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Review

Critical Points of Food Contamination with Human Noroviruses*

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Abstract

Human noroviruses are identified as the leading cause of foodborne diseases in many countries worldwide. Since 2011, in the EU the number of outbreaks caused by viruses has more than doubled, while in the US they cause 5.5 million foodborne illnesses (58%) annually. Contributing factors are some of the characteristics of human norovirus like ability to replicate to high titers, low infectious dose, persistence and stability in the environment. Noroviruses are typically spread by the fecal–oral route and transmitted by contaminated food, water or aerosols, person-to-person contact, cross-contamination from surfaces and contact with fomites. Some critical issues for contamination of foods at risk are: (i) Contamination of water sources with enteric viruses, particularly those used for agricultural practices (crop irrigation, food processing). Food at risk are fresh produce like salads and berries and bivalve shellfish due to filter-feeding what enables the concentration of norovirus from polluted water in the digestive glands. (ii) Norovirus is highly infectious and could be shed in feces at very high numbers (up to 10^{11} particles/g) for prolonged times (more than 3 weeks), which indicates ease of spread by food handlers. Infected foods handlers are the source of 53% of norovirus foodborne outbreaks. Foods at risk are those that need extensive handling and ready-to-eat foods that do not undergo further processing. (iii) Human noroviruses are able to attach to inert surfaces and persist for up to 28 days on common food-preparation surfaces. Likewise is their high survival on finger pads and transfer to, for example, stainless steel surfaces and successively to foods.

Key words: Noroviruses, food contamination, food handler, food contact surfaces.

Резюме

Човешките норовируси са определени като водещи причинители на хранителни болести в световен мащаб. От 2011 броят на разразилите се вирози в ЕС е нараснал повече от два пъти, а в САЩ те причиняват над 5.5 милиона (58%) случая годишно. Характеристиките на човешките норовируси, които допринасят за заразата, са: достигането на висок титър при репликацията, ниска инфекциозна доза, устойчивост в заобикалящата среда. За норовирусите е типично фекално–оралното разпространяване и пренасянето със заразена храна, вода, аерозоли, междуличностни контакти, замърсени повърхности и извержения. Някои спорни въпроси за замърсяването на храните в риск са (i) замърсяване на водните източници с ентеритни вируси, особено водите, употребявани в земеделските практики (поливане, преработка). Храните в риск са свежи салати, ягодоплодни, миди (хранещи се чрез филтрация в замърсени с норовируси води (ii) норовирусът е високо инфекциозен и може да достигне в изпражненията голяма численост (над 1011 частици / g) за продължително време (повече от 3 седмици), което показва улеснено предаване при рабогещите с храни. Те са източника при 53% от случаите на норовирусни заболявани. Рисковите храни са тези, при които има потребност от екстензивна обработка, както и готовите за ядене храни, неизискващи по-нататъшна обработка. (iii) Човешките норовируси са способни да се прикрепят към инертни повърхности и да устояват до 28 дена по повърхността на приготвената по обикновен начин храна. По същия начин те се запазват по пръстите, от там по неръждаемата стоманени остриета и по-нататък по храните.

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Introduction

From at least 320,000 different viruses that infect mammals (Anthony *et al.*, 2013), human noroviruses (NoV) attract attention due to their identification as the leading cause of acute gastroenteritis (diarrhea and vomiting illness) worldwide. About one out of every five cases is caused by NoV, so globally; it is estimated to be the most common cause of acute gastroenteritis responsible for 685 million cases every year (Lopman *et al.*, 2015). In the European Union in 2014, foodborne viruses were, for the first time, identified as the most commonly detected causative agent in the reported foodborne outbreaks, followed by *Salmonella*, bacterial toxins and *Campylobacter* (EFSA, 2015) (Table 1).

Since 2011, the number of outbreaks caused by viruses has more than doubled (from 525 in 2011 to 1,072 in 2014). In the United States, from estimated 9.4 million foodborne illnesses annually, viruses are at the leading position causing 58% (5.5 million) (Scallan *et al*., 2011). Although many different viruses are responsible for viral gastroenteritis (adenoviruses, coronaviruses, rotaviruses, parvoviruses and astroviruses), noroviruses are recognized as the main cause of epidemic and sporadic foodborne gastroenteritis, and represent a substantial public health burden (Head and Lopman, 2016).

