

HUMAN NOROVIRUS TRANSMISSION DUE TO CONTAMINATED FRESH FRUIT AND VEGETABLES

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Abstract - Almost any ready-to-eat fruit or vegetable that has been contaminated with pathogens, either from the environment, human or animal feces or through storage, processing and handling, could potentially cause disease. This problem is particularly associated with the occurrence of human intestinal viruses, especially noroviruses, which are of major epidemiological significance as a common cause of both epidemic and sporadic non-bacterial gastroenteritis in humans. Many outbreaks of viral gastroenteritis associated with fresh fruit and vegetables have been described. The rise in incidence of human norovirus outbreaks may be the result of increased consumption of fresh produce, availability of new commodities, export/import and transport around the globe, changes in production practices, improved reporting and detection methods.

Key words: Noroviruses, transmission, fruit, vegetables, gastroenteritis

INTRODUCTION

In recent years, it has become clear that noroviruses (NoVs) represent a significant cause of gastroenteritis in humans, in fact, they are among the most important causes of gastroenteritis in all age groups. Human NoVs are a primary cause of viral gastroenteritis throughout the world, and the principle cause of foodborne illness in Europe (Phillips et al., 2010) and the United States (Scallan et al., 2011). The overall burden of disease was estimated using the disability adjusted life year (DALY), which is a time-based measure that combines years of life lost due to premature mortality and years of life lost due to time lived in disability or states of less than full health (Murray and Lopez, 1996). The burden from diarrheal diseases is 72.8 million DALY and constitutes the second leading cause of burden of disease in the world (WHO, 2004).

Diarrheal diseases, of which a considerable proportion is foodborne, kill 2.2 million people globally every year (WHO, 2007). It is estimated that each year human NoVs alone cause 64,000 episodes of diarrhea requiring hospitalization and 900,000 clinical visits among children in industrialized countries, and up to 200,000 deaths of children under 5 years of age in developing countries (Patel et al., 2008). The WHO international review calculated the burden of acute gastroenteritis, foodborne diseases and pathogens commonly transmitted by food, and revealed that 20% of the population in England each year experience acute gastroenteritis with the NoV as the etiological agent; in the Netherlands, with 4.5 million cases of acute gastroenteritis, the most common pathogen at the community level was NoV; in Australia, foodborne transmission accounts for ~32% of a total 17.2 million cases per year of gastroenteritis; in Canada, from tested stool specimens

collected from gastroenteritis outbreaks, 19% were positive for NoV (Flint et al., 2005). A recent publication from the Centers for Disease Control and Prevention (CDC, Atlanta, USA) is the first comprehensive estimation since 1999 on foodborne illness acquired in the US and caused solely by foods eaten domestically (Scallan et al., 2011); revised episodes of foodborne illness caused by NoVs are estimated at 5.5 million annually.

The virus

Among human enteric viruses, NoVs are of a major epidemiological significance as a common cause of both epidemic and sporadic non-bacterial gastroenteritis in humans. They belong to the genus *Norovirus*, and together with four other genera (*Vesivirus*, *Lagovirus*, *Sapovirus* and *Nebovirus*) are members of the family *Caliciviridae* (ICTV, 2012). Virions are non-enveloped with icosahedral symmetry, and have a single-stranded, positive sense RNA genome of 7.4-8.3 kb. The genome is organized into three major open reading frames (ORFs): ORF1 encodes the non-structural polyprotein; ORF2 encodes the major structural capsid protein (VP1) and ORF3 encodes a small virion-associated protein (VP2).

Currently, the genus *Norovirus* consists of five genogroups (labeled GI to GV), which can differ as much as 40% with regard to the amino acid composition of the major capsid protein (VP1) (Zheng et al., 2006). Human NoV strains cluster within genogroups GI, GII, and GIV; however, two genogroups, GI and GII, are most commonly associated with enteric disease in humans. Each genogroup is further divided into genotypes defined by strains with a higher level of homology across the VP1 coding region (Zheng et al., 2006). NoV Genogroup GI contains 8 and GII 19 different genotypes (ICTV, 2012) that account for most human NoV illness cases.

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 myal-

gia. The incubation periods range from 1-3 days, and the virus is shed via stools and vomit, starting during the incubation period and lasting up to 10 days and even longer. NoV infections are highly contagious, resulting in a high rate of transmission to contacts, partially due to the very low infectious dose that is estimated to be 10-100 infectious viral particles (Sair et al., 2002).

