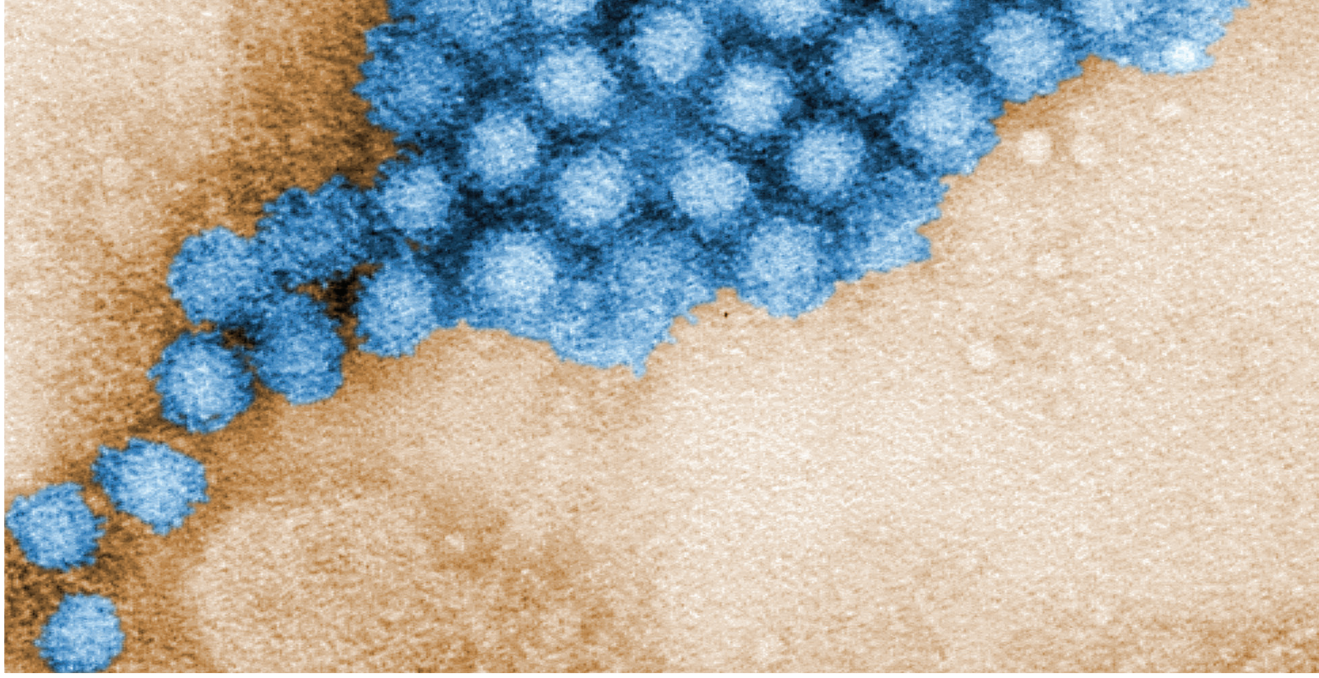


Global Burden of Norovirus and Prospects for Vaccine Development

Primary author

Ben Lopman

Centers for Disease Control and Prevention

Contributors and Reviewers

Robert Atmar, *Baylor College of Medicine*

Ralph Baric, *University of North Carolina*

Mary Estes, *Baylor College of Medicine*

Kim Green, *NIH; National Institute of Allergy and Infectious Diseases*

Roger Glass, *NIH; Fogarty International Center*

Aron Hall, *Centers for Disease Control and Prevention*

Miren Iturriza-Gómara, *University of Liverpool*

Cherry Kang, *Christian Medical College*

Bruce Lee, *Johns Hopkins University*

Umesh Parashar, *Centers for Disease Control and Prevention*

Mark Riddle, *Naval Medical Research Center*

Jan Vinjé, *Centers for Disease Control and Prevention*

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention, or the US Department of Health and Human Services.

This work was funded in part by a grant from the Bill & Melinda Gates Foundation to the CDC Foundation.

CDC
Centers for Disease Control and Prevention

CDC FOUNDATION
Helping CDC Do More, Faster

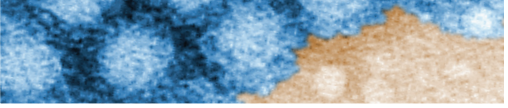


Table of Contents

1. Executive summary 3

2. Burden of disease and epidemiology 7
   a. Burden 7
      i. *Global burden and trends of diarrheal disease in children and adults* 7
      ii. *The role of norovirus* 8
   b. Epidemiology 9
      i. *Early childhood infections* 9
      ii. *Risk factors, modes and settings of transmission* 10
      iii. *Chronic health consequences associated with norovirus infection?* 11
   c. Challenges in attributing disease to norovirus 12

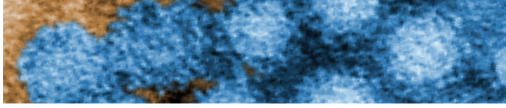
3. Norovirus biology, diagnostics and their interpretation for field studies and clinical trials. . 15
   a. Norovirus virology 15
      i. *Genetic diversity, evolution and related challenges for diagnosis* 15
      ii. *Evolutionary and public health importance of GII.4 viruses* 16
   b. Norovirus diagnostics and genotyping tools 17
   c. Detection of norovirus in healthy controls: implications for: 19
      i. *Disease attribution* 19
      ii. *Clinical trial design* 20
   d. Progress in cell culture systems for human norovirus 21

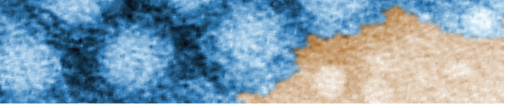
4. Acquired immunity and innate susceptibility to norovirus 23
   a. Patterns of acquired immunity and potential correlates of protection 23
   b. Genetic susceptibility 23
   c. Interaction of host immune response and viral evolution 25
   d. Candidate correlates of protection 26
   e. Evidence for early acquisition of immunity 26
   f. Innate immunity 28

5. Norovirus vaccines 29
   a. Developmental challenges and questions 29
      i. *Can a vaccine be developed that will elicit broad protection against multiple genotypes?* 29
      ii. *Will a norovirus vaccine have to be regularly updated in order to match viral evolution?* 29
      iii. *How will prior norovirus infection history affect vaccine immunogenicity and effectiveness?* 30
      iv. *Will the same vaccine formulation and schedule be effective in all groups?* 30
      v. *How will the genetic susceptibility affect vaccine outcomes?* 30
   b. Candidate vaccines 31
      i. *Transgenic plant-based norovirus vaccine* 31
      ii. *Norovirus P particle and combination vaccines* 31
      iii. *Trivalent norovirus /rotavirus combination vaccine* 32
      iv. *Takeda Pharmaceutical/Ligocyte VLP vaccine* 32

6. The road to a norovirus vaccine 36
   a. Specific age and risk groups 36
      i. *Age-based vaccination* 36
      ii. *Risk group-based vaccination* 36
   b. The potential population-level effects of a norovirus vaccine 38
   c. Economic impact of norovirus and potential cost-effectiveness of vaccination 38
   d. Economics and financing of a norovirus vaccine 38
   e. Bridging the developed and developing world markets 39

7. References 41





1. Executive summary

The burden of norovirus - is there a need for a norovirus vaccine?

Much progress had been made in the fight against diarrheal diseases. Global deaths have declined dramatically, from 2.6 million annually in 1990 to 1.3 million in 2013. Gains for children under the age of 5 years have been at least as impressive, with rates of decline in deaths at around 5% per year in absolute numbers. Largely, these gains have come about through improvements in water, sanitation and hygiene and use of oral rehydration solution, facilitated by economic development. Recently, rotavirus vaccination has offered a biomedical tool for further reducing child diarrheal deaths. Despite this progress, diarrheal disease remains the fourth most common cause of mortality and second most common cause of morbidity worldwide in children under the age of 5 years.

Norovirus is ubiquitous, associated with 18% (95% CI: 17-20%) of diarrheal disease globally, with similar proportions of disease in high- middle- and low- income settings. Norovirus is estimated to cause approximately 200,000 deaths annually worldwide, with 70,000 or more among children in developing countries. The entire age range is affected, with children experiencing the highest incidence. Severe outcomes, including hospitalization and deaths, are common among children and the elderly. In both high- and middle-income countries with mature rotavirus vaccination programs, norovirus is being unmasked as the most common cause of pediatric gastroenteritis requiring medical care.

Disease occurs across the age range in all settings, but incidence is highest in young children, with higher rates and an earlier age-distribution in lower income settings. Depending on setting, a person will experience an average of three to eight norovirus illness episodes in their lifetime, of which at least one will occur by 5 years of age. Noroviruses are transmitted by multiple routes but person-to-person spread predominates. Noroviruses remain a leading cause of sporadic disease and outbreaks of AGE even in industrialized settings, highlighting that improved hygiene and sanitation alone may not be fully effective in controlling norovirus.

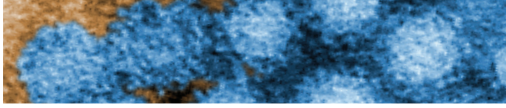
Considering the substantial disease burden and the difficulty in controlling norovirus, vaccines may be an attractive, and, perhaps, the only way to effectively control norovirus in the wider community. There are two overarching questions, answers to which will ultimately determine the fate of a norovirus vaccine: *Does the burden of norovirus represent a compelling need for a vaccination program? Can technical challenges be overcome to develop an effective norovirus vaccine for the populations that stand to benefit most?*

Challenges for quantifying disease burden

The current evidence is that disease burden of norovirus is high, second only to rotavirus as a cause of severe acute gastroenteritis and diarrhea-associated mortality worldwide. However there remains considerable uncertainty and some scientific controversy in defining the disease burden.

A number of factors complicate attributing a fraction of acute gastroenteritis to norovirus. First, data are lacking, especially from developing countries. Second, little routine testing is performed in ongoing surveillance platforms, because robust laboratory diagnostics have only recently been more widely available. Real-time quantitative RT-PCR (RT-qPCR) is the most sensitive and specific diagnostic assay for noroviruses, however, use of these assays is mainly restricted to public health and research laboratories in middle and high income settings.

With the use of sensitive RT-qPCR assays, norovirus can also be detected in stool of healthy individuals, complicating the interpretation of diagnostic results. Attributing a fraction of the overall diarrheal disease



burden to norovirus has been particularly challenging because reinfection is common and the virus can be shed for several weeks, detected at low concentration, and current diagnostics cannot readily discriminate between disease-causing and asymptomatic infection.

In a number of studies, particularly in low income countries, norovirus has been nearly as prevalent, and sometimes more so, in controls than in cases. This includes the recent Global Enterics Multi-Center Study (GEMS), the largest systematic assessment for understanding the etiology of childhood diarrhea in developing countries. GEMS and other case-control studies use the odds ratio of a microbe being present in cases versus healthy controls, to calculate an attributable or etiologic fraction. However, when a pathogen causes asymptomatic infection, the association between pathogen detection and disease weakens. But that does not mean that the pathogen is not causing disease when detected among cases. Many norovirus infections result in subclinical disease, virus shedding can last for weeks, and poor nutritional status could result in low level immunosuppression leading to chronic infection. So, we think it is inaccurate to conclude that norovirus is not an important pathogen because it is detected frequently in AGE cases and healthy controls. An alternative explanation is that high levels of asymptomatic infection are a result of frequent exposure, some of which will result in asymptomatic infection because of acquired immunity. Therefore, high prevalence of norovirus detection in healthy controls may be characteristic of 'hyper-endemicity' where burden is higher, not lower.

Challenges for norovirus vaccine development

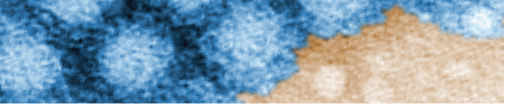
Noroviruses are a genetically and antigenically diverse group of ssRNA viruses, which present serious challenges both for creating broadly sensitive diagnostics and a broadly immunogenic vaccine. The norovirus strains that infect humans are found among 32 genotypes in GI (n=9), GII (n=19), and GIV (n=1) of which GI and GII viruses cause the vast majority of infections including the rapidly-evolving GII.4 viruses. Viruses from this genotype evolve in a boom-and-bust cycle with new GII.4 variant viruses emerging every 2-4 years replacing previous dominant viruses, a process driven by evasion of immunity in the human population. Apart from their evolutionary dynamic, there are other reasons that a successful norovirus vaccine must provide protection against GII.4 infections namely: i) they are the predominant cause of pediatric illness worldwide; ii) they predominate overwhelmingly as a cause of disease amongst the elderly in healthcare-associated outbreaks; iii)they result in more severe illness compared to other norovirus genotypes.

Understanding of natural immunity to norovirus is far from complete, but the current picture is that immunity is strain- or genotype-specific, with little or no protection conferred across genogroups. Immunity is not life-long, with estimates of duration ranging from 6 months to 9 years. Innate susceptibility to noroviruses is influenced by the host's genetics of glycan expression; individuals with a functional FUT2 gene (known as secretors) have greater susceptibility, at least to GII.4 and GI.1 viruses.

Genotype-specific immune responses and antigenic variation suggest that a polyvalent vaccine will be needed, which may require updating when new pandemic strains emerge. Vaccine trials and challenge studies have primarily been conducted among adults who have prior exposure, with little knowledge regarding how "unprimed" children (who have not experienced as many exposures) develop immunity and therefore respond to vaccination.

The lack of a robust cell culture system for human norovirus has hampered developing assays to measure protective neutralizing antibodies conferred by either natural or vaccine-induced immunity. However, important progress has been made very recently in the development of *in vitro* cell culture for norovirus as well as advances in the identification of candidate immune correlates of protection.

In addition to clinical efficacy, a number of key questions will determine the public health utility of a



vaccine, including:

- Can a vaccine elicit broad protection against multiple genotypes?
- What will be the duration of protection from vaccination?
- Will a norovirus vaccine have to be regularly updated in order to keep up with natural evolution of the virus?
- How will prior norovirus infection history affect vaccine immunogenicity and effectiveness?
- Will the same vaccine formulation be effective in all groups, including in low-income settings?
- How will the variation in human genetic susceptibility affect vaccine outcomes?

Progress with vaccine development

A number of norovirus vaccines are currently being developed. All of these products are based on the production of virus like particles (VLPs) or P particle subunit in various expression systems. Preclinical and early human studies have demonstrated safety and immunogenicity using various concentrations of monovalent or bivalent norovirus antigens, with and without adjuvants, and by various routes of administration. The only candidate vaccines with human efficacy data to date are being developed by Takeda Pharmaceuticals. An intranasal monovalent formulation (GI.1) was shown to be effective against infection and disease following GI.1 challenge. An intramuscular bivalent formulation (GI.1/GII.4) conferred a degree of protection against severe gastroenteritis outcomes following GII.4 challenge, sufficient to warrant further clinical development.

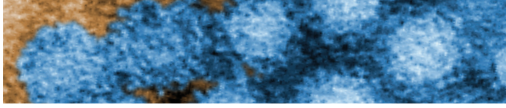
Target and risk groups: ensuring that vaccines benefits global public health

One of the challenges (and opportunities) in developing a norovirus vaccine is that many distinct population groups based on demographics (e.g. children, elderly) or risk (e.g. foodhandlers, military, travelers, healthcare workers) are affected. This can complicate the formulation a research agenda and clinical development plan. Such a plan for a target population of young children will look quite different than for older adults, or a specific risk group, such as healthcare workers or soldiers.

From a public health perspective, it is clear that young children experience the highest incidence of disease and severe outcomes are most common among young children and the elderly. Young children may also be the most important group in terms of driving transmission, and if so, vaccinating them could accrue direct protection and also prevent transmission, garnering the greatest benefits at the population level. However, severe disease burden is also clustered in elderly populations, so the individual benefits of vaccination in those groups should be considered as well.

Stimulating vaccine development

In the absence of an outside stimulus, such as a major donor, developed world markets are likely to provide the initial economic impetus for private industry to develop norovirus vaccines. To date, early-phase trials have been conducted among adults in high income settings, leaving a need for a pediatric development plan. Such a plan should include studies to generate data on the compatibility of norovirus vaccines with routine childhood immunizations, and, in particular, the Expanded Program on Immunization. Adding a vaccine to the EPI schedule involves great effort to demonstrate the added value of the vaccine, on both economic and health grounds and ensuring non-interference with other vaccines. The



economics of a norovirus vaccine which requires multiple doses and/or reformulation will be scrutinized carefully by policy makers.

At the earliest stage, clinical development plans should define a target product profile that will maximize public health gains by focusing on young children, with the aim of developing a vaccine that can be incorporated into the logistical arrangements of current immunization programs. Addressing these key issues will be vital to accelerate and achieve the development and implementation of interventions such as vaccines to control and prevent the tremendous global morbidity and mortality from norovirus.

Critical studies to be performed and questions to be answered to further vaccine development

1. Studies optimally designed for norovirus to more definitively quantify the incidence and burden, especially in lower income settings, including severe disease leading to hospitalization and death *(Section 2; 3)*
2. Development/optimization of diagnostics for use in etiological studies and clinical trials. *(Section 2; 3; 5)*
3. Birth cohort studies in low, middle and high income settings to further understand the acquisition of immunity. *(Section 2; 4)*
4. Development of a Global Norovirus Surveillance Network to monitor and characterize worldwide distribution and evolutionary dynamics. *(Section 3)*
5. Evaluations and reproducibility of *in vitro* cell culture system candidates. *(Section 4)*
6. Confirmation of currently proposed immune correlates of protection, and their validation in different populations. *(Section 4)*
7. Human clinical studies to characterize the safety, immunogenicity and efficacy of products not yet trialed in humans. *(Section 5)*
8. Pivotal, phase III field efficacy studies to demonstrate protection against disease among individuals in the community and herd protection in families. *(Section 5)*
9. Mathematical modeling studies to examine the direct and population level-effect of vaccinating different groups, defined by age or risk profile, including economic evaluation for developing country settings. *(Section 6)*
10. Development of a target product profile for a vaccine to be used in the EPI schedule, with updating as data become available. *(Section 6)*
11. A probe study, once a vaccine is available, to simultaneously define vaccine performance and disease burden. *(Section 5)*

2. Burden of disease and epidemiology

a. Burden

i. Global burden and trends of diarrheal disease in children and adults

A number of large international collaborations have estimated the global burden of disease, including diarrheal diseases, in both young children and adults. Despite important differences in methodology and results between projects, a clear pattern emerges. Child deaths and diarrheal deaths have declined in recent decades, but diarrheal disease remains the fourth most common cause of mortality and second most common cause of morbidity[i] worldwide in children under the age of 5 years.[1]

The Institute for Health Metrics and Evaluation's Global Burden of Disease (GBD) project estimated that diarrheal deaths fell from 2.579 (95% CI: 2.412 to 2.749) to 1.264 (1.151 to 1.383) million deaths, across all age groups from 1990 to 2013, a 60% decrease in age-standardized deaths rates[ii].[2] Over the same period, diarrheal deaths in children aged 0-4 years declined from an estimated 1.606 to 0.520 million, a decline of 68% over a 23-year period. The WHO's Child Health Epidemiology Reference Group (CHERG) estimated a similar rate of decline, over a somewhat narrower time window: 1.160 million (9 per 1,000 live births) to 0.558 million (6 per 1,000 live births) child diarrheal deaths from 2000 to 2013 represents an average annual rate of decline of nearly 5%.[3]

Globally, diarrhea morbidity has not changed at the same pace, with global diarrheal incidence at 46 and 37 episodes per 100 population in 1990 and 2013, respectively, for a total of 2.7 to 4 billion cases annually (2013).[4-6] Diarrheal diseases are the 25th top cause of years lived with disability (YLDs) at 6,854,000 in 2013.[4]

The global diarrhea burden and mortality among older children and adults has been less well-studied, and this demographic may be an important group specifically for norovirus, which affects all ages. The GBD estimated that 780,000 (54%) of the 1.45M diarrheal deaths in 2010 were among the older children and adults (Figure 1). Because years of life-lost are so influential for DALY estimation, only 32% of the DALY burden occurs outside the 0-4 year age group.

