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The Scenario of Norovirus Contamination in Food and Food Handlers

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Recently, many cases related to viral gastroenteritis outbreaks have been reported all over the world. Noroviruses are found to be leading as the major cause of outbreaks of acute gastroenteritis. Patients with acute gastroenteritis are normally found to be positive with norovirus when the stools and vomit are analyzed. This paper reviews various activities and previous reports that describe norovirus contamination in various food matrixes and the relationship between food handlers. Lately, a numbers of norovirus outbreaks have been reported that are involved with fresh produce (such as vegetables, fruits), shellfish, and prepared food. Food produce processed by infected food handlers may therefore become easily contaminated. In addition, foods that required much handling and had been eaten without heat treatment gave the high risk for getting foodborne illnesses. The standard method for detection of norovirus has already been available for stool samples. However, only a few methods for detection of norovirus in food samples have been developed until now.

Keywords: Norovirus, food, food handlers

Caliciviruses cause a variety of diseases in humans and animals [38] and are classified into four genera: Norovirus (NoV), Sapovirus (SaV), Vesivirus, and Lagovirus [40]. The noroviruses are single-stranded, positive-sense RNA viruses classified as the genus Norovirus. The norovirus genome is approximately 7.6 kb in length and comprises three open reading frames (ORFs). ORF 1 encodes the nonstructural polyproteins, which include the RNA helicase, protease, and polymerase (POL) proteins, ORF 2 encodes the major structural capsid protein, and ORF 3 encodes a small virion-associated protein [16].

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Noroviruses are genetically diverse and have been classified into genogroups based on sequence homology of the capsid gene. Of the five genogroups, viruses in GI, GII, and GIV are of the most significance to human health, as GIII and GV viruses have not been shown to infect humans. Virus strains in GI, GII, and GIV can be divided further into 8, 17, and 1 genetic clusters, respectively [95]

Noroviruses are the causative agents of viral gastroenteritis outbreaks worldwide [59, 74, 88] in all age groups and also cause sporadic cases of gastroenteritis [8, 32]. Primary human exposure usually results from the consumption of contaminated food or water [29]. Poor personal hygiene of infected food handlers is one of the major routes for viral contamination [35]. Despite the fact that human noroviruses remain uncultivable, the past decade has witnessed vast improvements in norovirus detection methods, surveillance, and awareness. Methods such as real-time RT–PCR have enabled rapid, broadly reactive, and highly sensitive screening [70].

Impact of Norovirus on Public Health

Noroviruses are recognized as the major cause of outbreaks of acute gastroenteritis and are common in settings such as hospitals, cruise ships, military and holiday camps, nursing homes, nurseries, hotels, and catered functions [6, 14, 22, 34, 65, 89, 92]. According to Vivancos *et al.* [93], norovirus outbreaks in Norfolk (East of England) are reported to the Health Protection Unit (HPU) throughout the year, with a peak in the winter months. The majority of cases occur in residential and nursing homes, and hospitals. More rarely, outbreaks have been reported in schools and food outlets.

These viruses are transmitted mainly *via* the fecal–oral route through person-to-person contact or consumption of contaminated food. Recently, norovirus has emerged as a significant etiologic agent and is recognized as the major cause of non-bacterial acute gastroenteritis in all age groups [38, 46]. In Europe, viral agents were responsible

for 10.2% of the foodborne outbreaks during 2006 and were pointed out as the second most common causative agent, after *Salmonella* [30]. Moreover, norovirus is the most common single foodborne agent in Sweden and causes 135,000–220,000 estimated cases of foodborne illnesses annually [58].

Mead *et al.* [64] reported that each year in the U.S.A., 67% of the foodborne acute gastroenteritis cases are attributed to viruses, representing the leading cause with an estimated 23 million cases occurring annually. Noroviruses, hepatitis A virus, rotavirus, astrovirus, and enteric adenoviruses were depicted as foodborne-associated viruses of which norovirus was by far the major causative agent [30, 64].

