Norovirus disease among older adults

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Abstract: Norovirus, a leading cause of gastroenteritis outbreaks worldwide, results in substantial direct and indirect healthcare costs. Adults older than 65 years of age bear a significant proportion of the disease burden, and the disease course in this population is often more severe and protracted. In this narrative review, we discuss the epidemiology of norovirus infection, mechanisms of pathogenesis, and transmission pertinent to outbreaks along with infection prevention and control efforts. We also describe the clinical manifestations of norovirus disease with a focus on individuals older than 65 years of age, diagnosis and available treatment options, and the challenges and progress within vaccine development.

Keywords: norovirus, older adults

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Introduction
A ‘winter vomiting disease’ characterized by primary symptoms of nausea and vomiting with a winter predilection was first described in 1929.\(^1,2\) This disease was later named ‘Norwalk virus’ after more than 50% of students and teachers of an elementary school in Norwalk, Ohio, United States were afflicted with nausea, vomiting, and abdominal cramps lasting 12–48 h in October 1968.\(^3,4\) A 27-nm virus-like particle known as ‘Norwalk virus’ or ‘small round-structured virus (SRSV)’ was later visualized in stool specimens using immune electron microscopy.\(^5,6\) Later, the genus of the virus was formally named Norovirus after the place of the original outbreak.\(^7\) Norwalk virus is the only species within the genus as per the International Committee on Taxonomy of Viruses classification.\(^8\)

The Norovirus genus is a group of non-enveloped, single-stranded positive-sense ribonucleic acid (RNA) viruses belonging to the Caliciviridae family. The human norovirus genome contains three open reading frames (ORFs) that encode eight viral proteins (VPs). The variable amino acid sequence of the major capsid protein (VP1) within ORF-2 determines the genogroup. There are 10 known genogroups (GI–GX),\(^2,9,10\) of which only genogroups GI, GII, and GIV cause human disease.\(^11\) Initially emerging in 2012, the GII.4 strain has become the most prevalent genotype and is implicated in approximately 80% of norovirus outbreaks.\(^10,12\)

Herein, we review norovirus epidemiology, describe its pathogenesis and immunity, and discuss norovirus transmission and its consequences for outbreaks and infection prevention and control efforts. We review clinical symptoms of norovirus infection, therapies and associated uncertainties, and vaccine development.

Epidemiology
Norovirus is the most commonly recognized foodborne gastroenteritis pathogen globally and is responsible for approximately 20% of all foodborne illnesses reported by the World Health Organization. Norovirus accounted for nearly 125 million cases of a total of 600 million cases of foodborne illnesses and 34,929 deaths globally in 2010.\(^13\) In the United States alone, norovirus is estimated to be responsible for 19–21 million total illnesses resulting in 1.7–1.9 million outpatient visits, 400,000 emergency room visits, 56,000–71,000 hospitalizations, and 570–800 deaths per year.\(^14\) These numbers likely underestimate the true incidence of norovirus gastroenteritis as not all individuals afflicted may seek or require medical care.\(^2\)
There are several patterns of norovirus infections that are of importance. Although norovirus infections are detected year-round, norovirus infection rates are highest during the winter months peaking in February and March in the northern hemisphere.\textsuperscript{15} Norovirus afflicts across age groups, but young children and elderly adults are more vulnerable to severe disease and associated morbidity and mortality. For instance, there is a notable bimodal age-related tendency of hospitalization from norovirus infection; young children and elderly populations are more susceptible to hospitalization compared to the other age groups (Table 1).\textsuperscript{16} Furthermore, norovirus has been reported as the etiological agent of gastroenteritis outbreaks in crowded, semi-closed environments, and healthcare-associated settings, such as long-term care facilities, hospitals, cruise ships, correctional facilities, restaurants, schools, child care centers, and parties.\textsuperscript{17} Hospitals and long-term care facilities bear the most outbreak burden with $>50\%$ of Norovirus outbreaks occurring in these settings.\textsuperscript{18} Its propensity to cause outbreaks is due to its environmental stability, low inoculum required for infection, and the potential high volume and long period of viral shedding.\textsuperscript{19} Hospitalizations and mortality rates due to norovirus gastroenteritis are higher during outbreaks than during non-outbreaks. In a comparison of hospitalization between norovirus outbreak versus non-outbreak periods of 308 nursing homes from 2009 to 2010, there were 124 hospitalizations per nursing home-year (95\% CI, 119.4–129.1) that occurred during the norovirus outbreak periods and 109.5 hospitalizations per nursing home-year (95\% CI, 108.6–110.3) during the non-outbreak periods, resulting in an adjusted rate ratio (RR) of 1.09 (95\% CI, 1.05–1.14).\textsuperscript{20} All-cause mortality rates during norovirus outbreaks were estimated to be 53.7 deaths per nursing home-year (95\% CI, 50.6–57.0) in contrast to 41.9 deaths per nursing home-year (95\% CI, 41.4–42.4) during non-outbreak periods, with an adjusted RR of 1.11 (95\% CI, 1.05–1.18).\textsuperscript{20}