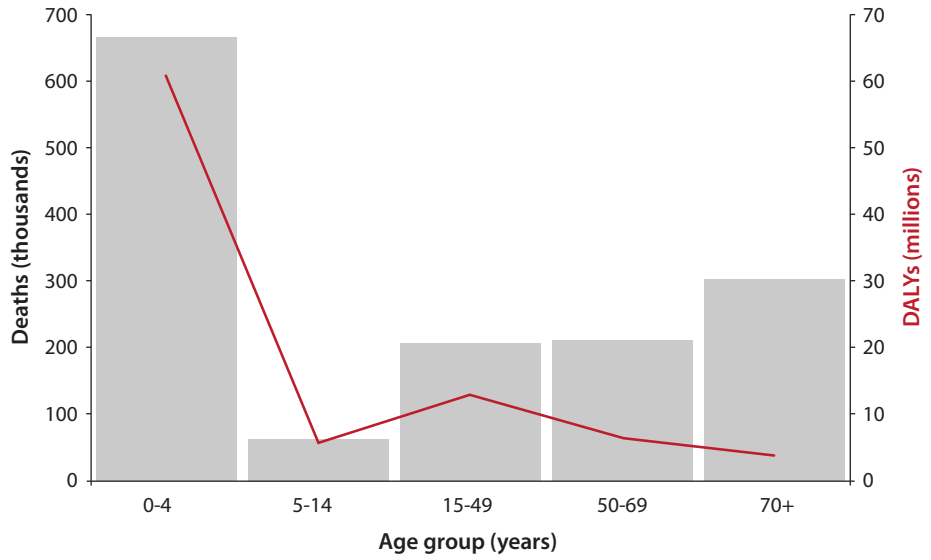
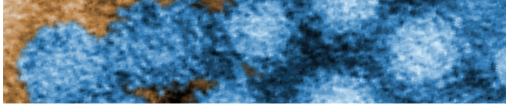


Figure 1.
Global Deaths and DALYs from diarrheal disease, 2010

[i] In terms of 'Years Lived with Disability'

[ii] All-cause mortality is estimated to have declined by 24% over the same time period, representing a shift away from diarrheal deaths and most other infectious diseases, with the exception of HIV.



ii. The role of norovirus

Based on a large systematic literature review of 175 studies, which, together, tested over 185,000 cases of acute gastroenteritis, norovirus is estimated to be associated with 18% (95% CI: 17-20%) of all diarrheal disease worldwide.[7] This percentage is higher among community cases (24%) than those presenting for outpatient (20%) or inpatient (17%) care, in line with the notion that norovirus is a more common cause of mild AGE, but still an important cause of more severe disease.[iii] As a fraction of all diarrheal disease, norovirus is more frequently detected in developed countries (20%) and low-mortality developing countries (19%) than those with high-mortality (14%). This lower prevalence in low-income settings likely indicates a more prominent role for other pathogens that are largely controlled through water and sanitation improvements, not that norovirus incidence is lower in developing country settings.

The data from this systematic review, as well as two previous ones, have been used to generate estimates of norovirus-associated mortality (Figure 2). For children under the age of 5 years, these estimates range from 71,000 (for 2011[13]) to 212,000 (for 2004[17]). These seemingly large differences are more of a result of changes in the 'envelope' of diarrhea deaths than of the percentage of those deaths attributed to norovirus, which is fairly consistent between studies, ranging from 9.9% to 12%. In this age group, noroviruses are the third most common etiological cause of diarrheal mortality, after rotavirus and, possibly, enteropathogenic *Escherichia coli*.[13]

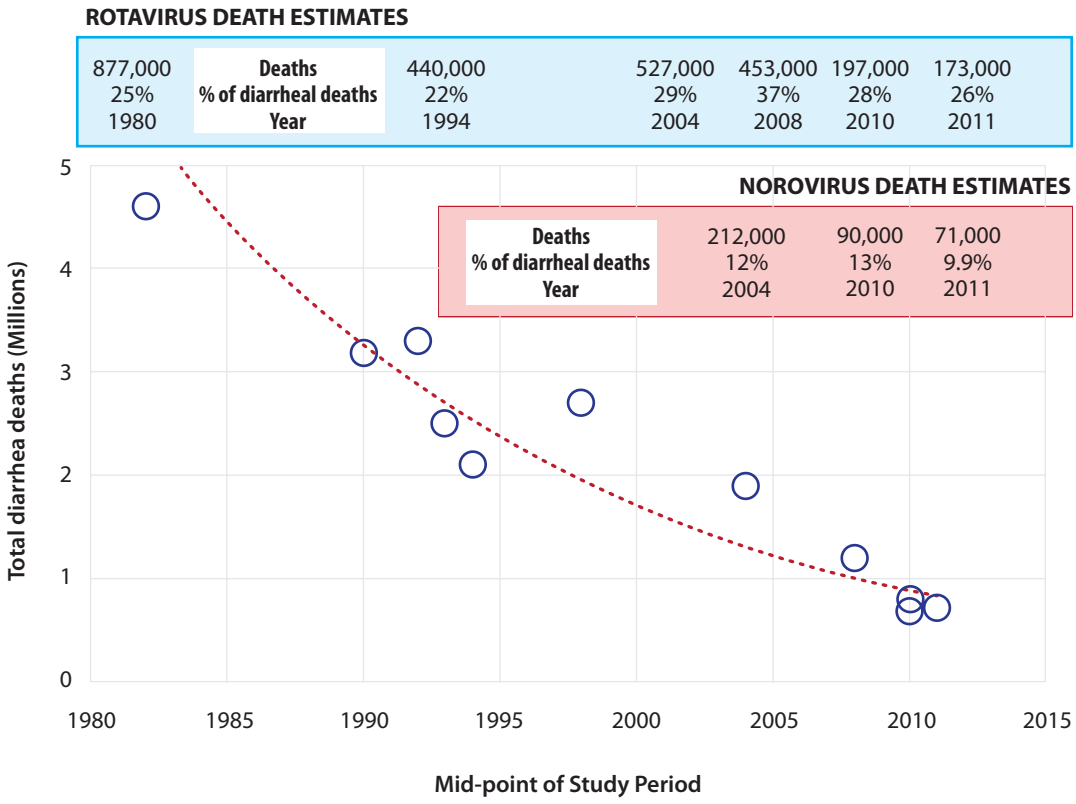
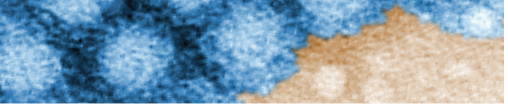


Figure 2.
Global child diarrhea deaths and estimates for rotavirus and norovirus: 1980-2015.

Sources include: [3, 8-16]

[iii] Note that detection of norovirus in stool (or any organism, for that matter) does not necessarily imply that it is the cause of disease. Case-control studies provide more interpretable data than case-only studies. This issue is discussed in more detail in 3.c.



It has been more challenging to estimate the global burden of norovirus for older children and adults since there are so little data on these ages from developing countries.[iv] The aforementioned systematic review [7] that was focused specifically on norovirus included 20 studies that reported on population groups aged 5 years and older. By meta-analysis, norovirus was associated with 18% (95% CI: 13-24%) of cases of AGE among those ages 5 years and older, the same percentage as in children 0-4 years of age. As the incidence of diarrhea is clearly higher in young children, the incidence of norovirus gastroenteritis is higher in this age group as well. Both the IHME and WHO estimate that, overall, there are more diarrhea deaths in those aged 5 years and older (780,000 – 868,000) than among under 5 years (666,000 – 692,000, respectively).[3, 6]

For norovirus, which causes mild disease in the majority of cases, much of the burden will be in the form of morbidity (as measured by YDLs and DALYs), not mortality. However, current global estimates are lacking. Updated age-specific mortality and morbidity estimates for norovirus and 8 other pathogens commonly transmitted by food are also forthcoming from the WHO Foodborne Epidemiology Reference Group. That study is using CHERG or national diarrhea incidence estimates together with a literature review to assign a fraction of disease to each pathogen and will also estimate the proportion due to food borne transmission.

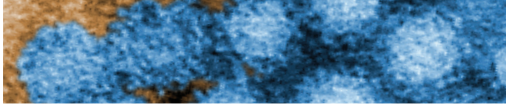
b. Epidemiology

Norovirus affects individuals across the age range, but the highest rates of norovirus gastroenteritis are among young children. Population-based national estimates of norovirus disease incidence across the age range are only available for select developed countries; these estimates range from 3.8% to 10.4% per year [20-25], with regional studies providing generally consistent results.[26, 27] This means that with a life expectancy of 80 years, a person will experience an average of approximately three to eight norovirus illness episodes in their lifetime, of which at least one will occur by 5 years of age.[23]

i. Early childhood infections

Norovirus disease incidence is approximately five times higher in children under the age of five years (21% per year) than among the whole population (4.5% per year), at least in high income countries (Figure 3).[23] Few studies have measured norovirus disease incidence across the age range in low or middle income countries, so comparing the age-specific incidence is difficult, but the few cohort studies in such settings point to a higher disease incidence in children in these settings compared to developed country settings. For example, in birth cohort from a Peruvian shantytown, norovirus disease incidence was over 50 cases per 100 person-years in the first 2 years of life.[28] Incidence was highest in the 6 – 23 month age group, suggesting a neonatal period of protection from maternal antibody and/or limited exposure. Preliminary incidence estimates from Guatemala and Kenya are 2.5 and 11 cases per 100 person-years for all ages with rates in < 5 year olds approximately 10 times higher than rates in ≥5 years. (Shioda, Bierhoff *in preparation*)

[iv] In one systematic review of the etiology of diarrhea in older children and adults, only a single paper presenting norovirus data from Hong Kong[18] met the inclusion criteria.[19]



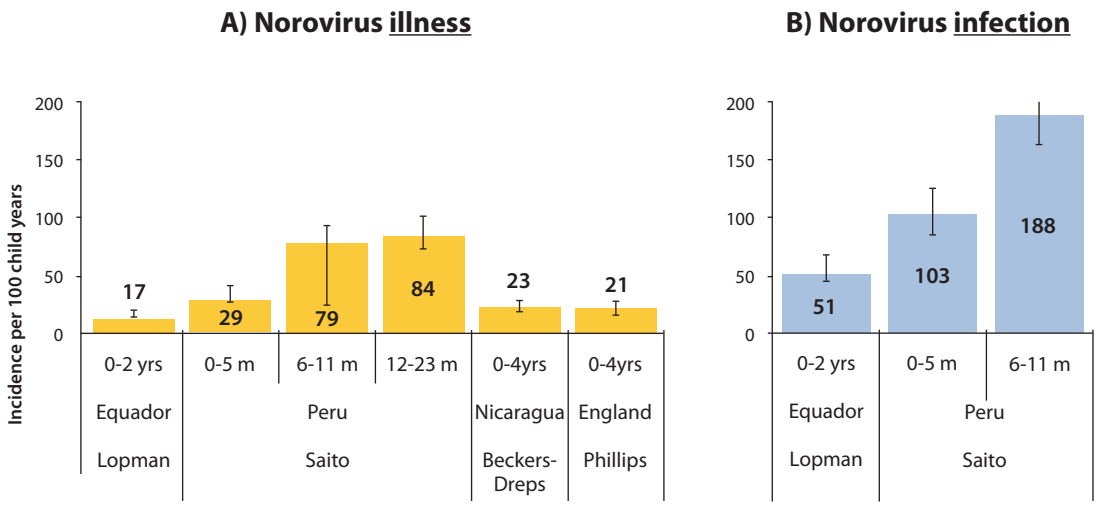


Figure 3.
Norovirus illness and infection rates in children, as measured in community-based cohort studies [23, 28, 37, 38]

Globally, approximately 70% of pediatric norovirus cases in the 0-4 year age group occur between 6-23 months of age.[29] A younger age distribution occurs in lower income countries as well as among cases in inpatient settings, consistent with the notion that the force of infection is greater in low income settings and that more severe disease results from primary infection at a young age. Almost all children will have at least one infection by the age of 5 years.[30-35] Multiple infections occur as a result of limited duration of immunity and limited cross-protection to the diverse range of noroviruses. Two birth cohort studies have concluded that GII infection confers a degree of protection against subsequent infection with the same genotype.[28, 36] One study with less frequent sampling did not find such patterns.[37] These observations are discussed in more detail in Section 4.a.

ii. Risk factors, modes and settings of transmission

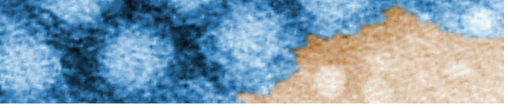
Norovirus is extremely contagious[vi] and humans are the only known reservoir for human norovirus. Transmission occurs via fecal-oral and vomit-oral pathways by four general routes: direct person-to-person, foodborne, waterborne or through environmental fomites; since humans are only known the source of all human infections, all transmission is ultimately person-to-person.

Consistently, the strongest risk factors for community disease are proxies for contact with an infectious person. For both young children and older children/adults, contact with a symptomatic household member, especially a child, is a strong predictor of disease.[27, 44-46] Young children appear to frequently bring infection into the household, and older children and adults acquire many of their infections within the household. [46, 47] Foreign travel is also a risk factor;[46, 48] with increased risk likely attributable to changes in behavior while traveling or exposure to a different spectrum of norovirus strains. Risk factor data on endemic disease in developing countries is extremely limited.

Investigations frequently attribute norovirus outbreaks to contamination of food during preparation by a range of mechanisms and in a range of settings, but food-related risk factors have not shown consistent associations with disease in community-based studies of endemic disease.[44, 46][vii] Foodborne transmission is estimated to account for 14% of norovirus outbreaks, globally, but, overwhelmingly, the data

[vi] The precise infectious dose may vary by strain, and there is some debate about how best to calculate the ID50 for norovirus[39-43] with estimates ranging from 18.2 (95% CI of 1.03–4350)[43] to 1320–2800[42] genomic equivalents. However, the extreme infectiousness and transmissibility is not in question.

[vii] The only foods consistently associated with disease are oyster and other shellfish harvested from areas where the seabeds are contaminated with sewage. However, in most populations, consumption of these products is not common and this exposure likely accounts for only a small fraction of disease.



sources for these estimates come from developed country settings. There is very little observational data on which to base assessments of the relative role of foodborne, waterborne or environmental transmission in low income settings, so some assessments are based on expert opinion.

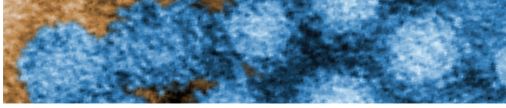
If one of the effects of development is improved sanitation and hygiene, economic development should coincide with a lower incidence of disease from food, water and environmentally-transmitted disease, possibly with an increasing proportion of disease transmitted directly from person-to-person in higher income settings. Put another way, individuals living in settings of poorer sanitation and hygiene likely have more exposure to norovirus from multiple routes. This notion of transmission is consistent with a higher overall disease incidence, younger age distribution,[29] and more common asymptomatic reinfection in lower income settings[viii].[49]

One of the few documented international differences in transmission patterns relates the frequency of acute-care hospital outbreaks. In highly developed countries, healthcare facilities, including nursing homes and hospitals, are the most common settings of norovirus outbreaks. These outbreaks are highly disruptive and costly to health services, can attract major media attention and affect already-vulnerable population. [50] In the United States, outbreaks in acute care hospitals are rarely reported, whereas in Europe, Australia, Canada and Japan outbreaks in long term care (e.g. nursing homes) and acute care (i.e. hospitals) are roughly equal in number.[ix] Health care-associated outbreaks are essentially not reported from low income settings, likely for a combination of reasons including lack of surveillance, absence of diagnostics and a younger population with few living in institutions for the elderly. Absence of hospital outbreaks in low income settings could also result from more frequent exposure, leading to high levels of immunity in adults; without robust surveillance, it's hard to know if this is a real phenomenon.

iii. Chronic health consequences associated with norovirus infection?

While more research is needed, there are recent data linking noroviruses with persistent long-term gastrointestinal health consequences and may represent preventable disease burden for which a vaccine may have benefit.[51-53] The most frequently identified long-term consequence studied has been irritable bowel syndrome (IBS) and prospective studies have shown that 3 to 36% of all enteric infections lead to an incident IBS diagnosis (e.g. post-infectious IBS or PI-IBS)[54]. Other chronic functional gastrointestinal disorders such as GERD, dyspepsia and constipation have shown variable association with antecedent IGE [55, 56]. While the majority of the studies to date have identified pathogenic bacteria as an etiologic agent in PI-IBS, recent data point to an association with other pathogens, including viruses and parasites [54]. Specific to norovirus there have been three studies looking at the link between norovirus infection and chronic functional gastrointestinal disorders (FGD) including IBS. A first report came from a small prospective study of IBS following an outbreak of purported (but not confirmed) norovirus-attributed severe gastroenteritis reported that 3 months after the outbreak, IBS was significantly higher in subjects with acute gastroenteritis than in control subjects after 3 months, but by 6 months the incidence was no different suggesting that PI-IBS following viral gastroenteritis was transient [57]. Following this initial report was a larger study which followed a massive outbreak of viral GE associated with contamination of municipal drinking water with norovirus that occurred in Italy during 2009.[53] In this study, the authors identified 40 patients (noroviruses exposed) with a new diagnosis of IBS (using Rome III criteria), in comparison with 3 subjects in the control cohort ($P < 0.0001$; OR 11.40; 95 % CI 3.44 – 37.82). The US military reported the largest study which was a cohort design in which 1,718 cases subjects from three confirmed norovirus outbreaks

[viii] However, at some point, incidence in low income settings may reach a critical threshold at which exposure becomes frequent enough to provide frequent immune boosting and, in turn, lower disease incidence.



were matched to non-exposed controls and followed through the DoD medical encounter database and showed increased risk for development of a number of functional gastrointestinal disorders[51]. While more research is needed to confirm the consistency of risk in other settings and advance understanding of disease mechanisms[58, 59], evidence to date suggests that the link between noroviruses and development of chronic FGD should not be dismissed, and investigative efforts to further delineate pathological changes explaining the various symptoms of post-infectious IBS, functional dyspepsia and GERD which include genetic, immunologic and microbiologic assays in varied patient populations with well characterized gastrointestinal infectious exposures and disease outcomes are needed. A more comprehensive understanding of the total attributable disease burden would be an important factor in valuing the benefit of vaccination.

c. Challenges in attributing disease to norovirus

There are a number of challenges that complicate attributing a burden of acute gastroenteritis disease to norovirus. These challenges include the following.

- Routine testing is rarely performed in ongoing surveillance platforms. Surveillance for disease provides essential local, national or regional data on disease burden and strain circulation, on which to base decisions. A number of high income countries conduct ongoing surveillance for outbreaks or special studies to estimate norovirus disease burden[ix] but, ongoing routine surveillance is rare in developing country settings.[x] Short-term surveillance studies have been conducted in many settings, mainly among inpatients under 5 years of age. Ongoing surveillance for endemic disease consistently employing the most appropriate diagnostics is particularly important for norovirus, considering its annual variability, irregular strain emergence and seasonal fluctuations. An international network should initially focus on children, since the burden is greatest among that age group, and could expand to adults and elderly, as appropriate.

The WHO Global Rotavirus Surveillance Network, in some ways, could be a model for a global norovirus surveillance network. The Global Rotavirus Surveillance Network had been coordinated by WHO since 2008 and by 2011/12 operated 79 sites from 37 countries.[62] The network includes sentinel hospitals and national laboratories working from common surveillance and laboratory protocols. Surveillance is exclusively for children 0-4 years of age and diagnostics are based on a relatively simple, but sensitive and specific, enzyme immunoassay (EIA). An informal norovirus global surveillance network (NoroNet) of scientists at public health institutes and universities sharing virologic, epidemiologic and sequence data on norovirus already exists which could be used as basis for a more formal collaboration such as the WHO Global Rotavirus Surveillance Network. Because norovirus affects the whole age range and simple diagnostics do not exist (both issues discussed in more detail in Sections 2.a.ii and 3.b), global surveillance for norovirus may require more intense resources and not be as widespread as for rotavirus, but the success of that network highlights the value of such data for making informed decisions about vaccine use, and, specifically, the importance of collecting these data before vaccine introduction.

- Sensitive assays have only recently been widely available. The genetic diversity of noroviruses is one of the main challenges in developing a broadly reactive EIA. Real-time quantitative RT-PCR (RT-qPCR) is the most sensitive and specific diagnostic for noroviruses, however, use of these assays is mainly restricted to laboratories in middle and high income settings and are rarely used in low-income countries because of cost of the assays and equipment required. Indeed, even the highly influential and comprehensive Global Enterics Multi-Center Study (GEMS) did not use RT-qPCR diagnostics for norovirus.[63]

[ix] These include the Infectious Intestinal Disease Studies [24, 60, 61] in England and Sensor in The Netherlands.

[x] A notable exception is the surveillance conducted by International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) at the rural Matlab Hospital and Urban Dhaka Hospital, where a selection of specimens from diarrhea admissions has been tested for norovirus since 2010. Overall, approximately 25% test positive for norovirus by real time RT-PCR. Uniquely, this surveillance platform covers the full age range.

- Challenges in interpretation of diagnostic results While norovirus is one the most frequently detected pathogens in stool of AGE cases, it is also commonly found in stool of healthy individuals. This may be for a number of reasons including: true asymptomatic infection, long duration post-symptomatic shedding of virus and, possibly, ingested non-replicating virus transiting the gut. Some studies have attributed a fraction of diarrheal disease based on the odds of detecting a given pathogen in cases compared to healthy controls, leading to the conclusion, incorrect in our view, that norovirus is not a major cause of AGE.[49] In any case, the high frequency of detection of viruses in healthy controls complicates the interpretation of norovirus diagnostics.

