.08	9.26
Green beans	15	29	52%	7	25	28%	1.85	0.90	3.80
Mashed potatoes	21	50	42%	2	6	33%	1.26	0.39	4.09
Pasta	9	19	47%	11	33	33%	1.42	0.72	2.79
Carrot cake	14	20	70%	9	36	25%	2.80	1.48	5.28
Chocolate cake	11	28	39%	12	28	43%	0.92	0.49	1.72
Lemon poppyseed cake	7	9	78%	15	45	33%	2.33	1.36	4.01
Vanilla cake	6	15	40%	15	38	39%	1.01	0.49	2.11

Table 2

Detection of norovirus RNA by RT-qPCR in nucleic acid extracts from left-over fruit salad samples. (A) samples from opened container (B) samples from a sealed container. Values are presented as average $\pm$ standard deviation of Cycle threshold (Ct) values and genome copies/ 50 g of food tested

(A)		
Sample ID	RT-qPCR Ct values	RNA copies/50 g
1	30.6 $\pm$ 0.1	(9.7 $\pm$ 0.3)10 ³
2	38.5 $\pm$ 2.4	(6.5 $\pm$ 7.0)10 ¹
3	31.5 $\pm$ 0.2	(1.2 $\pm$ 0.1)10 ⁴
4	35.4 $\pm$ 0.3	(3.5 $\pm$ 0.7)10 ²
(B)		
Sample ID	RT-qPCR Ct values	RNA copies/50g
1	39.9 $\pm$ 0.4	(1.1 $\pm$ 0.3)10 ¹
2	34.9 $\pm$ 0.3	(4.3 $\pm$ 0.8)10 ²
3	34.1 $\pm$ 0.1	(2.3 $\pm$ 0.2)10 ³
4	34.5 $\pm$ 0.3	(1.7 $\pm$ 0.3)10 ³
5	34.7 $\pm$ 0.8	(1.7 $\pm$ 0.9)10 ³
6	40.3 $\pm$ 4.4	(5.0 $\pm$ 6.8)10 ¹
7	33.9 $\pm$ 0.2	(1.0 $\pm$ 0.1)10 ³
8	39.8 $\pm$ 2.8	(0.2 $\pm$ 4.1)10 ¹