### Pathogenesis

Genogroup GII is responsible for most norovirus outbreaks and infections among older adults.\textsuperscript{21} Of outbreaks occurring in long-term care facilities in the United States from 2009 to 2018 with reported genogroups, genogroups GII and GI were responsible for 6370 (87.3\%) and 862 (11.8\%) outbreaks, respectively.\textsuperscript{22} The GII.4 strain was the causative agent in 81.8\% of the genogroup GII-associated outbreaks. This strain is characterized by antigenic variation every 2–5 years predisposing the virus to immune evasion,\textsuperscript{2,23} which confers important implications for vaccine development.

Norovirus genotypes are antigenically distinct from each other, and host immunity is therefore genotype-specific and not cross-protective against subsequent infection by multiple genotypes.\textsuperscript{24} The immune response against norovirus involves both cellular and humoral responses. Histo-blood group antigen (HBGA)-blocking assays are used as a surrogate marker of neutralization antibodies and HBGA-blocking antibodies are suggested to be associated with disease protection and lower viral shedding.\textsuperscript{25} Pre-existing norovirus-specific salivary immunoglobulin A (IgA) levels are inversely correlated to severity of vomiting and diarrhea, whereas pre-existing and post-infectious norovirus-specific fecal IgA levels are inversely correlated to peak viral load and duration of viral shedding.\textsuperscript{26} Norovirus-specific IgG memory B cells are also associated with protection from norovirus gastroenteritis and correlated with pre-existing serum HBGA-blocking antibodies.\textsuperscript{26}

Host genetic factors and immune responses are important determinants of norovirus susceptibility, infection severity, and degree of viral shedding. Individuals who carry a gene-encoding alpha-1,2-fucosyltransferase (FUT2) express

\begin{table}
\centering
\begin{tabular}{|c|c|}
\hline
Age (years) & No. of hospitalizations per 10,000 persons (cases) \\
\hline
0–4 & 9.4 \\
5–17 & 1.1 \\
18–64 & 1.0 \\
65–74 & 4.7 \\
75–84 & 9.2 \\
>84 & 18.5 \\
\hline
\end{tabular}
\caption{Seasonal mean number of hospitalizations from norovirus infection by age groups across July 1996/June 1997 to July 2006/June 2007.\textsuperscript{14}}
\end{table}
HBGAs on the surface of intestinal epithelial cells and these HBGAs antigens bind to VP1 facilitating norovirus attachment and entry, thus conferring susceptibility to infection.27 Individuals who do not encode FUT2 are known as ‘non-secretors’ and are resistant to GI.1 genogroup, the original Norwalk virus identified in 1969. However, some of the other strains, including GI.4, infect ‘non-secretors’ and demonstrate immune evasion by means of high antigenic variation, antibody-binding epitope blockade, and potentially binding to non-HBGA ligands.12,28–30