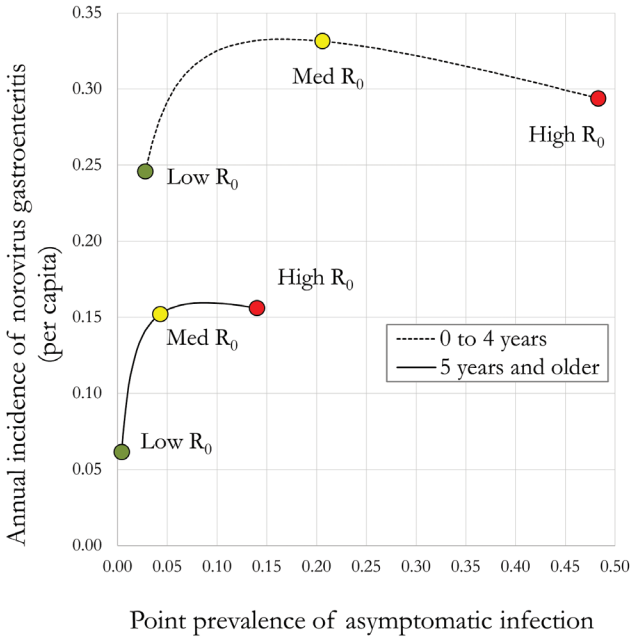


Figure 4.

Annual incidence of norovirus gastroenteritis and point prevalence of asymptomatic norovirus infection in children aged 0 to 4 years and the rest of the population, in settings of low to high basic reproductive numbers, adapted from [49].

- Some norovirus AGE cases present with vomiting, but without diarrhea. Most existing surveillance and etiological studies use a case definition for diarrheal disease. However, a classic symptom of norovirus infection is vomiting; norovirus infection may result vomiting without diarrhea in approximately 15% of cases. [64] These cases are typically excluded from the 'envelope' of the gastroenteritis disease burden and from the etiological studies used to attribute a fraction of this envelope to norovirus,[7] resulting in an underestimate of the norovirus disease burden.

- Community-based studies are expensive and challenging. As a high incidence, common infection, much of the disease burden of norovirus is in the community. However, community-based studies are logistically challenging and relatively expensive to conduct. Few such studies have been performed across the age range in high-income European settings [44, 60, 65] and for birth cohorts in middle-income Latin American populations. [28, 36, 37] Because these studies are so valuable for understanding disease burden, transmissions patterns and the acquisition of immunity, more studies in diverse settings will be highly informative.

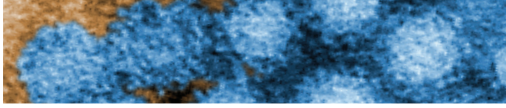


Table 1. Current knowledge, challenges and most critical studies for estimating norovirus disease burden

Current knowledge	Challenges	Studies needed

• Globally, norovirus is associated with 18% 18% (95% CI: 17-20%) of diarrheal disease
• Norovirus is estimated to cause approximately 200,000 deaths annually worldwide, about 70,000-100,000 of which are among children in developing countries.
• Disease occurs across the age range in all settings, but incidence is highest in young children.
• Noroviruses are transmitted by multiple routes
  • Person-to-person spread predominates
  • Foodborne transmission is estimated to account for approximately 15% of disease
• Policy makers are likely to be influenced to act based on:
  • pediatric disease burden in low/middle income settings
  • economic and severe disease burden in very young/old in high income settings
	• Etiological studies on the role of norovirus vary widely in terms of
	  • case definition
	  • inclusion criteria for controls
	  • diagnostics assays
	• Variable levels of detection in controls in different settings complicate interpretation of diagnostics and definition of burden
	• There is a dearth of incidence estimates
	• Current approaches of attributing mortality is based on extrapolation of inpatient norovirus prevalence but may face skepticism
	• There is little routine testing performed in endemic diarrheal disease ongoing surveillance platforms
		• Rigorously designed studies to more clearly identify the burden of norovirus disease, including:
		  • Population-based studies of norovirus incidence at inpatient, outpatient, and community levels
		  • Cohort studies with serology, to more clearly define true infections*
		  • Longitudinal studies with frequent sampling to compare norovirus-associated diarrhea episodes with a stool sample taken in a pre-symptomatic period (self-controlled case)*
		  • low/middle income settings
		  • and elderly (otherwise vulnerable) in high income
		• Global Norovirus Surveillance Network, modeled on the WHO Global Rotavirus Surveillance Network, based in sentinel hospitals
		  • Surveillance for severe disease – defined by hospital admission
		  • Relatively costly, but provides important data to policy makers

3. Norovirus biology, diagnostics and their interpretation for field studies and clinical trials

a. Norovirus virology

i. Genetic diversity, evolution and related challenges for diagnosis

Noroviruses are a group of non-enveloped, single-stranded RNA viruses with an icosahedral symmetry classified into the genus *Norovirus* of the family *Caliciviridae*. Other genera within this virus family include *Sapovirus*, which also causes AGE in humans, *Lagovirus*, *Vesivirus*, and *Nebovirus*, which are not pathogenic for humans.[66] The RNA genome of noroviruses consists of three open reading frames (ORF): ORF1 encodes six nonstructural proteins including the RNA dependent RNA polymerase (RdRp); ORF2 encodes the major capsid protein (VP1) that determines the antigenicity of the virus and consists of a shell domain (S) located at the base of the capsid and a protruding (P) domain, that is further subdivided into the P1 and P2 regions (Figure 5); and ORF3 which encodes the minor capsid (VP2) for which a functions has not been fully identified.

Until very recently, human norovirus could not be grown in cell culture, so a classification based on neutralization with anti-sera (serotypes) has not been possible. As such, noroviruses are classified based on phylogenetic clustering of the complete VP1 amino acid sequence. into genogroups (G)(currently 7, designated GI–GVII) [67], each of which is further divided into genotypes. The norovirus strains that infect humans are found in 32 genotypes in GI (n=9), GII (n=19), and GIV (n=1), whereas viruses in other genogroups infect other mammals including cows, mice, sheep, pigs and dogs. (Figure 6).[68]

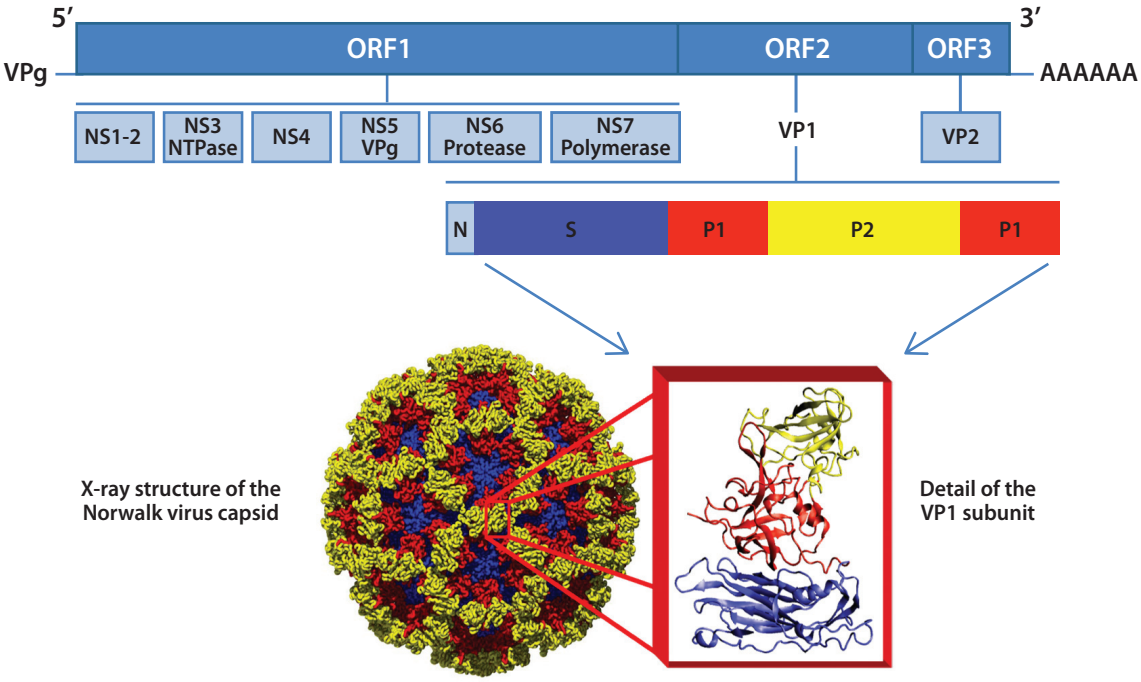


Figure 5. Human norovirus genome organization and virus encoded nonstructural and structural proteins.

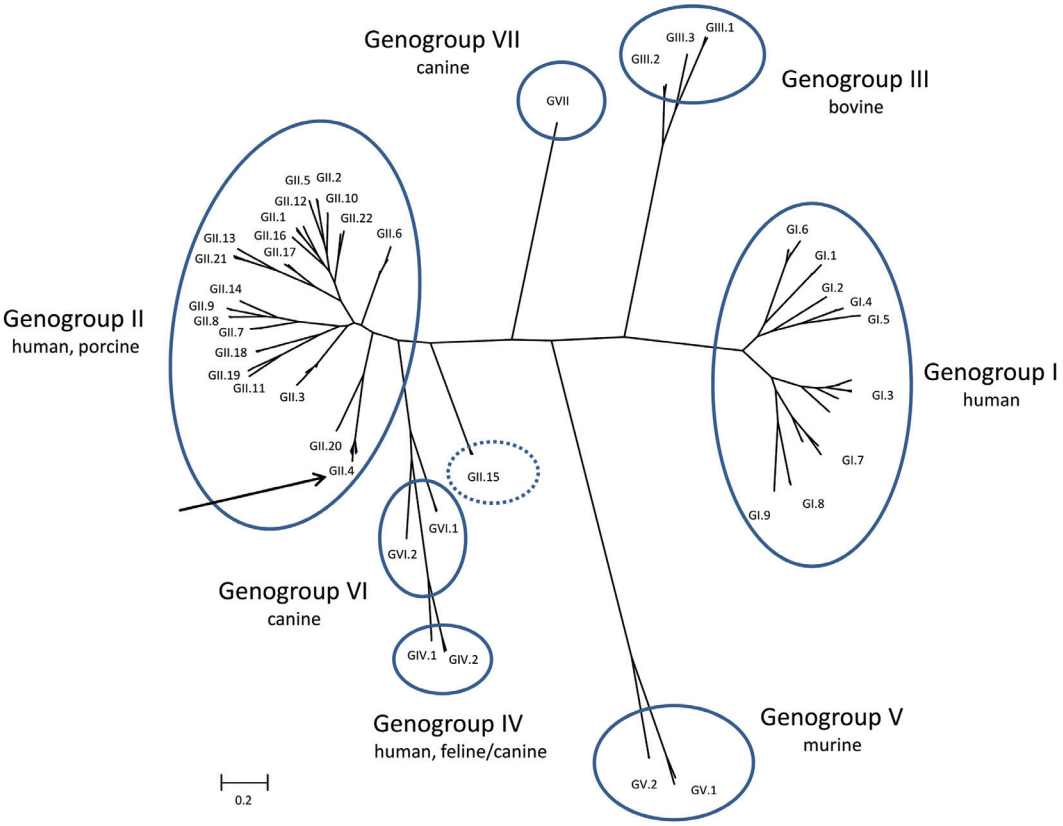


Figure 6.

Classification of noroviruses into 7 genogroups (GI to GVII) based on amino acid sequence diversity in the complete VP1 capsid protein. Adapted from Vinjé.[68]

ii. Evolutionary and public health importance of GII.4 viruses

Since the mid-1990s GII.4 viruses have caused the majority of norovirus outbreaks and sporadic cases worldwide. This genotype evolves in a boom-and-bust cycle with new GII.4 strains emerging every 2-3 years and replacing previous dominant strains, a process driven by evasion of immunity in the human population. [69-71]. GII.4 norovirus evolution and the emergence of new epidemic strains is principally driven by mutations in the P2 domain of ORF2, the hyper variable region of the capsid, which is the domain most exposed to the host cells [72-77]. Mutations in the P2 domain may lead to significant changes at key epitopes resulting in the virus being able to escape from antibodies generated by prior exposure to genetically related norovirus strains [77]. This process is known as epochal evolution, and was first described for influenza viruses to explain the emergence of epidemic strains.

In some cases, but not always, the emergence of novel GII.4 variant results in a global pandemic of norovirus. For example, both the 95/96_US [78] and Farmington Hills 2002 viruses were associated with an increased number of outbreaks worldwide [79, 80]. The emergences of New Orleans 2009 and Sydney 2012 viruses were not associated with an increased number of outbreaks in the US.[81, 82]

In addition to GII.4 viruses causing the majority of norovirus infections, they predominate overwhelmingly as a cause of disease amongst the elderly in healthcare-associated outbreaks. Even after accounting for the more vulnerable populations that they affect, GII.4 viruses result in more severe illness.[83] Once thought to be mainly a scourge of the elderly in hospital and nursing home outbreaks, the GII.4 viruses are also now recognized to be the primary cause of illness in children worldwide (Figure 7).[84, 85] Several other genotypes including GII.3, GII,6, and GII.12 viruses, have in different years been reported as the second most common genotype causing norovirus AGE in young children, but these viruses do not appear to share the evolutionary patterns of immune escape associated with GII.4 noroviruses.[86-88]

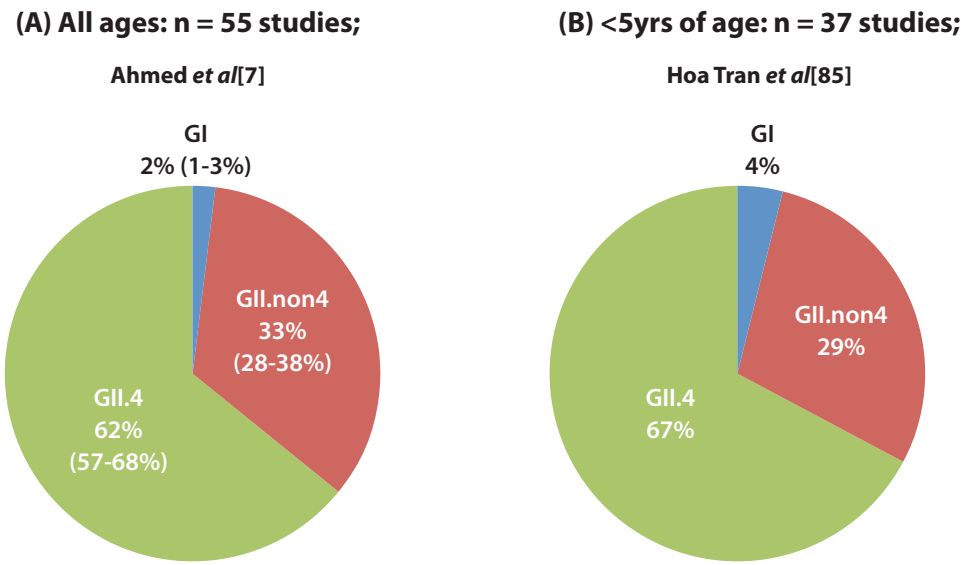


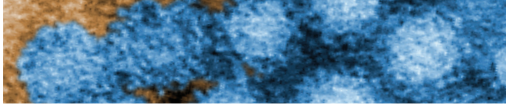
Figure 7. GII.4 noroviruses predominate across (A) all ages as well as among (B) children under 5 years of age.

b. Norovirus diagnostics and genotyping tools

The current appreciation of the major burden of norovirus AGE has been brought about by the use of molecular methods for the detection and characterization of noroviruses. In the 1970s and 1980s, electron microscopy (EM), immune-EM (IEM), and radioimmunoassays were used to detect norovirus; these methods were cumbersome, required highly skilled technicians and had limited sensitivity. In the 1990s, the prototype Norwalk virus and its close relative, Southampton virus, were cloned and sequenced,[89-91] paving the way for the development of new molecular diagnostic tests based upon reverse transcription-polymerse chain reaction (RT-PCR) for the detection and sequencing of the virus genome directly from fecal specimens.[92-94]

Employing similar virus cloning technology, expression of viral proteins and non-replicating virus-like particles (VLPs) led to the development of serologic assays with antigens that could be replenished in the laboratory and used for the generation of polyclonal sera and monoclonal antibodies.[89] This led to the development of antigen capture EIA assays and immunochromatograhic "near patient" tests which are also commercially available. However, given the large genetic and antigenic variation among noroviruses and variable viral loads in stool samples, these tests have limited sensitivity and may not capture the full range of circulating viruses. [95, 96] Consequently, EIAs or other antigen detection tests have limitations when used to establish a diagnosis of norovirus in individual patients but may be useful in outbreak settings where a large number of stool samples from patients may be available and some diminished diagnostic sensitivity may be acceptable [96-100]. Blockade assays offer a more robust measure of antigenic relatedness, employing epitope specific VLPs and also allow tracking of epitope specific responses/time (e.g [101]).

Among viruses of each genogroup, the ORF1-ORF2 junction is the most conserved region of the norovirus genome, making it the key target region for broadly-reactive oligonucleotide primers and probes for nucleic acid amplification and detection. Although several other formats have been developed with similar sensitivity, real-time RT-PCR assays using hydrolysis probes (TaqMan-assays) are the most widely used method for the detection of norovirus. Because of their high analytic sensitivity, real-time quantitative (RT-qPCR) assays can detect very small amounts of virus that might be present in samples from persons who are asymptomatically infected or have recovered within the past few weeks from norovirus AGE (discussed in more detail Section 3.c.i). While imperfect diagnostic accuracy is an issue for all assays, it is more problematic for RT-qPCR. Indeed, RT-qPCR is able to detect norovirus at a load as low as 103 copies per gram of stool, so its exquisite analytical sensitivity compromises diagnostic specificity. As such, low viral loads, perhaps even non-replicating virus particles, can be detected by RT-qPCR.

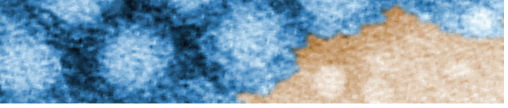


More recently, several platforms have been developed to simultaneously detect a range of enteric pathogens including viruses, bacteria and parasites. The U.S. Food and Drug Administration has cleared a number of these commercial multiplex molecular diagnostic kits for the simultaneous detection of enteric pathogens including the xTAG GPP (Luminex Corporation, Toronto, Canada), the FilmArray GI Panel (BioFire Diagnostics Inc., Salt Lake City, UT, USA), and the Verigene Enteric Pathogens Test (EP) (Nanosphere, Northbrook, IL, USA). All of these platforms are able to detect noroviruses and the Biofire and Luminex platforms distinguish between GI and GII noroviruses. While the comprehensiveness of these diagnostics is attractive, interpreting the cause of an individual's illness can be a challenge, especially when multiple pathogens are detected in a single stool (discussed in more detail below)[xi].

Table 2. Overview laboratory assays for detection of norovirus

Laboratory test(s)	Advantage	Disadvantage	Time *(sample to result)*	FDA (510k)-cleared test	Market
Electron microscopy	Ability to detect multiple viral pathogens	Expensive equipment and training; low throughput; insensitive	15 min		Reference laboratories
Immunological					
Enzyme immunoassay	High specificity, high throughput	57–76% sensitivity	60–90 min	R-Biopharm	Public health, clinical laboratories
Immuno-chromatographic	High specificity, no special equipment	35–52% sensitivity	15 min		Point of care
Molecular					
Conventional RT-PCR	PCR amplicons can be sequenced and used for typing	Results must to be confirmed by sequencing or hybridization	5–6 h		Reference laboratories
Real-time RT-PCR	High specificity, sensitivity and throughput; possibility to multiplex mulitple targets	PCR equipment required; reduced clinical specificity	3 h	Tests in pipeline	Public health, clinical laboratories
Molecular multiple enteric pathogen					
xTAG GPP	High sensitivity, high throughput; detects different enteric pathogens	Expensive equipment and kit format	5 h	Luminex Corporation	Public health, clinical laboratories
FilmArray GI Panel, Verigene Enteric Pathogens Test	Includes nucleic acid extraction; detects (FilmArray) and (Verigene) different enteric pathogens; single sample can be tested	Expensive equipment and kit format	2 h	Before Diagnostics Inc.; Nanosphere Inc.; tests from other companies pending 510k clearance	Clinical laboratories

[xi] A more complete discussion of norovirus diagnostics may be found in [68].



c. Detection of norovirus in healthy controls: implications for:

i. Disease attribution

A variety of factors may explain why individuals without diarrhea shed enteric pathogens in their stool. [102] Those most germane to norovirus are:

- Some individuals have true asymptomatic infection; in adult volunteer studies 15 to 35% of infections confirmed serologically or through detection of virus in stool were not associated with gastroenteritis.[64, 103-105] These asymptomatic infections may result from acquired immunity that is protective against disease but does not block infection.

