# Title

# Norovirus

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# Key words

# Norovirus, epidemiology, susceptibility, diversity

# Key concepts:

# Norovirus is the leading cause of acute gastroenteritis in humans;

# Transmission of norovirus is predominantly person to person, and is greatly amplified via contaminated food, water and the environment

# Virus attachment to glycans are important for infection and cell enty, and potentially drive host restriction.

# New in vitro systems have recently been developed for human norovirus replication: B cell and stem cell derived enteroids.

# Virus diversity and host genetic and acquired factors are important drives of susceptibility to infection

# The host microbiome has been recognized as an important factor for susceptibility to norovirus infection and clearance.

# Abstract

Norovirus infections are the leading cause of acute gastroenteritis globally. Despite the increasing recognition of norovirus infections in various animal species, there is to date no evidence of an animal reservoir for human norovirus, and transmission is primarily person-to-person. Contaminated food water and the environment can act as important amplifiers for infection. Human noroviruses are very diverse, but one genotype GII-4 predominates globally and is responsible for nearly half of all infections, and a higher proportion of the outbreaks. Recently *in vitro* systems for the propagation of human noroviruses have been developed, and there is an expectation that in the coming years these will provide new insights into the molecular biology of human noroviruses, and a better understanding of factors that determine susceptibility to and protection form infection. Host genetic factors, comorbidities in addition to potentially modifiable factors such as the microbiome and the use of certain treatment and drugs are increasingly recognised as important drivers of susceptibility to infection.

# Introduction

Noroviruses are the earliest documented viruses associated with acute gastroenteritis (AGE). These viruses were discovered by Albert Kapikian, who using electron microscopy, identified virus particles in a stool sample from an outbreak AGE in an elementary school in Norwalk, Ohio in 1968 (Kapikian, 2000). The virus was initially named ‘Norwalk virus’ and later became the prototype of the *norovirus* genus, in the *Caliciviridae* family. Noroviruses are often referred to as “winter vomiting disease” or “gastric ﬂu’ and are now recognized as the most common cause of AGE globally (Bartsch, Lopman, Ozawa, Hall, & Lee, 2016).

# Classification

*Norovirus* is one of four genera in the *Caliciviridae* family; All caliciviruses are small non-enveloped viruses with T=3 icosahedral symmetry and possess a positive-sense single stranded RNA genome (+)ssRNA. The norovirus particle is approximately 27-32nm in diameter, with 32 cup-shaped cavities across the virus surface.

The classification of the *Norovirus* genus is based on sequence diversity across the major capsid protein (VP1). There are currently seven recognised genogroups (GI – GVII) that are further subdivided into at least 40 genotypes (Table 1). Amino acid sequence diversity in the VP1 protein between genogroups can be greater than 40%, up to 20% between genotypes, and 3-5% between strains of the same genotype. Human noroviruses are associated with three of these genogroups: GI, GII and GIV, whilst animal noroviruses, include porcine (GII), bovine and ovine (GIII), feline (GIV) and canine (GIV and GVI and GVII) and murine (GV). The majority of human norovirus diarrhoeal cases are associated with GII, and in particular viruses of the genogroup II and the genotype 4 (GII-4), which are responsible for 70-80% of the outbreaks globally, and >50% of the sporadic cases in all ages (Lopman, Steele, Kirkwood, & Parashar, 2016). The GII-4 viruses cause periodic epidemics through a mechanism known as epochal evolution; GII-4 variants emerge from time to time, displacing previously circulating virus strains to become dominant globally due to a lack of herd protection in the population, similar to the phenomenon described fro influenza viruses (Debbink et al., 2013). It has recently been identified that GII-4 noroviruses have a faster rate of evolution compared to other genotypes, and this may in part at least contribute to their global dominance, although their emergence may have been relatively recent (Parra et al., 2017).

# Structure

The norovirus capsid is composed of 90 dimers of VP1, which consists of a shell (S) domain and a protruding (P) domain. The P domain, which is further subdivided into P1 and P2 (Figure 1; keep Fig 2 from previous edition). The P2 subdomain also known as the hypervariable region contains putative epitopes for immune recognition and is also responsible for binding to histo-blood group antigens (HBGAs), thought to function as receptors or co-receptors on susceptible cells (Donaldson, Lindesmith, Lobue, & Baric, 2010). The VP2 molecules associate with the S domain of VP1 in the interior surface of the capsid (Vongpunsawad, Venkataram Prasad, & Estes, 2013).