Clinical symptoms of acute NoV-associated gastroenteritis are characterized by the sudden onset of vomiting, watery diarrhea, or both. Additional symptoms include nausea, abdominal cramping and pain, malaise, anorexia, fever, chills, headache, and myalgias. After an incubation period of 10 – 51 h, there is an acute onset of symptoms which usually resolve in 1– 3 days. The human NoV replicate in the intestines of the infected person and are excreted via faeces and vomit, starting during the incubation period and lasting up to 3 weeks and longer. In persons who had been experimentally infected with NoV, virus shedding was first detected by RT-PCR 18 hours after inoculation and lasted a median of 28 days (range 13–56 days) (Atmar *et al*., 2008). NoV shed at high level in faeces (median 95×10^9 genomic copies/g) and vomit (10^9 genomic copies per single episode) are highly contagious (Teunis *et al*., 2008), resulting in a high rate of transmission to contacts, partially due to very low infectious dose. The 50% human infectious dose (HID50) is estimated to be in the range from 18 to 1,015 genome equivalents, though novel estimation is that the HID50 is more similar to those of other RNA viruses (1,320 to 2,800 particles) (Atmar *et al.,* 2014).

Gastroenteritis caused by the NoV is generally acute and self-limited; however NoV have been associated with severe clinical features other than gastroenteritis, including acute renal failure, arrhythmia and signs of acute graft organ rejection in renal transplant patients (Mattner *et* al., 2006). The most important at-risk groups are infants, elderly, and immunosuppressed persons since they are more susceptible to complications due to dehydration. Children aged < 5 years are at particular risk of foodborne diarrheal diseases, with 220 million illnesses and 96,000 deaths every year. The incidence of NoV among them is about 4 times higher than for children aged ≥ 5 years (O'Brien *et al*., 2016).

Noroviruses belong to the *Caliciviridae* family, which is composed of five genera, of which genera *Norovirus* and *Sapovirus* contain primarily human viruses, while the other genera contain animal viruses (Clark *et al*., 2012). Human noroviruses are non-enveloped RNA viruses, approximately 27 to 38 nm in diameter, have an icosahedral shape and contain single-stranded positive-sense RNA genomes ranging in size from 7.4 to 8.3 kb with 3 open reading frames (ORF1, ORF2, and ORF3). ORF1 encodes a polyprotein that is cleaved into seven nonstructural mature proteins (NS1 to NS7) that are involved in viral replication. ORF2 encodes the major structural protein (VP1) of approximately 60 kDa, and ORF3 encodes a minor structural protein (VP2). Noroviruses are genetically classified into 6 recognized genogroups (GI to GVI), while tentative genogroup VII is proposed by Vinjé (2015). GI and GII viruses are responsible for the majority of disease in humans, particularly GII.4 genotype which is the most detected genotype in NoV outbreaks. In NoV epidemiology, it is common the emergence of new GII.4 variant that replace previously predominant ones (Radin, 2013). Since 1995, new epidemic variants of GII.4 have emerged every two to three years. However, in the winter of 2014/15, a novel GII.P17-GII.17 NoV strain (GII.17 Kawasaki, 2014) emerged, as a major cause of gastroenteritis outbreaks in China and Japan, and have replaced the previously dominant GII.4 genotype Sydney 2012 variant in some areas in Asia (de Graaf *et al*., 2015).

Norovirus transmission and food contamination

Gastroenteritis viruses are typically spread by the faecal–oral route. Human NoV is transmitted indirectly by contaminated food or water, di-

Table 1. Overview of pathogens, causes of confirmed foodborne outbreaks in some countries

	US (Scallan *et al.*, 2011)	EU (EFSA, 2015)	Canada (GC, 2016)	UK (FSA, 2014)	New Zealand (Cressey and Lake, 2011)
1.	Norovirus	Viruses NoV and HAV	Norovirus	*Campylobacter*	Norovirus
2.	*Salmonella*	*Salmonella*	*Clostridium perfringens*	*Clostridium perfringens*	*Campylobacter*
3.	*Clostridium perfringens*	Bacterial toxins	*Campylobacter*	Norovirus	*Clostridium perfringens*
4.	*Campylobacter*	*Campylobacter*	*Salmonella*	*Salmonella*	*Yersinia enterocolitica*

Table 2. Sources of food contamination with viruses

Point of food contamination	Sources of viruses
Pre-harvest	Fecal pollution / water for irrigation or growing Contaminated water used for pesticide preparation Organic fertilizers Root intake of pathogen and plant internalization
Harvest	Contact with human feces Cross-contamination with equipment Poor hygiene of workers
Post-harvest	Hands of infected food handlers Contaminated water Cross-contamination during washing Cross-contamination from food contact surfaces and equipment
Sale/Consumption	Hands of infected food handlers Cross-contamination from food contact surfaces and equipment

rectly through person-to-person contact, and by cross-contamination from surfaces. Foods at risk include those which need extensive handling, mostly ready-to-eat foods that do not undergo further processing, and those exposed to environmental contamination, such as seafood and fresh produce (Radin, 2016). Contamination with human NoV can happen throughout the entire food production chain: at the pre-harvest stage (primary production), during harvest, at the post-harvest stage by inappropriate practices during handling, processing, preparation, storage, distribution and at the point of sale/consumption (Radin, 2014) (Table 2).