In Japan, noroviruses account for 28% of all cases of food poisoning and 99% of purely viral cases, which are attributed to the customs of food consumption [67]. Furthermore, strains belonging to the GII/4 cluster are recognized as the predominant genotype worldwide and, in a previous study, GII/4 strains accounted for 43.7% of the sporadic cases and 85.8% of the outbreaks [17]. In China, the GII/4 strains have continued to be the dominant strain identified in norovirus outbreaks [31].

Clinical norovirus infection generally has an incubation period of 24–48 h and is characterized by acute onset of nausea, vomiting, abdominal cramps, myalgias, and non-bloody diarrhea. Norovirus illness can present with relatively severe symptoms of vomiting and non-bloody diarrhea, with symptoms usually resolving in 2–3 days. Recent studies, however, have shown that the median duration of illness can be longer (*i.e.*, 4–6 days) in patients affected during hospital outbreaks and in children <11 years of age [73].

Consumption of even small numbers of norovirus particles can result in disease. The dose capable of causing infection has been estimated as approximately 10–100 viral particles, but consumption of only 1 virus particle in drinking water has also been reported to cause infection. Infected individuals produce large numbers of viruses in feces and vomit. The levels of viral particles shed in feces are 10^6–10^{11}/g, and approximately 10^7 potentially infectious doses can also be generated per vomiting incident [60]. According to Lindesmith *et al.* [58], the infectious dose of norovirus is estimated to be between 1 and 10 viral particles. Mark *et al.* [61] stated that the aerosolization of vomit presumably resulted in droplets contaminating surfaces of dish or cutlery and foods, which then were ingested. There is no drug or vaccine that exists to treat norovirus infections. This is partially a result of the absence of a robust tissue culture system [28, 87].

Food Handlers

There have been many reports regarding the monitoring of norovirus in facilities with outbreaks. However, little information is available about circulating viral strains in asymptomatic individuals in facilities without norovirus outbreaks [69]. Ozawa *et al.* [70] reported that the frequency of norovirus detection was 19% in outbreak facilities in Japan and that 73% of symptomatic food handlers and 7% of asymptomatic food handlers were positive for norovirus. They suggested that asymptomatic infections are common and contribute to the spread of the infection in areas of outbreak. Moreover, approximately 50% of foodborne norovirus outbreaks in the United States are linked to ill food handlers [94].

Norovirus outbreaks have been linked to fresh and frozen produce [75]. Such produce can become contaminated with noroviruses during either handling or preparation, often by infected food handlers, or at the source in the growing and harvesting areas [70]. According to Baert *et al.* [9], fresh produce and shellfish can therefore be considered as high-risk foods. At the post-harvest stage, infected food handlers not respecting hygienic regulations play a prominent role. The latter risk is actually associated with any food item handled manually that is not intended to be heated before consumption [63]. The source of infection in one outbreak was postulated to be a food handler who handled food 10 days after recovery. The overall public health consequences of prolonged viral shedding and asymptomatic infection and implications for outbreak control measures are unclear [73].

Ozawa *et al.* [70] concluded that norovirus infections were a common cause of gastroenteritis in the food-catering industry in Japan. Their results have also shown that asymptomatic infections with noroviruses, whether with a sequence identical to that infecting a symptomatic food handler or with a distinct sequence, were widespread in the food-catering industry. Much work is needed to curb the burden of this disease and reduce its transmission. A simple workplace policy that will protect ill workers and allow for paid leave may not be sufficient to stop transmission, since asymptomatic food handlers may continue to work.

Amar *et al.* [3] also indicated the occurrence of viral infection of intestinal disease in case patients as well as in asymptomatic individuals; however, quantification of the viral load may indicate a difference between symptomatic and asymptomatic individuals. Susceptibility to a particular genotype could differ among individuals, perhaps as a result of differences in innate and acquired immunities against norovirus. In the facility that they examined, norovirus outbreaks did not occur even though norovirus carriers were present. Several factors may contribute to this effect, such as pathogenicity of the virus, the number of individuals infected and the amounts of virus excreted, protective immune status of those exposed, and shut-off of infection routes [69].