The norovirus genome is a single strand of positive sense RNA (+ssRNA, Baltimore Class IV), and is approximately 7500nt in length. The RNA is covalently linked to a virus-encoded protein (VPg) at the 5′-end, and is polyadenylated at the 3′-end . The genome is organised into three open reading frames (ORFs) (Figure 2). The 5′-proximal ORF1 encodes a large polyprotein which is post-translationally processed by the virus protease into six non-structural proteins required for virus replication. These proteins include the VPg, protease, and RNA-dependent RNA polymerase. The remaining ORFs, ORF2 and ORF3 encode the major (VP1) and minor (VP2) capsid proteins, respectively. The MNV has an additional alternative fourth ORF. The ORF4 overlaps with ORF2 and is translated primarily from the sub-genomic RNA into the virulence factor 1 (VF1) protein (McFadden et al., 2011). An equivalent VF has not been identified for human noroviruses.

# Norovirus replication and pathogenesis

The virion attaches to the cell surface through interactions between VP1 and a cellular receptor. This attachment is thought to involve *HBGAs*, which are determinants of both the ABO blood group and Lewis blood group systems, and are expressed on the surface of specific cells and in saliva and other bodily secretions. Differences in the binding specificity of norovirus to different HBGAs result in differences in the susceptibility to specific strains of norovirus.

Animal noroviruses also use carbohydrates as attachment factors. Murine norovirus 1 (MNV‑1) binds to terminal sialic acids, but the glycan biding of murine noroviruses differ in a strain-dependent manner (Taube et al., 2009). Bovine noroviruses bind to α‑galactose (Zakhour et al., 2009), and some canine norovirus strains bind to H and A HBGAs (Caddy, Breiman, le Pendu, & Goodfellow, 2014).

The differences in the carbohydrate binding specificities observed between different species are likely to play a role in host restriction.

It is likely that other as yet unrecognised receptor molecules contribute to the attachment and/or entry of noroviruses into host cells. The mechanisms of internalisation and uncoating are not yet understood. Most of the available information derived from observations with MNV infections in macrophages or is extrapolated from feline calicivirus or picornaviruses. The (+)ssRNA is transcribed and translated in the cytosol; The non-structural virus protein VP recruited the host translation factors required for translation. The OFR1 encodes a polyprotein that is post-translationally cleaved by the virus protease, Pro (also known as NS6 or 3C-like), into proteins: p48 (also known as NS1/2 or N-term), NTPase (also known as NS3 or 2C-like), p22 (also known as NS4 or 3A-like), VPg, Pro and RNA-dependent RNA polymerase (RdRp).

The genomic (+)ssRNA is transcribed into negative-sense RNAs that are used as templates for the synthesis of new genomic and sub-genomic (+)ssRNAs. The sub-genomic (+)ssRNAs encompass ORF2 and ORF3, and are used for the synthesis of VP1 and VP2. Two models have been proposed for norovirus sub-genomic RNA synthesis. Both models are VPg dependent; The first involves premature termination during synthesis of the negative-sense transcribed form the genomic RNA, resulting in negative-sense sub- genomic RNA that serves as a template for the production of positive-sense sub-genomic RNA. The second proposes that an RNA secondary structure upstream of ORF2 in the negative-sense genomic RNA acts as a promoter for synthesis of positive-sense sub-genomic RNAs molecules.

# Norovirus cell tropism and *in vitro* systems

The discovery of a norovirus strain that was able to infect STAT1-deficient mice lead to the propagation MNV in macrophages and dendritic cells led for to the fist culture system for norovirus (Bailey, Thackray, & Goodfellow, 2008). This was followed by the establishment of reverse genetic systems which for the first time opened the door to studying virus replication and pathogenesis (van Beek et al., 2016). Attempt to culture human noroviruses in macrophages or other immune derived cells have however been largely unsuccessful, with the notable exception of the first reported reproducible human norovirus cell culture in human B cells (Jones et al., 2015). Immune cell tropism has also been described for human norovirus in mouse and chimpanzee models(Taube et al., 2013), despite the pathology associated with norovirus in humans demonstrating marked perturbations of the intestinal villi epithelial cells (Troeger et al., 2009). Bovine norovirus infection also produces symptoms compatible with those in humans, similar villus atrophy and epithelial cell damage, and a dual tropism by which norovirus could be detected by immunohistochemistry in the epithelial cells and also in the lamina propia of the villi. However, and despite numerous attempts to propagate noroviruses in intestinal cells, in the presence of HBGA receptors and in 3D systems (Papafragkou, Hewitt, Park, Greening, & Vinje, 2014; Takanashi et al., 2014), norovirus culture in epithelial cells has not been successful until very recently. In a recent breakthrough, stem-cell-derived intestinal enteroids have been successfully used as an in *vitro* culture system for human noroviruses (Ettayebi et al., 2016). The enterocytes in enteroid cultures derived from stem cells of the duodenum, jejunum, or ileum are susceptible to human various norovirus strains. In this in vitro system, unlike the B cell derived one, a cytopathic effect can be seen.