- Shedding of virus in stool continues long after the resolution of symptoms, with around 25% of cases shedding virus at detectable levels 3 weeks after onset of illness, which usually lasts for only 1-3 days. [106]

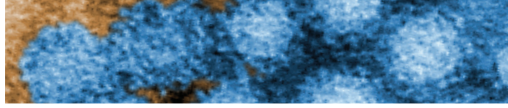
- It is possible that ingested virus may transit the gut without replicating while still being detectable with highly sensitive diagnostics.

Interpreting Global Enterics Multi-Center Study (GEMS)

For this combination of reasons, norovirus can frequently be detected in stools collected from healthy individuals. In fact, in a number of studies, particularly in low income countries, norovirus has been nearly as prevalent, and sometimes more so, in controls than in cases. This includes the recent Global Enterics Multi-Center Study (GEMS), the largest systematic assessment for understanding the etiology of childhood diarrhea in developing countries.[63] GEMS and other case-control studies use the odds ratio of a microbe being present in cases versus healthy controls, [107-109] which quantifies the magnitude of the association between microbe and disease, to calculate an attributable or etiologic fraction [108, 110]. We think it is inaccurate to conclude that norovirus is not an important pathogen because it is detected frequently in AGE cases and healthy controls. An alternative explanation is that high levels of asymptomatic infection are a result of frequent exposure, some of which will result in asymptomatic infection because of acquired immunity. Therefore, high prevalence of norovirus detection in healthy controls may be characteristic of 'hyper-endemicity' where burden is higher, not lower.

There are two study design improvements that may result in more accurate estimates of the attributable fraction of AGE for norovirus.

- Better inclusion criteria for healthy controls: Careful selection of healthy controls may help to minimize the influence of post-symptomatic shedding. Since norovirus shedding after an illness can persist (25% shedding virus at detectable levels after 3 weeks or longer for children in developing countries [28]) including only controls who have had a long period without any symptoms will help to exclude long-term shedders from a previous illness. A case in point is the inclusion criteria used in the CDC New Vaccine Surveillance Network where healthy controls had "no symptoms of acute gastroenteritis within 14 days before enrollment, and no symptoms of acute respiratory infection (cough, congestion, sore throat, runny nose, or wheezing) within 3 days before enrollment." In that study, norovirus was detected in stool of 21% of cases and 4% of controls.[111] . Another approach could be longitudinal cohort studies with frequent sampling. Data from such studies could be used to compare norovirus-associated diarrhea episodes with a stool sample taken in a pre-symptomatic period, such that a child acts as her 'own control'.



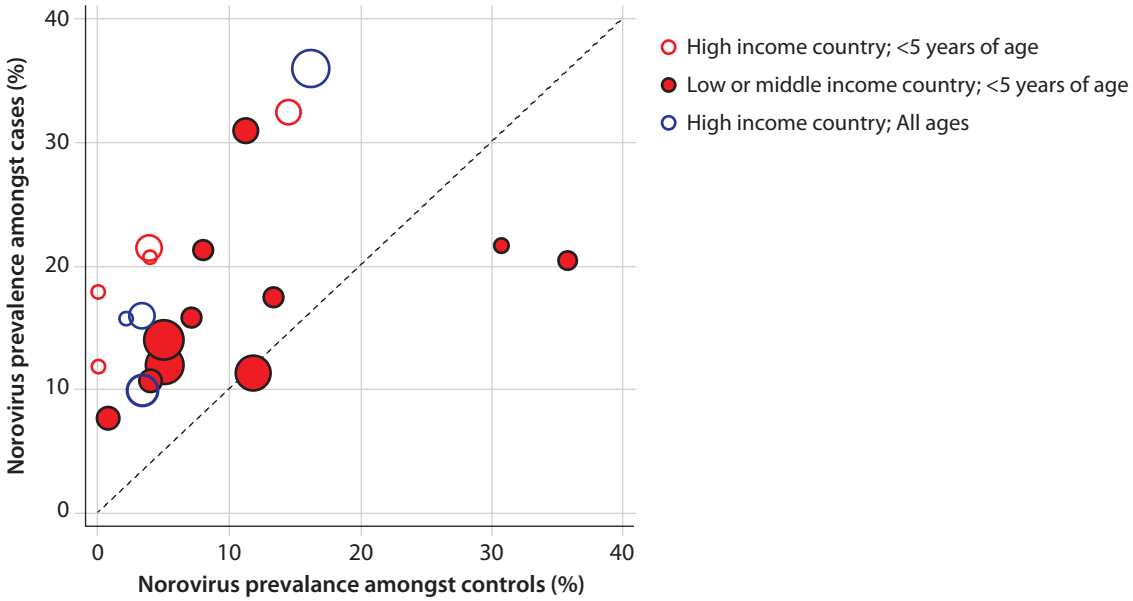


Figure 8.
Norovirus prevalence amongst diarrhea/gastroenteritis cases and healthy controls.

Based on a literature review of published studies, 1998 to 2013. Adapted from[49]
** Size of circle proportional to number of subjects in study*

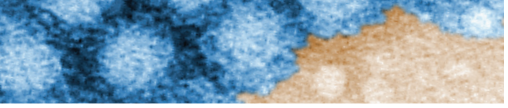
- Use of quantitative viral load data from RT-qPCR: It has been proposed that cycle threshold values, as an indicator of viral load, can be used to discriminate between disease-causing infections and asymptomatic infections. The quantity of virus in stool specimens decreases after 3-4 days post-infection, making viral load (based on cycle threshold value from quantitative reverse transcription- polymerase chain reaction (RT-qPCR) a potential indicator of disease-causing infection.[112] One large study in England used this approach to calculate the probability that viral detection at a certain Ct value was disease-causing and use these probabilities to 'readjust' incidence. However, this approach has not been extensively employed for a number of reasons including: (a) there is not a clear cut-off of viral load associated with symptoms, (b) asymptomatically-infected individuals appear to initially shed at levels similar to norovirus gastroenteritis cases and (c) other factors including age, setting of infection and genotype are related to Ct value, complicating the relationship between Ct value as a proxy for viral load and disease.[64]

However, we caution that - even with the optimal design - the natural history of norovirus may make it impossible to accurately estimate its disease burden using observational studies. Norovirus does not confer lifelong sterilizing immunity and therefore reinfection is common. Modeling studies illustrate that frequent reinfection weakens the relationship between norovirus detection and disease and predict that prevalence of asymptomatic shedding will increase as the force of infection increases (i.e. in low income countries and especially in children).[xii] However, disease incidence may not rise to the same degree. Accordingly, the relationship between virus detection and disease is diluted, even though the pathogen may be causing the same disease incidence. In the absence of an assay that can discriminate between true disease and background shedding, comparing prevalence in cases with prevalence in controls will be of limited value.

ii. Clinical trial design

Clinical trials for norovirus vaccines will be faced with the same diagnostic challenges as etiological studies. To make the most accurate assessment of protection afforded by a vaccine, it will be critical to accurately

[xii] These patterns are not unique to norovirus. A recent sero-epidemiological study, for example, found that the incidence of asymptomatic infection of *Campylobacter* is orders of magnitude greater than reported disease rates. [113] Indeed, using *Campylobacter* as an model, Swart et al have illustrated that due to the interplay between waning and boosting of immunity, reducing the force of infection does not necessarily lead to reduction in disease incidence.[114]



identify cases of norovirus gastroenteritis. For trials among adults in developed countries (the likely setting and population for initial Phase III trials), where background levels of asymptomatic infection are comparatively low, this will be less of a problem. However, for trials among children in low income settings, interpretation of diagnostic norovirus data may have to be considered more carefully.

In addition to defining vaccine performance, clinical trials may also provide the best opportunity to characterize the burden of norovirus disease. Probe studies are those designed to characterize the disease, whereas traditional vaccine studies focus on characterizing the vaccine.[115, 116] In addition to the usual vaccine efficacy (VE) end points, vaccine probe studies can also be used to characterize etiological fraction or vaccine-preventable disease incidence (VPDI). Given the complications in defining norovirus burden, such a study may be the best way to both determine the true etiological fraction of norovirus and the effect of a vaccine. Clearly, for such an approach to be useful, an effective vaccine must exist, and its performance must already be known, to an extent.[xiii] Candidate vaccines are discussed in more detail in Section 4.

d. Progress in cell culture systems for human norovirus

The lack of a robust cell culture system for human norovirus is fundamental technological barrier hampering many areas of research. These areas include development of assays to measure protective neutralizing antibodies conferred by either natural or vaccine-induced immunity as well as the development of infectivity assay to measure the effectiveness of disinfectants and sanitizers. Over the last few decades a number of laboratories have made many unsuccessful efforts to culture norovirus using a different range of cell lines an inoculation and culture methods and conditions, [117] Previous reports of promising 3-D in vitro cell culture systems that turned out to not be reproducible in other labs have created an air of skepticism.

Accordingly, there is considerable, albeit cautious, enthusiasm about recent progress from two groups in developing *in vitro* cell culture systems. First, Jones *et al* (Dr. Stephanie Karst laboratory) acheived replication of a GII.4 Sydney virus in a particular human B cell line. Interestingly, replication of norovirus when filtered through a 0.22 µm filter, could be restored by adding heat-killed HBGA-expressing enteric bacteria, demonstrating the importance of HBGA receptors, in this system.[118] Even more recently, the laboratory of Dr. Mary Estes and colleagues have developed an infectivity model of human intestinal epithelial cells, reportedly reproducible within the laboratory. Details of this system are yet to be published and efforts are underway to reproduce both of these results in different laboratories. A robust cell culture system will permit a number of advancements, possibly leading to improved diagnostic and infectivity assays which will facilitate confirmation of the current correlates of protection, each with a bearing on vaccine development.

[xiii] More detail on vaccine probe studies can be found in Feikin et al[116] and Mullholland.[115]

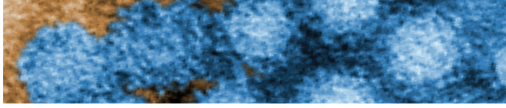
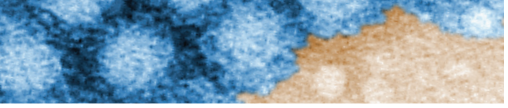


Table 3. Current knowledge, challenges and most critical studies for norovirus diagnostics and characterization

Current knowledge	Challenges	Studies needed

• Noroviruses are a highly diverse group of ssRNA viruses.
• GII.4 norovirus:
  • is the most common genotype causing cases and outbreaks across the age range
  • evolves in a boom-and-bust cycling of epochal evolution and escape population immunity with new variants emerge every 2-4 years
  • cause more severe disease and affect both young and elderly vulnerable populations.
• Real time RT-PCR is
  • the gold standard for norovirus diagnostics
  • exquisitely sensitive and frequently detects virus in the stool of healthy individuals
• There has been important recent progress in *in vitro* cell culture for norovirus.
	• A norovirus vaccine will need to protect against fast evolving GII.4 viruses.
	• Detection of virus in controls may obscure the apparent relationship between virus and disease.
	• The attributable fraction, as calculated from observational studies such as GEMS, MAL-ED and others may not provide an accurate of the role of norovirus.
	• Viral loads may be influenced by virus type and age of infection.
	• A cell culture system should be robust and reproducible
		• Rigorous assessments of how viral quantitation (Ct value from RT-qPCR) can be used to assess disease severity and burden.
		• Development/optimization of diagnostics for use in etiological studies and clinical trials, including:
		  • refined serological assays
		• Ultimately, a probe study, once a vaccine is available. Such a study will define the vaccine performance and disease burden.
		• Global Norovirus Strain Surveillance Network
		• Evaluations and reproducibility of *in vitro* cell culture system candidates.



4. Acquired immunity and innate susceptibility to norovirus

a. Patterns of acquired immunity and potential correlates of protection

Understanding of immunity to norovirus remains limited, in part because the lack of a permissive cell culture system has hampered scientific progress. Despite this, a number of key features have been characterized through analysis of human challenge and observational studies.

First, immunity seems to be of limited duration, so infection and disease occurs throughout life. By adulthood, antibody seroprevalence to norovirus exceeds 80%, but some adults continue to experience a high degree of susceptibility to infection and disease. [23, 119] This has been observed for both naturally-occurring and experimentally-administered noroviruses, demonstrating that immunity is incomplete and not lifelong. A pattern of short-term, acquired immunity was clear from the earliest volunteer human challenge studies, which were conducted in the 1970s.[xiv] Evidence from these studies suggested that protection against re-challenge with the same norovirus strain lasted for at least 6 months to two years. [121-124] Estimates from a recently published transmission modeling study indicate that the duration of homotypic immunity may last on the order of 4 to 9 years.[49]

Second, immunity is generally to homotypic strains, with some degree of protection to other viruses within the genogroups, but does not extend to other genogroups. Re-challenged volunteers have been observed to be susceptible to heterologous strains, indicating a lack of cross-protection between genogroup I (GI) and genogroup II (GII) noroviruses.[124]

Third, there is a genetic component to susceptibility to norovirus. Because pre-existing antibodies among challenged volunteers did not confer immunity in all individuals, and because some persons remain uninfected despite the absence of antibody and significant exposure, both innate host factors and acquired immunity have been hypothesized to contribute to the susceptibility to infection since the earliest studies. [123] It is now known that this predisposition for norovirus susceptibility is governed, at least in part, by the expression of histo-blood group antigens (HBGAs), which are hypothesized to serve as attachment factors through which noroviruses initiate infection.

b. Genetic susceptibility

Noroviruses recognize and bind to HBGAs, which are oligosaccharides found on the surface of epithelial cells of the gastrointestinal and respiratory tracts, as well in saliva and other secretions [125, 126]. The expression of HBGAs on the gut surface epithelium is controlled, in part, by the FUT2 gene, which encodes an alpha (1, 2) fucosyltransferase to generate H-antigens. In turn, H-antigens are catalyzed by enzymes to produce A or B blood group antigens. Numerous polymorphisms exist on the FUT2 gene; for example, the 428 (G>A) nonsense mutation is most commonly found in European populations, while the missense mutation found at nucleotide 385 (A>T) predominantly occurs in Asian populations [127]. Individuals with such polymorphisms are known as "non-secretors" based upon the absence of ABH glycans 'secreted' into their bodily fluids. Non-secretors make up about 20% of the European population; the remaining 80% have

[xiv] One of the concerns with all the classic challenge studies is that the dose of virus given to volunteers was several-thousand fold greater than the small dose of virus capable of causing human illness, estimated to be as few as 18 to 1000 virus particles. [42, 43] Thus, immunity to a lower challenge dose, similar to what might be encountered in the community, might be more robust and more broadly protective than the protection to artificial doses encountered in these volunteer studies. Contemporary volunteer studies have clearly demonstrated a dose-response relationship whereby individuals challenged with a higher noroviruses dose have greater risk of illness.[42, 120]

a functional FUT2 gene, and are known as "secretors". Similarly, the FUT3 gene encodes and alpha (1, 3) or (1, 4) fucosyltransferase to generate Lewis antigens [128]. About 6-8% of the European population is Lewis negative, compared to about one-third of the African population [129, 130].

Results from studies of human volunteers who were challenged with Norwalk virus (GI.1) demonstrated that non-secretors were not infected by the virus and none of the volunteers showed an increase in anti-Norwalk virus antibodies or had detectable RNA in feces while secretors (FUT2+/+ or FUT2+/–) excreted the virus and developed a strong antibody response.[103] A large number of observational and challenge studies have now clearly shown that the expression of HBGAs are associated with strain-specific susceptibility to norovirus infection (Figure 9).[103, 131-134] Strain specificities for binding different HBGAs also have been demonstrated using in vitro studies of VLP binding to HBGAs [135, 136] and may even vary within a genotype [137]. The association with GII.4 and GI.1 viruses with infection of secretor positive individuals in challenge studies is strong and consistent, while the association of GI.non-1 and GII.non-4 is weaker and/or inconsistent among studies, likely reflecting different HBGA binding specificities among the non-GII.4 genotypes. For GII.4 viruses, single amino acid replacements drastically alter the binding capacity of the VLP.[101]

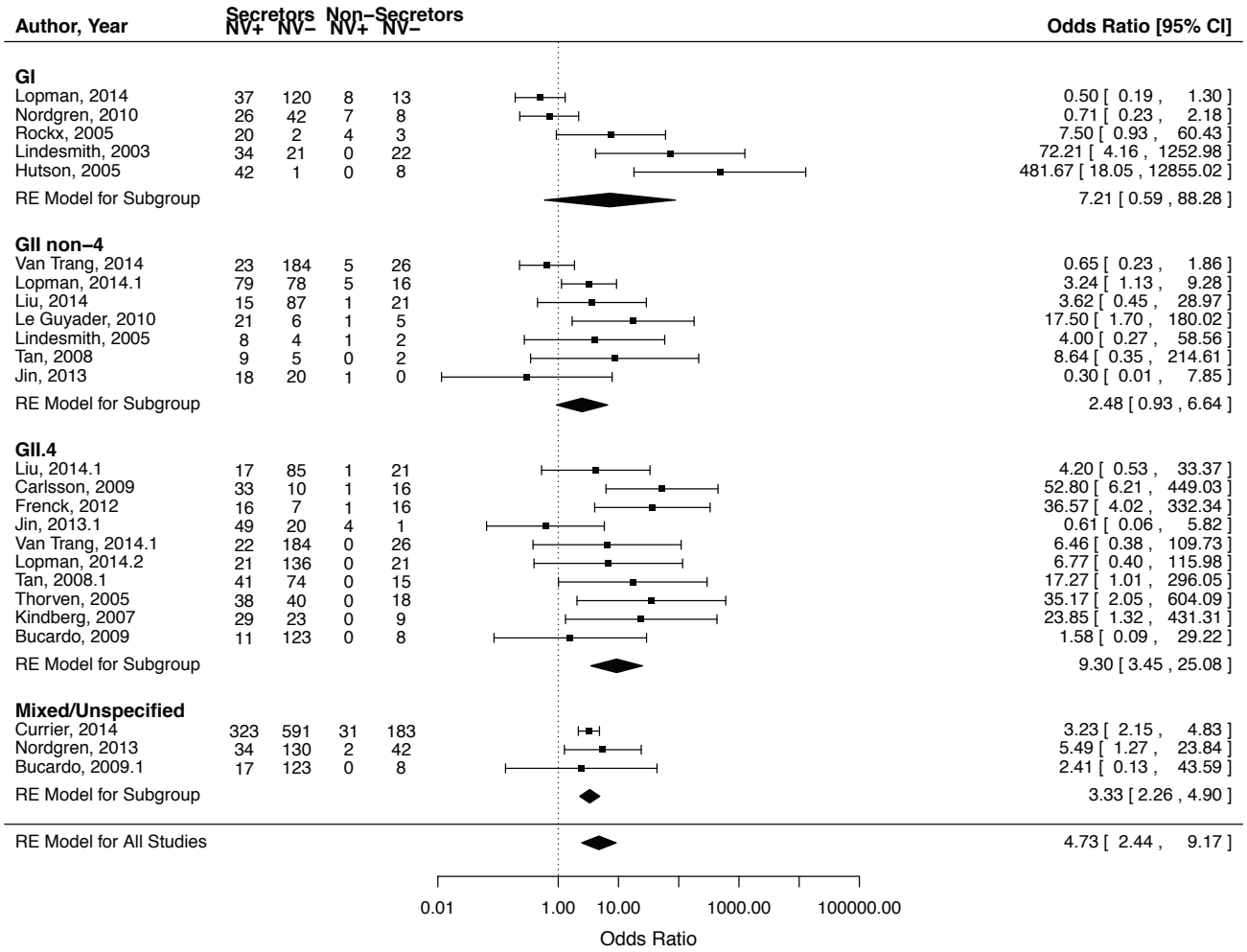


Figure 9.

Meta-analysis of effect of secretor status on susceptibility to norovirus infection, by genotype. Random effect model, including 22 studies

c. Interaction of host immune response and viral evolution

The public health and diagnostic implications of GII.4 norovirus evolution have been discussed previously (3.a.ii. Here, we highlight the implications of immune-driven evolution on immunity and vaccine design. The GII.4 capsid (ORF2) shifts antigenicity over time through a process of epochal evolution.[138] Amino acid changes in the P2 domain confer antigenic changes and affect HBGA binding affinity. GII.4 viruses exhibit a greater breadth of binding patterns than non-GII.4 viruses, and there are clear differences between GII.4 strains governed by microvariation in the P2 domain.[139] Three GII.4-specific, evolving blockade Ab epitopes have been identified. As such, in addition to the need for a noroviruses vaccine to protect against a large number of antigenic variants, it would ideally also elicit a response to currently circulating noroviruses. The viruses circulating at any given time are likely to be antigenically distinct from the VLPs on which the vaccine is based.