Knowledge of host immune responses to norovirus infections is primarily derived from challenge studies due to the historic lack of an in vitro cell culture model.27 Early challenge studies suggested norovirus immunity lasted only 6 months to 2 years; however, mathematical modeling using observational evidence of community transmission estimated the duration of norovirus immunity to be 4.1–8.7 years.31

Efforts to elucidate pathophysiology and explore vaccine and antiviral targets have been made by studying various in vitro models. For instance, Chang et al.32 described a norovirus replicon model in which they observed stable expression of self-replicating norovirus RNA in human and hamster cells, generated by transfection. The authors also reported dose-dependent reduction of RNA expression in the model during the presence of interferon alpha, indicating the role of innate immunity in norovirus replication. Although the replicon model is potentially a very powerful tool in studying norovirus pathophysiology, the replication level is reportedly low.32,33 Jones et al.34 suggested in 2014 that human and murine norovirus were able to infect B cells in the presence of HBGA-expressing enteric bacteria. Their subsequent study in 2015 illustrated human norovirus replication in human B cells in vitro.35 In contrast, Brown et al.36 found that among pediatric patients with severe combined immune deficiency, 60% of B cell-positive and 63% of B cell-negative patients developed norovirus infection, suggesting that the viral replication in B cells is not essential for human norovirus infection. More recently, Ettayebi et al.37 used the stem-cell derived human intestinal enteroids (HIEs) as a realistic in vitro experimental model to show replication of several strains of human norovirus, facilitating a deeper understanding of norovirus pathophysiology.

### Transmission
Norovirus is primarily transmitted fecal-orally, through person-to-person contact or food or waterborne contamination.38 An analysis of norovirus outbreaks in the European Network from July 2001 to June 2006 demonstrated that person-to-person transmission is the major mode of transmission, accounting for 55% of non-GII strain cases and 90% of GII strain cases.39 Similarly, a 10-year surveillance study of long-term care facilities in the United States from 2009 through 2018 by Calderwood et al.22 revealed that 90.4% of the norovirus cases occurred from person-to-person transmission, 0.7% from foodborne and environmental transmission, and the remainder from unknown sources.

Norovirus, which has a more than 30% secondary attack rate in long-term care facilities among contacts of infected individuals,40 is one of the most frequent causes of healthcare-associated infectious disease outbreaks because of several characteristics: low infectious dose, effective transmission via multiple modes, environmental stability, long duration of viral shedding, and short duration of prior immunity. The inoculum required to cause infection is as few as 18–1000 virions.41 Although the main transmission mode is fecal-oral through contact of infected persons or contaminated food or water and secondary attacks are sustained via person-to-person and environmental transmission,42 norovirus transmission also occurs through aerosolization of particles from vomitus, diarrhea, and toilet flushing.43 Norovirus RNA has been detected in hospital dust and air samples collected during an outbreak, primarily from recent vomiting, suggesting this is a possible route of infection.43 Norovirus can withstand a range of temperatures and survives on surfaces for extended periods of time. One study estimated that norovirus particles remain infectious for up to 28 days on stainless steel and polyvinyl chloride surfaces at 20°C.44

Viral shedding is another significant contributor to transmissibility, occurring in both asymptomatic and symptomatic individuals. Viral shedding occurs prior to symptom onset in nearly 30% of those infected and persists for several days to weeks depending on host age and immune status.18 In a challenge study, norovirus was detected in stool of healthy hosts 4–8 weeks after inoculation.45 In immunocompromised hosts, symptoms and viral shedding may occur for 6–1004 days.21 The role of persistent viral shedding in secondary...
transmission and the time necessary for an infected person to be deemed no longer infectious are largely unknown. Further study is needed to understand the relationship between norovirus infectivity and viral shedding. In vitro experimental models using HIEs have shown promise. For instance, Ettayebi et al. adopted the HIE model on a study with GII.3 and GII.4 strains after 6 days post-infection.