# Epidemiology

Norovirus is estimated to cause 18 percent of AGE disease globally (Bouwknegt et al., 2015). Norovirus is responsible for a lower proportion of AGE in countries where there is higher mortality associated with AGE disease (14 percent). This is likely due to the higher proportion of illness caused by pathogens controlled by better sanitation in low mortality countries rather than a lower burden of norovirus (Bouwknegt et al., 2015).

In the UK the incidence of norovirus is estimated at 47/1000 person years which translates to an estimated 3 million cases of norovirus annually (IID2), this estimate reflects the likely burden of disease, or symptomatic cases. A recent analysis suggests the burden of infection for norovirus is 59/1000 person years, and nearer 4 million cases, when you allow for those who are likely to be infected but remain asymptomatic (Harris, Iturriza-Gomara, & O'Brien, 2017). Higher rates are reported from another prospective study in the Netherlands (Bernard, Hohne, Niendorf, Altmann, & Stark, 2014) which suggested norovirus is responsible for 11 percent of AGE disease. In the USA estimates of around 21 million cases occur a year with a significant attributable impact attributable to norovirus alone (9,900 DALYS) (Scallan, Hoekstra, Mahon, Jones, & Griffin, 2015). A review of the prevalence of norovirus in studies in Africa suggests around 11 percent of GI is due to norovirus (Kabue, Meader, Hunter, & Potgieter, 2016).

Norovirus infects people of all ages, however, in the prospective studies in the UK and The Netherlands the highest rates of norovirus infection were found in those aged less than five years (O'Brien, Donaldson, Iturriza-Gomara, & Tam, 2016).

Generally norovirus AGE is a mild and self-limiting, with an infection lasting around one or two days; recovery is normally rapid and no treatment is required. However, some people are more susceptible and more severe outcomes can occur, particularly in those with underlying serious illness. A large study of outbreaks in hospitals and care homes suggested hospitalised patients were ill for longer than those in care homes or staff working in the hospital (Lopman et al., 2005). Furthermore, norovirus infection has been associated with mortality, particularly in elderly patients (Harris, Edmunds, Pebody, Brown, & Lopman, 2008). Norovirus can result in hospitalisation, with a study in the UK estimating 0.1 percent of emergency admissions in adults were associated with norovirus infection (Haustein, Harris, Pebody, & Lopman, 2009) and in the U.S. 0.03 percent hospitalisations due to foodborne illness were associated with norovirus infections (Scallan et al., 2011). Norovirus infection can also result in in severe complications and chronic infections in severely immunosuppressed or immunodeficient individuals (Brown, Clark, Brown, Breuer, & Lowe, 2017).

Understanding the epidemiology of norovirus is challenging partly because the majority of people affected by the pathogen do not contact health care services. Severity of symptoms has been shown to affect the likelihood of seeking medical attention (Blazevic, Malm, & Vesikari, 2015). Norovirus infections have a rapid onset and recovery therefore the majority of people will be well before they can seek medical help. Furthermore, the public health advice is for people affected with a sudden onset of diarrhoea and or vomiting is to remain at home, because visiting hospitals or a family doctor risks spreading the infection to other individuals some of whom might be at higher risk of complications.

Perhaps the greatest burden for norovirus is in health care settings. Outbreaks in hospitals and care homes are common, and are reported from all around the world. These outbreaks affect patients and staff and can cause considerable disruption. In the UK it has been estimated that norovirus outbreaks cost the NHS around £115 million, in lost bed days, staff illness and cancelled operations (Lopman et al., 2005). More recently a study looking at the both direct and indirect costs to patients and the health service suggested norovirus causes a greater economic burden than Campylobacter and rotavirus combined at £83 million (Tam & O'Brien, 2016). Most norovirus illness occurs in the winter months. A review of outbreaks from the outbreak reporting scheme at Public Health England (PHE) showed almost 4000 outbreaks had been reported in a three year period 2009-2011. These outbreak affected around 13000 patients and 3400 staff becoming ill annually and leading to almost 15000 bed-days lost each year (Harris, 2016).