Encouragingly, it has been observed that noroviruses VLP-based vaccines can induce broadly reactive IgG and blockade Ab (which blocks virus-HBGA interactions, see 4.d below) responses to antigenically diverse noroviruses VLPs administered to adults with a history of exposure to norovirus and a multivalent vaccine appears to expand the antibody response, depending on exposure history.[141, 142] There is even evidence to suggest that VLP vaccines can elicit a response against GII.4 viruses that had not yet circulated GII.4 2012 (also known as GII.4 Sydney) which would not emerge until at least one year after subjects were vaccinated with a VLP vaccine based on strains that circulated >5 years prior.[143] While these observations provide some optimism that rational vaccine design may induce a broad response, our understanding of human immunity to norovirus, its interaction with viral evolution and, particularly, the development of immunity in naïve children remains far from complete.

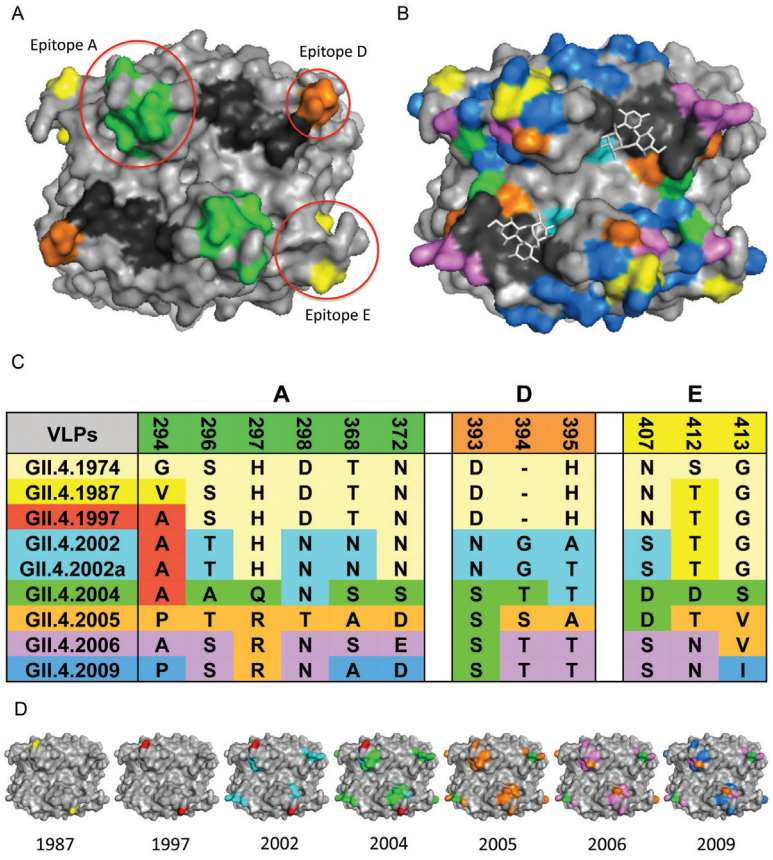
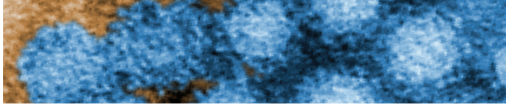


Figure 10. GII.4 noroviruses Variation over Time.

Figure originally published in [140]



d. Candidate correlates of protection

In addition to evaluating candidate vaccines, clinical trials have proven useful for characterizing immune responses to exposure and potential correlates of protection. Pre-challenge HBGA-blocking antibody titers are associated with protection against infection and disease,[105, 144] and a threshold of ≥200 was associated with a significant lower risk of illness in one study.[105] More recently, both pre-challenge NV-specific salivary IgA and pre-challenge NV-specific memory IgG cells have also been correlated with protection against NV challenge; both of these immune markers also correlate with HBGA blocking antibody titers.[145] Early mucosal IgA responses correlated with protection against NoV GI.1 infection[103] and fecal IgA at 7 days post-infection correlates with shorter duration of virus shedding, suggesting a role for mucosal immunity in protection and recovery.[138] These potential correlates are promising and, if validated, can further understanding of natural immunity and serve to accelerate vaccine development. However, all of the aforementioned data come from observations in adults and there is much less understanding on norovirus immunity in children.

e. Evidence for early acquisition of immunity

Birth cohort studies can be vital for understanding the acquisition of protective immunity against a pathogen. Such studies have led to fundamental improvements in understanding rotavirus, for example, and, in turn, have supported the development of vaccination strategies. There have been three birth cohort studies that have analyzed the acquisition of immunity to norovirus in young children. All of these were conducted in South America, with one study each in Chile,[36] Peru,[28] and Ecuador.[37] The study by Saito *et al* in Peru had the most robust design for understanding acquisition of immunity, as it involved twice-weekly home visits for surveillance of diarrheal episodes and weekly stool sampling (Table 4). A primary norovirus infection was associated with a 26% reduction in subsequent infection risk, with stronger protection against GII infection (45% and 77% reduction in disease risk following one or multiple GII infections). No protection against GI was observed, but this genogroup was far less common. The Chile study was consistent with the Peru study, in that there were data to support acquisition of immunity against GII.4. In the Ecuador study, on the other hand, there was no decrease in incidence following previous infections. However, sampling was less frequent, so infections were likely to have been missed in that study. All three studies observed a high incidence of infection and disease, with considerable genetic diversity among infecting genotypes. Birth cohort studies with intense sampling schemes, including serology, are needed, preferably from other parts of the world, to better understand the early acquisition of immunity.

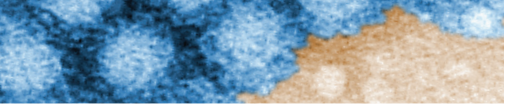
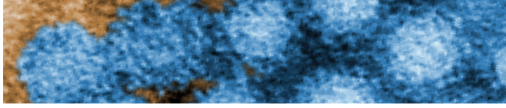


Table 4. Summary of norovirus birth cohort studies

	Chile[36]	Peru[28]	Ecuador[37]
Setting	Middle-low socioeconomic Metropolitan Region	Lima Shantytown	Peri-urban tropical
Number of children followed up/recruited (%)	198/246 (80%)	220/291 to one year 189/291 to two years	193/194 to one year (99%) 183/194 to two years (94%) 135/194 to two years (70%)
Duration of follow-up (actual mean)	18 months (15.7)	2 years	3 years
Frequency of visits	Self-reporting for diarrhea and monthly well-child visits	Twice weekly visits, weekly stool collection and during diarrhea episodes	Twice weekly phone calls and self-reporting for diarrhea
Frequency of stool sampling and testing	Monthly + episodes of diarrhea	All diarrhea specimens, one non-diarrheal specimen per month in the first year of life, two in the second year of life	All diarrhea specimens, 3,7,13,18,24,30 and 36 months for asymptomatic samples.
Time to infection	Not reported	80% with at least 1 norovirus infection at 1 year; 71% with at least 1 norovirus-associated diarrhea at 1 year	33% with at least 1 norovirus infection at 1 year; 20% with at least 1 norovirus-associated diarrhea at 1 year
Protection against subsequent infection following infection	Not reported	One infection; 0.74 (.57–.95) Two or more: 0.58 (.38–.90)	None found
Protection against same genogroups following primary infection	100% (no GII.4 infections following a previous G.II)	One GII infection; 0.55 (.41–.74) Two or more GII: 0.23 (.11–.48)	None found
Protection against other genogroups following primary infection	Not reported	Previous GI infection: 0.93 (.40–2.18)	None found
Other key findings		Repeat infections by the same genogroup were common, but repeat infections by the same genotype were rare; longer duration of GII shedding; association of norovirus infection w/ growth	GII.4 infections were exclusively detected in secretor-positive children

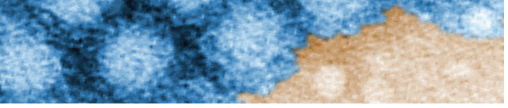


f. Innate immunity

Recent studies have reported that innate immunity plays an important role in the control of murine norovirus infection, but little is known about cell-mediated immune responses against noroviruses.[146, 147] A study using oral immunization of human volunteers with Norwalk virus-like particles showed an increase in interferon- γ (IFN-γ) in the absence of IL-4 production, suggesting a dominant Th-1 pattern of cytokine production.[148] This dominant Th1 response was confirmed in a study of 15 volunteers infected with Snow Mountain virus, who experienced significant increases in serum IFN-γ and IL-2, but not IL-6 or IL-10, on day 2 after challenge.[133] Interestingly, in an in vitro study using a Norwalk virus replicon-bearing cells, IFN-α efficiently cleared the NV replicon in a dose-dependent manner at comparable levels to hepatitis C virus, indicating a potential therapeutic application of IFNs to norovirus infection.[149]

Table 5. Current knowledge, challenges and most critical studies for understanding immunity and susceptibility to norovirus

Current knowledge	Challenges	Studies needed
• Immunity to norovirus is strain-specific (to GII.4 viruses) and genotype-specific. There appears to be little protection across genogroups. • Immunity to norovirus is not life-long, with estimates of duration ranging from 6 months to 9 years. • Susceptibility to noroviruses is affected by a host's glycan expression; individuals with a functional FUT2 gene (secretors) have greater susceptibility to GII.4 and GI.1 strains. • Some surrogates for protection have been proposed (HBGA blockage, IgA, memory B cells).	• Genotype-specific immune responses and antigenic variation suggest that a polyvalent vaccine will be needed, which may require updating when new strains emerge • Vaccine trials and challenge studies have primarily been conducted among adults, there is much to learn about how "unprimed" children (who have not experienced as many exposures) develop immunity.	• Birth cohort studies in low, middle and high income setting to further understanding on the acquisition of immunity. • Globally representative strain surveillance data to address whether host population genetics affects local viral diversity. • Clear guidance on types of samples to collect in challenge and observational studies. • Confirmation of currently identified correlates of immunity in different populations



5. Norovirus vaccines

Ever since recombinant norovirus capsid proteins were expressed as virus like particles (VLPs), they have been considered as potential antigens for norovirus vaccines. VLPs are morphologically and antigenically indistinguishable to native viruses, but lack genetic material, so are non-replicating. Early studies showed that VLPs can elicit a humoral and mucosal response in mice and humans by oral, intranasal or parenteral administration [150-154]. A number of norovirus vaccines are now under development, and all are based on expressed VLPs or similar technology.

In general, a norovirus vaccine will be subject to the same regulatory process as other vaccine candidates. In the United States, this will include consideration for licensure by the Food and Drug Administration, followed by consideration for a recommendation by the Advisory Committee for Immunization Practices. Issues that may be specific to norovirus include (a) the potential need for reformulation in the event of the emergence of novel antigenic variants, as with current influenza vaccines, and (b) addition of other viral antigens (e.g., sapovirus VLP). Because of the biology of and human immunity to noroviruses, there are a number of characteristics that will determine the success and public health value of a norovirus vaccine. We summarize these issues below.

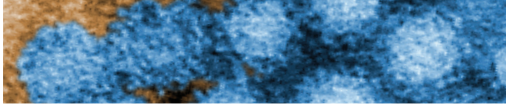
a. Developmental challenges and questions

i. Can a vaccine be developed that will elicit broad protection against multiple genotypes?

Noroviruses are genetically and antigenically diverse. Acquired immunity is of limited duration against homotypic strains, and there is some degree of protection against viruses in the same genogroup, but there is little or no heterotypic protection across genogroups. Accordingly, a norovirus vaccine would likely need to be at least bivalent to protect against GI and GII viruses. So far, all vaccine trials have involved challenge with a genotype included in the vaccine. Recently, Lindesmith and colleagues analyzed sera from human volunteers who had been immunized with a bivalent norovirus vaccine containing GI.1 and GII.4 virus-like particles (VLPs)[141] of which the GII.4 component is a consensus (GII.4C) based on major capsid protein sequences from three GII.4 variants (GII.4 Houston/2002, GII.4 Yerseke/2006a and GII.4 DenHaag/2006b). At day 7-post vaccination substantial rises of IgG and blockade Abs were only observed against GI.1 and GII.4 and also against GI.3, GII.3,and GII.14 VLPs , suggesting broad and rapid antibody response. That GI.1 and GII.4 VLPs would elicit a response to other genotypes in humans was hardly a foregone conclusion given the extreme antigenic diversity of noroviruses. This observation is consistent with observations of humans infected during experimental GI.1 Norwalk virus studies who exhibited surrogate neutralizing antibody responses to homotypic GI.1 VLPs but also heterotypic response to GI.2, GI.3, GI.4, GI.7 and GII.4 VLPs including the novel Sydney variant of GII.4 that had not yet circulated at the time of the infections.[143, 155]

ii. Will a norovirus vaccine have to be regularly updated in order to match viral evolution?

As discussed in Section 3.a.ii, GII.4 noroviruses undergo epochal evolution with new GII.4 variants emerging every 2-3 years and replacing previous dominant strains, a process driven by evasion of immunity in the human population. As such, vaccines against norovirus are likely to face similar obstacles as those faced by seasonal influenza vaccines.[156] Ideally, a norovirus vaccine will protect against viruses not included in the formulation, including both heterotopic genotypes and antigenically-novel GII.4 variants. With GII.4 viruses evolving to avoid human population immunity, a VLP based on any specific GII.4 variant is likely to lose its antigenic 'match' with contemporary strains at some point. A strategy taken by one vaccine developer was to engineer the GII.4 antigen from a consensus (GII.4C) based on major capsid protein sequences from three GII.4 variants (GII.4 Houston/2002, GII.4 Yerseke/2006a and GII.4 DenHaag/2006b).[105] Immunization with this vaccine resulted in responses (both IgG and blockade Ab titers) not only across a panel of



different GII.4 viruses that circulated prior to the time the human volunteers were vaccinated (September 2010 until April 2011), but also elicited a response against two GII.4 viruses that had *not yet circulated*, most importantly against GII.4 2012 (also known as GII.4 Sydney) which would not emerge until at least one year later. These results are also consistent with experimental infection studies where a single GI.1 strain elicited antibodies against GII.4 Sydney virus.[143] This raises the question: could a norovirus vaccine protect against future (GII.4) strains? These are promising results but, in the end, these questions will only be settled with a phase III randomized controlled trial conducted in the community where participants are exposed to naturally-circulating viruses. Those viruses will inevitably be antigenically distinct from the VLPs on which the vaccine is based. To prepare for vaccines, a surveillance network with good global representation will be needed to characterize global strain distribution and identify the emergence of new strains.

iii. How will prior norovirus infection history affect vaccine immunogenicity and effectiveness?

By the age of five years, nearly every child will have had at least one norovirus infection and many will have had multiple infections and disease episodes.[28] Therefore, nearly all older children and adults have pre-existing antibodies from natural infection.[33, 157, 158] Immunizing these age groups will involve boosting pre-existing immunity. Therefore, a single dose or limited number of doses, may be sufficient to elicit a protective immune response in those with pre-existing antibodies. Infants and young children will generally not have antibody from a previous infection. Therefore a vaccine would need to generate immunity *de novo* in immunologically naïve children and, accordingly, multiple doses are likely to be required.

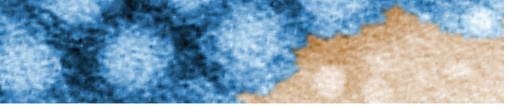
Clearly, there is a need to assess infection history separately in children and adults. Specifically, birth cohort studies can be used to assess severity of and immune response to primary and subsequent infections. While there is some limited data on the development of immunity following natural infection in children, there are no data on vaccine induced immunity in this age group. So while data among adults demonstrate promising outcomes, we still need data among children, as well as more research on the degree of cross protection. In addition, the role of maternal antibodies, and their effect on infant vaccine response require further study. For this combination of reasons, different developmental plans are needed for pediatric and adult vaccines.

iv. Will the same vaccine formulation and schedule be effective in all groups?

A related question is: will the same vaccine formulation be effective in all groups (adults versus children) and populations (high-income versus low income)? For the reasons noted above, it is quite possible that the vaccine formulation will have to be altered for children in terms of number of doses, the amount and genotype of antigen in the vaccine and presence of other (non-norovirus) antigens. Since infants will be immunologically naïve, a prime-boost vaccination strategy may be needed to generate protective immunity. Combination vaccines that include antigens to target other common causes of childhood diarrhea may increase the attractiveness and cost-effectiveness compared to a stand-alone norovirus vaccine. Indeed, a rotavirus-norovirus vaccine is being developed (see section 5.b.iii), which would target the top 2 causes of pediatric diarrhea. Given its genetic similarity to norovirus, a combination with sapovirus may also be an attractive formulation, but such as vaccine has not been currently developed. Influenza and Hepatitis E antigens have been combined with norovirus P particles, in preclinical studies, as noted in Section 5.b.ii.

v. How will the genetic susceptibility affect vaccine outcomes?

Noroviruses recognize and bind to histo-blood group antigens (HBGA) (as described in more detail in the previous chapter).[125, 126] Non-secretors (who make up ~20% of the European population) lack a functional FUT2 gene, do not express certain HBGAs on their gut epithelia and are genetically resistant to (at least some) noroviruses. Genetic susceptibility clearly affects norovirus disease risk and there may also be



implications for vaccine development and vaccine study design. Non-secretors do appear to respond to parental immunization with the bivalent VLPs vaccine without diminished affect immunogenicity, compared to secretors.[159] Some norovirus vaccine trials and challenge studies only included secretor positive individuals as a criteria for eligibility to ensure susceptibility to the challenge virus [105, 160], however field trials will not be able to exclude non-secretors. As such, results of vaccine efficacy studies should be interpreted while bearing in mind the proportion of individuals in the study population with FUT2 polymorphisms. Population-level differences in secretor distribution (associated with race or ethnicity) could result in different strain distributions affecting certain populations. As such, it's conceivable that the optimal vaccine formulation (in terms of VLP genotype antigens) varies from population to population.

b. Candidate vaccines

There are at least four different norovirus vaccines that are under development based on the manufacture of virus proteins in various expression systems.[xv] Each of these vaccines is reviewed in brief below.

i. Transgenic plant-based norovirus vaccine

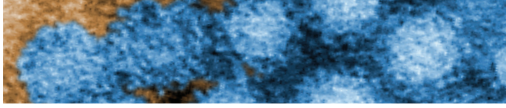
No vaccines developed from plant-based expression systems have yet reached the market in the United States but, in principle, these systems provide an alternative to traditional cell-culture systems that could produce safe vaccines at comparatively low cost. Norwalk virus VLPs have been expressed in transgenic tobacco as well as potato expression systems. [162] In preclinical studies, these plant-expressed rNV have been immunogenic in mice.[162, 163] As a proof of principle, eating raw potato genetically modified to express NV capsid protein have been shown to be immunogenic for humans.[164] A more efficient system has since been engineered whereby VLPs are expressed in a tobacco mosaic virus (TMV)-derived transient expression system,[165] with further improvements gained in a bean yellow dwarf virus (BeYDV)-derived vector and Rep/RepA-supplying vector.[166] These candidate vaccines have not yet been evaluated in either challenge or field-based clinical efficacy studies.

ii. Norovirus P particle and combination vaccines

The norovirus P particle is a nanoparticle formed by copies of the protruding (P) domain of the norovirus capsid protein. These particles are readily produced in *E. coli*, and have authentic host receptor binding properties. Since the P particle is formed by the surface antigen of norovirus and contains all the required elements to interact with the viral receptors. P particles have been shown to be highly immunogenic, able to tolerate a wide range of pH and temperatures, and are stable and therefore, and therefore have been proposed as a vaccine candidate. The norovirus P particle activates both the innate immune system and elicits humoral and cellular immunity. Intranasal delivery of the P particle provides a degree of protection against human GII.4 norovirus diarrhea in gnotobiotic piglets.[167] Hoever, not all human monoclonal antibodies recognize P-particle A epitope, suggesting some conformational variance between VLP and P particles.[139]

P-particle vaccines may also serve as a platform for incorporation of additional antigens: dual vaccines against norovirus with influenza, Hepatitis E and rotavirus have all been engineered.[168, 169] The chimeric (norovirus) P- (rotavirus) VP8 particle could be particularly attractive dual vaccine for children against the two leading causes of pediatric gastroenteritis. One preclinical study in mice suggests VLPs, but not P particles, prime T cells for interferon-γ production and induce cross-reactive B and T cells.[170] Norovirus P particle vaccines are yet to be evaluated in either challenge or field-based clinical efficacy studies.