A notable proportion of those infected with norovirus remain asymptomatic and these individuals play a significant role in transmission and outbreaks. Data for the rates of asymptomatic infection come primarily from volunteer infection challenges performed for norovirus vaccine studies and through outbreak analyses. Statistical modeling performed by Miura et al. using food-borne norovirus outbreak data from 2005 to 2006 of 55 outbreaks in Japan estimated an asymptomatic ratio of 32.1%, similar to the 30% asymptomatic infection rate found in infection challenge studies. Norovirus was detected in the stool of 32% of asymptomatic individuals between 2 and 6 days after inoculation in a 1994 challenge study and in all five asymptomatic participants in a 2008 challenge study. Furthermore, the mean viral load detected in stool is similar between symptomatic and asymptomatic individuals infected with Norovirus. This suggests that norovirus-infected individuals who are asymptomatic or have subclinical disease may potentiate transmission if infection prevention and control efforts are not directed toward this population.

Infection prevention and control

The goals of infection prevention and control for norovirus gastroenteritis are to prevent and minimize the scale of outbreaks. This is achieved by a multipronged approach of patient isolation or cohorting, hand hygiene, personal protective equipment for standard and contact precautions, environmental cleaning and disinfection, diagnostics, patient transfers, consideration of unit closures as need be, staff cohorting, and finally, visitor restrictions. Infection prevention and control measures particularly pertinent for norovirus infections are mitigating potential aerosolization of particles, optimizing duration of isolation, and preferential hand hygiene with soap and water over alcohol-based sanitizers, owing to the poor virucidal activity of alcohol to non-enveloped viruses. Because norovirus particles may be aerosolized, the Society of Healthcare Epidemiology of America (SHEA) recommends the use of a surgical or procedure mask and eye or face protection if there may be anticipated splashes to the face, such as through vomitus. During outbreaks, contact precautions are continued for patients for a minimum of 48 h after resolution of symptoms. One area of uncertainty is the optimal duration of isolation or precautions for patients at risk for prolonged or relapsing disease course and prolonged viral shedding. SHEA suggests a longer period of isolation may be considered, but no specific duration has been defined. Isolation for the duration of hospitalization has been proposed for immunocompromised patients, but the applicability of this to individuals residing in long-term care facilities is unclear.

Although it is difficult to determine which infection prevention and control interventions are most effective at preventing and curtailing a norovirus outbreak, hand hygiene is the cornerstone. During norovirus outbreaks, active promotion of hand hygiene particularly with soap and water, rather than alcohol-based hand sanitizers are recommended. Norovirus is difficult to eradicate using alcohol-based sanitizers because it does not have a lipid envelope. Thus, alcohol-based hand sanitizers are not a suitable primary hand hygiene method in long-term care settings during norovirus outbreaks. A multivariable analysis conducted by Blaney et al. of norovirus outbreaks in long-term care facilities identified that facilities in which staff were equally or more likely to use alcohol-based hand sanitizer than soap and water was associated with a 6.06 odds ratio of a norovirus outbreak. Liu et al. measured norovirus cDNA before and after hand washing with commercial alcohol-based hand sanitizer (62% ethyl alcohol), antimicrobial soap (0.5% triclosan), and water rinse only. Their experiment revealed higher norovirus genomic copy reduction with antimicrobial soap (0.67–1.20 log10) and water rinse only (0.58–1.58 log10), whereas alcohol-based hand sanitizer resulted in reduction of 0.14–0.34 log10. A more recent experimental model by Costantini et al. using HIEs was in agreement with the above findings. In total, 10% fecal filtrates were treated with 70% alcohol (ethanol or isopropyl alcohol), and the RNA count was suppressed initially, however, recurred by the third day post infection.
Disinfection of surfaces is another key element in preventing norovirus transmission. Liu et al.\textsuperscript{54} compared in vitro reduction of Norwalk virus concentration using sodium hypochlorite and ethyl alcohol in varying concentrations. The in vitro reduction was greater for sodium hypochlorite, whereas ethyl alcohol failed to display a concentration-dependent norovirus genome reduction.\textsuperscript{54} An in vitro experimental model by Costantini et al.\textsuperscript{56} using HIE showed complete suppression of norovirus GII strains in fecal specimens with as low as 50 parts per million (ppm) of chlorine solution.\textsuperscript{56} Comparison of a commercial-grade alcohol-based surface disinfectant (‘PSS’) containing 29.4% ethanol (pH 12.6–12.9) with sodium hypochlorite solutions of various concentrations (1000–5000 ppm) was performed by Escudero-Abarca et al.\textsuperscript{57} In vitro assays (suspension and soil on stainless steel surface) consistently showed higher genome-equivalent copy number reduction for sodium hypochlorite as opposed to PSS, although PSS showed similar efficacy comparable to PSS, although PSS showed similar efficacy comparable to sodium hypochlorite and ethyl alcohol in varying concentrations. The Center for Disease Control and Prevention (CDC) currently recommends using 1000–5000 ppm of chlorine solution.\textsuperscript{56} The authors suggested loss of attachment when 60-s contact time was applied with high soil efficacy comparable to 1000–5000 ppm chlorine, opposed to PSS, although PSS showed similar number reduction for sodium hypochlorite as ethyl alcohol in varying concentrations. The deterioration of host immunity, known as ‘immunosenescence’ and the presence of other medical comorbidities are thought to predispose older individuals to a more severe and protracted disease course.\textsuperscript{21} A study of norovirus outbreaks

