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CONFERENCE PROCEEDINGS* - REVIEW
Human Noroviruses and Food Safety

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ABSTRACT

Foodborne disease outbreaks occur each year in the United States, and the most common etiological agent is human norovirus causing an estimated 58% of all illnesses. Key characteristics of human norovirus (NoV) (i.e. resistance to environmental degradation and high concentration of viral shedding) allow food to be vulnerable to contamination with NoV at each step of the supply chain: pre-harvest, post-harvest, processing, and preparation. This review will highlight key characteristics of NoV, the sources and routes of contamination in particularly susceptible food items (i.e. bivalve mollusks, fresh produce, and ready-to-eat products), and the ways to potentially control and prevent the transmission of NoV in the farm to fork supply chain.

Keywords: Human norovirus, food safety, acute gastroenteritis, minimally processed, ready-to-eat, prevention, calicivirus, person-to-person, environmental transmission

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INTRODUCTION

Human noroviruses (NoV) are estimated to cause 21 million cases of acute gastroenteritis each year—more than 90% of all nonbacterial outbreaks of gastroenteritis—and are the primary cause of foodborne disease outbreaks in the United States (Patel *et al.*, 2009; Scallan *et al.*, 2011). The socio-economic burden of a single nosocomial NoV outbreak in a healthcare setting costs nearly $660,000 in lost revenue, sick leave and cleaning expenses (Johnston *et al.*, 2007). The majority of NoV cases are caused by transmission via contaminated foodstuffs such as leafy vegetables, salads, sandwiches, oysters, baked goods, frosting, and fresh berries (Centers for Disease Control and Prevention, 2010). These foods may become contaminated with NoV: 1) at the source due to environmental inputs such as poor quality irrigation water, estuarine water, as well as organic fertilizers (i.e. municipal biosolids and compost) (Berger *et al.*, 2010; Gentry *et al.*, 2009; Wei and Kniel, 2010); 2) during manufacturing or packaging of final product (i.e. deli meats, packaged salad greens) (Malek *et al.*, 2009); and 3) during preparation of a food item by an infected food handler (Tuan Zainazor *et al.*, 2010). A recent report by Scallan *et al.* (2011) identified human norovirus as the ma-

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jor etiologic agent in foodborne illnesses acquired each year in the United States causing an estimated 58% of reported illnesses. This review highlights the 1) key characteristics of NoV; 2) transmission of NoV along the farm to fork supply chain; and 3) control and prevention of NoV within both the natural environment and food handling environments.

KEY CHARACTERISTICS OF HUMAN NOROVIRUS

Virus structure and classification

As members of the *Caliciviridae* family, noroviruses are a group of evolutionarily related single-stranded, positive-sense RNA viruses—some causing gastroenteritis in humans. Noroviruses are 27 to 35 nm diameter in size, and their RNA genome (~7.5 kb) is surrounded by a nonenveloped, icosahedral protein capsid (Green, 2007). The capsid is composed of two viral proteins (VP)—a major protein capsid known as VP1 and a smaller basic structural protein known as VP2 (Hutson *et al.*, 2004). Similar to other enteric viruses, NoV are divided into genogroups on the basis of genetic similarity across areas of the genome that are highly conserved, such as the RNA-dependent RNA polymerase (RdRp) (i.e. an essential enzyme that catalyzes the replication of RNA) and the VP1 capsid protein or shell domain (Green, 2007). To be classified in the same genogroup, NoV strains share at least 60% amino acid sequence identity in the major capsid protein VP1 (Hutson *et al.*, 2004). There are five genogroups (GI, GII, GIII, GIV, GV) that have been identified along with more than 40 recognized genetic clusters, or genotypes, designated as GI.1 indicating genotype 1 within genogroup I (Atmar, 2010; Koopmans, 2008). Each genotype identified may also contain variant or recombinant strains which have been most recently outlined by Bull *et al.* (2007). The genogroups associated with human illnesses are GI, GII, and GIV with GII being the most common followed by GI (Atmar, 2010). Genogroup II and GIV also contain porcine-specific genotypes (GII.11, GII.18, GII.19) and a feline-specific genotype of norovirus, respectively, while GIII and GV are associated with bovine and murine hosts, respectively (Glass *et al.*, 2009).