[xv] Vectored vaccines such as VSV and alphavirus VRP-based vaccines are also possible and both have been used in human trials for other systems. Both technologies owned by companies that could potentially take them into the marketplace.[142, 161]



iii. Trivalent norovirus /rotavirus combination vaccine

A trivalent norovirus/rotavirus combination vaccine, which includes norovirus GII.4 VLPs, GI.3 VLPs, and rotavirus VP6, is being developed by the Vaccine Research Center in Tampere, Finland. The rationale for this product is that a combination noroviruses + rotavirus vaccine has the potential to eliminate most of severe acute GE in children. In animal models, this combination vaccine has been observed to elicit antibodies that block binding to HBGAs (a) of homologous (GII.4 and GI.3 VLP) strains included in the vaccine as well as (b) heterologous (GII.4 New Orleans/2009 VLP or GI.1/2001 strains not included in the vaccine.[171] Both intramuscular and intranasal immunization induce robust serum IgG responses in the mouse model. The combination of RV and noroviruses antigens in the vaccines does not appear to result in any inhibition of noroviruses-or RV-specific immune responses in the trivalent combination compared to the single vaccines.[171] A high level of noroviruses and RV type-specific and cross-reactive serum antibodies are elicited. Regarding the rotavirus components, the RV VP6-specific immune response (IgA) protects from heterologous RV challenge in mice [172] and there is some evidence that the RV VP6 component has an adjuvant effect on the noroviruses response, potentially resulting in noroviruses VLP dose-sparing.[172] When delivered with RV VP6, the cross-reactive antibody responses and blocking activity to noroviruses is increased. In 2012, a Licensing and Development Agreement was entered into with UMN Pharma (Japan). To date all studies have been performed in mice; the next step for this vaccine will be Phase I human trials. It is intended as a vaccine for infants or toddlers.

iv. Takeda Pharmaceutical/Ligocyte VLP vaccine

The candidate furthest along in the development pipeline is a bivalent, intramuscular VLP vaccine. This vaccine has been shown to confer a degree of protection against disease when human volunteers were vaccinated and subsequently challenged. In preclinical studies, early versions of this product were well-tolerated and immunogenic when delivered orally [151], intranasally [173] or intramuscularly.[89] The vaccine has been administered to humans as both a monovalent (GI.1 VLP) and bivalent (GI.1 + GII.4 VLP) formulation. The GI.1 VLP is derived from the prototype Norwalk virus (1968) while the GII.4 is a consensus sequence containing epitopes from Houston/TCH186/2002/US, Yerseke 38/2006/NL and Den Haag 89/2006/NL, along with adjuvants alum and MPL (3-O-desacyl-4' monophosphoryl lipid A).[174] The GI.1 component vaccine was efficacious against homotypic GI.1 challenge when given intranasally.[105] Vaccine reduced the risk of Norwalk virus gastroenteritis by 47% (95% CI, 15%–67%) and Norwalk virus infection by 26% (95% CI, 1%–45%). In that study, as well as in a previous Phase I immunogenicity study, approximately 75% of adult subjects mounted a Norwalk virus–specific serum IgA antibody response.[xvi] More recently, volunteers were administered 2 doses of the GI.1/GII.4c intramuscular vaccine, followed by experimental challenge with a heterologous GII.4 virus.[175] Vaccines failed to significantly prevent acute gastroenteritis with 13 (26.0%) cases among vaccinees and 16 (33.3%) in placebo recipients. However, reduced severe disease, diarrhea and vomiting was experienced by vaccine recipients. This bivalent vaccine was well-tolerated and immunogenic, generated rapid serum response (peaking at 7 days), elicited HBGA binding blocking activity[159] and a robust mucosal-homing antibody-secreting B cell response.[176]

Adolescents and adults are likely to be administered one dose; it is unclear what dosing will be required in infants, and a prime-boost strategy may be trialed. Phase I/II studies are planned in different age groups in order to confirm dosage, composition, number of doses, schedule as well as to assess safety and immunogenicity.[177] Efficacy clinical trials are planned to start in military recruits, followed by trials in the elderly and infants.

[xvi] This trial was also the first demonstration that an IN-delivered vaccine could prevent human illness due to an enteric pathogen.

Table 6. Summary of candidate norovirus vaccines: technology, composition and stage of development

	1. Transgenic plant-based norovirus vaccine	2. Norovirus P particle and combination vaccines	3. Trivalent norovirus/ rotavirus combination vaccine	4. Bivalent norovirus VLP vaccine
Principal inventor	Charles Arntzen, PhD *Arizona State University*	Xi Jason Jiang, PhD *Cincinnati Children's Hospital*	Timo Vesikari, MD PhD *University of Tampere, Finland*	Mary K. Estes, PhD *Baylor College of Medicine*
Norovirus antigen(s) (all based on VP1)	Norwalk virus (GI.1) VLP	Two to three noroviruses P domains representing different GI and GII strains	GII-4 and GI-3 VLP	GI.1 and GII.4 consensus VLP
Other antigen(s)	None	Chimeric norovirus P-rotavirus VP8* particle, other experimental formulations include influenza, Hepatitis E	Rotavirus VP6	None
Adjuvant(s)	gardiquimod [178] or none when delivered with GelVac	5 mg chitosan, 50 µg MPL, and TNC buffer 24920797	None, but some evidence that the RV VP6 component has offers an adjuvant effect on the noroviruses response homologous and heterologous blocking ("neutralizing") activity of noroviruses-specific sera	alum and MPL (3-O-desacyl-4' monophosphoryl lipid A)
Expression system	tobacco mosaic virus (TMV)-derived transient expression system using leaves of Nicotiana benthamiana[165]	Baculovirus vector in *E. coli* or yeast system	Baculovirus expressed noroviruses VLPs	Baculovirus expressed noroviruses VLPs
Route of administration	Intranasal by GelVac dry powder[163] and oral by ingestion of raw potato[179]	Intranasal	Intramuscular and intranasal	Intramuscular, previously intranasal and oral
Summary of safety studies	None	None	None	Range of studies with various concentrations monovalent (GI.1) or bivalent (+ GII.4c) VLP, with and without adjuvants, and various routes of immunization.[xvii] Generally safe and well-tolerated with some local reactogenicity, esp. when delivered with MPL adjuvant. [105, 151, 152, 159, 175, 181]

[xvii] For a complete review, see Ramani et al[180]

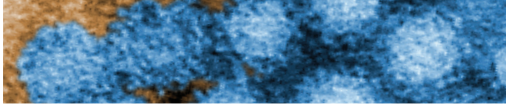


Table 6. Summary of candidate norovirus vaccines: technology, composition and stage of development (continued)

	1. Transgenic plant-based norovirus vaccine	2. Norovirus P particle and combination vaccines	3. Trivalent norovirus/ rotavirus combination vaccine	4. Bivalent norovirus VLP vaccine
Summary of immunogenicity studies	In mice: humoral and mucosal immune responses when delivered via the enteric or intranasal route [162, 165, 178, 182]	Stable, with authentic host receptor binding properties, highly immunogenic. In mice: humoral and cellular immune responses [183]	In mice: Combination of two noroviruses VLPs induces cross-reactive antibodies to genotypes not included in the trivalent vaccine. Both IM and IN immunization induce robust serum IgG response. noroviruses and RV type-specific and cross-reactive serum antibodies are elicited.	Range of studies with various concentrations monovalent (GI.1) or bivalent (+ GII.4c) VLP, with and without adjuvants, and various routes of immunization[xviii] Studies have generally shown rises in serum IgA and IgG, on the order of 4 to 25 8 GMFR.[xix] Also hemagglutination inhibition and histoblood group antigens blocking activity. Mucosal-homing antibody-secreting B cell responses consistent with B cell memory response. [105, 151, 152, 159, 175, 176, 181]
Summary of efficacy studies	None	None in humans In gnotobiotic pigs: protection against diarrhea (47%) but not against virus shedding in GII.4/2006b-challenged[167]	None in humans In mice: Both IM and IN routes induced protective VP6-specific immunity against live RV challenge No norovirus efficacy data	**In human challenge study VE of:** 47% (95% CI, 15%–67%) against AGE Norwalk virus infection by 26% (95% CI, 1%–45%) following GI.1 challenge; Non-significant reduction in AGE (26.0% among vaccinees; 33.3% among placebo recipients) Reductions of more severe disease and diarrhea and vomiting in vaccine recipients following GII.4 challenge. [105, 184]
Next likely development stage	Human immunogenicity and safety studies	Human immunogenicity and safety studies	Phase I in adults Phase I/II in older children Phase II/III Proof-of-concept study in young children	Phase I/II in different age groups to confirm dosage, composition, number of doses, schedule Phase III field efficacy trials in adults (US military recruits), followed by children in LMIC
Commercial partner			UMN Pharma (Japan).	Takeda Pharmaceuticals

[xviii] For a complete review, see Ramani *et al*.[180]

[xix] Geometric mean fold rise

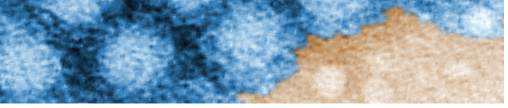
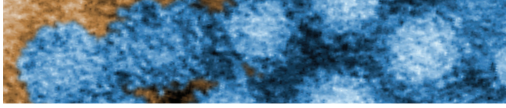


Table 7. Current knowledge, challenges and most critical studies for norovirus vaccine development

Current knowledge	Critical questions	Studies needed

• A number of noroviruses vaccines being developed.
• These products are based on the expression of VP1 leading to the production of VLPs or P particle subunit in various expression systems.
• Preclinical and early human studies of various concentrations of monovalent or bivalent norovirus antigens, with and without adjuvants, and various routes of immunization have shown safety and immunogenicity.
• The products with human efficacy data are being developed by Takeda Pharamaceuticals.
  • An intranasal monovalent formulation was shown to be effective against infection and disease following GI.1 challenge.
  • An IM bivalent formulation showed a degree of protection against disease following GII.4 challenge sufficient to warrant further clinical development.
	• Can a vaccine be developed that will elicit broad protection against multiple serotypes?
	• Will a norovirus vaccine have to regularly updated in order to match viral evolution?
	• How will prior norovirus infection history affect vaccine immunogenicity and effectiveness?
	• Will the same vaccine formulation be effective in all groups?
	• How will the genetic susceptibility affect vaccine outcomes?
		• Human clinical studies to characterize the safety and immunogenicity of products not yet studied in humans.
		• Pivotal, phase III field efficacy studies to demonstrate protection against natural infection.
		• Separate clinical development is required for adults and children to define immunogenicity related to:
		  • number of doses
		  • timing of doses
		  • antigen concentration
		  • need for adjuvants
		• Probe studies, using an efficacious vaccine, to help to define the burden of norovirus.



6. The road to a norovirus vaccine

a. Specific age and risk groups

One of the challenges (and opportunities) in developing norovirus vaccines is that so many distinct population subgroups are affected which complicates the formulation a research agenda and clinical development plan. Such a plan would look quite different for a target population of young children than it would for older adults, or a specific risk group, such as healthcare workers.

Table 8. Epidemiological and economics characteristics of various age groups for considering norovirus vaccines

	Incidence	Health care utilization[1]	Hospitalization	Deaths	Societal costs	Healthcare costs	Role/risk in transmission	Challenges in vaccinating: immunological	Challenges in vaccinating: programmatic
Children *(<5 years)*	High	High	High	Med.[2]	High	High	High	Naïve: may need multiple doses	Interaction with other routine immunizations
Older children *(5-14 years)*	Med.	Low	Low	Low	Low	Low	Med.	History of exposure	
Adults *(15-64 years)*	Med.	Low	Low	Low	Low	Low	Med.	History of exposure	Generally low coverage
Older adults *(≥65 years)*	Low	Med.	High	High	Low	High	Low	History of exposure;	

[1] Outpatient, emergency and ambulatory services
[2] Little data from lower income settings.

i. Age-based vaccination

Thinking about norovirus disease, the population can be broken up by demographic or risk groups (Table 8). A product developed with a pediatric market should have a target product profile (in terms of number doses, antigen concentration and adjuvants) most useful for young children in developing countries to prevent hospitalizations and deaths. Regarding demographics, young children have the highest disease incidence [23] and have the highest rates of overall healthcare utilization. They also appear to be the primary drivers of transmission, as discussed below in Section 6.b. However, for severe disease there is a shifting burden to the older age groups; young children and elderly have similar hospitalization rates, and >95% of deaths are estimated to occur among those 65 years and older.[21]

ii. Risk group-based vaccination

Other groups may be targets for vaccination based on their risk profile. Travelers and military personnel are potential targets mainly in an effort to protect them from travelers' diarrhea (TD), a leading cause of illness during and after international travel. 20-40% of travelers to low-income regions experience TD and norovirus is associated with 3-17% of TD.[185] In the military, norovirus is common among deployed troops, especially in the early phase of an operation [186], aboard Navy ships which are uniquely confined and transmission-favorable environments [187], and is also a frequent cause of large outbreaks in recruit training populations.[188]

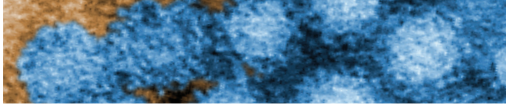
The immunocompromised may be subject to higher rates of infection, have prolonged and severe illness and may even be a source of novel virus strains.[189] For these reasons, these disparate groups may be important to vaccinate, but each group also comes with a specific set of challenges (Table 9).

Based on the risks of transmission associated with their profession, healthcare workers could be vaccinated to prevent healthcare outbreaks and food handlers could be targeted with the aim of preventing food borne disease. Predicting the impact of these strategies requires knowledge of the group's role in transmission. For healthcare workers, this knowledge is limited, but current evidence suggests that their role in transmitting norovirus in hospital outbreaks is minor compared to the role of patients.[190]

Norovirus accounts for 58% of all domestically-acquired foodborne illness from known agents in the U.S. and food workers are identified as source in 70% of outbreaks where contributing factors are reported.[191] Therefore, vaccinating food handlers may be one way to control food borne disease. The potential for reducing disease burden through vaccination has not been explored yet in modeling studies. There are considerable challenges to vaccinating healthcare workers, food handlers, or any specific risk groups.

Table 9. Epidemiological, economic and programmatic considerations for specific sub-population risk groups.

	Healthcare workers	Travelers	Military personnel	Immuno compromised	Food service workers
Incidence	Affected in outbreaks	High	High	Unknown	Likely same as general population
Severe disease	Low risk	Can have limited access to care while traveling	Outbreaks may occur under extreme temperature & exertional conditions	High risk, including chronic infection and death	Low risk
Economic losses and disruption	Productivity losses; compromised patient care from missed work	Loss of personal travel funds, sometimes loss to operators (e.g. cruise industry)	Impact on training, mission readiness and operations	Costly extended length of hospitalization	Productivity losses from missed work; impact on business of product recall, store closure or brand impact
Role in transmission and potential indirect benefits of vaccination	May transmit to patients, but current evidence suggests low rates	Generally low, but potential for transmitting on aircraft, buses, hotels, etc.	Potentially high for those resident in barracks, on ships, or on missions	Unknown, put potential risk due to prolonged shedding	High: food handlers implicated in the majority of foodborne norovirus outbreaks
Challenges in vaccinating: immunological	May have extensive history of exposure	Unfamiliar strains during foreign travel	Unfamiliar strains during foreign deployment	Poor immune response	None
Challenges in vaccinating: programmatic	Has taken many years to achieve reasonable flu vaccine coverage in the U.S. Many healthcare workers do not get vaccinated.	Need to be vaccinated in a travel clinic, with sufficient time to mount immune response before departure.	None, but if given in recruit setting, interference with other concomitant immunizations should be assessed	May be difficult to identify in advance of exposure	Hard to reach population with high turnover; unwillingness of employer to pay



b. The potential population-level effects of a norovirus vaccine

In addition to the direct effects of immunologically protecting individuals from infection and/or disease, vaccines may also offer indirect benefits to the population at large.[192] The current generation of candidate norovirus vaccines are inactivated (so vaccine virus is not transmissible itself), and that is likely to be the case at least until robust norovirus cell culture systems are available to produce live-attenuated vaccines. Accordingly, any indirect protection from a norovirus vaccine program will be from reduced transmission and exposure. This may come about via two mechanisms. The first is by preventing infections, thereby removing those individuals as a source for onward transmission to other susceptible individuals in the community. The potential for this mechanism was demonstrated in the monovalent, intranasal vaccine-challenge trial, were Atmar *et al* observed it is possible to prevent norovirus infection by vaccination.[105] A second mechanism is by reduction of infectiousness (or the risk of transmission) given infection. For norovirus, this could be by reduced severity/duration of symptoms or reduced magnitude and duration of viral load. Again, there is a suggestion from vaccine trials with human challenge that severe disease symptoms are reduced as well as some evidence of a reduction in the proportion shedding at 10 days post-infection.[175] It is notable that there is evidence of reduced vomiting with vaccination, important since vomiting is a key route by which norovirus is transmitted.[46]

Assuming that norovirus vaccination can reduce the force of infection, a critical question is how to maximize the population level impact of a vaccination strategy, considering practical constraints of how a vaccine program may be implemented. To address this issue, it is critical to understand which groups have the highest burden of disease and which groups play important roles in transmission. For the latter, direct observation is not usually possible, so mathematical models are useful tools to estimate the roles of various groups in transmission and to explore the potential impacts of a control strategy, including vaccination. We have fit such models to age-specific incidence for the U.S. and U.K. using realistic contact patterns, and have found that children likely play a much larger role in transmission than do older children or adults.[193] Therefore vaccinating children is likely to garner greater direct and indirect effects compared to vaccinating the elderly. Based on preliminary analysis, we predicted that a pediatric vaccine program (90% coverage, 50% efficacy) prevents as many hospitalizations in the elderly (aged 65 years and older) than directly vaccinating the elderly age group (65% coverage, 50% efficacy) (Figure 11). However, there is considerable uncertainty in these projections, especially where transmission is concerned. These models are yet to be applied to developing country settings where transmission patterns may differ considerably from to high income settings.

c. Economic impact of norovirus and potential cost-effectiveness of vaccination

Estimates of the economic burden of norovirus are limited and most are for the U.S, where hospitalizations are estimated to cost US$500 million annually and foodborne disease results in a total cost (including healthcare and productivity losses) of US$2 billion with overall disease (including all modes of transmission) in the range of US$8 billion.[194, 195] The economic value will be determined by many factors, and without a product on the market, it is preliminary to make decisions based on economics. However, based on scenario analysis, we projected that a low cost vaccine (<$50) given to young children could be cost-saving. A more expensive vaccine led to costs per case averted at levels comparable to other vaccines in the US.[196] Overall, norovirus vaccination could provide substantial health benefits to developed countries, but would likely incur additional net costs to society in most scenarios.

Economic evaluations are needed for middle and low income countries and to evaluate the potential economic consequences of norovirus vaccination in specific high-risk populations such as travelers, nursing home residents, and military personnel and groups important for transmission such as healthcare workers and food handlers.

d. Economics and financing of a norovirus vaccine

It will be important to develop economic models as early as possible in vaccine development when vaccine

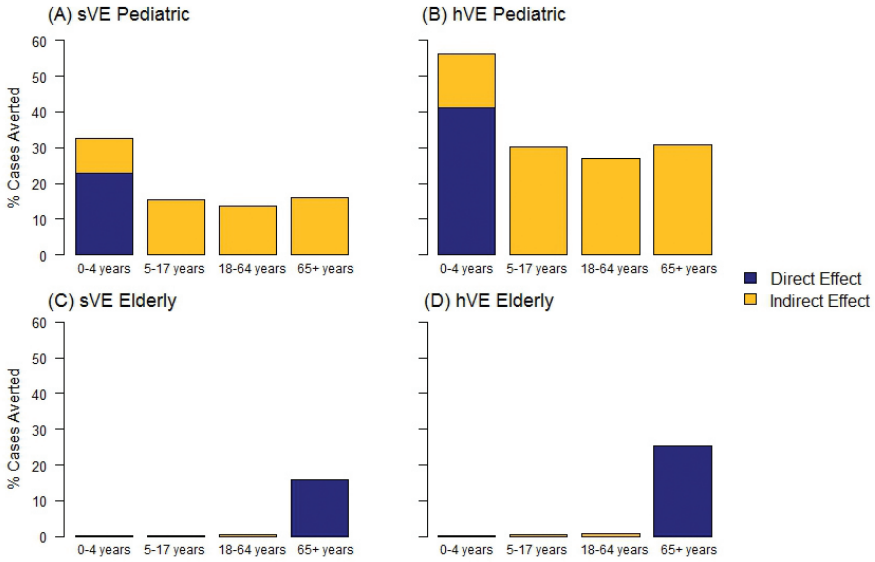


Figure 11.

Comparison of the direct (blue) and indirect (yellow) effects of vaccines for vaccine scenarios in the U.S.

A: *infant vaccine with 90% coverage and 50% efficacy.*
B: *elderly vaccine with 65% coverage and 50% efficacy.*
C: *infant vaccine with 90% coverage and 90% efficacy.*
D: *elderly vaccine with 65% coverage and 90% efficacy.*

characteristics and implementation plans can be more readily adjusted to match market, policy, and population needs. Such work can substantially help various key stakeholders (e.g., scientists and developers, manufacturers, funders, policy makers and public health officials, third party payers, healthcare workers, vaccine suppliers and distributors) better construct and adjust vaccine target product profiles (TPPs), tailor vaccine development accordingly, determine price points, allocate funding and other resources, forecast returns-on-investments, establish timelines, select target populations, determine pricing and reimbursement policies, plan logistics, and assist in other important decisions.

