New Interventions Against Human Norovirus: Progress, Opportunities, and Challenges

Jianrong Li,[1,2] Ashley Predmore,[1] Erin Divers,[1] and Fangfei Lou[1]

[1]Department of Food Science and Technology, College of Food, Agricultural and Environmental Sciences, The Ohio State University, Columbus, Ohio 43210

[2]Division of Environmental Health Sciences, College of Public Health, The Ohio State University, Columbus, Ohio 43210; email: li.926@osu.edu

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Abstract

Human norovirus (HuNoV) is the leading causative agent of foodborne disease outbreaks worldwide. HuNoV is highly stable, contagious, and only a few virus particles can cause illness. However, HuNoV is difficult to study because of the lack of an efficient in vitro cell culture system or a small animal model. To date, there is very limited information available about the biology of HuNoV, with most data coming from the study of surrogates, such as HuNoV virus-like particle (VLP), murine norovirus (MNV), and feline calicivirus (FCV). High-risk foods for HuNoV contamination include seafood, fresh produce, and ready-to-eat foods. Currently, there is no effective measure to control HuNoV outbreaks; thus, development of food-processing technologies to inactivate HuNoV in these high-risk foods is urgently needed. Although a VLP-based vaccine induces humoral, mucosal, and cellular immunities in animals and currently is in human clinical trials, development of other new vaccine candidates, such as live vectored vaccines, should be considered. Recent evidence suggests that blockage of virus-receptor interaction may be a promising antiviral target. To enhance our capability to combat this important agent, there is an urgent need to develop multidisciplinary, multi-institutional integrated research and to implement food virology education and extension programs nationwide.

INTRODUCTION TO FOODBORNE VIRUS

Foodborne illness can be caused by viruses, bacteria, fungi, parasites, toxins, or prions, but viruses are the major causative agent of foodborne gastroenteritis. In 1999, Mead et al. published a comprehensive statistical analysis of outbreak data regarding foodborne illness in the United States. There are approximately 76 million estimated cases of foodborne illness, 323,914 hospitalizations, and 5,194 deaths each year in the United States alone. It should be emphasized that more than 67% of foodborne illnesses can be attributed to viruses (Mead et al. 1999). Most recently, public health officials at the Centers for Disease Control and Prevention revised their estimates of U.S. foodborne illnesses (Fox 2011). It is now estimated that 48 million individuals, or about 17% of the population, are sickened each year, leading to approximately 128,000 hospitalizations and 3,000 fatalities (Centers for Disease Control and Prevention 2010, Fox 2011). Remarkably, norovirus alone causes nearly 60% of estimated illnesses (Fox 2011). A foodborne virus is defined as any virus that may be transmitted by food or water and is able to cause illness via the fecal-oral route. Examples of foodborne viruses include human norovirus (HuNoV), hepatitis A (HAV) and hepatitis E viruses, sapovirus, astrovirus, rotavirus, adenovirus, poliovirus, and enterovirus 71. Among these foodborne viruses, HuNoV has long been considered the most prominent cause of illness (Cliver 1997, Mead et al. 1999, Fox 2011). HuNoV causes more than 90% of nonbacterial acute gastroenteritis (Mead et al. 1999, Estes et al. 2006, Centers for Disease Control and Prevention 2010). Approximately 23 million people suffer from norovirus-induced gastroenteritis each year in the United States (Centers for Disease Control and Prevention 2010). The total number of annual outbreaks may even be underestimated because of underreporting of outbreaks and lack of rapid detection of the virus. The good news is that viruses are unable to amplify in food, water, or during storage because they are strict intracellular parasites and only replicate inside of host cells. The bad news is that foodborne viruses are extremely stable, and only a few virus particles can cause illness (Duizer et al. 2004, Teunis et al. 2008). Most common foodborne viruses are much more resistant to heat, pH, and disinfection than bacteria (Koopmans & Duzier 2004, Duizer et al. 2004, Teunis et al. 2008). As a result, current procedures to prevent bacterial infections in food processing, preservation, and storage may not be fully effective against viral pathogens (Baert et al. 2009). This review focuses on HuNoV, the major foodborne virus that has a significant impact on food safety and public health.

HUMAN NOROVIRUS: VIROLOGY, EPIDEMIOLOGY, AND MOLECULAR BIOLOGY

Clinical Features and Epidemiology of Human Norovirus

The first outbreak of HuNoV was reported in Norwalk, Ohio in 1968 (Kapikian et al. 1972). It was not until 1972, though, that Kapikian et al. determined the etiology of this disease. Electron microscopy analysis identified small round-structured viral particles ranging from 27–38 nm in diameter. This is where norovirus received its former name of Norwalk-like virus. Other names used to refer to norovirus are winter vomiting disease, viral gastroenteritis, food poisoning virus, small round-structured virus, and the stomach flu (Adler & Zickl 1969, Kapikian et al. 1972, Koopmans & Duzier 2004). Symptoms of HuNoV infection include diarrhea, vomiting, nausea, abdominal cramping, chills, headache, dehydration, and a high-grade fever. The virus has a 1–3 day incubation period with symptoms normally persisting for 2–3 days (Koopmans 2008). It has been estimated that the stool of an individual with an active norovirus infection may shed up to 100 billion virus particles per gram of feces (Centers for Disease Control and Prevention

2011). The duration of virus shedding is increased in the immunocompromised, the elderly, and children (Rockx et al. 2002, Harris et al. 2008). Although the virus may cause severe discomfort and embarrassment, the clinical manifestation is normally relatively mild with an average mortality rate of only about 0.003% (Centers for Disease Control and Prevention 2011). Outbreaks typically occur in any area where a large number of people are in close contact with each other, such as restaurants, swimming pools, cruise ships, hospitals, nursing homes, schools, daycare centers, hotels, prisons, military installments, and sports stadiums (Goodgame 2006, Rockx et al. 2002, Becker et al. 2000, Marks et al. 2004, Estes et al. 2006).

Fecal-oral transmission is the most important and most common mode of transmission for noroviruses (Atmar & Estes 2001, Koopmans & Duzier 2004). Transmission through infectious vomit or feces either by direct contamination (through hand and/or mouth contact) or by indirect contamination (such as by aerosolization) may also account for the rapid transmission of the virus in closed settings (Marks et al. 2004). Most commonly, contaminated food or water is the primary source of infection, and person-to-person spread further disseminates the outbreak (Becker et al. 2000). The virus is extremely contagious and infectious, with only a few particles (usually less than 10) being sufficient to cause infection (Teunis et al. 2008, Koopmans & Duizer 2004). Recent human volunteer studies and mathematical modeling showed the average probability of infection for a single norovirus particle is close to 0.5 (Teunis et al. 2008). There is no long-term immunity to norovirus; therefore, repeated infections may occur throughout a person's life.