Transmission of Norovirus

NoVs are easily transmitted from person to person through the fecal-oral route, either directly or indirectly via contaminated surfaces, food or water and aerosols of vomit. Food is a frequent vehicle for human NoV transmission through contamination with human fecal material that may have occurred at any step during production (Gerba and Kayed, 2003; Atmar, 2010), poor personal hygiene of food workers and virus survival on/in food and the environment (Rzezutka and Cook, 2004). Once present in the environment, human NoV, being a non-enveloped virus (a characteristic that has an effect on survival), has higher resistance to drying or desiccation methods. Therefore, they are thought to spread more easily than enveloped viruses, which are less stable in the environment (Vasickova et al., 2010). According to data reported through the Foodborne Viruses network in Europe for over 10,000 viral gastroenteritis outbreaks between 2001 and 2007, the proportion of food-borne outbreaks varied greatly between countries. Using predictive models, foodborne outbreaks were estimated to be about 50% (Verhoef et al., 2009). A similar estimation has been reported in the USA, where 40 to 57% of all NoV outbreaks were foodborne (Fankhauser et al., 2002).

Many food items have been associated with NoV outbreaks. In addition to filter-feeding shellfish, a well-known source of foodborne viral infections, many other foods, such as desserts, fruits, vegetables, salads, deli meat, sandwiches, etc. have been implicated. However, it is important to keep in mind that any food that has been handled manually and not sufficiently heated may be a source of infection. Raw and minimally processed fruits and salad vegetables

Table 1. Foodborne viral outbreaks related to fruit and vegetables

Product	Year	Organism	No. of cases	Country	Reference
Green salad	1979	NoV	63	USA	Tauxe et al. 1997
Melon	1987	NoV	-		
Raspberries	1988	NoV	108	Finland	Ponka et al. 1999
Lettuce	1988	HAV	202		
Celery	1991	NoV	1440	USA	Tauxe et al. 1997
Strawberries	1992	HAV	28		
Tomatoes	1994	HAV	-		
Raspberries	1997	NoV	300	Canada	Gaulin et al. 1999
Strawberries	1997	HAV	153	USA	CDC, 1997
Strawberries	1997	HAV	126	USA	Hutin et al. 1999
Green onions	1998	HAV	43	USA	Dentinger et al. 2001
Carrot, tomato	2000	NoV	-	UK	ACMSE, 2000
Raspberries	2001	NoV	30	Sweden	Le Guyader et al. 2004
Rucola lettuce	2001	HAV	80	Sweden	Nygaard et al. 2001
Blueberries	2002	HAV	39	New Zealand	Calder et al. 2003
Green onions	2003	HAV	600	USA	Wheeler et al. 2005
Raspberries	2005	NoV	75	France	Cotterelle et al. 2005
Raspberries	2005	NoV	276	Denmark	Korsager et al. 2005
Raspberries	2005	NoV	6 outbreaks/1,043	Denmark	Falkenhorst et al. 2005
Raspberries	2006	NoV	4 outbreaks/43	Sweden	Hjertqvist et al. 2006
Tomatoes	2007	NoV	400	Sweden	Zomer et al. 2010
Raspberries	2009	NoV	200	Finland	Maunula et al. 2009
Lettuce	2010	NoV	260	Denmark	Ethelberg et al. 2010
Green/Salad		NoV	139 outbreaks/5,139		
Lettuce/Veg	1998-2005	NoV	42 outbreaks/1,470	USA	DeWall and Bhuiya,
Fruit/Salad		NoV	30 outbreaks/1,502		(CSPI, 2007)

are typically consumed in a ready-to-use or ready-to-eat form, and rarely undergo any heat processing prior to consumption. Lately, an increased number of foodborne NoV outbreaks have been linked to fruits and vegetables (Table 1). In the USA, the proportion of all foodborne outbreaks associated with raw produce increased from 0.7% in the 1970s to 6% in the 1990s, while outbreak-associated illnesses accounted for by fresh produce increased from <1% to 12% (Sivapalasingam et al., 2004). Between 1990 and 2005, fresh produce outbreaks caused more illnesses on average than beef, poultry and seafood, with human NoVs, as the major cause of fresh produce-associated illnesses, accounting for more than 40% of all outbreaks (DeWall and Bhuiya, 2007).

The Center for Science in the Public Interest (CSPI, Washington DC) that maintains a database of only those foodborne illnesses outbreaks with an

identified etiology and food vehicle, revealed the most common food-pathogen combinations are green-based salads and lettuce contaminated with human NoV (DeWall and Bhuiya, 2007). The CDC has recognized that leafy vegetables are among the top three single commodity items associated with human NoV foodborne outbreaks (MMWR, 2009, 2010). Therefore, the expert scientific advice by the Codex Alimentarius indicates that human NoV and hepatitis A virus in bivalve mollusk shellfish, fresh produce or prepared foods are among the virus-commodity combinations for which prevention and control measures should be considered.

A good example is Finland, where out of a total 117 outbreaks reported as food- or waterborne, 55% were NoV positive (Maunula et al., 1999). Among them, 15 outbreaks were related to imported frozen berries, mainly raspberries. A recommendation

for all catering and other large-scale kitchens not to serve unheated frozen berries was implemented (Ponka et al., 1999).