# Modes of transmission.

Humans are the reservoir for human noroviruses, the infection is not spread via animals. Other AGE pathogens such as Salmonella or Campylobacter can be considered zoonotic foodborne infections because the reservoir for these pathogens are animals. Similarly for rotavirus, interspecies transmission has been frequently documented, and is believed contributes to the emergence of new strains. Despite many years of hypothesising that norovirus is a potentially zoonotic infection, possibly facilitated by recombination of human and animal noroviruses, or by intermediates such as oysters that may specifically bind and concentrate human and animal noroviruses a large scoping review of evidence found no studies reporting zoonotic transmission of norovirus (Wilhelm et al., 2015). The predominant method of transmission for norovirus is person to person through the faeco-oral route. Many outbreaks occur in settings where people are in close contact with each other such as Hospitals (Harris, Lopman, Cooper, & O'Brien, 2013), care homes (Petrignani, van Beek, Borsboom, Richardus, & Koopmans, 2015) , and cruise ships. Outbreaks in these settings are often associated with vomiting events. This is largely because the infectious dose is so low, estimated at around 10-100 virions, although it may vary according to strain. Environmental contamination can give rise to further cases (Cheesbrough, Green, Gallimore, Wright, & Brown, 2000). Contamination of food also leads to the transmission of norovirus. In the US it is estimated that 26 percent of indigenous norovirus infections are caused by the consumption of food. Food can be contaminated at source or by a food handler contaminating food during preparation. Either route can lead to a large number of people being infected. A large outbreak occurred in Germany affecting 11000 people in which the consumption of contaminated strawberries was implicated (Bernard et al., 2014). One outbreak associated with a single food handler is estimated to have affected 3000 people (Kuritsky et al., 1984). Oysters are a common food implicated in norovirus outbreaks (Centers for Disease & Prevention, 1993). Other foods that can be contaminated during irrigation have also been implicated such as salad vegetables (Gandhi, Mandrell, & Tian, 2010; Showell, Sundkvist, Reacher, & Gray, 2007).

# Susceptibility to infection

Host blood group and expression of HBGA antigen types appear confer resistance to infection with particular norovirus types to infection. This suggests that there is a genetic resistance to infection and together with strains diversity could have important implications for vaccine development (Atmar et al., 2015).

In recent years increasing evidence suggests that infection by enteric viruses, including noroviruses, is influenced by the commensal microbiota (Kuss et al., 2011). For norovirus culture in B cells in vitro, a strain of *Enterobacter* directly enhances infection with GII-4, and this effect is mediated, at least in part, by increasing cell attachment via bacterially expressed HBGA-like molecules (Jones et al., 2014; Karst, 2015). Bacterial species that HBGA-like structures can also increase virus stability and protect virions from heat stress enhancing or facilitating transmission (Li, Breiman, le Pendu, & Uyttendaele, 2015). *Lactobacillus,* may play a protective role against norovirus infections: Consumption of probiotic fermented milk was correlated with a reduction in some symptoms associated with norovirus among the elderly (Nagata et al., 2011), modulation of the microbiota via administration of retinoic acid (Vitamin A) inhibited MNV infection, correlating with an increase in the in the abundance of *Lactobacillus* (Lee & Ko, 2016).

Susceptibility to norovirus infection and disease may also be altered by other factors and treatments associated with comorbidities, an in particular those associated with age. Recent studies have suggested that there is an increased susceptibility to infection among people who are taking statins (Rondy et al., 2011). Furthermore, studies in gnotobiotic pigs showed increased human norovirus replication and increased infectivity in pigs treated with statins.

# Summary

Noroviruses represent a diverse and important group of human and animal pathogens. The study of noroviruses has until recently lagged behind that of other positive- stranded RNA viruses however, progress in the ﬁeld has been rapid and new promising in vitro systems are likely to drive new discoveries and understanding of the biology of these important pathogens. There is an increasing understanding of the role of genetic as well as acquired host factors on susceptibility to norovirus infection and disease, and this important area of research that is likely to lead to the design of targeted treatments and preventative interventions.