Classification and Molecular Biology

Noroviruses belong to the family *Caliciviridae* under the genus *Norovirus*. There are five other genera in this family: *Vesivirus*, *Lagovirus*, *Becovirus*, *Recovirus*, and *Sapovirus*. Caliciviruses are nonenveloped, single-stranded, positive-sense RNA viruses. The outer shell of the virus particle is a highly stable protein capsid that exhibits icosahedral symmetry. The genome of HuNoV is approximately 7.5–7.7 kb and encodes three open reading frames (ORFs) (Jiang et al. 1990, Jiang et al. 1993). The first ORF (ORF1) encodes for a nonstructural polyprotein, which is proteolytically cleaved into six nonstructural proteins, including N-terminal protein (designated p48 for Norwalk virus), NTPase, 3A-like protein (designated p22 for Norwalk virus), VPg, viral protease ($3CL^{pro}$), and RNA-dependent RNA polymerase (RdRp) (Belliot et al. 2003, Green 2007). The second ORF (ORF2) encodes the major capsid protein VP1, and ORF3 encodes the minor structural capsid protein VP2 (Jiang et al. 1990, Jiang et al. 1993, Hardy 2005). VP1 ranges normally from 530–555 amino acids in length and has an approximate molecular weight of 58–60 kDa. VP1 forms the capsid of the virus particle and plays many essential roles in the viral life cycle. First, VP1 protein binds to its functional receptor, the histo-blood group antigen (HBGA) on the surface of host cells, which mediates virus entry (Hutson et al. 2002, 2003, 2004; Tan et al. 2003). Second, VP1 protein determines the antigenicity and strain specificity of norovirus (Katayama et al. 2002, Prasad et al. 1999, Huston et al. 2004). Third, VP1 protein is the host-protective antigen that is responsible for eliciting neutralizing antibody, cellular, and mucosal immunities (Ball et al. 1998). Lastly, VP1 may play many other roles in the virus life cycle, such as uncoating, assembly, and release (Hardy 2005). VP2 is the minor capsid protein that plays a role in RNA packaging and regulation of the synthesis of VP1 proteins (Glass et al. 2000, Bertolotti-Ciarlet et al. 2003). Furthermore, VP2 increases VP1 stability and protects VP1 from disassembly and protease degradation (Bertolotti-Ciarlet et al. 2003).

Human Norovirus Genogroups and Genotypes

Since the discovery of norovirus, thousands of norovirus strains have been identified worldwide. Noroviruses are very diverse, both antigenically and genetically (Zheng et al. 2006). Based on

the phylogenetic analysis of viral capsid genes (*VP1*), five genogroups of noroviruses, GI, GII, GIII, GIV, and GV, have been assigned. Within a genogroup, noroviruses are further divided into genotypes, or genetic clusters (Zheng et al. 2006). To date, there are at least 33 norovirus genotypes. Genogroup II contains the majority of HuNoVs, and genogroups I and IV also infect humans (Green 2007, Zheng et al. 2006). The prototype norovirus strain, the Norwalk virus, belongs to genogroup I and genotype 1, and is designated as GI.1. In contrast, genogroup III is found in cows, and genogroup V includes the murine norovirus (MNV), which infects mice. Additionally, porcine strains are found in genogroup II, and strains that infect feline and canine species are found in genogroup IV. There are a total of 19 genotypes assigned to GII noroviruses. Currently, the most prevalent HuNoV belongs to genogroup II, genotype 4 (GII.4). The evolution of GII.4 noroviruses has resulted in many new variants. In the past ten years, more than three global pandemics have occurred, all of which were due to strains of GII.4 (Green 2007). To date, GII.4 is the most studied genotype of norovirus. Currently, there is no evidence to support that noroviruses are zoonotic. Humans infected by animal strains have not been reported, suggesting that noroviruses are species specific.

Human Norovirus Receptors and Host Susceptibility

The HBGAs have been identified as the functional receptors for HuNoV (Hutson et al. 2002, 2003; Tan et al. 2003). HBGAs are carbohydrate complexes that are present on the surface of erythrocytes as well as the intestinal, genitourinary, and respiratory epithelia. They also exist as free oligosaccharides in saliva, blood, milk, and in the contents of the intestine. There are three major families of HBGAs, Lewis, ABO, and secretor, and each is specifically recognized by different norovirus strains. The mechanism of the interaction between norovirus virus-like particles (VLPs) and HBGA receptors has been extensively studied. It has been demonstrated that amino acid residues in the P domain of the VP1 protein are responsible for the specificity of receptor binding (Tan et al. 2003, Tan & Jiang 2005a, Tan et al. 2008). The discovery of human HBGAs as norovirus receptors also resolved many historical puzzles about the infectivity of HuNoV. In a human volunteer study, it was found that individuals with O blood type were easily infected by some norovirus strains, whereas those with B blood type had the lowest risk of infection (Hutson et al. 2002, Huston et al. 2005). Presumably, volunteers with B blood type never became infected with those specific norovirus strains following challenge simply because they lacked the matched viral receptors on their intestinal epithelium. The specificity of virus-receptor interactions also contributed to the observation that some individuals with a high level of antibodies against norovirus were even more susceptible to norovirus challenge than those with lower levels of antibodies or no antibodies (Parrino et al. 1977). Clearly, receptor specificity plays an important role in host susceptibility, although acquired immunity is also involved in resistance to norovirus infection.

HUMAN NOROVIRUS IN HIGH-RISK FOODS

Human Norovirus in Fresh Produce

Fresh produce is considered a major vehicle of norovirus contamination because it normally undergoes little to no processing before consumption. This allows for contamination anywhere from the preharvest to postharvest stages (Cliver 1997, Heaton & Jones 2008, Lynch et al. 2009). It has been reported that norovirus accounted for more than 40% of outbreaks in fresh produce from 1998–2005 in the United States (DeWaal & Bhuiya 2007). These outbreaks of norovirus

occurred in fresh produce, such as lettuce, tomatoes, melons, green onions, strawberries, raspberries, blueberries, peppers, and fresh-cut fruits (Allwood et al. 2004, Heaton & Jones 2008, Lynch et al. 2009, Seymour & Appleton 2001). Mixed fruits, mixed vegetables, puree, salad, and salsa are also high-risk foods for norovirus contamination (Centers for Disease Control and Prevention 2010, Le Guyader et al. 2004, Doyle & Erickson 2008, Lynch et al. 2009). One major route with a high probability of contamination is the use of contaminated water for irrigation, fertilizing, or washing (Doyle & Erickson 2008, Lynch et al. 2009). Contamination may also occur through infected workers handling the food during harvesting, processing, or distribution (Doyle & Erickson 2008, Lynch et al. 2009). With an increasing number of people striving to eat healthier by increasing their consumption of fruits and vegetables, this has emerged as a major public health concern.