Food Contamination

Noroviruses are commonly identified in foodborne outbreaks. Prepared foods, such as salads, sandwiches, and bakery items, are frequently associated with outbreaks of viral foodborne disease [35]. Butot *et al.* [18] reviewed outbreaks associated with salad vegetables and fruits in England and Wales between 1992 and 2000. Of 83 outbreaks, 13 (15.7%) were caused by noroviruses. Twenty-three outbreaks (28%) were caused by unknown agents, but in these the clinical and epidemiological features suggested that the majority were also caused by noroviruses. Twenty percent of these outbreaks were associated with fruits and vegetables. In Finland between 1998 and 2001, about 15 berry-related outbreaks were reported. This situation caused the ban on use of unheated berries in all catering and large-scale kitchens [18, 75]. Six norovirus outbreaks that involved up to 1,100 people in Europe were associated with the consumption of frozen berries imported from Poland [18]. In the U.S.A., noroviruses are associated with 40% of foodborne outbreaks and 96% of all reported outbreaks of viral gastroenteritis [41].

Typical food items implicated in norovirus outbreaks are raw or poorly cooked meat or seafood, such as shellfish, ready-to-eat food, and commodities such as fruits and vegetables, which are associated with a high risk of infection [12, 25, 40, 45, 54, 76–78] because they are consumed typically without additional heat treatment. However, norovirus contamination of food is demonstrated rarely during foodborne outbreaks, either because appropriate detection methods are lacking or because the culpable food samples are unavailable [24].

Viruses cannot grow on food and thus the contamination level cannot increase during processing or storage, but survival should be considered owing to a low infectious dose [20, 50]. Thus, introduction of noroviruses in a community (seeding) often results in secondary cases because of the highly infectious nature of these viruses, and can sometimes lead to an outbreak. Outbreaks associated with food consumption, particularly fresh fruits (or frozen fresh fruits), vegetables, oysters, and ready to eat food, are frequent [44, 55, 57].

Foodborne outbreaks have also been described in the literature, mainly associated with shellfish, frozen berries, and salads [93]. In addition, Carter [20] stated that fruits and vegetables can be contaminated at the pre-harvest stage through contact with fecally contaminated irrigation water or organic-based fertilizer in the field. Many types of fruit, such as grapes, raspberries, and strawberries, are increasingly recognized as the vehicles of many norovirus outbreaks [18, 43, 48].

Seafood has also contributed to norovirus outbreaks. The foods affected can be classified into two distinct groups based on the route of contamination: one group includes bivalve shellfishes such as oysters, which are contaminated with norovirus in their sea life [13, 23, 67, 68, 80, 90], and the other group includes various kinds of foods other than bivalve shellfishes, which are secondarily contaminated with norovirus from infected food handlers during food processing and/or food serving. Furthermore, there are many different ways in which food can become contaminated with viruses including by infected food handlers, contaminated food preparation surfaces, irrigation and fertilization of crops with animal and human waste, and sewage contamination of shellfish-growing waters [19, 50].

Of the norovirus outbreaks reported to the Centers for Disease Control and Prevention (CDC) between 2000 and 2004, 5% (n=184) were attributed to waterborne transmission. Noroviruses are thought to cause many waterborne outbreaks listed as being of unknown etiology, and individual outbreaks of norovirus have been linked directly to water as the vehicle of transmission through genomic sequence comparisons obtained from both clinical and environmental samples [5, 10, 72]. Furthermore, the genomic sequences of noroviruses have been detected in drinking water supplies [1, 33, 52].

The majority of foodborne infections originate from fecal–oral contact and transmission occurs in two ways. A primary contamination arises when food materials are already contaminated before they are harvested (*e.g.*, shellfish grown in contaminated waters or soft fruits irrigated or sprayed with contaminated water). A secondary contamination occurs at harvest or during processing and emphasizes the role of the food handler in food preparation for other individuals, concerning not only viral transfer from infected people, but also the use of polluted water or materials in processing [20, 50].

Method of Analysis

To date, cell culture has been used for the discovery of many viruses [27]; however, its use is limited by an inability to propagate many viruses in cell culture systems [14]. Because no readily available cell culture system exists for norovirus, the characterization and classification of norovirus is based on reverse transcription–polymerase chain reaction (RT–PCR), genomic sequencing, and phylogenetic analysis [95].