Economic models and evaluations for low and middle income countries will have additional key considerations, given their limited resources, the time lag between expenditure and benefits, the frequent need for partners and intermediaries to broker financing and other operational needs, concomitant disease control programs (e.g., other disease control programs may compete for resources but also may benefit from a new vaccine), and potential obstacles to delivering and administering the vaccine (e.g., bottlenecks in the supply chains and a dearth of health care workers). Moreover, the challenges of health systems for vaccine delivery should not to be overlooked, the economics of vaccines change over time (changing disease incidence), and there are interactions between disease, economic status, and political and social climate. Economic studies can also help guide target product profiles (TPPs), which are important for vaccine success. TPPs can help by tailoring vaccine design and implementation plan to market and understand how vaccine characteristics impact vaccine adoption, distribution, and administration. TPPs can also incorporate how vaccine technological characteristics may interact with a wider array for strategic, marketing, operational, epidemiological, and public health market issues.

e. Bridging the developed and developing world markets

It is useful to take a step back to consider the current vaccine development paradigm and interrelated domains and processes which inform the development and, ultimately, the success of a vaccine. Broadly speaking there five areas to consider including (1) the defining of the public health need, (2) the making of an investment case, (3) the understanding and optimization of probability of success, (4) identifying and aligning sponsors, and (5) conducting an analysis of alternatives. Each of these development considerations are independently important, but also are interdependent and are briefly discussed. For example, the extent to which the public (and private) health need can be defined, will directly inform the demand side of an

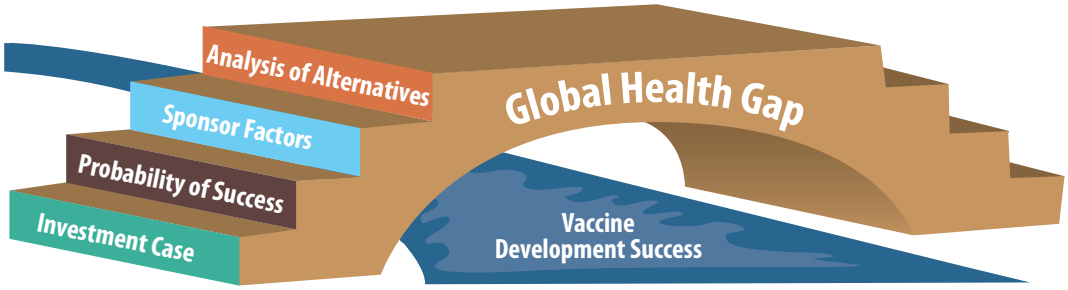
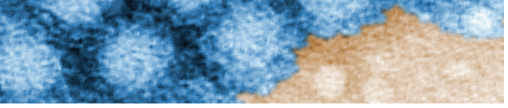


Figure 12.
Framework to consider vaccine development

investment case. As described earlier, additional epidemiological studies which identifies the important (direct and indirect) health impacts in both developed and developed world settings are keys to success, particularly in a competitive landscape of multiple global infectious disease targets for which vaccines are being developed. The investment cases that are to be made from these data on public health need are important drivers of sponsor commitment and alignment of resources. But such investment cases also serve as important information that will drive vaccination policy and introduction into the schedules of the variety of target populations. The appropriate (and different) payer perspectives need to be considered in these investment cases so that when a vaccine is developed, decision makers who will drive vaccine utilization will have a good fundamental base of knowledge. The probability of success of a vaccine being developed will depend upon many factors known (and unknown) including the use of proven technology, scalability, cost of goods, and whether the vaccine construct and delivery will meet the required target product profiles for each target population. With respect to a norovirus vaccine, as identified, the probability of success may not be equal given unique challenges both in the response to and investment case for the various target populations. However, as with other vaccines for global health like malaria, dengue and HIV, norovirus has the advantage of attracting a diversity of sponsors from industry, governmental organizations, and not-for-profit institutions to form a product development partnership (e.g. International AIDS Vaccine Initiative, European Vaccine Initiativ, Dengue Vaccine Initiative). Additionally, sponsor factors will have an important influence on probability of success through commitment to development and enabling technologies which are brought to bear on the development, delivery and policy challenges a norovirus vaccine will face. In summary, it is useful to consider the development of a norovirus vaccine through a framework of the individual and connected factors of the current vaccine development paradigm. Through consideration of each we may better understand potential gaps and opportunities to ensure ultimate success in a vaccine for all (Figure 12).

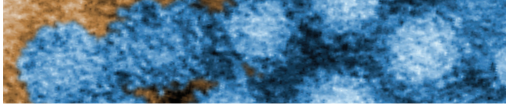
Table 10. Current knowledge, challenges and most critical studies for developing a norovirus vaccine program

Current knowledge	Challenges	Studies needed
• Young children experience the highest incidence of disease • Severe disease outcomes are most common among young children and the elderly • Young children are likely the most important group • A multivalent vaccine that targets several pathogens in a single dose is likely to be a more attractive proposition that the introduction of additional single target vaccine. • Developed world markets are likely to provide the initial economic impetus for private industry to develop vaccines.	• Adding a vaccine to the EPI schedule involves great efforts to prove the added value of inclusion, for both economic and health reasons • The economics of a norovirus vaccine that requires multiple doses and/or reformulation will be scrutinized carefully by policy makers. • A vaccine developed specifically for high income populations (especially adults) is unlikely to be the optimal product for children in low income settings.	• The role of these different groups in transmission and the transmission-blocking potential of a vaccine should be better understood. • Economic evaluation of norovirus vaccines, including combination products for developing country settings • Development of a target product profile for a vaccine to be used in the EPI schedule.



7. References

1. *Global Health Data Exchange.* 2013; Available from: http://vizhub.healthdata.org/irank/arrow.php.
2. GBD Causes of Death Mortality Collaborators, *Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013.* Lancet, 2015. **385**(9963): p. 117-71.
3. Liu, L., et al., *Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis.* Lancet, 2015. **385**(9966): p. 430-40.
4. Global Burden of Disease Study Collaborators, *Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013.* Lancet, 2015.
5. Fischer Walker, C.L., et al., *Diarrhea incidence in low- and middle-income countries in 1990 and 2010: a systematic review.* BMC Public Health, 2012. **12**: p. 220.
6. Lozano, R., et al., *Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010.* Lancet, 2012. **380**(9859): p. 2095-128.
7. Ahmed, S.M., et al., *Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis.* Lancet Infect Dis, 2014.
8. Snyder, J.D. and M.H. Merson, *The magnitude of the global problem of acute diarrhoeal disease: a review of active surveillance data.* Bull World Health Organ, 1982. **60**(4): p. 605-13.
9. Bern, C., et al., *The magnitude of the global problem of diarrhoeal disease: a ten-year update.* Bull World Health Organ, 1992. **70**(6): p. 705-14.
10. Parashar, U.D., et al., *Global illness and deaths caused by rotavirus disease in children.* Emerg Infect Dis, 2003. **9**(5): p. 565-72.
11. Parashar, U.D., et al., *Global mortality associated with rotavirus disease among children in 2004.* J Infect Dis, 2009. **200 Suppl 1**: p. S9-S15.
12. Tate, J.E., et al., *2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis.* Lancet Infect Dis, 2012. **12**(2): p. 136-141.
13. Lanata, C.F., et al., *Global causes of diarrheal disease mortality in children <5 years of age: a systematic review.* PLoS One, 2013. **8**(9): p. e72788.
14. Institute of Medicine, *The prospects of immunizing against rotavirus, in New vaccine development: diseases of importance in developing countries.* 1986: Washington.
15. Bank, W., *World Development Report 2003 : Sustainable Development in a Dynamic World--Transforming Institutions, Growth, and Quality of Life.* 2003, World Bank.
16. Hall, A., et al. *Burden of disease: The global burden of norovirus. in American Society of Tropical Medicine and Hygiene: 63rd Annual Meeting.* 2014. New Orleans, LA.
17. Patel, M.M., et al., *Systematic literature review of role of noroviruses in sporadic gastroenteritis.* Emerg Infect Dis., 2008. **14**(8): p. 1224-1231.
18. Lau, C.S., et al., *High rate and changing molecular epidemiology pattern of norovirus infections in sporadic cases and outbreaks of gastroenteritis in Hong Kong.* J.Med.Virol., 2004. **73**(1): p. 113-117.
19. Fischer Walker, C.L., D. Sack, and R.E. Black, *Etiology of diarrhea in older children, adolescents and adults: a systematic review.* PLoS Negl Trop Dis, 2010. **4**(8): p. e768.
20. Scallan, E., et al., *Foodborne illness acquired in the United States--major pathogens.* Emerg Infect Dis, 2011. **17**(1): p. 7-15.
21. Hall, A.J., et al., *Norovirus disease in the United States.* Emerg Infect Dis, 2013. **19**(8): p. 1198-205.
22. Verhoef, L., et al., *The estimated disease burden of norovirus in The Netherlands.* Epidemiol Infect, 2012: p. 1-11.
23. Phillips, G., et al., *Community incidence of norovirus-associated infectious intestinal disease in England: improved estimates using viral load for norovirus diagnosis.* Am J Epidemiol, 2010. **171**(9): p. 1014-22.
24. Tam, C.C., et al., *Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice.* Gut, 2012. **61**(1): p. 69-77.
25. Thomas, M.K., et al., *Estimates of the burden of foodborne illness in Canada for 30 specified pathogens and unspecified agents, circa 2006.* Foodborne Pathog Dis, 2013. **10**(7): p. 639-48.
26. Hall, A.J., et al., *Incidence of acute gastroenteritis and role of norovirus, Georgia, USA, 2004-2005.* Emerg Infect Dis, 2011. **17**(8): p. 1381-8.
27. Karsten, C., et al., *Incidence and risk factors for community-acquired acute gastroenteritis in north-west Germany in 2004.* Eur J Clin Microbiol Infect Dis, 2009. **28**(8): p. 935-43.
28. Saito, M., et al., *Multiple norovirus infections in a birth cohort in a Peruvian Periurban community.* Clin Infect Dis, 2014. **58**(4): p. 483-91.
29. Shioda, K., et al., *Global Age Distribution of Pediatric Norovirus Cases* Vaccine, 2015. **In press.**
30. Liu, X., et al., *Seroepidemiology of Norovirus GII.3 and GII.4 Infections in Children with Diarrhea in Xi'an, China.* Foodborne Pathog Dis, 2015.
31. Carmona-Vicente, N., et al., *Norovirus infections and seroprevalence of genotype GII.4-specific antibodies in a Spanish population.* J Med Virol, 2015. **87**(4): p. 675-82.
32. Son, H., et al., *Seroepidemiology of predominant norovirus strains circulating in Korea by using recombinant virus-like particle antigens.* Foodborne Pathog Dis, 2013. **10**(5): p. 461-6.
33. Menon, V.K., et al., *Comparison of age-stratified seroprevalence of antibodies against norovirus GII in India and the United Kingdom.* PLoS One, 2013. **8**(2): p. e56239.
34. Nurminen, K., et al., *Prevalence of norovirus GII-4 antibodies in Finnish children.* J Med Virol, 2011. **83**(3): p. 525-31.
35. Kobayashi, S., et al., *Seroepidemiological study of norovirus infection in Aichi Prefecture, Japan.* Microbiol Immunol, 2009. **53**(6): p. 356-9.
36. O'Ryan, M.L., et al., *Symptomatic and asymptomatic rotavirus and norovirus infections during infancy in a Chilean birth cohort.* Pediatr Infect Dis J, 2009. **28**(10): p. 879-84.
37. Lopman, B.A., et al., *Norovirus Infection and Disease in an Ecuadorian Birth Cohort: Association of Certain Norovirus Genotypes With Host FUT2 Secretor Status.* J Infect Dis, 2014.
38. Becker-Dreps, S., et al., *Etiology of childhood diarrhea after rotavirus vaccine introduction: a prospective, population-based study in Nicaragua.* Pediatr Infect Dis J, 2014. **33**(11): p. 1156-63.
39. Schmidt, P.J., *Norovirus Dose-Response: Are Currently Available Data Informative Enough to Determine How Susceptible Humans Are to Infection from a Single Virus?* Risk Anal, 2014.
40. Kirby, A.E., P.F. Teunis, and C.L. Moe, *Two human challenge studies confirm high infectivity of Norwalk virus.* J Infect Dis, 2015. **211**(1): p. 166-7.
41. Atmar, R.L., et al., *Reply to Kirby et al.* J Infect Dis, 2015. **211**(1): p. 167.
42. Atmar, R.L., et al., *Determination of the 50% human infectious dose for Norwalk virus.* J Infect Dis, 2014. **209**(7): p. 1016-22.
43. Teunis, P.F., et al., *Norwalk virus: how infectious is it?* J Med Virol, 2008. **80**(8): p. 1468-76.
44. de Wit, M.A., et al., *Sensor, a population-based cohort study on gastroenteritis in the Netherlands: incidence and etiology.* Am.J.Epidemiol., 2001. **154**(7): p. 666-674.
45. Fretz, R., et al., *Risk factors for infections with Norovirus gastrointestinal illness in Switzerland.* Eur.J.Clin.Microbiol.Infect.Dis., 2005. **24**(4): p. 256-261.
46. Phillips, G., et al., *Risk factors for symptomatic and asymptomatic norovirus infection in the community.* Epidemiol Infect, 2010: p. 1-11.



47. Gotz, H., et al., *Clinical spectrum and transmission characteristics of infection with Norwalk-like virus: findings from a large community outbreak in Sweden.* Clin.Infect.Dis., 2001. **33**(5): p. 622-628.

48. Chapin, A.R., et al., *Prevalence of norovirus among visitors from the United States to Mexico and Guatemala who experience traveler's diarrhea.* J.Clin. Microbiol., 2005. **43**(3): p. 1112-1117.

49. Lopman, B., et al., *Epidemiologic implications of asymptomatic reinfection: a mathematical modeling study of norovirus.* Am J Epidemiol, 2014. **179**(4): p. 507-12.

50. Kambhampati, A., M. Koopmans, and B.A. Lopman, *Burden of norovirus in healthcare facilities and strategies for outbreak control.* J Hosp Infect, 2015. **89**(4): p. 296-301.

51. Porter, C.K., et al., *Postinfectious gastrointestinal disorders following norovirus outbreaks.* Clin Infect Dis, 2012. **55**(7): p. 915-22.

52. Mearin, F., *Editorial: From the acute infection to the chronic disorder "Don't worry it's just a viral gastroenteritis".* Am J Gastroenterol, 2012. **107**(6): p. 900-1.

53. Zanini, B., et al., *Incidence of post-infectious irritable bowel syndrome and functional intestinal disorders following a water-borne viral gastroenteritis outbreak.* Am J Gastroenterol, 2012. **107**(6): p. 891-9.

54. Spiller, R. and K. Garsed, *Postinfectious irritable bowel syndrome.* Gastroenterology, 2009. **136**(6): p. 1979-88.

55. Mearin, F., et al., *Dyspepsia and irritable bowel syndrome after a Salmonella gastroenteritis outbreak: one-year follow-up cohort study.* Gastroenterology, 2005. **129**(1): p. 98-104.

56. Porter, C.K., et al., *The Incidence and Gastrointestinal Infectious Risk of Functional Gastrointestinal Disorders in a Healthy US Adult Population.* Am J Gastroenterol.

57. Marshall, J.K., et al., *Postinfectious irritable bowel syndrome after a food-borne outbreak of acute gastroenteritis attributed to a viral pathogen.* Clin Gastroenterol Hepatol, 2007. **5**(4): p. 457-60.

58. Kindt, S., et al., *Intestinal immune activation in presumed post-infectious functional dyspepsia.* Neurogastroenterol Motil, 2009. **21**(8): p. 832-e56.

59. Tack, J., et al., *Clinical and pathophysiological characteristics of acute-onset functional dyspepsia.* Gastroenterology, 2002. **122**(7): p. 1738-47.

60. Wheeler, J.G., et al., *Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. The Infectious Intestinal Disease Study Executive.* BMJ, 1999. **318**(7190): p. 1046-1050.

61. de Wit, M.A., et al., *Sensor, a population-based cohort study on gastroenteritis in the Netherlands: incidence and etiology.* Am J Epidemiol, 2001. **154**(7): p. 666-74.

62. Agocs, M.M., et al., *WHO global rotavirus surveillance network: a strategic review of the first 5 years, 2008-2012.* MMWR Morb Mortal Wkly Rep, 2014. **63**(29): p. 634-7.

63. Kotloff, K.L., et al., *Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study.* Lancet, 2013. **382**(9888): p. 209-222.

64. Atmar, R.L., et al., *Norwalk virus shedding after experimental human infection.* Emerg Infect Dis, 2008. **14**(10): p. 1553-7.

65. Tam, C.C., et al., *Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice.* Gut, 2011. **61**(1): p. 69-77.

66. Clarke, I.N., et al., *Caliciviridae, in Virus taxonomy: the classification and nomenclature of viruses. The ninth report of the International Committee on Taxonomy of Viruses,* A. King, et al., Editors. 2012, Springer-Verlag: Walham, MA, USA. p. 977-986.

67. Zheng, D.P., et al., *Norovirus classification and proposed strain nomenclature.* Virology, 2006. **346**(2): p. 312-323.

68. Vinje, J., *Advances in Laboratory Methods for Detection and Typing of Norovirus.* J Clin Microbiol, 2015. **53**(2): p. 373-381.

69. Lopman, B.A., et al., *Clinical manifestation of norovirus gastroenteritis in health care settings.* Clin Infect Dis, 2004. **39**(3): p. 318-24.

70. Hasing, M.E., et al., *Emergence of a new norovirus GII.4 variant and changes in the historical biennial pattern of norovirus outbreak activity in Alberta, Canada, from 2008 to 2013.* J Clin Microbiol, 2013. **51**(7): p. 2204-11.

71. Fonager, J., L.S. Hindbaek, and T.K. Fischer, *Rapid emergence and antigenic diversification of the norovirus 2012 Sydney variant in Denmark, October to December, 2012.* Euro Surveill, 2013. **18**(9).

72. Tan, M., et al., *Mutations within the P2 domain of norovirus capsid affect binding to human histo-blood group antigens: evidence for a binding pocket.* J Virol, 2003. **77**(23): p. 12562-71.

73. Chen, R., et al., *Inter- and intragenus structural variations in caliciviruses and their functional implications.* J Virol, 2004. **78**(12): p. 6469-79.

74. Lindesmith, L.C., et al., *Mechanisms of GII.4 norovirus persistence in human populations.* PLoS Med, 2008. **5**(2): p. e31.

75. Boon, D., et al., *Comparative evolution of GII.3 and GII.4 norovirus over a 31-year period.* J Virol, 2011. **85**(17): p. 8656-66.

76. Bull, R.A., et al., *Rapid evolution of pandemic noroviruses of the GII.4 lineage.* PLoS Pathog, 2010. **6**(3): p. e1000831.

77. Bull, R.A. and P.A. White, *Mechanisms of GII.4 norovirus evolution.* Trends Microbiol, 2011. **19**(5): p. 233-40.

78. Noel, J.S., et al., *Identification of a distinct common strain of "Norwalk-like viruses" having a global distribution.* J Infect Dis, 1999. **179**(6): p. 1334-44.

79. Lopman, B., et al., *Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant.* Lancet, 2004. **363**(9410): p. 682-688.

80. Vinje, J., S.A. Altena, and M.P. Koopmans, *The incidence and genetic variability of small round-structured viruses in outbreaks of gastroenteritis in The Netherlands.* J Infect Dis, 1997. **176**(5): p. 1374-8.

81. Yen, C., et al., *Impact of an emergent norovirus variant in 2009 on norovirus outbreak activity in the United States.* Clin Infect Dis, 2011. **53**(6): p. 568-71.

82. Leshem, E., et al., *Effects and clinical significance of GII.4 Sydney norovirus, United States, 2012-2013.* Emerg Infect Dis, 2013. **19**(8): p. 1231-8.

83. Desai, R., et al., *Severe outcomes are associated with genogroup 2 genotype 4 norovirus outbreaks: a systematic literature review.* Clin Infect Dis, 2012. **55**(2): p. 189-93.

84. Payne, D.C., et al., *Norovirus and Medically Attended Gastroenteritis in U.S. Children.* New England Journal of Medicine, 2013. **368**(12): p. 1121-1130.

85. Hoa Tran, T.N., et al., *Molecular epidemiology of noroviruses associated with acute sporadic gastroenteritis in children: global distribution of genogroups, genotypes and GII.4 variants.* J Clin Virol, 2013. **56**(3): p. 185-93.

86. Mahar, J.E., et al., *Identification and characterization of antibody-binding epitopes on the norovirus GII.3 capsid.* J Virol, 2014. **88**(4): p. 1942-52.

87. Rackoff, L.A., et al., *Epidemiology and evolution of rotaviruses and noroviruses from an archival WHO Global Study in Children (1976-79) with implications for vaccine design.* PLoS One, 2013. **8**(3): p. e59394.