Fresh produce are considered high-risk food commodities for human NoV contamination. They can become contaminated whilst growing in fields at the pre-harvest stage through contact with fecally contaminated irrigation water or organic-based fertilizers (Carter, 2005), as well as during harvest or at the post-harvest stage (handling, processing, storage, distribution, preparation and use), where infected food handlers who do not follow proper/adequate hygienic practices, play an important role (Baert et al., 2009). These data are supported by the results of virus persistence on finger pads and food preparation surfaces, which can act as vehicles for human NoV transmission long after the initial contamination event has occurred (D'Souza et al., 2006; Liu et al., 2009; Leon-Felix et al., 2010).

Recent studies have reported internalization and transport of enteric viruses in lettuce plants grown under hydroponic conditions or during irrigation (Urbanucci et al., 2009; Wei et al., 2011). These findings indicate another 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. Therefore, the identification of HAV inside green onion tissues by Chancellor et al. (2006) and the conclusion that simple washing of the surface of a food may be insufficient to identify viruses responsible for outbreaks of disease, should be taken seriously.

The rise in incidences of human NoV outbreaks may be the result of increased consumption of fresh produce, with the consumer demand for fresh produce year around, and their export/import and transport around the globe (Lynch et al., 2009). The FAO statistical database (FAOSTAT) confirms that global fruit and vegetable consumption increased by an average of 4.5% per annum between 1990 and 2004. Other factors that contribute to the increase of foodborne illnesses associated with fruit and vegetables include availability of new commodities, changes in

production practices, improved reporting and detection methods, etc.

Detection of the Norovirus in fresh fruits and vegetables

The detection of foodborne NoVs in fresh produce has become increasingly important because of the number of outbreaks being reported. Currently, in the absence of available cell-culture-based systems for human NoVs, detection using molecular techniques remains the method of choice. Therefore, the detection of NoV relies on molecular methods, with (real-time) reverse transcription (RT)-PCR considered the gold standard due to its high sensitivity and specificity. Generally, efficient virus detection methods present several challenges, such as the typically low viral load in fruits and vegetables and the presence of food components and substances that are able to inhibit the molecular assays used for the detection and quantification of viral genomic material. As mentioned earlier, NoVs have a very low infectious dose, so a sensitive method should be sensitive enough to detect a single viral particle per sample (Radin, 2011; Radin and D'Souza, 2011a, b). Furthermore, as NoVs are genetically extremely heterogeneous, the challenge remains in detecting so many diverse strains of human NoV genogroups, and this complicates the design of protocols to detect all strain variants. For these reasons, it is obvious that the detection methods must be very sensitive as well as specific.

Generally, the detection of viral pathogens from food samples comprises several consecutive steps such as (1) sample preparation in order to separate and concentrate the agents from the food matrix; (2) nucleic acid extraction and purification; (3) detection (in this case mainly using a molecular approach) and confirmation or typing. It is difficult to generalize due to the large differences in composition between soft fruit and hard surface vegetables. Nevertheless, the methods that have been developed and optimized for virus detection in fruit and salad vegetables focus on elution of the virus from the surface. A number of washing procedures and buffer systems has been implemented, as well as rinsing with thiocyanate com-

pounds, which directly release viral RNA from the virus particle (Baert et al., 2008; Stals et al., 2011a, 2012). After elution, the viruses must be concentrated by one of the frequently used concentration methods such as precipitation by PEG, ultrafiltration, and ultracentrifugation (Rutjes et al., 2006). Subsequent to virus elution or concentration, a variety of nucleic acid extraction and purification protocols may be employed, followed by the detection of viral genomes by molecular amplification techniques.

CONCLUSION

Fresh produce contributes to the transmission of NoVs infections. Foodborne outbreaks related to fruit and vegetable products are probably mainly transmitted via two routes. These products can be contaminated by pre-harvest manipulation (contaminated irrigation water) or by post-harvest contamination (infected food handlers, contaminated equipment, and process water used).

Viruses can be detected in fresh produce, but prevalence studies are limited, and quantitative data on viral load are insufficient for the establishment of microbiological criteria (food safety criteria) for these food categories. There have been suggestions that fecal coliforms on fresh produce may be an indicator of the probable presence of enteric viruses. However, no significant correlation has been found and in studies that have investigated the prevalence of NoV in fruit and vegetables, despite good bacteriological quality, an unexpected high prevalence of NoV was observed by RT-qPCR, particularly in raspberries, strawberries and cherry tomatoes (Stals et al., 2011b). However, qPCR detects only genomic material and it cannot distinguish infectious and noninfectious NoV particles. Therefore, the development of methods able to discriminate infectious and noninfectious NoV particles in food may be of crucial significance.

The obvious conclusion is that the control of food-borne viruses cannot rely solely on the testing and removal of contaminated food items, but rather on the development of validated controlled produc-

tion. The most efficient way to improve the safety of fruit and vegetables is to rely on a proactive system reducing risk factors during production and handling.

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