Epidemiology

Human noroviruses enter the body primarily through the fecal-oral route, though transmission via aerosol droplets due to vomiting has also been reported (Marks *et al.*, 2000; 2003). Based on limited volunteer studies and numerous epidemiologic studies, the incubation period for NoV ranges from 10 to 51 hours followed by an average of 2 to 3 days of illness (Glass *et al.*, 2009). Symptoms of NoV infection include acute onset of nausea, vomiting, abdominal cramps, general malaise, and non-bloody diarrhea. Human noroviruses infect people of all ages, though recent outbreaks demonstrate that children under 5 years of age and elderly may experience more severe symptoms (i.e. fever and dehydration) requiring hospitalization (Patel *et al.*, 2009). For the most part, infection with NoV is less severe than other diarrheal infections (such as those caused by *Escherichia coli* O157:H7 and *Campylobacter*). Asymptomatic infections are estimated to occur in one-third of all infected persons (Glass *et al.*, 2009). Both outbreaks of NoV and sporadic cases can occur year-round, though they tend to peak in the colder months. In addition, NoV outbreaks have been reported most frequently in association with scenarios or environments that favor person-to-person contact such as nursing homes, hospitals, cruise ships, military, camping trips, and schools (Isakbaeva *et al.*, 2005; Malek *et al.*, 2009; Wadl *et al.*, 2010; Wu *et al.*, 2005).

Immunity

Host susceptibility and specific immunological responses related to infection with NoV are not well understood due to the lack of a reproducible *in vitro* cell culture systems or small animal models for the cultivation of NoV (Duizer *et al.*, 2004). Thus, the study of NoV has relied on immune electron microscopy and molecular methods such as reverse transcription PCR (RT-PCR) for detection. As a result,

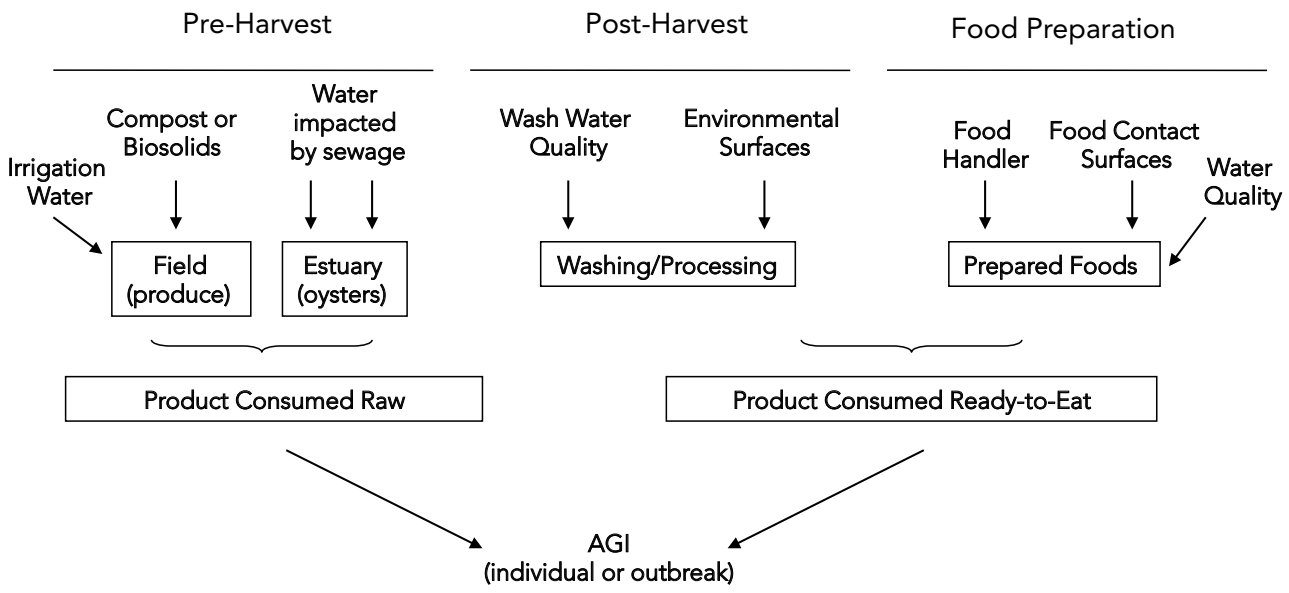

Figure 1. Potential points of human norovirus contamination at each node in the farm to fork supply chain. AGI = acute gastrointestinal illness.

the cellular receptors for NoV attachment had not been characterized until recently. Marionneau *et al.* (2002) hypothesized that NoV use carbohydrates (i.e. histo-blood group antigens) present on human gastroduodenal epithelial cells as ligands—similar to the attachment of rabbit hemorrhagic disease virus, also a member of the *Caliciviridae* family—and revealed that NoV do in fact bind to specific carbohydrates found on the exterior epithelial cell surfaces. Carbohydrate binding is a common method used by many viruses and other microorganisms to attach to their host cells (Hutson *et al.*, 2004). In the case of NoV, the capsid (VP1 and VP2) binds to histo-blood group antigens (HBGA)—a group of structurally related carbohydrates found in secretions and on mucosal surfaces (Huang *et al.*, 2003). Certain enzymes are important in the synthesis of HBGAs including fucosyl transferase-2 (FUT-2, secretor enzyme), FUT-3 (Lewis enzyme) and the A and B enzymes. Research has demonstrated that the FUT-2 enzyme plays a particularly important role in host susceptibility to NoV infection as individuals who are non-secretors (i.e. do not secrete FUT-2) do not become infected after challenge with NoV (Lindesmith *et al.*, 2003).