Clinical manifestations
Investigations have provided insights on the norovirus incubation period, although exact calculations are challenging because primary and secondary cases can be difficult to distinguish when the incubation period is brief. In the 1968 index outbreaks occurring in Norwalk, Ohio and Columbus, Ohio (US), evaluation of secondary cases found an average incubation period of 48 h. A 2013 meta-analysis of 2540 observations from 23 studies estimated the median incubation period for norovirus genogroups I and II to be 1.2 days with 5% and 95% of cases exhibiting symptoms 0.5 and 2.6 days after infection, respectively.\textsuperscript{60} This estimate, however, included estimates from uncited sources or source estimates, of which 54% originally drew from one or two of the same articles.\textsuperscript{60} The CDC analyzed norovirus foodborne outbreaks from 1998 to 2013 reported to the national Foodborne Disease Outbreak Surveillance System (FDOSS).\textsuperscript{61} Of the 2172 norovirus foodborne outbreaks evaluated, the median incubation time period was 32 h with median outbreak incubation period ranges being 27–37 h (70% of outbreaks, 15th–85th percentile) and 12–47 h (95% of outbreaks, 2.5th–97.5th percentile). This incubation time of 12–47 h can assist in differentiating norovirus from other bacterial causes of gastroenteritis, except for Salmonella enterica (median incubation time of 32 h, $p=0.19$). The incubation periods of Staphylococcus aureus, Bacillus cereus, Clostridium perfringens, and Vibrio parahaemolyticus are significantly shorter ($p<0.0001$) and the incubation periods of Shigella species and Campylobacter species are significantly longer ($p<0.001$) compared to that of norovirus.\textsuperscript{61}

Mild gastrointestinal symptoms of nausea, vomiting, diarrhea, and abdominal pain occur following a brief incubation period.\textsuperscript{2} These symptoms may be accompanied by fever, chills, headache, generalized malaise, and myalgias.\textsuperscript{21} Most patients, especially healthy young adults, experience resolution of symptoms within 2–4 days.\textsuperscript{21} Complications rarely occur, although norovirus infection does confer increased odds of post-infectious irritable bowel syndrome, dyspepsia, constipation, and gastroesophageal reflux disease.\textsuperscript{62} Older adults residing at long-term care facilities are at an increased risk of exposure, and if infected, vulnerable to longer and more severe symptoms causing complications and hospitalizations. The deterioration of host immunity, known as ‘immunosenescence’ and the presence of other medical comorbidities are thought to predispose older individuals to a more severe and protracted disease course.\textsuperscript{21}
from 2002 to 2004 evaluated clinical characteristics and symptom duration among hospitalized patients and nursing home residents with median ages of 81 and 87.5 years, respectively. Individuals \( \geq 85 \) years of age had the slowest recovery, with 40% still symptomatic after 4 days. Costantini et al. found similar results in a prospective study of norovirus outbreaks in long-term care facilities with those aged \( \geq 70 \) years experiencing a significantly longer illness duration (median 4 days) than those \(< 70\) years of age, and a higher proportion of patients \( \geq 70 \) years of age reporting a perceived severe illness.