Study by Scherer *et al.* [81] shows that the higher recovery rate was detected from grape and oyster (both achieved 80%) by using the QIAamp viral RNA Mini Kit. Norovirus was not just being detected in food but also on the food surface. In order to detect norovirus on the food surface, the swab sampling method was used. This method was rapid and simple to perform and also can be applied to various food surfaces. The highest recovery rate of norovirus from food surfaces at both inoculum levels could be detected with cucumber (77.9±6.7% and 31.6±9.8% for 2×10^5 and 2×10^4 RT–PCRU, respectively) followed by apple (38.6±22.8% and 23.8±4.8%) and pepper (22.2±

15.9% and 20.5±19.2%). Minimum recovery was observed for ham (1.5±1.3% and 2.4±2.1%). Physical properties of the surface can influence and reduce the recovery rate because virus particle are trapped within the matrix, especially if the surface is porous and smooth surfaces can facilitate virus recovery. This explained the difference of recovery, which may be due to different abilities of viruses to adhere to the respective surface.

Park *et al.* [71] used immunomagnetic separation (IMS) to concentrate norovirus from fresh strawberry. Conventional RT–PCR and Taqman real time RT–PCR were used to detect norovirus. IMS combined with Taqman real time RT–PCR can be used to isolate and concentrate the virus and will efficiently detect low copy numbers of a viral genome present in contaminated food [71]. Table 1 shows that by using conventional RT–PCR, a 5% recovery rate was achieved for both GI and GII noroviruses. The mean recovery rate of GI (29.50%) was higher compared with GII (14.14%) by using IMS combined with Taqman real-time RT–PCR. These data demonstrate that IMS combined with real time Taqman RT–PCR is a useful method to detect norovirus in food. According to Myrmel and Rimstad [66], IMS has been applied for detecting various microorganisms in different environmental samples. The specificity and sensitivity of IMS methods rely on antibody specificity, incubation time and temperature, washing condition, and subsequent RT–PCR assays. The important advantage of using IMS methods is that the sample volume is reduced for the subsequent RT–PCR assay.

Many types of fruits (grapes, raspberries, and strawberries) are being identified as the vehicles of many norovirus outbreaks [18, 48]. Table 1 shows the recovery rates achieved for grapes and raspberry using five different viral RNA extraction methods. The highest recovery rate was achieved for grapes (80%) by using the QIAamp Viral RNA Mini kit, followed by the methods using heat release (14%), immunomagnetic separation (8.6%), Toyobo magnetic beads (1.8%), and TRIzol (0.4%). The recovery rate for raspberry was lower than those from grapes. The QIAamp Viral RNA Mini kit and heat release recovered 6% for both methods, followed by Toyobo magnetic beads (4.2%); Immunomagnetic separation (3%) and TRIzol achieved the lowest recovery rate (<0.3%). The factors that determine the usefulness of viral RNA extraction methods are their compatibility with prior elution and concentration steps and ability to remove inhibitors of RT–PCR. Of the extraction methods tested in this study, the best recovery rate was obtained with the QIAamp viral RNA Mini kit, followed by immunomagnetic separation [48].

Virus can contaminate berries and vegetables through contaminated surface water and through sewage. During harvesting, packaging, or food preparation, contamination by food handlers also can occur, where the viruses are likely to be on the food surface. Table 1 shows the that average recoveries for the different types of berries were calculated to be 6.8% (from 19.6% to 0.5%) and the average recoveries from the fresh vegetables were calculated to be 25% (from 46.3% to 9.5%) by using the QIAamp viral RNA Mini kit for extraction. Virus recovery with berry samples was low compared with that of vegetables. These data confirm the findings of Dubois *et al.* that a pH drop can inactivate viruses on the berry surface [18, 49].

Lettuce and cheese are recognized as sources of infection of norovirus gastroenteritis. Both are ready-to-eat foods and can be consumed raw in salads and sandwiches that can be contaminated by asymptomatic infected food handlers [84]. The recovery percentage detected ranged from 5.2% to 72.3% and 6% to 56.3% for lettuce and cheese samples by using the same extraction kit as used for berries. The results for recovery rate of norovirus suggest that a large number of viral particles in the fecal suspension used to seed the food samples could reduce the rate of norovirus recovery, mainly in cheese samples.