Currently, the attachment, penetration, uptake, internalization, dissemination, and persistence of viruses in plants are poorly understood. One possible mechanism for the attachment of HuNoV to produce is the presence of carbohydrate moieties that resemble HBGAs, the functional receptors for HuNoV attachment to host tissues. Carbohydrate moieties, the analogs of HuNoV receptors, such as glucose and glycan, are highly abundant in vegetables and fruits. Using HuNoV VLPs as a model, Gandhi et al. (2010) found recently that these VLPs attached to the surface of romaine lettuce and were localized primarily on the leaf veins. In fact, an extract of romaine lettuce leaves binds to VLPs in a dose-dependent manner. Extracts of cilantro, iceberg lettuce, spinach, celery, green onions, clover sprouts, and raspberries also bind to VLPs. However, these extracts did not bind VLPs by HBGAs, nor was it competitive with VLPs in binding to porcine gastric mucin. Further research needs to be done in order to gain more knowledge about whether receptors are indeed involved in HuNoV attachment to fresh produce.

It is well established that bacterial pathogens can be internalized and disseminated in fresh produce via stomata or wounds in the cuticula (Doyle & Erickson 2008, Lynch et al. 2009). Generally, viruses are approximately 1,000 times smaller than bacteria. Therefore, theoretically, it is easier for a smaller pathogen to enter and disseminate in plants. The feasibility of internalization of human enteric viruses by plants is supported by the ability of plants to internalize their own viral pathogens, which can be taken up from the soil and water (Ward & Mahler 1982, Oron et al. 1995). It is likely that HuNoV may also be taken into the plant through the roots, canal systems, and/or leaves because viruses may be present in sewage-contaminated soil and water (Ward & Mahler 1982, Oron et al. 1995, Wei et al. 2010). There have been very few studies that have actually investigated norovirus internalization in produce. Urbanucci et al. (2009) performed the first norovirus internalization in plants. The roots of lettuce, growing either in hydroponic culture or in soil, were exposed to both canine calicivirus (CaCV) and a HuNoV genogroup 2. For CaCV, it was found that the internalization occurred in both seedling and mature plants, in both hydroponic and soil cultures, and whether the roots were intact or damaged. However, the frequency of positive results was low. Interestingly, internalization of HuNoV was not detected although the plants were exposed to a high concentration of HuNoV. These results suggested that viral contamination of lettuce plants via roots cannot be excluded, but may not be an important transmission route for viruses in plants. Wei et al. (2011) reported that MNV can be internalized via roots in romaine lettuce (*Lactuca sativa*). MNV genomic RNA was detected in some lettuce leaf samples when the roots of lettuce were inoculated with both high and low doses of MNV. However, infectious MNV was found in lettuce samples challenged with high virus inocula grown hydroponically and in soil but not in lettuce grown with low virus inocula. This evidence suggests that MNV can become internalized via plant roots and disseminated into the leaf portion. Taken together, these results suggest that norovirus can be potentially internalized and disseminated, although the detailed mechanism is still unclear.

Human Norovirus in Seafood

Another group of high-risk food for norovirus contamination is seafood, particularly bivalves such as mussels, oysters, and clams. This is due to the fact that they are filter-feeding animals that sieve liters of water per day. If viruses or contaminants are present in the water, the seafood can easily become contaminated. High numbers of norovirus particles can be detected in shellfish and oyster tissues (Atmar et al. 1995, Le Guyader et al. 2000, Cheng et al. 2005). Worldwide, a large number of outbreaks were associated with consumption of raw or undercooked shellfish. The percentage of HuNoV-positive oysters depends on the location where oysters are grown, seasonality, and the sensitivity of detection methods. A study from France showed that the HuNoV-positive rate of oyster samples is approximately 23% (Le Guyader et al. 2000). However, mussel samples, which are collected in areas routinely impacted by human sewage, were more highly contaminated by HuNoV (35%) (Le Guyader et al. 2000). Costantin et al. (2006) reported a relatively high occurrence of human and animal enteric caliciviruses in the U.S. market oysters. Oysters were collected from 45 bays along the U.S. coast during the summers and winters of 2002 and 2003. Nine samples (20%) were positive for HuNoV genogroup II. Animal enteric caliciviruses were detected in 10 samples (22%). A study in the United Kingdom showed that the percentage of norovirus positive oysters in two locations was 77% and 95% from October to March. However, lower positive rates (32% and 48%) were found from April to September (Lowther et al. 2008). Companies producing oysters often implement recalls because of norovirus contamination (Dore et al. 2010).

Interestingly, it was found that HuNoV VLPs and native virions bound to the midgut, the main and secondary ducts of the digestive diverticula, and tubules of oyster by HBGA-like molecules, similar to what is seen in humans (Le Guyader et al. 2006). Binding of HuNoV VLPs to oyster digestive cells was specifically inhibited by human saliva and antibodies to carbohydrates and lectins. Furthermore, amino acid substitutions in receptor binding sites of VLPs inhibited the binding of these mutant VLPs to oyster tissues. These results provided compelling evidence that HBGA-like molecules were involved in bioaccumulation of HuNoV in oysters. It was shown that VLPs derived from different HuNoV genogroups exhibited different binding affinity to oyster tissues (Maalouf et al. 2010). GI.1 VLPs bound readily to digestive tissues but not to the gills or the mantle. GII.4 VLPs bound strongly to digestive tissues but also to the gills and the mantle. GI.1 bound to the oyster digestive tract via an A-like carbohydrate structure indistinguishable from human blood group A antigen. However, the GII.4 strain recognized a sialic acid-containing ligand that is present in all tissues (Maalouf et al. 2010). In a study to determine the rate of bioaccumulation of norovirus by oysters, three different strains of HuNoV (GI.1, GII.3, and GII.4) were compared (Maalouf et al. 2011). It was found that strain differences in combination with seasonality play a large role in norovirus bioaccumulation in oysters. The GI.1 strain was the most efficiently concentrated strain. Bioaccumulation specifically occurred in digestive tissues in a dose-dependent manner. The bioaccumulation levels in the digestive tissue were significantly higher during the winter months (January to March). The GII.4 strain was poorly bioaccumulated and a low level of virus was recovered in almost all tissues without seasonal influence. The GII.3 strain showed an intermediate bioaccumulation without seasonal effect. However, GII.3 strain had less bioaccumulation efficiency than that of the GI.1 strain during the cold months.