In those \( \geq 65 \) years of age, the hospitalization and mortality rates from norovirus infection are striking. Hospitalization rates are estimated to be 0.5%–6%, and of those hospitalized, 36% were admitted to intensive care units. Case fatality rates are 0.3%–1.6% compared to 0.03% for those 18–64 years of age with 90% of deaths from norovirus-associated illness occurring in persons \( \geq 65 \) years of age.

**Diagnosis**

Rapid diagnosis of norovirus infection is crucial to facilitate swift implementation of infection control measures aimed at reducing transmission. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR), such as TaqMan-based RT-qPCR, is the most sensitive method and can distinguish GI, GII, and GIV genotypes. It can be performed on stool, vomitus, and food or environmental specimens and detects as few as 10–100 virus copies, but requires specific equipment. The BioFire FilmArray gastrointestinal (GI) panel, a multiplex nucleic acid test that detects bacterial, viral, and parasitic agents of gastroenteritis, is more widely available and offers rapid results. It only detects norovirus GI/GII genotypes and does not distinguish between them. The FilmArray GI panel has a positive percent agreement of 94.5% and negative percent agreement (NPA) of 98.8% compared to PCR with bidirectional sequencing and has a limit of detection of \( 1 \times 10^4 \) norovirus copies/ml.

Electron microscopy assays, enzyme immunoassays (EIA), and immunochromatogenic lateral flow assays are other diagnostic methods less commonly used because of their notable limitations. Although electron microscopy assays can detect other viral pathogens including norovirus based on morphological characteristics, it is expensive, labor-intensive, has low sensitivity and specificity. This technique may also not be readily available for use in clinical practice settings. EIA and immunochromatogenic lateral flow assays which detect norovirus antigens in stool have poor sensitivity, 50%–75% and 35%–76%, respectively, and therefore negative results require confirmation with a second diagnostic method.

In the absence of or delays in diagnostic testing, diagnostic criteria are available to assist in outbreak management. Kaplan et al. analyzed symptomatology from norovirus outbreak investigation descriptions in the 1970s and devised criteria to distinguish norovirus from other bacterial etiologies of gastroenteritis when laboratory confirmation of norovirus cannot be obtained. The Kaplan criteria include a short incubation period (24–60h), a short infection duration (12–60h), high frequency of vomiting (\( > 50\% \)), and no enteric bacteria found. If all four criteria are met, the likelihood of norovirus being the etiologic agent is high. Subsequent analysis of the Kaplan criteria performed by Lively et al. demonstrated a specificity of 99% but sensitivity of 68%. Furthermore, only 3.3% of norovirus outbreaks reported all criteria. Re-evaluation of norovirus outbreak characteristics using classification and regression tree modeling resulted in revised proposed criteria that offer greater sensitivity (87.5%) and excellent specificity (92.4%). The set of revised criteria includes a greater proportion of cases with vomiting than with fever, bloody diarrhea in less than 10% of cases, and vomiting in greater than 25% of cases. However, the criteria devised by Kaplan and Lively used analysis of outbreak investigations and may not be fully reflective of norovirus symptomatology in those \( \geq 65 \) years of age.