The viral gastroenteritis outbreaks linked to shellfish have been increasing but it is difficult to relate the shellfish consumption with the outbreaks using molecular techniques. This is because it is difficult to collect or analyze the specific food items implicated [26]. The recovery rate for oyster was detected after seeding the oyster suspension with 2.0×10^6 copies of GII RNA transcript. Table 1 shows that the recovery rate of oyster using the silica bead method is 0.175% (3.5×10^3 copies of RNA), which is lower than the amount seeded. The Qiagen RNeasy Mini Kit recovered 80% (estimated 1.6×10^6 copies) [36]. This result shows that the Qiagen RNeasy Mini Kit had a higher total recovery compared with the silica bead method (80% versus 0.175% correspondingly). Even though both extraction results are silica based, the extraction efficiency increased by using the Qiagen kit owing to its one-tube technology and less nucleic acid loss compared with silica bead. The Silica bead method undergoes several washing and centrifugation steps that can cause nucleic acid loss.

Discussion

Noroviruses have been found to be a leading cause of outbreaks of gastroenteritis worldwide. At present, norovirus genogroup II, genotype 4 (GII/4) strains are the most prevalent in many countries [70]. Seymour and Appleton [86] stated that fresh produce is reported to be a major vehicle in foodborne outbreaks. Raw and minimally processed produce and mixed salads are part of the ready-to-eat (RTE) group. Besides fruits and vegetables, RTE dishes, such as catered meals, are considered as a possible source of viral contamination owing to food handlers [6]. Vivancos *et al.* [93] reported that norovirus was detected in a fecal sample from a food handler who had prepared the salads

Table 1. Method for norovirus analysis in different types of food matrixes.

Samples	No. of ref.	Genogroup	Surface inoculated	Recovery rate	Extraction methods	Detection methods
Food surface						
Pepper		Genogroup II.3	10 cm^2	22.2±15.9%	QIAamp Viral RNA Mini Kit	
Cucumber	82	2.0×10^5 (RT	10 cm^2	77.9±6.7%	QIAamp Viral RNA Mini Kit	Real-Time RT–PCR
Apple		PCRU/10cm^2 n=3)	10 cm^2	38.6±22.8%	QIAamp Viral RNA Mini Kit	
Ham			10 cm^2	1.5±1.3%	QIAamp Viral RNA Mini Kit	
Pepper		Genogroup II.3	10 cm^2	20.5±19.2%	QIAamp Viral RNA Mini Kit	RT–PCR
Cucumber	82	2.0×10^5 RT	10 cm^2	31.6±9.8%	QIAamp Viral RNA Mini Kit	
Apple		(PCRU/10cm^2 n=3)	10 cm^2	23.8±4.8%	QIAamp Viral RNA Mini Kit	
Ham			10 cm^2	2.4±2.1%	QIAamp Viral RNA Mini Kit	
Fruits						
Strawberries (fresh)	72	4×10^3 to 10^4				IMS and
		Genogroup I.1	20 g	5%	QIAamp Viral RNA Mini Kit	conventional
		Genogroup II.4	20 g	5%	QIAamp Viral RNA Mini Kit	
Strawberries (fresh)	72	4×10^3 to 10^4				Real-Time (Taqman)
		Genogroup I.1	20 g	29.50%	QIAamp Viral RNA Mini Kit	RT–PCR
		Genogroup II.4	20 g	14.14%	QIAamp Viral RNA Mini Kit	
Grape	48	Genogroup II.4	20 g	80%	QIAamp Viral RNA Mini Kit	RT–PCR
				14%	Heat release	
				1.8%	Toyobo Magextractor	
				0.4%	Trizol reagent	
				8.6%	Immunomagnetic separation	
Raspberry	48	Genogroup II.4	20 g	6%	QIAamp Viral RNA Mini Kit	RT–PCR
				6%	Heat release	
				4.2%	Toyobo Magextractor	
				<0.3%	Trizol reagent	
				3%	Immunomagnetic separation	
Berries (strawberries, raspberries, blueberries, blackberries, blackcurrents	18	Genogroup I. Veletta strain	15 g	6.8% (from 19.6% to 0.5%)	QIAamp Viral RNA Mini Kit	Real-Time RT–PCR
Fresh vegetables (lettuce, green onions, mint, parsley, basil)	18	Genogroup I. Veletta strain	15 g	25% (from 46.3% to 9.5%)	QIAamp Viral RNA Mini Kit	Real-Time RT PCR
Lettuce	35	Genogroup II.4			QIAamp Viral RNA Mini Kit	Real-Time RT PCR
		$1913.24\times10^4 (10^0)$	15 g	6%	QIAamp Viral RNA Mini Kit	(Taqman)
		$180.27\times10^4 (10^{-1})$		9.1%	QIAamp Viral RNA Mini Kit	
		$4.45\times10^4 (10^{-2})$		56.3%	QIAamp Viral RNA Mini Kit	
		$0.95\times10^4 (10^{-3})$		-		
Cheese	35	Genogroup II.4				
		$1504.66\times10^4 (10^0)$	15 g	5.2	QIAamp Viral RNA Mini Kit	Real-Time RT PCR
		$168.25\times10^4 (10^{-1})$		8.3	QIAamp Viral RNA Mini Kit	(Taqman)
		$4.12\times10^4 (10^{-2})$		72.3	QIAamp Viral RNA Mini Kit	
		$0.89\times10^4 (10^{-3})$		53.7	QIAamp Viral RNA Mini Kit	
Oyster	36	Genogroup II RNA copies 2.0×10^6	4 oysters	3.5×10^3 copies (0.175%)	Silica bead	Real-Time RT PCR
				1.6×10^6 copies (80%)	Qiagen Rneasy Mini kit	Real-Time RT PCR