Filter-feeding shellfish is the most common food contaminated at source (Table 3), but a wide range of different cooked and fresh foods take part in secondary contamination (Table 4).

As already mentioned, one of the well-established vehicles of transmission and principal source of food-borne virus in outbreaks are bivalve shellfish such as oysters, mussels, clams and cockles responsible for large, occasionally international, outbreaks (Le Guyader *et al*., 2006). There are several reasons why shellfish are at risk, but primarily due to their natural habitat and filter-feeding large volumes of water (up to 24 l of water/h) that enables the accumulation and concentration of norovirus particles from polluted water in the digestive glands. Their lifestyle leads to virus concentrations which can be hundreds or even thousands times higher than that in the surrounding water. It was determined that 76% of samples of oysters analyzed at harvest from UK harvesting areas over a two-year period tested positive for norovirus (CEFAS,

Table 3. Rapid Alert System for Food and Feed: Virus alerts in Europe 2015 – April, 2017

Year	2015	2016	2017
# virus alerts	15	13	14
Virus type			
NoV	100 %	69%	93%
HAV	-	31%	7%
Product type			
Strawberries	7%	-	22%
Raspberries	20%	16%	7%
Blueberries	7%	-	-
Spinach	-	-	15%
Dried tomato	-	-	7%
Oysters	26%	46%	35%
Clams	40%	38%	7%
Mussel	-	-	7%

2014). Another very important safety point is that people in their diet frequently consume uncooked or only with a light heat treatment prepared shellfish, which is not sufficient to guarantee virus inactivation. For commercial harvesting, some measures like the depuration process, offer good protection against harmful bacteria such as *E. coli*, but are not efficient in the elimination of viruses from live shellfish.

Generally, contamination of water sources with enteric viruses is a common event to some extent due to the fact that current wastewater treatments do not guarantee complete virus removal (Blatchley, 2007); therefore, non-enveloped RNA or DNA enteric viruses become environmental contaminants and a public health concern. Water-related diseases are associated not only with drinking and recreational water but also with those used for agricultural practices such as crop irrigation and food processing, which may result in foodborne outbreaks (Li, 2012). Index viruses of human or animal fecal contamination (human and porcine AdV, bovine PyV), and human pathogenic viruses (HAV, HEV, and NoV GI/GII) were found in irrigation

Table 4. Examples of confirmed norovirus foodborne outbreaks in the US reported by FOOD tool (CDC, 2016)

Year	Food Vehicle	No. of outbreaks	No. of illness	Place of outbreak
2014	Salad/carrots, cucumber, green peppers, tomato	1	44	Restaurant
	Guacamole, sour cream, sauces	2	429	
	Macaroni salad, Caesar salad, breads	2	133	
	Potato salad Green beans	2	97	Banquet Facility
	Sandwiches	1	40	Caterer
2015	Chips, rolls, pastries	1	86	Banquet Facility
	Salad/ Lettuce	1	48	
	Strawberry sauce	1	123	
	Cheese and crackers, fruit, green beans	1	170	
	Cupcakes	1	170	Hospital
	Sandwiches	1	54	School/college
	Cake frosting/icing	1	73	Private home

Table 5. Norovirus transfer efficiency (%)

Donor	Recipient						Reference
	Lettuce	Deli meat	Stainless steel	Blueberries, grapes		Raspberries	
				dry conditions	wet conditions	wet conditions	
Solid surfaces	0-26	55-95					Escudero *et al.,* 2012
Hands			58-60	4-12	20-70	4	Sharps *et al.,* 2012
Stainless steel to hands				2-11	1-50		

water samples from the leafy green vegetables and berry fruit production chain (Kokkinos *et al*., 2017). Foodborne disease outbreaks associated with fresh fruits and vegetables are widespread in the European Union and United States, with NoV and *Salmonella* as the most common pathogens. Noroviruses are primarily linked with the consumption of salad in the US and of berries in the EU (Callejon *et* al., 2015). In the production of fresh fruits and vegetables even contaminated water used to dilute pesticides could be a source of human noroviruses, since their infectivity was unaffected when combined with diluted pesticide (Verhaelen *et al*., 2013a). Moreover, recent studies have reported internalization and transport of enteric viruses in lettuce plants cultivated hydroponically or during irrigation (Wei, 2011). These findings indicate a possible route of contamination by uptake of the virus through the root system and subsequent transport of the virus into edible portions of the plant via the vascular tissue.