Based on a combination of human challenge studies, carbohydrate binding assays with NoV virus like particles (VLP; NoV capsid proteins VP1 and VP2) and HBGA phenotyped salivary samples, and inoculation of inbred mice with NoV VLP (e.g., mice cannot be infected with human norovirus but an immune response can be induced), researchers have been able to piece together key aspects of NoV immunity. With respect to the human challenge studies, researchers demonstrated that immunity to NoV is short-lived (e.g., partial immunity retained for 6 to 14 weeks) making persons susceptible to repeated NoV infections with both different and identical genotypes throughout one's life (LoBue *et al.*, 2010). As explained previously, HBGA receptors on the mucosal surfaces of the gastrointestinal (GI) tract play a role in NoV infection; however, this only holds true for certain genotypes (i.e. susceptibility to some genotypes of NoV can be independent of secretor status) (Marionneau *et al.*, 2002; Nordgren *et al.*, 2010). In addition, resistance to NoV infection stems from a combination of genetic factors (i.e. non-secretors vs. secretors of certain HBGA carbohydrate receptors) and acquired immunity (i.e. recent infection) (Donaldson *et al.*, 2010). Finally, research has shown that NoV evolves through the synergistic effects of antigenic drift and HBGA receptor switching—there is an immense range of similar, yet distinct HBGA receptors available on the GI tract surfaces that can interface with the NoV protein capsid carbohydrate

binding domain (Lindesmith *et al.*, 2008). Recent papers by Lindesmith *et al.* (2010a,b), Donaldson *et al.* (2010) and Teunis *et al.* (2008) provide more in depth examinations of the host susceptibility and immunological aspects of NoV infection.

TRANSMISSION OF HUMAN NOROVIRUS

Transmission of NoV through food, water, fomite (or inanimate) surfaces, and person-to-person is relatively easy owing primarily to the low infectious dose (median, approximately 18 viral particles) and the high concentrations shed in feces (10^{11} genomic copies per gram) over a prolonged period—virus particles can be shed up to 4 weeks after exposure with peak amounts shed usually *after* physical signs of infection (Chan *et al.*, 2006; Teunis *et al.*, 2008; Tu *et al.*, 2008). In addition, viral shedding of GII has been reported to be 100-fold higher than GI therefore possibly explaining GII dominance in outbreaks and persistence in the population (Chan *et al.*, 2006). For the purposes of this review, transmission of NoV at critical nodes along the farm to fork supply chain will be addressed (Figure 1).

Pre-harvest

Contamination of fruit and vegetable crops and bivalve mollusks with NoV may occur during the initial phase of the supply chain during pre-harvest. With respect to fruits and vegetables, NoV may be introduced to crops via contaminated irrigation water and agricultural lands (Mara and Sleigh, 2010; Wei and Kniel, 2010). Soil and source water used for irrigation may become contaminated by leakage of onsite sewage systems (septic systems) or sewer pipes and runoff of municipal biosolids or contaminated soil from nearby land due to flooding or heavy rain. Because of these contamination scenarios, several studies have investigated the ability of NoV to survive in the environment (Dawson *et al.*, 2005), adsorb to biosolids and food surfaces, and to be internalized by produce, specifically leafy vegetables (Wei *et al.*, 2010a; 2010b). In general, NoV survival in the environment or on plant surfaces is dependent on the type of fruit or vegetables (e.g., increased survival on lettuce), ambient temperature, relative humidity, and type of soil (i.e. faster movement through a soil column to groundwater source if coarse or through a finger-flow soil) (McLeod *et al.*, 2001). A recent review paper by Wei and Kniel (2010) provides an overview of the potential vehicles of pre-harvest viral contamination of fresh produce crops and additional information about current research involving virus fate and transport in the environment.