88. Mahar, J.E., et al., *The importance of intergenic recombination in norovirus GII.3 evolution.* J Virol, 2013. **87**(7): p. 3687-98.

89. Jiang, X., et al., *Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein.* J Virol, 1992. **66**(11): p. 6527-32.

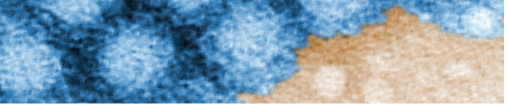
90. Lambden, P.R., et al., *Sequence and genome organization of a human small round-structured (Norwalk-like) virus.* Science, 1993. **259**(5094): p. 516-519.

91. Xi, J.N., et al., *Norwalk virus genome cloning and characterization.* Science, 1990. **250**(4987): p. 1580-1583.

92. Jiang, X., et al., *Detection of Norwalk virus in stool by polymerase chain reaction.* J.Clin.Microbiol., 1992. **30**(10): p. 2529-2534.

93. De Leon, R., et al., *Detection of Norwalk virus in stool specimens by reverse transcriptase-polymerase chain reaction and nonradioactive oligoprobes.* J.Clin.Microbiol., 1992. **30**(12): p. 3151-3157.

94. Green, J., et al., *Norwalk-like viruses: demonstration of genomic diversity by polymerase chain reaction.* J.Clin.Microbiol., 1993. **31**(11): p. 3007-3012.



95. Bruggink, L.D., M.G. Catton, and J.A. Marshall, *Evaluation of the Bioline Standard Diagnostics SD immunochromatographic norovirus detection kit using fecal specimens from Australian gastroenteritis incidents.* Diagn Microbiol Infect Dis, 2013. **76**(2): p. 147-52.

96. Gray, J.J., et al., *European multicenter evaluation of commercial enzyme immunoassays for detecting norovirus antigen in fecal samples.* Clin Vaccine Immunol, 2007. **14**(10): p. 1349-55.

97. Kirby, A., et al., *An evaluation of the RIDASCREEN and IDEIA enzyme immunoassays and the RIDAQUICK immunochromatographic test for the detection of norovirus in faecal specimens.* J Clin Virol, 2010. **49**(4): p. 254-7.

98. Morillo, S.G., et al., *Norovirus 3rd Generation kit: an improvement for rapid diagnosis of sporadic gastroenteritis cases and valuable for outbreak detection.* J Virol Methods, 2011. **173**(1): p. 13-6.

99. Derrington, P., et al., *Norovirus Ridaquick: a new test for rapid diagnosis of norovirus.* Pathology, 2009. **41**(7): p. 687-8.

100. Bruins, M.J., et al., *Evaluation of a rapid immunochromatographic test for the detection of norovirus in stool samples.* Eur J Clin Microbiol Infect Dis, 2010. **29**(6): p. 741-3.

101. Donaldson, E.F., et al., *Viral shape-shifting: norovirus evasion of the human immune system.* Nat Rev Microbiol, 2010. **8**(3): p. 231-41.

102. Levine, M.M. and R.M. Robins-Browne, *Factors that explain excretion of enteric pathogens by persons without diarrhea.* Clin Infect Dis, 2012. **55 Suppl 4**: p. S303-11.

103. Lindesmith, L., et al., *Human susceptibility and resistance to Norwalk virus infection.* Nat.Med., 2003. **9**(5): p. 548-553.

104. Graham, D.Y., et al., *Norwalk virus infection of volunteers: new insights based on improved assays.* J.Infect.Dis., 1994. **170**(1): p. 34-43.

105. Atmar, R.L., et al., *Norovirus vaccine against experimental human Norwalk Virus illness.* N Engl J Med, 2011. **365**(23): p. 2178-87.

106. Rockx, B., et al., *Natural history of human calicivirus infection: a prospective cohort study.* Clin. Infect.Dis., 2002. **35**(3): p. 246-253.

107. Valentiner-Branth, P., et al., *Cohort study of Guinean children: incidence, pathogenicity, conferred protection, and attributable risk for enteropathogens during the first 2 years of life.* J Clin Microbiol, 2003. **41**(9): p. 4238-45.

108. Blackwelder, W.C., et al., *Statistical methods in the Global Enteric Multicenter Study (GEMS).* Clin Infect Dis, 2012. **55 Suppl 4**: p. S246-53.

109. Sethi, D., et al., *A study of infectious intestinal disease in England: plan and methods of data collection.* Commun Dis Public Health, 1999. **2**(2): p. 101-107.

110. Bruzzi, P., et al., *Estimating the population attributable risk for multiple risk factors using case-control data.* Am.J.Epidemiol., 1985. **122**(5): p. 904-914.

111. Payne, D.C., et al., *Norovirus and medically attended gastroenteritis in U.S. children.* N Engl J Med, 2013. **368**(12): p. 1121-30.

112. Phillips, G., et al., *Diagnosing norovirus-associated infectious intestinal disease using viral load.* BMC Infect Dis, 2009. **9**: p. 63.

113. Teunis, P.F., et al., *Campylobacter seroconversion rates in selected countries in the European Union.* Epidemiol Infect, 2012. **141**(10): p. 1-7.

114. Swart, A.N., et al., *The protective effects of temporary immunity under imposed infection pressure.* Epidemics, 2012. **4**(1): p. 43-7.

115. Mulholland, E.K., *Use of vaccine trials to estimate burden of disease.* J Health Popul Nutr, 2004. **22**(3): p. 257-67.

116. Feikin, D.R., J.A. Scott, and B.D. Gessner, *Use of vaccines as probes to define disease burden.* Lancet, 2014. **383**(9930): p. 1762-70.

117. Duizer, E., et al., *Laboratory efforts to cultivate noroviruses.* J Gen Virol, 2004. **85**(Pt 1): p. 79-87.

118. Jones, M.K., et al., *Enteric bacteria promote human and mouse norovirus infection of B cells.* Science, 2014. **346**(6210): p. 755-9.

119. Pelosi, E., et al., *The seroepidemiology of genogroup 1 and genogroup 2 Norwalk-like viruses in Italy.* J.Med.Virol., 1999. **58**(1): p. 93-99.

120. Teunis, P., et al., *The dose-response relation in human volunteers for gastro-intestinal pathogens.* 1998, National Institute of Public Health and the Environment (RIVM): Bilthoven.

121. Baron, R.C., et al., *Serological responses among teenagers after natural exposure to Norwalk virus.* J.Infect.Dis., 1984. **150**(4): p. 531-534.

122. Johnson, P.C., et al., *Multiple-challenge study of host susceptibility to Norwalk gastroenteritis in US adults.* J.Infect.Dis., 1990. **161**(1): p. 18-21.

123. Parrino, T.A., et al., *Clinical immunity in acute gastroenteritis caused by Norwalk agent.* N.Engl.J.Med., 1977. **297**(2): p. 86-89.

124. Wyatt, R.G., et al., *Comparison of three agents of acute infectious nonbacterial gastroenteritis by cross-challenge in volunteers.* J Infect Dis, 1974. **129**(6): p. 709-14.

125. Huang, P., et al., *Spike protein VP8* of human rotavirus recognizes histo-blood group antigens in a type-specific manner.* J Virol, 2012. **86**(9): p. 4833-43.

126. Marionneau, S., et al., *Norwalk virus binds to histo-blood group antigens present on gastroduodenal epithelial cells of secretor individuals.* Gastroenterology, 2002. **122**(7): p. 1967-77.

127. Ferrer-Admetlla, A., et al., *A natural history of FUT2 polymorphism in humans.* Mol Biol Evol, 2009. **26**(9): p. 1993-2003.

128. Henry, S., R. Oriol, and B. Samuelsson, *Lewis Histo-Blood Group System and Associated Secretory Phenotypes.* Vox Sanguinis, 1995. **69**(3): p. 166-182.

129. Vestergaard, E.M., et al., *Reference values and biological variation for tumor marker CA 19-9 in serum for different Lewis and secretor genotypes and evaluation of secretor and Lewis genotyping in a Caucasian population.* Clin Chem, 1999. **45**(1): p. 54-61.

130. Nordgren, J., et al., *Host genetic factors affect susceptibility to norovirus infections in Burkina Faso.* PLoS One, 2013. **8**(7): p. e69557.

131. Hutson, A.M., et al., *Norwalk virus infection associates with secretor status genotyped from sera.* J Med Virol, 2005. **77**(1): p. 116-20.

132. Larsson, M.M., et al., *Antibody prevalence and titer to norovirus (genogroup II) correlate with secretor (FUT2) but not with ABO phenotype or Lewis (FUT3) genotype.* J Infect Dis, 2006. **194**(10): p. 1422-7.

133. Lindesmith, L., et al., *Cellular and humoral immunity following Snow Mountain virus challenge.* J.Virol., 2005. **79**(5): p. 2900-2909.

134. Hutson, A.M., et al., *Norwalk virus-like particle hemagglutination by binding to h histo-blood group antigens.* J Virol, 2003. **77**(1): p. 405-15.

135. Huang, P., et al., *Norovirus and histo-blood group antigens: demonstration of a wide spectrum of strain specificities and classification of two major binding groups among multiple binding patterns.* J.Virol., 2005. **79**(11): p. 6714-6722.

136. Shirato, H., et al., *Noroviruses distinguish between type 1 and type 2 histo-blood group antigens for binding.* J Virol, 2008. **82**(21): p. 10756-67.

137. de Rougemont, A., et al., *Qualitative and quantitative analysis of the binding of GII.4 norovirus variants onto human blood group antigens.* J Virol, 2011. **85**(9): p. 4057-70.

138. Siebenga, J.J., et al., *Epochal evolution of GGII.4 norovirus capsid proteins from 1995 to 2006.* J.Virol., 2007. **81**(18): p. 9932-9941.

139. Lindesmith, L.C., et al., *Immunogenetic mechanisms driving norovirus GII.4 antigenic variation.* PLoS Pathog, 2012. **8**(5): p. e1002705.

140. Debbink, K., et al., *Norovirus immunity and the great escape.* PLoS Pathog, 2012. **8**(10): p. e1002921.

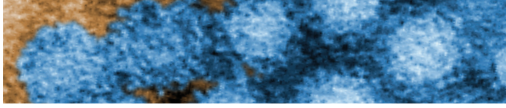
141. Lindesmith, L.C., et al., *Broad blockade antibody responses in human volunteers after immunization with a multivalent norovirus VLP candidate vaccine: immunological analyses from a phase I clinical trial.* PLoS Med, 2015. **12**(3): p. e1001807.

142. LoBue, A.D., et al., *Multivalent norovirus vaccines induce strong mucosal and systemic blocking antibodies against multiple strains.* Vaccine, 2006. **24**(24): p. 5220-5234.

143. Czako, R., et al., *Experimental human infection with Norwalk virus elicits a surrogate neutralizing antibody response with cross-genogroup activity.* Clin Vaccine Immunol, 2015. **22**(2): p. 221-8.

144. Reeck, A., et al., *Serological Correlate of Protection against Norovirus-Induced Gastroenteritis.* J Infect Dis. **202**(8): p. 1212-8.

145. Ramani, S., et al., *Mucosal and Cellular Immune Responses to Norwalk Virus.* J Infect Dis, 2015.



146. Karst, S.M., et al., *STAT1-dependent innate immunity to a Norwalk-like virus.* Science, 2003. **299**(5612): p. 1575-1578.

147. Wobus, C.E., et al., *Replication of Norovirus in cell culture reveals a tropism for dendritic cells and macrophages.* PLoS.Biol., 2004. **2**(12): p. e432.

148. Tacket, C.O., et al., *Humoral, mucosal, and cellular immune responses to oral Norwalk virus-like particles in volunteers.* Clin.Immunol., 2003. **108**(3): p. 241-247.

149. Chang, K.O., et al., *Stable expression of a Norwalk virus RNA replicon in a human hepatoma cell line.* Virology, 2006. **353**(2): p. 463-473.

150. Herbst-Kralovetz, M., H.S. Mason, and Q. Chen, *Norwalk virus-like particles as vaccines.* Expert Rev Vaccines, 2010. **9**(3): p. 299-307.

151. Ball, J.M., et al., *Recombinant Norwalk virus-like particles given orally to volunteers: phase I study.* Gastroenterology, 1999. **117**(1): p. 40-48.

152. Tacket, C.O., et al., *Humoral, mucosal, and cellular immune responses to oral Norwalk virus-like particles in volunteers.* Clin Immunol, 2003. **108**(3): p. 241-7.

153. Ball, J.M., et al., *Oral immunization with recombinant Norwalk virus-like particles induces a systemic and mucosal immune response in mice.* J.Virol., 1998. **72**(2): p. 1345-1353.

154. Guerrero, R.A., et al., *Recombinant Norwalk virus-like particles administered intranasally to mice induce systemic and mucosal (fecal and vaginal) immune responses.* J.Virol., 2001. **75**(20): p. 9713-9722.

155. Lindesmith, L.C., et al., *Heterotypic humoral and cellular immune responses following Norwalk virus infection.* J Virol, 2010. **84**(4): p. 1800-15.

156. Koelle, K., et al., *Epochal evolution shapes the phylodynamics of interpandemic influenza A (H3N2) in humans.* Science, 2006. **314**(5807): p. 1898-903.

157. Liu, X., et al., *Seroepidemiology of Norovirus GII.3 and GII.4 Infections in Children with Diarrhea in Xi'an, China.* Foodborne Pathog Dis, 2015. **12**(6): p. 500-5.

158. Pelosi, E., et al., *The seroepidemiology of genogroup 1 and genogroup 2 Norwalk-like viruses in Italy.* J Med Virol, 1999. **58**(1): p. 93-9.

159. Treanor, J.J., et al., *A novel intramuscular bivalent norovirus virus-like particle vaccine candidate--reactogenicity, safety, and immunogenicity in a phase 1 trial in healthy adults.* J Infect Dis, 2014. **210**(11): p. 1763-71.

160. Bernstein, D.I., et al., *Norovirus vaccine against experimental human GII. 4 virus illness: a challenge study in healthy adults.* J Infect Dis, 2014. **doi:10.1093/infdis/jiu497**

161. Ma, Y., et al., *Heat shock protein 70 enhances mucosal immunity against human norovirus when coexpressed from a vesicular stomatitis virus vector.* J Virol, 2014. **88**(9): p. 5122-37.

162. Mason, H.S., et al., *Expression of Norwalk virus capsid protein in transgenic tobacco and potato and its oral immunogenicity in mice.* Proc Natl Acad Sci U S A, 1996. **93**(11): p. 5335-40.

163. Velasquez, L.S., et al., *Intranasal delivery of Norwalk virus-like particles formulated in an in situ gelling, dry powder vaccine.* Vaccine, 2011. **29**(32): p. 5221-31.

164. Tacket, C.O., et al., *Human immune responses to a novel norwalk virus vaccine delivered in transgenic potatoes.* J.Infect.Dis., 2000. **182**(1): p. 302-305.

165. Santi, L., et al., *An efficient plant viral expression system generating orally immunogenic Norwalk virus-like particles.* Vaccine, 2008. **26**(15): p. 1846-54.

166. Huang, Z., et al., *A DNA replicon system for rapid high-level production of virus-like particles in plants.* Biotechnol Bioeng, 2009. **103**(4): p. 706-14.

167. Kocher, J., et al., *Intranasal P particle vaccine provided partial cross-variant protection against human GII.4 norovirus diarrhea in gnotobiotic pigs.* J Virol, 2014. **88**(17): p. 9728-43.

168. Xia, M., et al., *A candidate dual vaccine against influenza and noroviruses.* Vaccine, 2011. **29**(44): p. 7670-7.

169. Tan, M. and X. Jiang, *Norovirus P particle: a subviral nanoparticle for vaccine development against norovirus, rotavirus and influenza virus.* Nanomedicine (Lond), 2012. **7**(6): p. 889-97.

170. Tamminen, K., et al., *A comparison of immunogenicity of norovirus GII-4 virus-like particles and P-particles.* Immunology, 2012. **135**(1): p. 89-99.

171. Tamminen, K., et al., *Trivalent combination vaccine induces broad heterologous immune responses to norovirus and rotavirus in mice.* PLoS One, 2013. **8**(7): p. e70409.

172. Lappalainen, S., et al., *Immune responses elicited against rotavirus middle layer protein VP6 inhibit viral replication in vitro and in vivo.* Hum Vaccin Immunother, 2014. **10**(7): p. 2039-47.

173. Guerrero, R.A., et al., *Recombinant Norwalk virus-like particles administered intranasally to mice induce systemic and mucosal (fecal and vaginal) immune responses.* J Virol, 2001. **75**(20): p. 9713-22.

174. Parra, G.I., et al., *Immunogenicity and specificity of norovirus Consensus GII.4 virus-like particles in monovalent and bivalent vaccine formulations.* Vaccine, 2012. **30**(24): p. 3580-6.

175. Bernstein, D.I., et al., *Norovirus vaccine against experimental human GII.4 virus illness: a challenge study in healthy adults.* J Infect Dis, 2015. **211**(6): p. 870-8.

176. Sundararajan, A., et al., *Robust mucosal-homing antibody-secreting B cell responses induced by intramuscular administration of adjuvanted bivalent human norovirus-like particle vaccine.* Vaccine, 2015. **33**(4): p. 568-76.

177. Health, U.S.N.I.o. *ClinicalTrials.gov.* 2015; Available from: https://clinicaltrials.gov/ct2/home.

178. Velasquez, L.S., et al., *An intranasally delivered Toll-like receptor 7 agonist elicits robust systemic and mucosal responses to Norwalk virus-like particles.* Clin Vaccine Immunol, 2010. **17**(12): p. 1850-8.

179. Tacket, C.O., et al., *Human immune responses to a novel norwalk virus vaccine delivered in transgenic potatoes.* J Infect Dis, 2000. **182**(1): p. 302-5.

180. Ramani, S., R.L. Atmar, and M.K. Estes, *Epidemiology of human noroviruses and updates on vaccine development.* Curr Opin Gastroenterol, 2014. **30**(1): p. 25-33.

181. El-Kamary, S.S., et al., *Adjuvanted intranasal Norwalk virus-like particle vaccine elicits antibodies and antibody-secreting cells that express homing receptors for mucosal and peripheral lymphoid tissues.* J Infect Dis, 2010. **202**(11): p. 1649-58.

182. Huang, Z., et al., *Virus-like particle expression and assembly in plants: hepatitis B and Norwalk viruses.* Vaccine, 2005. **23**(15): p. 1851-1858.

183. Fang, H., et al., *Norovirus P particle efficiently elicits innate, humoral and cellular immunity.* PLoS One, 2013. **8**(4): p. e63269.

184. Bernstein, D.I., R.L. Ward, and G.M. Schiff, *Preliminary Studies of Rotavirus Challenge in Adult Volunteers.* Clinical Research, 1985. **33**(2): p. A562-A562.

185. Ajami, N.J., et al., *Seroepidemiology of norovirus-associated travelers' diarrhea.* J Travel Med, 2014. **21**(1): p. 6-11.

186. Thornton, S.A., et al., *Gastroenteritis in US Marines during Operation Iraqi Freedom.* Clin Infect Dis, 2005. **40**(4): p. 519-25.

187. Riddle, M.S., et al., *Epidemic infectious gastrointestinal illness aboard U.S. Navy ships deployed to the Middle East during peacetime operations--2000-2001.* BMC Gastroenterol, 2006. **6**: p. 9.

188. Armed Forces Health Surveillance, C., *Gastrointestinal infections, active component, U.S. Armed Forces, 2002-2012.* MSMR, 2013. **20**(10): p. 7-11; discussion 11.

189. Bok, K. and K.Y. Green, *Norovirus gastroenteritis in immunocompromised patients.* N Engl J Med, 2012. **367**(22): p. 2126-32.

190. Sukhrie, F.H., et al., *Nosocomial transmission of norovirus is mainly caused by symptomatic cases.* Clin Infect Dis, 2012. **54**(7): p. 931-7.

191. Hall, A.J., et al., *Vital signs: foodborne norovirus outbreaks - United States, 2009-2012.* MMWR Morb Mortal Wkly Rep, 2014. **63**(22): p. 491-5.

192. Fine, P., K. Eames, and D.L. Heymann, *"Herd immunity": a rough guide.* Clin Infect Dis, 2011. **52**(7): p. 911-6.

193. Simmons, K., et al., *Duration of immunity to norovirus gastroenteritis.* Emerg Infect Dis, 2013. **19**(8): p. 1260-7.

194. Lopman, B.A., et al., *Increasing rates of gastroenteritis hospital discharges in US adults and the contribution of norovirus, 1996-2007.* Clin Infect Dis, 2011. **52**(4): p. 466-74.

195. Batz, M., S. Hoffmann, and J.G. Morris, Jr., *Ranking the risks: The 10 pathogen-food combinations with the greatest burden on public health.* 2011.

196. Bartsch, S.M., et al., *The potential economic value of a human norovirus vaccine for the United States.* Vaccine, 2012. **30**(49): p. 7097-104.

August 2015