Table 1: Norovirus classification and host range according to the classification.

|  |  |  |
| --- | --- | --- |
| Genogroup | Host Range | Genotypes |
| GI | Human | GI-1 - GI-9 |
| GII | Human and Porcine | GII-1 - GII-22 |
| GIII | Bovine | GIII1 - GIII-3 |
| GIV | Human, Canine and Feline | GV-1 - GV-2 |
| GV | Murine | GV-1 - GV-2 |
| GVI | Canine and Feline | GVI-1 - GVI-2 |
| GVII | Canine | GVII-1 |
| Unclassified | Bat |  |
|  | Sea Lion |  |
|  | Harbour Porpoise |  |

References:

1. Atmar, R. L., Bernstein, D. I., Lyon, G. M., Treanor, J. J., Al-Ibrahim, M. S., Graham, D. Y., . . . Mendelman, P. M. (2015). Serological Correlates of Protection against a GII.4 Norovirus. *Clin Vaccine Immunol, 22*(8), 923-929. doi:10.1128/CVI.00196-15
2. Bailey, D., Thackray, L. B., & Goodfellow, I. G. (2008). A single amino acid substitution in the murine norovirus capsid protein is sufficient for attenuation in vivo. *J Virol, 82*(15), 7725-7728. doi:10.1128/JVI.00237-08
3. Bartsch, S. M., Lopman, B. A., Ozawa, S., Hall, A. J., & Lee, B. Y. (2016). Global Economic Burden of Norovirus Gastroenteritis. *PLoS One, 11*(4), e0151219. doi:10.1371/journal.pone.0151219
4. Bernard, H., Hohne, M., Niendorf, S., Altmann, D., & Stark, K. (2014). Epidemiology of norovirus gastroenteritis in Germany 2001-2009: eight seasons of routine surveillance. *Epidemiol Infect, 142*(1), 63-74. doi:10.1017/S0950268813000435
5. Blazevic, V., Malm, M., & Vesikari, T. (2015). Induction of homologous and cross-reactive GII.4-specific blocking antibodies in children after GII.4 New Orleans norovirus infection. *J Med Virol, 87*(10), 1656-1661. doi:10.1002/jmv.24237
6. Bouwknegt, M., Verhaelen, K., Rzezutka, A., Kozyra, I., Maunula, L., von Bonsdorff, C. H., . . . de Roda Husman, A. M. (2015). Quantitative farm-to-fork risk assessment model for norovirus and hepatitis A virus in European leafy green vegetable and berry fruit supply chains. *Int J Food Microbiol, 198*, 50-58. doi:10.1016/j.ijfoodmicro.2014.12.013
7. Brown, L. K., Clark, I., Brown, J. R., Breuer, J., & Lowe, D. M. (2017). Norovirus infection in primary immune deficiency. *Rev Med Virol*. doi:10.1002/rmv.1926
8. Caddy, S., Breiman, A., le Pendu, J., & Goodfellow, I. (2014). Genogroup IV and VI canine noroviruses interact with histo-blood group antigens. *J Virol, 88*(18), 10377-10391. doi:10.1128/JVI.01008-14
9. Centers for Disease, C., & Prevention. (1993). Multistate outbreak of viral gastroenteritis related to consumption of oysters--Louisiana, Maryland, Mississippi and North Carolina, 1993. *MMWR Morb Mortal Wkly Rep, 42*(49), 945-948.
10. Cheesbrough, J. S., Green, J., Gallimore, C. I., Wright, P. A., & Brown, D. W. (2000). Widespread environmental contamination with Norwalk-like viruses (NLV) detected in a prolonged hotel outbreak of gastroenteritis. *Epidemiol Infect, 125*(1), 93-98.
11. Debbink, K., Lindesmith, L. C., Donaldson, E. F., Costantini, V., Beltramello, M., Corti, D., . . . Baric, R. S. (2013). Emergence of new pandemic GII.4 Sydney norovirus strain correlates with escape from herd immunity. *J Infect Dis, 208*(11), 1877-1887. doi:10.1093/infdis/jit370
12. Donaldson, E. F., Lindesmith, L. C., Lobue, A. D., & Baric, R. S. (2010). Viral shape-shifting: norovirus evasion of the human immune system. *Nat Rev Microbiol, 8*(3), 231-241. doi:10.1038/nrmicro2296
13. Ettayebi, K., Crawford, S. E., Murakami, K., Broughman, J. R., Karandikar, U., Tenge, V. R., . . . Estes, M. K. (2016). Replication of human noroviruses in stem cell-derived human enteroids. *Science, 353*(6306), 1387-1393. doi:10.1126/science.aaf5211
14. Gandhi, K. M., Mandrell, R. E., & Tian, P. (2010). Binding of virus-like particles of Norwalk virus to romaine lettuce veins. *Appl Environ Microbiol, 76*(24), 7997-8003. doi:10.1128/AEM.01566-10
15. Harris, J. P. (2016). Norovirus Surveillance: An Epidemiological Perspective. *J Infect Dis, 213 Suppl 1*, S8-S11. doi:10.1093/infdis/jiv452
16. Harris, J. P., Edmunds, W. J., Pebody, R., Brown, D. W., & Lopman, B. A. (2008). Deaths from norovirus among the elderly, England and Wales. *Emerg Infect Dis, 14*(10), 1546-1552. doi:10.3201/eid1410.080188