Contamination of bivalve mollusks, specifically oysters, with NoV during production has been well-documented (Bosch and Le Guyader, 2010). Inherent to the way oysters are produced, bay and estuary environments impacted by fecal matter through land runoff, sanitary sewer overflows, or wastewater effluent discharge (Gentry *et al.*, 2009; Shieh *et al.*, 2003) are the primary vehicles of contamination. The susceptibility of oysters to contamination with NoV can also be attributed to the fact that oysters are filter feeders and tend to accumulate and concentrate viruses and other microorganisms within their digestive system over time. A recent article by Le Guyader (2006) helped to further elucidate the association of oysters with NoV by demonstrating that oysters were found to have A-like carbohydrate structures along their digestive ducts which are indistinguishable from human blood group A antigens (Le Guyader *et al.*, 2006). The research by Le Guyader and others (2006) indicates that NoV-specific binding may occur in oysters thus making control of NoV contamination in oysters even more challenging. In addition, strain dependent NoV bioaccumulation in oysters has also been demonstrated recently in which GI.1 strain bioaccumulates very efficiently in oysters while the GII.4 strain (i.e. the NoV strain which predominantly circulates within the population) bioaccumulates very poorly (Maalouf *et al.*, 2011). Maalouf *et al.* (2011) indicate the difference in binding is due to ligand expression in the oyster digestive tissues.

Post-harvest and processing

Post-harvest food product contamination is mostly related to on-farm harvesting practices

as well as the efficacy of the methods used for washing and sanitizing fresh produce. Some of the harvesting practices that may allow fresh produce to become contaminated include: 1) barehand harvesting combined with a lack of personal hygiene (i.e. hand washing); 2) continuous use of disposable (latex) gloves (e.g., accumulation of organic matter contaminated with NoV could allow for wide-spread distribution within a crop) without appropriate sanitation (LGMA, 2010); and 3) contaminated harvest containers and tools (Luo, 2011). After harvesting in the field, fresh produce may also become contaminated through contact with wash water used for cleaning and sanitation.

Food preparation

Human norovirus contamination during food preparation is reportedly the most common cause of NoV outbreaks with a known food commodity. Within the food preparation environment, NoV may be transferred to food by contaminated surfaces, a food handler infected with NoV (symptomatic or asymptomatic) and not utilizing best practices (i.e. hand washing, glove use), or the use of sanitizing agents ineffective against NoV (Newell *et al.*, 2010). A nine part review series on food workers and spread of foodborne disease published in Journal of Food Protection from 2007 to 2010 highlights NoV as the primary etiologic agent in these scenarios and discusses the factors contributing to outbreaks, the transmission and survival of pathogens in the food preparation environment, and reduction of contamination (Greig *et al.*, 2007). Because of the low infectious dose, high number of viruses shed during infection and non-enveloped structure, NoV can spread easily and persist for extended periods of time in the food preparation environment even if proper hygiene and sanitation procedures are followed.

CONTROL AND PREVENTION OF HUMAN NOROVIRUS

Because of their nonenveloped structure, NoV is presumed to be relatively resistant to chemical inactivation (i.e. chlorination) and environmental degradation (temperature, pH, ultraviolet radiation, desiccation) which aids in the ease of transmission (Green, 2007). However, the persistence of infectious NoV in water sources, on food contact surfaces and in food products under various conditions (i.e. temperature, pH, ultraviolet radiation) has been difficult to study due to the lack of reproducible cell culture systems for propagation and detection of viable NoV (Duizer *et al.*, 2004). Thus viral surrogates including murine norovirus (MNV), feline calicivirus (FCV), and MS2 bacteriophage have been utilized for studying the physicochemical properties of human norovirus (Bae and Schwab, 2008; Belliot *et al.*, 2008; Nappier *et al.*, 2008). Both FCV and MNV are members of the *Caliciviridae* family; however, FCV (a feline respiratory virus) belongs to the *Vesivirus* genus whereas MNV is located within the *Norovirus* genus (genogroup V) making it morphologically and genetically similar to human norovirus. Until recently, FCV was the predominant surrogate used for studying NoV, and as a result, many guidelines and recommendations for NoV are based on the characteristics of FCV with respect to control and prevention in the environment and in food products (e.g., recommended sanitizing agents, disinfection of drinking water, thermal inactivation, etc.).