**Treatment**

The mainstay of treatment is supportive care and targeted treatments for associated complications, such as electrolyte imbalances, dehydration, and acute kidney injury. There are no US Food and Drug Administration (FDA)-approved treatments for norovirus infection including no antiviral agents, although nitazoxanide and immunoglobulins have been used off-label in primarily immunocompromised hosts with varying efficacy (Table 2).
Nitazoxanide and its active metabolite tizoxanide are thiazolide compounds with broad anti-infective activity against bacteria, protozoa, parasites, and viruses. Formally, it is approved by the US FDA only for the treatment of gastroenteritis caused by Cryptosporidium parvum and Giardia lamblia but has been shown to have in vitro and in vivo activity against norovirus, rotavirus, and adenovirus. The specific mechanism of action against norovirus is thought to involve interference of protein synthesis and the activation of host cellular antiviral response pathways. The efficacy of nitazoxanide for the treatment of norovirus gastroenteritis in adults has been evaluated in only one randomized controlled double-blinded trial. In this trial, 50 persons who were at least 12 years of age with clinical gastroenteritis and positive stool EIA testing for either norovirus, adenovirus, or rotavirus were enrolled to receive either nitazoxanide twice daily or placebo for 3 days. Of the 13 participants with norovirus detected in stool, the median time to resolution of illness for the nitazoxanide group (n=6) was 1.5 days, a statistically significant reduction compared to the median time to resolution of illness for the placebo group (n=7), which was 2.5 days.

For immunocompromised hosts including those with solid organ or hematopoietic stem cell transplants (HSCT), hematological malignancies, and primary immunodeficiencies, the use of nitazoxanide to treat norovirus infection has been described in case reports and case series with varying success. Siddiq et al. described a patient with relapsed refractory acute myeloid leukemia (AML) and allogeneic HSCT with chronic graft-versus-host disease (GVHD), and Ghusson et al. described three renal transplant recipients who were treated with nitazoxanide for norovirus infection with resolution of diarrhea within 4 days and 1 week, respectively. Other studies have suggested nitazoxanide treatment in immunocompromised patients with norovirus infection that did not result in improvement in diarrhea. In a study of 195 solid organ transplant (SOT) recipients with norovirus infection that accounted for changes in immunosuppression and intravenous immunoglobulin (IVIG) use, SOT recipients treated with nitazoxanide for 3 days reported a significant symptom improvement (59.6%) compared to SOT recipients who were not treated with nitazoxanide (42.3%), but there was no statistically significant difference in median time to symptom improvement (6 versus 23 days, p=0.170). An important factor to consider in this population is incidence of chronic or relapsing norovirus infection, the pathophysiology of which is poorly understood but nitazoxanide treatment may not be effective. There is an ongoing double-blind,
placebo-controlled trial studying the efficacy and safety of nitazoxanide in SOT and HSCT recipients for treatment of norovirus diarrhea.

The efficacy of nitazoxanide for norovirus gastroenteritis has not been specifically studied in older individuals (i.e. \( \geq 65 \) years of age), and thus, its clinical benefit for symptom resolution or impact on transmission is unknown. Although duration of symptoms from norovirus infection may be longer for older adults compared to their younger counterparts, there is no evidence to suggest that chronic or relapsing norovirus infection occurs. The role of nitazoxanide is uncertain but warrants further study given the disproportionate burden of norovirus infection among older adults.

**Other antiviral agents**

There are no licensed antiviral agents for norovirus treatment or prophylaxis, but several potential agents are currently under study. Discovery of antiviral therapies has been limited by the absence of *in vitro* cell culture models for human norovirus. Murine norovirus *in vitro* cell cultures have been useful surrogates, and a norovirus replicon system, a cell line containing the human norovirus genome, has been a tool that facilitated evaluation of antiviral targets. Current norovirus life cycle targets under investigation are protease inhibitors, polymerase inhibitors, and non-nucleoside inhibitors. Targeting host cell proteins required for viral replication is an attractive option as norovirus, an RNA virus, is prone to replication errors resulting in potential antiviral drug resistance.

**Immunoglobulins**

Enterically administered anti-norovirus immunoglobulins are proposed to bind to viral particles in the intestine inhibiting viral adhesion and replication and may also modulate pro-inflammatory and anti-inflammatory cytokines. Its use for norovirus treatment has only been described in small, retrospective studies of SOT recipients and reported efficacy has been variable. In addition to uncertain clinical benefit and preclusive cost, this therapy is limited by the lack of standard dosing regimen for commercially prepared antinorovirus immunoglobulins.