17. Harris, J. P., Iturriza-Gomara, M., & O'Brien, S. J. (2017). Re-assessing the total burden of norovirus circulating in the United Kingdom population. *Vaccine, 35*(6), 853-855. doi:10.1016/j.vaccine.2017.01.009
18. Harris, J. P., Lopman, B. A., Cooper, B. S., & O'Brien, S. J. (2013). Does spatial proximity drive norovirus transmission during outbreaks in hospitals? *BMJ Open, 3*(7). doi:10.1136/bmjopen-2013-003060
19. Haustein, T., Harris, J. P., Pebody, R., & Lopman, B. A. (2009). Hospital admissions due to norovirus in adult and elderly patients in England. *Clin Infect Dis, 49*(12), 1890-1892. doi:10.1086/648440
20. Jones, M. K., Grau, K. R., Costantini, V., Kolawole, A. O., de Graaf, M., Freiden, P., . . . Karst, S. M. (2015). Human norovirus culture in B cells. *Nat Protoc, 10*(12), 1939-1947. doi:10.1038/nprot.2015.121
21. Jones, M. K., Watanabe, M., Zhu, S., Graves, C. L., Keyes, L. R., Grau, K. R., . . . Karst, S. M. (2014). Enteric bacteria promote human and mouse norovirus infection of B cells. *Science, 346*(6210), 755-759. doi:10.1126/science.1257147
22. Kabue, J. P., Meader, E., Hunter, P. R., & Potgieter, N. (2016). Norovirus prevalence and estimated viral load in symptomatic and asymptomatic children from rural communities of Vhembe district, South Africa. *J Clin Virol, 84*, 12-18. doi:10.1016/j.jcv.2016.09.005
23. Kapikian, A. Z. (2000). The discovery of the 27-nm Norwalk virus: an historic perspective. *J Infect Dis, 181 Suppl 2*, S295-302. doi:10.1086/315584
24. Karst, S. M. (2015). Identification of a novel cellular target and a co-factor for norovirus infection - B cells & commensal bacteria. *Gut Microbes, 6*(4), 266-271. doi:10.1080/19490976.2015.1052211
25. Kuritsky, J. N., Osterholm, M. T., Greenberg, H. B., Korlath, J. A., Godes, J. R., Hedberg, C. W., . . . White, K. E. (1984). Norwalk gastroenteritis: a community outbreak associated with bakery product consumption. *Ann Intern Med, 100*(4), 519-521.
26. Kuss, S. K., Best, G. T., Etheredge, C. A., Pruijssers, A. J., Frierson, J. M., Hooper, L. V., . . . Pfeiffer, J. K. (2011). Intestinal microbiota promote enteric virus replication and systemic pathogenesis. *Science, 334*(6053), 249-252. doi:10.1126/science.1211057
27. Lee, H., & Ko, G. (2016). Antiviral effect of vitamin A on norovirus infection via modulation of the gut microbiome. *Sci Rep, 6*, 25835. doi:10.1038/srep25835
28. Li, D., Breiman, A., le Pendu, J., & Uyttendaele, M. (2015). Binding to histo-blood group antigen-expressing bacteria protects human norovirus from acute heat stress. *Front Microbiol, 6*, 659. doi:10.3389/fmicb.2015.00659
29. Lopman, B. A., Andrews, N., Sarangi, J., Vipond, I. B., Brown, D. W., & Reacher, M. H. (2005). Institutional risk factors for outbreaks of nosocomial gastroenteritis: survival analysis of a cohort of hospital units in South-west England, 2002-2003. *J Hosp Infect, 60*(2), 135-143. doi:10.1016/j.jhin.2004.10.021
30. Lopman, B. A., Steele, D., Kirkwood, C. D., & Parashar, U. D. (2016). The Vast and Varied Global Burden of Norovirus: Prospects for Prevention and Control. *PLoS Med, 13*(4), e1001999. doi:10.1371/journal.pmed.1001999
31. McFadden, N., Bailey, D., Carrara, G., Benson, A., Chaudhry, Y., Shortland, A., . . . Goodfellow, I. (2011). Norovirus regulation of the innate immune response and apoptosis occurs via the product of the alternative open reading frame 4. *PLoS Pathog, 7*(12), e1002413. doi:10.1371/journal.ppat.1002413
32. Nagata, S., Asahara, T., Ohta, T., Yamada, T., Kondo, S., Bian, L., . . . Nomoto, K. (2011). Effect of the continuous intake of probiotic-fermented milk containing Lactobacillus casei strain Shirota on fever in a mass outbreak of norovirus gastroenteritis and the faecal microflora in a health service facility for the aged. *Br J Nutr, 106*(4), 549-556. doi:10.1017/S000711451100064X
33. O'Brien, S. J., Donaldson, A. L., Iturriza-Gomara, M., & Tam, C. C. (2016). Age-Specific Incidence Rates for Norovirus in the Community and Presenting to Primary Healthcare Facilities in the United Kingdom. *J Infect Dis, 213 Suppl 1*, S15-18. doi:10.1093/infdis/jiv411