Fresh Produce

For the control of NoV contamination from farm to fork, food safety guidelines need to be revamped to include both viral and bacterial pathogens. Traditional parameters (i.e. pH, temperature, water activity) for control and inactivation of microorganisms during food processing have historically focused on bacterial pathogens, such as *E coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp., and not viral pathogens (Grove *et al.*, 2006; Hirneisen *et al.*, 2010; Koopmans and Duizer, 2004). Additionally, most of the engineering processes or interventions along the supply chain are also focused on the control of bacterial pathogens and should be adjusted to target viruses as well (Mormann *et al.*, 2010). With respect to on-farm food safety, irrigation water should be tested for more than just bacterial indicators (i.e. fecal co-

liforms) as previous studies have demonstrated that these bacteria poorly correlate with the presence of human enteric viruses (Gerba *et al.*, 1979; Gibson *et al.*, 2011; Harwood *et al.*, 2005). In addition, harvesting practices related to fresh produce such as washing products with a sanitizing agent should be validated for efficacy against enteric viruses—chlorine bleach is most commonly used though the concentration and contact time may be ineffective against viruses (Hirneisen *et al.*, 2010). More advanced technologies such as high pressure processing (HPP) have been reported as effective against MNV inoculated in fresh vegetables and produce; however, HPP may affect the quality of the product and may only be suitable for fruits intended for frozen storage (Lou *et al.*, 2011).

Oysters

For the control and prevention of NoV contamination in oysters, the primary goal is to maintain good water quality in estuaries. Some regulations such as the Clean Vessel Act (33 U.S.C. 1322, 106 Stat 5039) have been put in place to prevent the discharge of sewage in oyster harvesting areas (USFWS, 1992). In addition, estuary sites should be located away from wastewater effluent discharge (i.e. upstream instead of downstream), and these sites should be in areas protected against the impacts of potential sanitary sewer overflows, septic system failures, and stormwater runoff. Post-harvest, oysters are subjected to a practice called depuration. During depuration, oysters are placed in tanks of clean seawater and allowed to resume normal pumping (filtration) activity for a period of time that may range from a few hours to days in order to expel microbial contaminants (Lee *et al.*, 2008). However, research involving bioaccumulation and depuration of NoV in oysters demonstrates that there is a selective retention mechanism for NoV within oysters possibly due to the similarity in NoV binding sites between humans and oysters indicating attachment of NoV rather than simple sequestering of the virus (Nappier *et al.*, 2008; Schwab *et al.*, 1998; Ueki *et al.*, 2007). Oysters may also undergo HPP during whole oyster processing to inactivate bacterial and viral pathogens that have been sequestered in the oyster. During HPP, the oysters are killed by the high pressure treatment therefore this intervention would only be applicable to oysters sold as meat without the shell (Grove *et al.*, 2006).

Food Preparation

In the food preparation environment, control and prevention of NoV starts with good handling practices (GHP) and strict personal hygiene. Regular and consistent hand washing by food handlers can be a very effective tool in preventing the spread of microbial contaminants when promoted effectively (Chapman *et al.*, 2010). Education and training, positive incentives, and reinforcement from managers may increase the frequency and quality of hand washing by food handlers (Moe, 2008). In addition, food handlers who experience an episode of acute gastrointestinal illness should communicate this information to their employer and proper precautions should be taken such as exclusion of ill workers during the period of illness—two to three days has been recommended; however, viral shedding occurs over a much longer period of time (Parashar *et al.*, 2001). In general, minimal bare-hand contact during preparation of foodstuffs and proper disinfection of environmental surfaces is crucial to prevention. A list of antimicrobial products effective against NoV is available through the USEPA Office of Pesticide Programs; however, it should be noted that most of the products listed have only been proven effective against FCV and not specifically against NoV(USEPA, 2009).

CONCLUSIONS

In the United States there is currently no systematic surveillance for human norovirus—only a select number of bacterial and parasitic pathogens are actively monitored (Centers for Disease Control and Prevention, 2010). Passive monitoring is primarily due to the short duration and overall nature (i.e. nonfebrile, no bloody diarrhea) of illness caused by NoV as well as the lack of routine clinical tests for NoV

available in hospitals. Therefore, NoV is usually diagnosed only when an outbreak occurs as opposed to sporadic, individual cases. This passive approach to monitoring NoV in the United States presents a wide knowledge gap with respect to the endemic nature of NoV as well as the true magnitude that contaminated foodstuffs may have in the spread of NoV. Enhancing the capacity of state and local laboratories would significantly increase our knowledge about the prevalence of NoV and would help capture unreported outbreaks due to NoV. In addition to monitoring the population for NoV, steps should be taken to monitor for NoV in high-risk foodstuffs (i.e. fresh produce and oysters). Methods for the detection of NoV have improved dramatically over the past decade by using techniques such as real time quantitative RT-PCR as well as advanced methods for concentration of NoV from food and water. To do this, a standard protocol for the isolation and detection of NoV from food, water, and fomite surfaces should be established. Overall, we should begin to shift the approach used for monitoring and control strategies and move from being reactive to being proactive and focus on prevention. This can be done through understanding of the key characteristics of human noroviruses.

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