involved in an outbreak. However, the food handler claimed that she had no diarrhea and vomiting until later that day. Moreover, they were also able to identify the same norovirus genotype (GII-6) in both the outbreaks and the food handler who prepared the salads. Therefore, they hypothesized that the food handler was infected with norovirus and, because of mild or no symptoms at the time, inadvertently contaminated the salads during the preparation process.

After the introduction of an index case caused by contaminated food or a contaminated healthcares, worker or visitor, the propagation is usually explosive by the fecal–oral route or by contact transmission [91]. The advent of molecular diagnostic microbiological methods has allowed the detection of enteric viruses previously undetected through their inability to grow in conventional cell culture systems. Although viruses such as norovirus, sapovirus, and astrovirus were initially detected by electron microscopy, the concentration of virus present in clinical samples and the sensitivity of electron microscopy allowed only 30–50% of cases to be identified. The introduction of PCR and RT–PCR has improved the detection of enteric pathogens and 75% of cases are now identified [3, 14]. The diagnostic gap has been difficult to reduce even with continued increases in the sensitivity of detection of molecular methods [14].

Almost all of the food was extracted using extraction kits. Most researchers prefer to use the QIAamp Viral RNA Mini Kit to extract viral RNA. This Kit was chosen because of its sensitivity to recover the virus in food samples. This kit also shows higher recovery rates compared with other methods. According to Scherer *et al.* [81], methods for virus detection in food are mainly based on elution concentration, ultrafiltration or ultracentrifugation procedures, and combinations of these systems. These protocols are laborious and time consuming. The results of virus recovery also differ depending on the food matrices and the methods used [81]. Real-time RT–PCR is one of the molecular methods that are normally used to detect norovirus in foods. In detecting norovirus, elution buffers are normally necessary for eluting norovirus from food [40, 85]. Various PCR inhibitors that are present in elution buffers can interfere with successive molecular assays [71, 82]. In the past, investigations of shipboard outbreaks of viral gastroenteritis were limited by the lack of adequate molecular methods for detecting and characterizing viruses [15].

Conclusion

Based on the literature that we reviewed, we found that various types of food matrixes contributed to norovirus outbreaks. Food that is eaten raw without heat treatment contributed a high risk to these outbreaks. Everyone who is involved in the catering business, especially food handlers, should play their role strictly to prevent norovirus outbreaks. Many researchers concluded that there is a strong correlation between norovirus outbreaks and food handlers who prepared the foods. Therefore, minimal hands handling for preparation of foodstuffs can minimize the risk of bacterial and viral contaminations.

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