The Role of Food Handlers in Human Norovirus Transmissison

The overwhelming evidence suggests that the fecal-oral route is the most common cause of contamination of food by an infected food handler (Cliver 1997, Mead et al. 1999, Koopmans &

Duzier 2004, Duzier et al. 2004). It usually involves transmission of the pathogen to food by the food handler's fecally contaminated hands. Other means of pathogen contamination include nasopharyngeal secretions, respiratory inhalation (aerosolization of vomitus), and fomites. Processed food items exposed to food handlers are another high-risk food for norovirus contamination. Good examples of high-risk foods are the ready-to-eat foods, such as deli sandwiches, appetizers, street foods, dips, bakery items, and finger foods (foods eaten directly with the hands). It was reported that food handlers are key players in norovirus outbreaks because they directly handle packaged or unpackaged food, food equipment and utensils, and food contact surfaces (Koopmans 2008, Barrabeig et al. 2010). Many of these foods become contaminated through the poor hygienic practices of food handlers. The virus can be isolated from people who have become ill after consumption of the same food items. Sometimes an outbreak may be traced to a food handler who also harbors norovirus. It has also been demonstrated that approximately 30% of norovirus infections are asymptomatic, but in fact these infected individuals may actively be shedding the virus while appearing healthy (Centers for Disease Control and Prevention 2011). These asymptomatic carriers can still shed the virus and pass norovirus to other people or to foods that they handle (Koopmans & Duzier 2004, Barrabeig et al. 2010). To improve food safety, food handlers should strictly follow food service and food handling regulations.

CHALLENGES IN HUMAN NOROVIRUS RESEARCH

Current Challenges

Noroviruses are classified as category B biodefense agents by the National Institute of Allergy and Infectious Diseases (NIAID) because they are highly contagious, extremely stable, resistant to common disinfectants, have a low infectious dose, and are associated with debilitating illness. Despite the fact that HuNoV causes significant health, emotional, social, and economical burdens worldwide, research on this biodefense agent has been seriously hampered. Currently, many aspects of human norovirus, such as molecular biology, gene expression, replication, pathogenesis, and immunology, are poorly understood. The stability and sensitivity of HuNoV to food-processing technologies are not understood. Two major challenges have been encountered in HuNoV research. First, HuNoV cannot be propagated in cell culture. Thus, research relying on cell culture, such as survival, replication, and gene expression, cannot be conducted. Second, there are no small animal models for HuNoV. All questions that must rely on animal models, such as pathogenesis and immunology, cannot be studied. Despite the tremendous efforts over the past forty years, little progress has been made on overcoming these two challenges. To date, there is limited information about the infectivity and immunity of HuNoV from human volunteer subjects (Parrino et al. 1977, Hutson et al. 2002, Tacket et al. 2003). Studies on volunteers not only require human subject protocols, but it is also very uncommon and impractical to have people voluntarily become infected with a highly infectious and contagious agent that causes acute gastroenteritis.

Recent Laboratory Efforts to Cultivate Human Norovirus

Since the discovery of HuNoV, many laboratories have devoted research to attempt to cultivate this virus. To date, none of these endeavors have been successful. In an effort to replicate HuNoV, Duizer et al. (2004) systematically evaluated a variety of cell lines and laboratory methods. The authors created an in vitro cell culture system that mimicked the intestinal epithelium, using gastric cells, duodenal cells, and small intestinal enterocyte-like cells that were allowed to differentiate. None of the cell culture combinations were successful. HuNoV also does not

replicate in macrophages or dendritic cells that support the replication of MNV (Lay et al. 2010). In 2007, Straub et al. first reported an in vitro cell culture infectivity assay for HuNoV. The authors showed that HuNoV can infect and replicate in a physiologically relevant three-dimensional (3D) organoid model of human small intestinal epithelium. It was found that cytopathic effect and HuNoV RNA were detected at each of the five cell passages for genogroup I and II viruses. This report is very encouraging; however, the level of virus replication and the amount of newly synthesized viruses have been questioned (Chan et al. 2007). In response to this question, Straub et al. (2007) claimed that norovirus titer did increase in their culture system. However, the magnitude and time course of these increases is dependent on both virus strain and multiplicity of infection. Recently, Leung et al. (2010) reported an ex vivo culture system to cultivate HuNoV using freshly collected human duodenal tissue that closely mimics the primary site of viral replication in vivo. They found that viral genomic RNA levels in cell-free culture supernatants increased over time as measured by real time polymerase chain reaction. Notably, in situ hybridization of viral RNA and immuno-histochemical staining of VP1 as well as newly synthesized viral protease revealed that HuNoV displays an apparent tropism for glandular epithelial cells. It appears that ex vivo culture can support key stages of complete HuNoV replication, ranging from virus adsorption and internalization to viral RNA replication and protein synthesis. Although these findings are promising, the robustness of these cultivation systems remains to be determined.

Recent Efforts to Develop an Animal Model for Human Norovirus

Although there is no small animal model for HuNoV, it has been recently shown that gnotobiotic pigs, gnotobiotic calves, and chimpanzees support the replication of HuNoV. Cheetham et al. (2006) first developed a gnotobiotic pig model to study the pathogenesis of HuNoV and to determine the target cells for viral replication. Seventy-four percent (48 out of 65) of animals developed mild diarrhea after oral inoculation with norovirus GII.4 strain. Immunofluorescent microscopy revealed sporadic infection of duodenal and jejunal enterocytes with a few stained cells in the ileum in 18 out of 31 HuNoV-inoculated pigs. However, only one out of seven pigs showed mild histopathologic lesions in the proximal small intestine. Transmission electron microscopy of intestines from HuNoV-inoculated pigs showed disrupted enterocytes, with cytoplasmic membrane vesicles containing calicivirus-like particles of 25 to 40 nm in diameter. These data demonstrated that norovirus replicates in gnotobiotic pigs. In addition, HuNoV infection induced low levels of antibodies and low numbers of antibody-secreting cells, both systemically and in the gut mucosa, and 65% seroconversion in pigs (Souza et al. 2007a). Using this model, Souza et al. (2007b) further demonstrated that a HuNoV VLP-based vaccine adjuvanted with ISCOM or mutant *E. coli* LT toxin induces cytokine and antibody responses and protection of the homologous GII.4 HuNoV strain. It was also reported that gnotobiotic calves support norovirus replication (Souza et al. 2008). The HuNoV GII.4 strain HS66 caused diarrhea (five out of five calves tested) and intestinal lesions (one out of two calves) in the proximal small intestine (duodenum and jejunum) of gnotobiotic calves (Souza et al. 2008). All inoculated calves shed virus in feces (five out of five calves), and one out of five had viremia. Most recently, Bok et al. (2011) established a chimpanzee model for the study of norovirus replication and immunity. Seronegative chimpanzees inoculated intravenously with the HuNoV-strain Norwalk virus did not show clinical signs of gastroenteritis, but the onset and duration of virus shedding in stool and serum antibody responses were similar to those observed in humans. Furthermore, chimpanzees vaccinated intramuscularly with VLPs derived from norovirus GI strain were protected from norovirus infection when challenged 2 and 18 months after vaccination. These experiments are very exciting although the robustness of these animal models remains to be determined. It is known that only a few HuNoV particles can cause

illness in humans. It will be interesting to determine the minimum number of particles that can replicate in these animal models. In addition, gnotobiotic animals are extremely expensive, and there are not many institutions that have such facilities or the expertise to maintain these animals.