34. Papafragkou, E., Hewitt, J., Park, G. W., Greening, G., & Vinje, J. (2014). Challenges of culturing human norovirus in three-dimensional organoid intestinal cell culture models. *PLoS One, 8*(6), e63485. doi:10.1371/journal.pone.0063485
35. Parra, G. I., Squires, R. B., Karangwa, C. K., Johnson, J. A., Lepore, C. J., Sosnovtsev, S. V., & Green, K. Y. (2017). Static and Evolving Norovirus Genotypes: Implications for Epidemiology and Immunity. *PLoS Pathog, 13*(1), e1006136. doi:10.1371/journal.ppat.1006136
36. Petrignani, M., van Beek, J., Borsboom, G., Richardus, J. H., & Koopmans, M. (2015). Norovirus introduction routes into nursing homes and risk factors for spread: a systematic review and meta-analysis of observational studies. *J Hosp Infect, 89*(3), 163-178. doi:10.1016/j.jhin.2014.11.015
37. Rondy, M., Koopmans, M., Rotsaert, C., Van Loon, T., Beljaars, B., Van Dijk, G., . . . Verhoef, L. (2011). Norovirus disease associated with excess mortality and use of statins: a retrospective cohort study of an outbreak following a pilgrimage to Lourdes. *Epidemiol Infect, 139*(3), 453-463. doi:10.1017/S0950268810000993
38. Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., . . . Griffin, P. M. (2011). Foodborne illness acquired in the United States--major pathogens. *Emerg Infect Dis, 17*(1), 7-15. doi:10.3201/eid1701.P11101
39. 10.3201/eid1701.091101p1
40. Scallan, E., Hoekstra, R. M., Mahon, B. E., Jones, T. F., & Griffin, P. M. (2015). An assessment of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years. *Epidemiol Infect, 143*(13), 2795-2804. doi:10.1017/S0950268814003185
41. Showell, D., Sundkvist, T., Reacher, M., & Gray, J. (2007). Norovirus outbreak associated with canteen salad in Suffolk, United Kingdom. *Euro Surveill, 12*(11), E071129 071126.
42. Takanashi, S., Saif, L. J., Hughes, J. H., Meulia, T., Jung, K., Scheuer, K. A., & Wang, Q. (2014). Failure of propagation of human norovirus in intestinal epithelial cells with microvilli grown in three-dimensional cultures. *Arch Virol, 159*(2), 257-266. doi:10.1007/s00705-013-1806-4
43. Tam, C. C., & O'Brien, S. J. (2016). Economic Cost of Campylobacter, Norovirus and Rotavirus Disease in the United Kingdom. *PLoS One, 11*(2), e0138526. doi:10.1371/journal.pone.0138526
44. Taube, S., Kolawole, A. O., Hohne, M., Wilkinson, J. E., Handley, S. A., Perry, J. W., . . . Wobus, C. E. (2013). A mouse model for human norovirus. *MBio, 4*(4). doi:10.1128/mBio.00450-13
45. Taube, S., Perry, J. W., Yetming, K., Patel, S. P., Auble, H., Shu, L., . . . Wobus, C. E. (2009). Ganglioside-linked terminal sialic acid moieties on murine macrophages function as attachment receptors for murine noroviruses. *J Virol, 83*(9), 4092-4101. doi:10.1128/JVI.02245-08
46. Troeger, H., Loddenkemper, C., Schneider, T., Schreier, E., Epple, H. J., Zeitz, M., . . . Schulzke, J. D. (2009). Structural and functional changes of the duodenum in human norovirus infection. *Gut, 58*(8), 1070-1077. doi:10.1136/gut.2008.160150
47. van Beek, J., de Graaf, M., Xia, M., Jiang, X., Vinje, J., Beersma, M., . . . Koopmans, M. P. (2016). Comparison of norovirus genogroup I, II and IV seroprevalence among children in the Netherlands, 1963, 1983 and 2006. *J Gen Virol, 97*(9), 2255-2264. doi:10.1099/jgv.0.000533
48. Vongpunsawad, S., Venkataram Prasad, B. V., & Estes, M. K. (2013). Norwalk Virus Minor Capsid Protein VP2 Associates within the VP1 Shell Domain. *J Virol, 87*(9), 4818-4825. doi:10.1128/JVI.03508-12
49. Wilhelm, B., Waddell, L., Greig, J., Rajic, A., Houde, A., & McEwen, S. A. (2015). A scoping review of the evidence for public health risks of three emerging potentially zoonotic viruses: hepatitis E virus, norovirus, and rotavirus. *Prev Vet Med, 119*(1-2), 61-79. doi:10.1016/j.prevetmed.2015.01.015
50. Zakhour, M., Ruvoen-Clouet, N., Charpilienne, A., Langpap, B., Poncet, D., Peters, T., . . . Le Pendu, J. (2009). The alphaGal epitope of the histo-blood group antigen family is a ligand for bovine norovirus Newbury2 expected to prevent cross-species transmission. *PLoS Pathog, 5*(7), e1000504. doi:10.1371/journal.ppat.1000504