**Vaccines**

There are no FDA-approved norovirus vaccines, but there are several intranasal, oral, and intramuscular vaccines in pre-clinical development and one vaccine in clinical development (Table 3). The development of norovirus vaccines has been complicated by the lack of *in vitro* cell culture models, hence precluding detailed evaluation of viral pathogenesis and mechanisms of immunity. The HIE *in vitro* model shown by Ettayebi *et al.* may serve as an invaluable tool to evaluate the targets of human norovirus. Most vaccines target the major capsid protein (VP1), which differs among genogroups or the P-particle subunit using viral recombinant technology. A recent study by Ford-Siltz *et al.* used an *in vitro* neutralization assay using HIEs to demonstrate that norovirus GII.4 neutralization was primarily mediated by binding the protruding (P) domain of the major capsid protein. Another recent work by Green *et al.* demonstrated that negative-sense of norovirus RNA was predominantly found in intestinal epithelial cells in a study with four immunocompromised patients. Specifically, enteroendocrine epithelial cells were permissive targets in norovirus patients.

Due to the significant genetic variation among genogroups, a vaccine against norovirus may not be broadly protective and therefore, a multivalent vaccine against the major genogroups implicated in human disease may be more useful. Frequent genetic shifts occur within genogroups with GII.4 variants emerging every 2–4 years, thus a norovirus vaccine may require serial reformulation and boosting or periodic readministration.
Even after the development of an effective norovirus vaccine, the decision to adopt the vaccine in public health programs requires analysis of potential benefits, costs, and efforts targeted to specific populations at high risk of disease or severe disease and high risk of transmission. Steele et al. developed a model to analyze the clinical impact of norovirus vaccination among individuals ≥ 65 years of age using an estimated 65% vaccine coverage with revaccination every 5 years. A low vaccine efficacy of 22%–43% averted 430 outpatient visits, 130 emergency department (ED) visits, 61 hospitalizations, and 2 deaths per 100,000 doses. A high vaccine efficacy of 50% averted 840 outpatient visits, 270 ED visits, 145 hospitalizations, and 4 deaths per 100,000 doses.

Norovirus vaccines in development are listed in Table 3. Notably, Kim et al. studied 66 adults who received an oral (tablet formulation) non-replicating adenoviral vector-based Norovirus vaccine and compared them against placebo recipients, reporting substantial mucosal and systemic immune responses along with mild-to-moderate adverse events and a generally acceptable tolerance. Vaxart has also reported positive preliminary data from its phase Ib oral Norovirus vaccine trial among adults 55–80 years of age, citing robust immune responses across all doses, a dose-dependent production of IgA-secreting cells and mild-to-moderate adverse events.

Additional analysis on the potential clinical benefits of vaccination for individuals ≥ 65 years of age living in high-risk settings and if vaccination impacts viral shedding, an important mode of transmission during outbreaks would be valuable. There are several unanswered questions regarding norovirus vaccines including the lack of clear clinical correlates of protection, immunologic efficacy in preventing disease or severe disease within and among genogroups, duration of protection, influence on viral shedding, the cost and feasibility of periodic reformulation and administration, and potential vaccine hesitancy.

**Conclusion**

First described in 1929 and then again in 1968, norovirus is a significant pathogen for vulnerable populations and settings. Although norovirus gastroenteritis is typically brief and self-limiting, older adults, young children, and immunocompromised individuals are prone to a more severe and longer disease course in addition to higher hospitalization and mortality rates. Its highly effective viral transmission characteristics contribute to its propensity to cause gastroenteritis outbreaks in healthcare settings and long-term care facilities conferring significant infection prevention and control consequences. Advances in the understanding of viral pathogenesis have helped to elucidate genetic and host factors important to disease susceptibility and immune responses. Areas of continued research and uncertainty are the role of norovirus-specific treatments, the efficacy of norovirus vaccines, and their public health implications.

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Not applicable.

**Author contributions**

**Helen Tsai:** Conceptualization; Writing – original draft; Writing – review & editing.

**Philip Yune:** Conceptualization; Writing – original draft; Writing – review & editing.

**Mana Rao:** Conceptualization; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing – review & editing.

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