HUMAN NOROVIRUS SURROGATES FOR FOOD SAFETY RESEARCH

Virus-Like Particle As a Surrogate

It is known that expression of norovirus VP1 results in the formation of VLPs that are antigenically and morphologically similar to native virions. Therefore, the VLPs have been an essential tool to study the epidemiological, immunological, structural, and biochemical properties of HuNoV. It would be lethal to the virus if receptor binding activity were disrupted. Hence, the receptor binding of VLPs can be used as an indicator for virus survival. Alternatively, we can examine the damage of VLPs by electron microscope and SDS-PAGE. Feng et al. (2011) demonstrated that gamma irradiation disrupted the structure of VLPs and degraded VP1 protein. In addition, the capsid of HuNoV has a similar stability compared with MNV-1 after exposure to gamma irradiation (Feng et al. 2011). VLPs can also be used as a surrogate for studying the interaction of norovirus with high-risk foods, such as fresh produce and seafood.

Murine Norovirus As a Surrogate

MNV was first isolated from severely immunocompromised mice that were deficient in recombination-activating gene 2 (RAG2) and signal transducer and activator of transcription 1 (STAT-1) (RAG2/STAT1$^{-/-}$ mice) (Karst et al. 2003). MNV causes systemic infection and lethal disease in STAT1$^{-/-}$ mice (Karst et al. 2003). However, MNV does not cause the typical symptoms of HuNoV infection, such as vomiting and diarrhea. Interestingly, MNV can also be found in feces of infected mice. Since its discovery, MNV has been widely used as a surrogate for HuNoV (Wobus et al. 2006). There are many advantages to using MNV as a surrogate. First, MNV-1 shares many biochemical features with HuNoV. MNV-1 and HuNoV are similar in size (28-35 nm), shape (icosahedral), and also buoyant density (Karst et al. 2003, Wobus et al. 2006). Second, MNV-1 is genetically closely related to HuNoV. Both MNV-1 and HuNoV belong to genus *Norovirus*. MNV and HuNoV have a similar genome size and gene organization. Third, MNV is resistant to acid and heat, and highly stable and persistent in the environment (Cannon et al. 2006, Wobus et al. 2006, Taube et al. 2009). However, MNV differs in many aspects from HuNoV, such as viral receptor binding, pathogenesis, and immunity (Karst et al. 2003, Wobus et al. 2006). MNV does not cause the clinical manifestation of gastroenteritis that the human counterpart does. In addition, MNV uses sialic acid as a functional receptor (Wobus et al. 2006). MNV has tissue tropism in macrophage and dendritic cells. In comparison, HuNoV utilizes HBGAs as cellular receptors for infection (Tan & Jiang 2005a,b). HuNoV infects intestinal cells in vivo. Cannon et al. (2006) directly compared the viabilities of MNV and feline calicivirus (FCV) as surrogates for HuNoV. It was found that MNV was stable across the pH range of 2–10, whereas FCV was rapidly inactivated at a pH less than 3 and greater than 9. FCV was more stable than MNV at 56°C, but they both exhibited similar stability at both 63°C and 72°C. Long-term persistence studies found that MNV was more stable than FCV at room temperature when both viruses were suspended in a fecal matrix and inoculated onto stainless steel coupons (Cannon et al. 2006). Taken together, these studies show that MNV is currently the most relevant and promising surrogate for HuNoV.

Feline Calicivirus As a Surrogate

FCV was first discovered in the 1950s. It is one of the leading causative agents of infectious upper respiratory tract disease in cats, but it does not infect humans (Doultree et al. 1999). FCV does not cause gastroenteritis as HuNoV does. FCV belongs to the genus *Vesivirus* within the family of *Caliciviridae*. Because of its genetic relatedness to HuNoV, FCV has been widely used as a surrogate for HuNoV. However, FCV has distinct biochemical properties as compared with HuNoV. FCV is less stable and less persistent in the environment. It can be rapidly inactivated under acidic conditions (Cannon et al. 2006). Therefore, its ability to serve as a HuNoV surrogate was compromised. With the discovery of MNV in 2003, MNV has replaced FCV as a HuNoV surrogate in most laboratories.

Porcine Sapovirus As a Surrogate

Porcine sapovirus is a member of the genus *Sapovirus* within the family of *Caliciviridae* (Wang et al. 2005, Wang et al. 2007). Other members in the genus *Sapovirus* include human sapovirus that causes gastroenteritis in human. Unfortunately, human sapovirus is also noncultivable. A breakthrough in the cultivation of porcine sapovirus was made by Saif and her colleagues in 1980 (Saif et al. 1980). Porcine sapovirus Cowden strain has been successfully adapted to cell culture by serial passage in a continuous cell line (LLC-PK) (Flynn & Saif 1988). The growth of this virus depends on the presence of bile acid or intestinal content fluid filtrate obtained from uninfected gnotobiotic pigs (Chang et al. 2004). In addition to genetic relatedness to HuNoV, a distinct advantage of using porcine sapovirus as a surrogate is that this virus is enteric and causes gastroenteritis in pigs (Flynn et al. 1988, Guo et al. 2001). It was found that gnotobiotic pigs infected with porcine sapovirus developed symptoms of gastroenteritis, such as diarrhea (Guo et al. 2001). Histopathology studies found that porcine sapovirus replicates in intestinal cells of pigs. The physicochemical properties of porcine sapovirus and HuNoV were recently compared (Wang et al. 2011). The infectivity of sapovirus was not significantly changed after exposure to pH 4–8 at room temperature for 1 h and less than tenfold virus reduction was observed at pH 3. It was also shown that porcine sapovirus and HuNoV shared similar resistance to heat and chlorine treatment. These data suggest that porcine sapovirus may be an improved surrogate for HuNoV. However, the stability of porcine sapovirus to food-processing technologies remains to be investigated.