Further Reading

1. Bartfeld S. Modeling infectious diseases and host-microbe interactions in gastrointestinal organoids. Dev Biol. 2016 Dec 15;420(2):262-270. doi:10.1016/j.ydbio.2016.09.014. Epub 2016 Sep 14. Review. PubMed PMID: 27640087.
2. Bartnicki E, Cunha JB, Kolawole AO, Wobus CE. Recent advances in understanding noroviruses. F1000Res. 2017 Jan 26;6:79. doi: 10.12688/f1000research.10081.1.eCollection 2017. Review. PubMed PMID: 28163914; PubMed Central PMCID:PMC5270584.
3. Brown LK, Clark I, Brown JR, Breuer J, Lowe DM. Norovirus infection in primary

immune deficiency. Rev Med Virol. 2017 Mar 8. doi: 10.1002/rmv.1926. PubMed PMID: 28271593.

1. de Graaf M, van Beek J, Koopmans MP. Human norovirus transmission and evolution in a changing world. Nat Rev Microbiol. 2016 Jul;14(7):421-33. doi:10.1038/nrmicro.2016.48. Epub 2016 May 23. Review. PubMed PMID: 27211790.
2. Heggelund JE, Varrot A, Imberty A, Krengel U. Histo-blood group antigens as mediators of infections. Curr Opin Struct Biol. 2017 May 22;44:190-200. doi:10.1016/j.sbi.2017.04.001. [Epub ahead of print] Review. PubMed PMID: 28544984.
3. Karst SM, Tibbetts SA. Recent advances in understanding norovirus pathogenesis. J Med Virol. 2016 Nov;88(11):1837-43. doi: 10.1002/jmv.24559. Epub 2016 May 5. Review. PubMed PMID: 27110852; PubMed Central PMCID: PMC5203933.
4. Mans J, Armah GE, Steele AD, Taylor MB. Norovirus Epidemiology in Africa: A Review. PLoS One. 2016 Apr 26;11(4):e0146280. doi: 10.1371/journal.pone.0146280. eCollection 2016. Review. PubMed PMID: 27116615; PubMed Central PMCID:PMC4846019.
5. Iturriza-Gomara M, O'Brien SJ. Foodborne viral infections. Curr Opin InfectDis. 2016 Oct;29(5):495-501. doi: 10.1097/QCO.0000000000000299. PubMed PMID:27454403.
6. Rocha-Pereira J, Van Dycke J, Neyts J. Norovirus genetic diversity and evolution: implications for antiviral therapy. Curr Opin Virol. 2016 Oct;20:92-98. doi: 10.1016/j.coviro.2016.09.009. Epub 2016 Oct 11. Review. PubMed PMID: 27736665.
7. Schroten H, Hanisch FG, Hansman GS. Human Norovirus Interactions with Histo-Blood Group Antigens and Human Milk Oligosaccharides. J Virol. 2016 Jun 10;90(13):5855-9. doi: 10.1128/JVI.00317-16. Print 2016 Jul 1. Review. PubMedPMID: 27122582; PubMed Central PMCID: PMC4907220.