Food contact surfaces

Essentially, all foodborne viruses are shed with feces and vomit, therefore easily transmitted from person to person, via food, water and different surfaces (e.g. stainless steel). Human NoV is able to attach to inert surfaces, vegetal and other food matrices, as well as hands. Moreover, the survival on finger pads is high and its transfer from hand to stainless steel surfaces and from stainless steel surfaces to vegetable surface could occur relatively easily (Table 5).

Furthermore, it has been demonstrated that viruses are transferred at different levels from gloved fingertips to produce. Viruses are more easily transferred from fingertips to lettuce as compared to fingertips and soft berries (Verhaelen *et al*., 2013b). Not surprisingly, infected food handlers are the source of 53% of NoV foodborne outbreaks and may have contributed even to 82% of outbreaks (Hall *et al.*, 2012). A very important point is that viruses maintain the infectivity for 28 days at room temperature on almost all food contact surfaces (ceramic, wood, rubber, glass, stainless steel, plastic) which are commonly used (Bae *et al*., 2014). To assess the cross-contamination with equipment, virus transfer during slicing deli meat type bologna, as a model of ready-to-eat foods, the knife blade was artificially contaminated with NoV particles. For bologna deli, the first two slices contained initial NoV level, while transfer to slice 3-12 occurred with approx. 50% reduction per slice (Radin and Velebit, 2015).

It is obvious that cleaning followed by disinfection is of top priority. A very good example of significance of disinfection in reducing the presence NoV particles on surfaces is the outbreak that happened in a summer camp (Soule, 2014). The timetable of the events was as follows: July 5 - three people were sick, July 6 - 38 people reported sick; July 21 and 22 - 40% of the surfaces tested positive for NoV; July 30 - surface cleaning with soap and water; Aug. 1 - 73% of surfaces positive for NoV; Aug. 14 - surface cleaning followed by disinfection and on Aug.15 - 33% of surfaces positive for NoV.

Chemical disinfection of food-contact surfaces and rinsing food items with sanitizers is currently applied in order to prevent food-borne outbreaks. Among different household disinfectants, sodium hypochlorite (3%) was the most effective for viral inactivation from food contact surfaces (Girard, 2010). Treatment with hydrogen peroxide (200-1000 ppm), quaternary ammonium compounds (100-2000 ppm), iodine (25-500 ppm), and 10-70% ethanol exhibited no disinfection effect (Ha *et al*., 2016). Enteric viruses are highly resistant to many biocides commonly used on fresh produce so washing with tap water and chlorine solution (200 ppm) provides on average 1log reductions in virus titer. Enhanced efficiency in removing a norovirus surrogate from produce (strawberries, raspberries, lettuce and cabbage) is achieved by combining chlorine with surfactants like sodium dodecyl sulfate, Nonidet P-40, Triton X-100, and polysorbates (Predmore and Li, 2011).

A chemical treatment of foods (when applicable, e.g. fresh cut produce) with e.g. chlorine, in order to inactivate viruses is well established. However, due to the possible bad impact on the environment and production of chlorine health risk by-products (Meireles *et al*., 2016) and the fact that some European countries (Germany, Switzerland, Belgium, Netherlands, Denmark, United Kingdom) introduced a ban on the use of hypochlorite in the fresh cut industry, food industry is looking for potential alternatives. One of the candidates is ozone, a strong oxidizer, like chlorine. Ozone is environmentally friendly and can inactivate a wide range of foodborne pathogens, including viruses, bacteria, and protozoa. It can be effective in the gaseous state or dispersed in water, and does not form residues or by-products. The efficacy of ozone was tested on viruses contaminating seeds intended for production of sprouts (Wang *et* al., 2015). Biological alternative disinfection methods include bacteriocins, bacteriophages, enzymes and phytochemical natural antiviral compounds (Radin, 2017).

Conclusion

Viruses are transmitted by contaminated food or water, through person-to-person contact, and by cross-contamination from surfaces. Foods at risk includes those which need extensive handling, mostly ready-to-eat foods that do not undergo further processing, and those exposed to environmental contamination, such as seafood and fresh produce. Strategies to reduce human noroviruses in the food chain should, beside good hygiene practice, engage interventions to prevent their adsorption to food/contact surfaces and inactivation on/within the products using successful processing techniques.

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