Tulane Virus As a Surrogate

The Tulane virus, also known as monkey calicivirus, was first isolated in stools of rhesus macaques at the Tulane National Primate Research Center (Farkas et al. 2008). The genome of the Tulane virus is 6,714 nt long, which is the shortest known calicivirus genome. Phylogenetic analysis revealed that Tulane virus clustered into a new genus within the *Caliciviridae*. Farkas and his colleagues proposed the name *Recovirus* (*r*hesus *e*nteric *Calicivirus*) for this new genus represented by Tulane virus. The buoyant density, size, and morphology of Tulane virions are similar to those of other caliciviruses. Tulane virus has been since isolated from stool samples of animals without symptoms of diarrhea; however, the clinical disease linked to Tulane virus infection and its pathogenesis and immunology remain to be investigated. The Tulane virus has been successfully adapted to monkey kidney cells (LLC-MK2 cells). Notably, Tulane virus recognizes the type A and B HBGAs similar to HuNoV (Farkas et al. 2010). This unique characteristic, combined with robust replication in cell culture, suggests that Tulane virus could serve as an improved surrogate

for HuNoV, although the stability of Tulane virus to pH, heat, disinfectants, and food-processing technologies has not been reported.

PROMISING FOOD-PROCESSING TECHNOLOGIES IN INACTIVATION OF HUMAN NOROVIRUS IN HIGH-RISK FOODS

The Choice of Food-Processing Technology

An effective food-processing technology is a key step to minimize and eliminate HuNoV in high-risk foods. The choice of different food-processing technology depends on the type of food, the location of the pathogens in food, and the effectiveness of the technology. For fresh produce, thermal processing technologies, such as heating, cooking, boiling, and microwave, are not practical. Washing and sanitization may be the best option for fresh produce. For internalized viruses, technologies, such as UV and cold plasma, targeting food surface areas are not effective. For seafood, thermal and nonthermal processing technologies may be ideal. Some technologies, such as pasteurization and pulsed electric field, are only suitable for liquid foods. Irradiation may be highly effective at destroying bacterial pathogens in food, but is ineffective with foodborne viruses.

New Approaches for Removal of Norovirus in Fresh Produce

In current practices for processing of leafy greens, chlorine (up to 200 ppm) is the most commonly used sanitizer for washing fresh produce. Unfortunately, chlorine solution is not effective (usually less than 1.2-log virus reductions) in removing viral contaminants from fresh produce (Bae & Schwab 2008, Baert et al. 2009, Baldev et al. 2001, Dawson et al. 2005). Other sanitizers, such as organic acid (peroxyacetic acid), chlorine dioxide, and hydrogen peroxide, are also not effective in removing virus from fresh produce (Baert et al. 2009, Thurston-Enriquez et al. 2005).

Recently, several approaches that can enhance norovirus sanitization in fresh produce have been reported. Su & D'Souza (2011) reported that trisodium phosphate (TSP) significantly enhanced virus sanitization in fresh produce. MNV-1 was inoculated onto lettuce and jalapeño peppers and washed with 2% TSP and 5% TSP, respectively, for 15s or 30s. After washing with 2% TSP, MNV-1 at inoculation level of 5- and 7-log plaque-forming unit (PFU) ml^{-1} were decreased by 2–3- and 2–3.4-log PFU ml^{-1}, respectively. MNV-1 was reduced to undetectable levels by 5% TSP. No significant difference between the two contact times on viral reduction was observed. These results suggest that TSP at 5% appears suitable as an alternative treatment to chlorine washes for virus reduction on produce, without any noticeable visual quality changes. Recently, Predmore & Li (2011) found that surfactants, including sodium dodecyl sulfate (SDS), Nonidet P-40 (NP-40), Triton X-100, and polysorbates (Tween 20), significantly enhanced the sanitization of a HuNoV surrogate (MNV-1) from fresh fruits and vegetables. The use of 50 ppm of surfactant solution alone resulted in 3-log reduction of virus in strawberry and approximately a 2-log reduction of virus in lettuce, cabbage, and raspberry. Moreover, approximately 3-log reduction of virus was observed in all the tested fresh produce after sanitization with a combination of 50 ppm of each surfactant and 200 ppm of chlorine solution. These results demonstrated that the combination of a surfactant with a commonly used sanitizer enhanced the efficiency in removing viruses from fresh produce by approximately 100 times. Since SDS is an FDA-approved food additive, and polysorbates are FDA recognized GRAS (generally recognized as safe) products, implementation of this novel sanitization strategy in industry would be a feasible approach to reduce the virus load in fresh produce.

The Effectiveness of Food-Processing Technologies on Inactivation of Human Norovirus

Overall, the effectiveness of food-processing technologies on inactivating HuNoV is poorly understood because HuNoV is noncultivable. Most of our understanding of the effectiveness of these technologies comes from the studies of surrogates. To date, it has been shown that food irradiation [gamma irradiation and electron beam (E-beam)] is not effective in inactivation of a HuNoV surrogate. Only 1.7- to 2.4-log virus (MNV) reduction was observed in fresh produce (strawberries, romaine lettuce, and spinach) at the dose of 5.6 kGy gamma irradiation (Feng et al. 2011). Sanglay et al. (2011) found that less than 1-log MNV-1 reduction was observed in either cabbage or strawberries after 4 kGy of E-beam irradiation. Su et al. (2010) showed that high-intensity ultrasound is not sufficient to inactivate HuNoV surrogates. UV may not be suitable for fresh produce and seafood because it only kills pathogens on the surface. It is not well understood why these HuNoV surrogates are highly resistant to irradiation. However, it is known that the mechanism underlying viral inactivation by irradiation is involved in disrupting virion structure, damaging viral proteins, and degrading viral genetic materials (Feng et al. 2011). Probably, virus particles are too small to be hit by irradiaiton (Sullivan et al. 1971, Thomas et al. 1982). Because all foodborne and waterborne viruses are nonenveloped viruses, it is possible that the capsids of these viruses are highly resistant to irradiation. In fact, it was found that enveloped viruses are usually more senstitive to gamma irradiation than nonenveloped viruses (Feng et al. 2011).

High pressure processing (HPP) is one of the most promising technologies to control viruses in foods because it effectively inactivates both bacterial and viral pathogens but has minimal effects on the taste, flavor, texture, appearance, and nutritional value of food. The effectiveness of HPP is influenced by many factors, such as processing parameters (pressure, temperature, and holding time) and nonprocessing parameters (the nature of the virus, food matrix, and pH of foods) (Chen et al. 2005, Grove et al. 2006, Grove et al. 2008, Lou et al. 2011a,b). It was reported that more than 5-log reductions were observed for MNV-1 and FCV at 300 MPa at 4°C for 2 min, and 250 MPa at 20°C for 5 min, respectively (Lou et al. 2011a, Kingsley et al. 2002, Murchie et al. 2007). Lou et al. (2011a) demonstrated the feasibility of HPP on inactivating MNV in fresh produce and related products such as juice and puree. More than a 5-log-PFU g^{-1} reduction was achieved in all tested fresh produce when pressurized at 400 MPa for 2 min at 4°C. This pressure had a minimal effect on the sensorial quality of fresh produce. In oyster tissue, it was shown that 4.05-log PFU MNV reductions were achieved at 400 MPa at 5°C for 5 min (Kingsley et al. 2007). Two cultivable foodborne viruses (HAV and rotavirus) can be efficiently inactivated by HPP between 500 and 600 MPa (Khadre & Yousef 2002, Calci et al. 2005, Kingsley et al. 2005, Kingsley & Chen 2009, Lou et al. 2011b). In fact, HPP-processed oysters are commercially available, although it is unknown whether HuNoV is completely inactivated.

VACCINES AGAINST HUMAN NOROVIRUS

Virus-Like-Particle-Based Vaccine

Vaccination is the most effective strategy to protect humans from infectious diseases. Jiang et al. (1992) first found that the expression of VP1 alone in cell culture yields self-assembled VLPs that are structurally and antigenically similar to native virions. Consequently, most HuNoV vaccine studies have focused on VLPs. To date, HuNoV VLPs have been expressed in *E. coli*, yeast, insect cells, mammalian cell lines, tobacco, and potatoes (Jiang et al. 1992, Mason et al. 1996, Zhang et al. 2006). Immunization with VLPs orally or intranasally induced variable humoral, mucosal, and cellular immunities (Ball et al. 1998, Guerrero et al. 2001, Souza et al. 2007b, Bok

et al. 2011). In 1999, Ball and colleagues performed the first clinical study to demonstrate that baculovirus-expressed HuNoV VLPs were safe and immunogenic in humans when administered orally (Ball et al. 1999). Tacket et al. (2000) performed a human volunteer study of a transgenic potato-based VLP vaccine. The 19 out of 20 (95%) volunteers who ingested transgenic potatoes developed significant increases in the number of specific IgA antibody-secreting cells. Four of 20 (20%) volunteers developed specific serum IgG, and 6 of 20 (30%) volunteers developed specific stool IgA. Overall, 19 of 20 volunteers developed an immune response. In the United States, LigoCyte Pharmaceuticals Inc. licensed two VLP-based vaccine candidates, dry powder formulation (intranasal delivery) and liquid formulation (intramuscular delivery). The vaccines include an adjuvant, GSK's MPL® Toll-4 Agonist, for enhancing the efficacy of vaccine. In human clinical trials, volunteers that received the dry powder VLP vaccine reduced the risk of illness by 47% after exposure to HuNoV (LigoCyte Pharmaceuticals Inc. 2010). There were significant reductions in clinical norovirus illness, infection, and severity of illness in individuals who received the vaccine compared with those who received the placebo (LigoCyte Pharmaceuticals Inc. 2010). Although these studies are very promising, there are several limitations of VLP-based vaccine candidates. Preparation of VLPs in vitro is time consuming and expensive. Immunization usually requires a high dosage of VLPs and multiple booster immunizations. The efficacy of VLP-based vaccines relies on the addition of mucosal adjuvants such as cholera toxin and *E. coli* toxin. Also, the duration of the antigen stimulation may be limited because VLPs are actually proteins, nonreplicating immunogens.

Protrusion Particle–Based Vaccine

The structure of the capsid protein can be divided into two principal domains, shell (S) and protrusion (P), linked by a flexible hinge region. Tan & Jiang (2005b) first found that expression of a P domain (without the hinge region) of VP1 protein resulted in the formation of ring- or pentagon-shaped structures with a diameter of 5 nm. This small particle was named the P particle. Interestingly, the P particles exhibited enhanced binding ability to HBGAs. One important advantage of using the P particle as a HuNoV vaccine candidate is that the P particle can be easily produced in *E. coli*, it is extremely stable, and it is highly immunogenic (Tan & Jiang 2005b). Structure studies revealed that the P particle is an octahedral nanoparticle composed of 24 copies of the P domain of the norovirus capsid protein (Tan et al. 2011). There are three surface loops per P domain, making a total of 72 loops per particle. Notably, the P particle can also be used as a platform to deliver other antigens. To demonstrate this novel concept, Tan et al. (2011) inserted rotavirus VP8 gene into one of the loops. Interestingly, the insertion of this foreign antigen did not affect P particle formation. The resulting VP8 chimeric P particles triggered a high level of neutralization antibody titer against rotavirus in mice. Furthermore, the sera blocked norovirus VLPs from binding to HBGAs. Thus, the P particle-VP8 chimeras may serve as a dual vaccine against both rotavirus and norovirus. Similarly, the Matrix protein (M2) of influenza A virus was inserted into one of the loops in the P particle, which resulted in the formation of M2e-P particles. Mice immunized with M2e-P particles were fully protected from the influenza virus challenge (Xia et al. 2011). These findings demonstrated that the P particle is not only an excellent vaccine candidate for HuNoV but also a novel platform for antigen presentation.

The Needs for Developing Live-Vectored Vaccines Against HuNoV

Generally, a live attenuated virus vaccine stimulates strong systemic immunity and provides durable protection because replication in vivo results in a high level of intracellular synthesis of the full

complement of viral antigens over a prolonged period. However, such a vaccine is not realistic for viruses that cannot be grown in cell culture. Given this limitation, the virus cannot be attenuated, and even if an attenuated strain were available, it could not be mass produced. In this situation, a vectored vaccine may be ideal to overcome this obstacle.

Venezuelan Equine Encephalitis Virus–Vectored HuNoV Vaccine

Venezuelan equine encephalitis (VEE) virus is a member of *Alphavirus* subfamily within *Togaviridae* family. VEE is the first viral vector that has been used for a HuNoV vaccine study. It is a replicon-based vector that only contains a partial VEE genome encoding nonstructural proteins required for transcription and replication. The vectored vaccine is constructed by co-transfection of a replicon that contains the norovirus capsid gene and helper plasmids that encode VEE structural genes. Mice inoculated with this single-cycle vectored vaccine triggered a high level of norovirus-specific systemic, mucosal, and heterotypic immunity (Harrington et al. 2002). Co-administration of multivalent norovirus VLP vaccines with alphavirus adjuvant particles to mice resulted in homotypic and heterotypic humoral and protective immunity to HuNoV and MNV strains. Furthermore, inclusion of alphavirus adjuvants in the inoculum significantly augmented VLP-induced systemic and mucosal immunity (LoBue et al. 2009). Using MNV as a model, it was shown that a VEE-adjuvanted vaccine protected mice from the MNV challenge (LoBue et al. 2009). These studies demonstrated the feasibility of using a vectored vaccine against HuNoV. However, there are some potential disadvantages to the use of VEE as a vector. Although the VEE replicon is a single-cycle replicating vector, the biosafety of VEE has been questioned because VEE is a biodefense pathogen, and the use of functional VEE genes is restricted.

Adenovirus-Vectored HuNoV Vaccine

Adenovirus is a double-stranded DNA virus that allows for insertion of exogenous genes without affecting efficient viral replication or packaging. It was reported that intranasal immunization of a recombinant adenovirus-expressing capsid protein of HuNoV stimulated systemic, mucosal, and cellular Th1/Th2 immune responses in mice (Guo et al. 2008). It was also shown that mice primed with recombinant adenovirus-vectored vaccine and boosted with traditional VLP-based vaccine triggered much stronger humoral, mucosal, and interferon-gamma responses than those immunized with adenovirus-based or VLP vaccine alone (Guo et al. 2009). Such a prime-boost vaccination may be a promising strategy to improve current VLP-based HuNoV vaccine development. However, one of the major drawbacks of the adenovirus-vectored vaccines is that a large portion of the global population has preexisting immunities against the adenovirus vector (Sekaly 2008). Thus, in vivo delivery of the adenovirus-vectored vaccine may be hampered by the host immune response.

Vesicular Stomatitis Virus–Vectored HuNoV Vaccine

Vesicular stomatitis virus (VSV) is a nonsegmented negative-sense RNA virus that belongs to the virus family *Rhabdoviridae*. VSV has been shown to be an excellent vector to deliver foreign antigens for live vaccines, oncolytic therapy, and gene therapy (Li et al. 2005, Li et al. 2006). Recently, Ma & Li (2011) constructed a recombinant VSV (rVSV-VP1) that expresses the major capsid protein of HuNoV. The yield of VLPs by the VSV expression system is approximately ten times higher than that of the baculovirus expression system. Recombinant rVSV-VP1 was attenuated in cell culture as well as in mice models. Mice inoculated with a single dose of

rVSV-VP1 through intranasal and oral routes stimulated a significantly stronger humoral and cellular immune response than baculovirus-expressed VLP vaccination. Moreover, it was shown that mice inoculated with rVSV-VP1 triggered a comparable level of fecal and vaginal IgA antibody. A VSV-based vaccine offers a number of other distinctive advantages, including genetic stability, expression of multiple antigens, simplicity of production, multiple routes of administration, and ease of manipulation. Unlike the adenovirus vector, human infection with VSV is very rare, and the general population is free of preexisting immunity against VSV. Therefore, these advantages will facilitate the clinical trials of a VSV-vectored vaccine in the future. The VSV recombinant system not only provides a new approach to generate HuNoV VLPs in vitro but also a new avenue for the development of vectored vaccines against HuNoV.

ANTIVIRAL DRUGS AGAINST HUMAN NOROVIRUS

No antiviral drug is currently available for HuNoV. For healthy individuals, norovirus-associated illness is usually self limited, and no treatment is needed or is limited to supportive care (such as rehydration). However, an effective antiviral drug may be beneficial for some severe cases. Particularly, there are many lethal cases associated with infants and young children in developing countries. Given that the virus cannot be grown, approaches to screen effective drugs are very limited. It was reported that ribavirin and interferons inhibited viral replication in a replicon system (Chang & George 2007). Using the theory of norovirus-receptor interaction, Feng & Jiang (2007) developed a saliva-based receptor binding assay to screen a compound library for inhibition of norovirus binding to HBGA receptors. Among the 5,000 compounds screened, 153 revealed inhibitory activities against VLPs binding to the A antigen, and 14 of the 153 compounds revealed strong inhibition, with a 50% effective concentration less than 15 μM. Using a similar assay, Zhang et al. (2011) screened 50 Chinese herbal medicines and identified that two medicines (Chinese gall and pomegranate) were highly effective in blocking receptor binding. More interestingly, the authors identified tannic acid in these medicines as a strong inhibitor in binding norovirus P particles to both A and B saliva with an IC50 of 0.1μM. It seems possible that antiviral drugs targeting the first step of viral infection, the virus-receptor binding, may be developed. To date, no clinical data are available to evaluate the effectiveness of these compounds against norovirus infection.

FUTURE DIRECTIONS

The Bottleneck: Cultivation of HuNoV

HuNoVs are difficult to study because of the lack of an efficient in vitro cell culture system. Recent efforts in 3D cell culture and ex vivo cell culture systems are encouraging. However, they still may not be robust enough to study HuNoV. Ultimately, the understanding of the survival of HuNoV during food processing depends on the successful cultivation of this virus. One important future direction is to identify which steps are critical for HuNoV replication. For example, HuNoV recognizes HBGAs as cellular receptors. So, what is the expression level of HBGAs on cell surfaces? Are the amounts of receptors sufficient for viral attachment and entry? If viruses do enter the cells, why don't they replicate? Is there any defect in viral assembly or release?

Develop Food Virology Program for Food Safety Higher Education

Current food safety education programs are almost exclusively devoted to bacterial pathogens. Foodborne viral pathogens are typically not mentioned in textbooks, curricula, or classrooms.

There is a critical need to recruit more virologists for food safety research. Furthermore, new curricula specifically addressing foodborne viruses should be developed. Recently, the Department of Food Science and Technology at The Ohio State University has started to offer two food virology courses, Food Safety and Public Health-Food Virology (undergraduate level), and Advanced Food Microbiology II: Food Virology and Immunology (graduate level). Incorporation of food virology courses into the education system will improve food safety and train the next generation of food safety professionals.

Develop Food Virology Module for Extension and Outreach

No major extension and outreach effort has focused on the highly relevant challenge of preventing and minimizing virus contamination in high-risk foods. There is an urgent need to bridge the significant knowledge and practice gap in the transmission, prevention, control, and minimization of enteric foodborne viruses in the food chain. Outreach to all sectors of the farm-to-table continuum is needed because virus contamination occurs anywhere from preharvest to postharvest. It is important to deliver and disseminate virological knowledge and preventive practices to stakeholders and incorporate major research findings as they become available to target audiences and stakeholders. These extension and outreach activities will likely reduce the incidence of foodborne viral outbreaks.

Establish Interdisciplinary Collaborations Between Calicivirologists and Food Safety Experts

The good news is that there are many excellent calicivirologists in medical and veterinary schools, and many outstanding food safety experts in the food science departments across the country. The bad news is that there is little interaction and collaboration between these calicivirologists and food safety experts. To achieve success, both basic and applied virological technologies are needed to prevent and eliminate noroviruses from the food chain. To enhance the collaborative opportunities, calicivirologists may invite food safety professionals to their society conferences, such as the American Society for Virology, the Positive-Sense RNA Virus conference, and the Calicivirus conference. On the other hand, food scientists may invite calicivirologists to attend their society meetings, such as those of the International Association for Food Protection (IAFP) and Institute of Food Technologists (IFT). Multidisciplinary and multi-institutional collaborations will undoubtedly enhance our capability to combat this important biodefense agent.

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