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What is the duration of immunity to norovirus? A mathematical modeling study

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B.S., B.A., North Carolina State University, 2008

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An abstract of

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Environmental Health
2012

What is the duration of immunity to natural norovirus infection?
A mathematical modeling study

Background

The duration of immunity to norovirus (NoV) is traditionally believed to be on the order of 2-6 months. However, at any point in time, approximately 30% of the population has acquired immunity. Since community incidence rates in the general population are approximately 5% per year, we hypothesize that duration of immunity may be substantially longer.

Methods

We developed a compartmental mathematical model of community NoV transmission based on the natural history of the disease. Parameter values were obtained from a literature review. The model was fit to incidence data from England and Wales and seasonality data from the United States using maximum likelihood. Since there are a range of unknowns regarding NoV transmission, we developed several scenarios to determine the effect of unknown factors on our estimate of the duration of immunity.

Results

Our baseline model estimates the duration of immunity to NoV to be 5.1 (95% CI: 2.9 – 9.6) years. In other scenarios, the duration of immunity ranges from 5.1 to 6.5 years.

Conclusion

Our analysis suggests that the duration of immunity to NoV is significantly longer than previously thought. This finding argues for the continued development of vaccines for NoV, since a short duration of immunity has been considered a major obstacle.

Keywords: norovirus, duration of immunity, mathematical model, vaccine development

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Finally, I'd like to thank my friends and family for their support of my goals and endeavors. And my dogs for keeping me from spending hours on end at the computer and making me laugh when things got tough.

Role

For this manuscript, the author collaborated with Ben Lopman to write the model and analyze the output. She was responsible solely for the writing of all sections and the development of tables and figures. Data supporting the model was collected by other researchers on unrelated projects and adapted to the uses described herein by Ben Lopman.

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Introduction

Acute gastroenteritis is defined as a condition causing rapid onset of diarrhea, often accompanied by nausea, vomiting, fever or abdominal pain. Worldwide, acute gastroenteritis cases cause 1.8 million deaths in children under five each year and is responsible for an estimated 72.8 million disability adjusted life years (DALYs). (1,2) It is estimated that 179 million episodes of acute gastroenteritis occur annually in the US alone, resulting in approximately 600,000 hospitalizations and 5,000 deaths. (3)

There are numerous causes of acute gastroenteritis, the most common of which are viruses. Norovirus (NoV), in particular, is thought to be responsible for 50% of all-cause epidemic gastroenteritis and 12% of sporadic cases, for a total of over 20 million cases per year, worldwide. (1,4)

For most healthy individuals, a case of NoV means an unpleasant few days with no lasting consequences. After an incubation period of 12-24 hours, patients experience a rapid onset of nausea, vomiting and diarrhea, which clears on its own after 24-48 hours. The virus is then shed in stool for 7-21 days. (5)

For vulnerable populations, however, the consequences of a norovirus infection can be more severe, including complications such as Guillen-Barre syndrome, hemolytic uremic syndrome, or death. (6) High risk groups include young children, the elderly, and immunocompromised patients. (7)

NoV outbreaks are more likely to occur in semi-closed environments such as among military personnel, cruise ship passengers, nursing homes and long term care facilities. (7,8)

Disease Burden

Worldwide, NoV is currently thought to be the behind about 12% of all-cause gastroenteritis cases. (9) When it comes to gastroenteritis outbreaks, however, NoV is considered to be responsible for at least 50%. (10) However, the majority of NoV cases occur in non-epidemic situations in the general community. Part of the difficulty in obtaining reliable incidence estimates lies in the currently limited diagnostic capabilities. Prior to

1993, definitive diagnosis consisted of examination of fecal specimens under an electron microscope, a method which can only detect norovirus with concentrations $\geq 10^5$ -10^6 viral particles per ml of stool suspension. (7,11) ELISA assays for specific strains of NoV became available during the 80s and were used in some outbreak investigations, though accurate diagnosis depended on the outbreak strain matching the detection ability of the ELISA. (12–14) Work is ongoing on a strain neutral ELISA assay with a high enough sensitivity and specificity for widespread use. (15)

Currently, NoV can only be detected definitively using PCR, however limitations of time, finance and equipment make routine diagnoses with this method impractical. In the absence of an electron microscope, PCR, or a viral detection assay, a diagnosis of viral gastroenteritis can only be made based on epidemiological criteria – watery diarrhea, vomiting, fever, chills, headache and/or abdominal pain. (16) This type of diagnosis excludes specific NoV diagnosis, but instead broadly fingers the viruses causing gastroenteritis. (17)

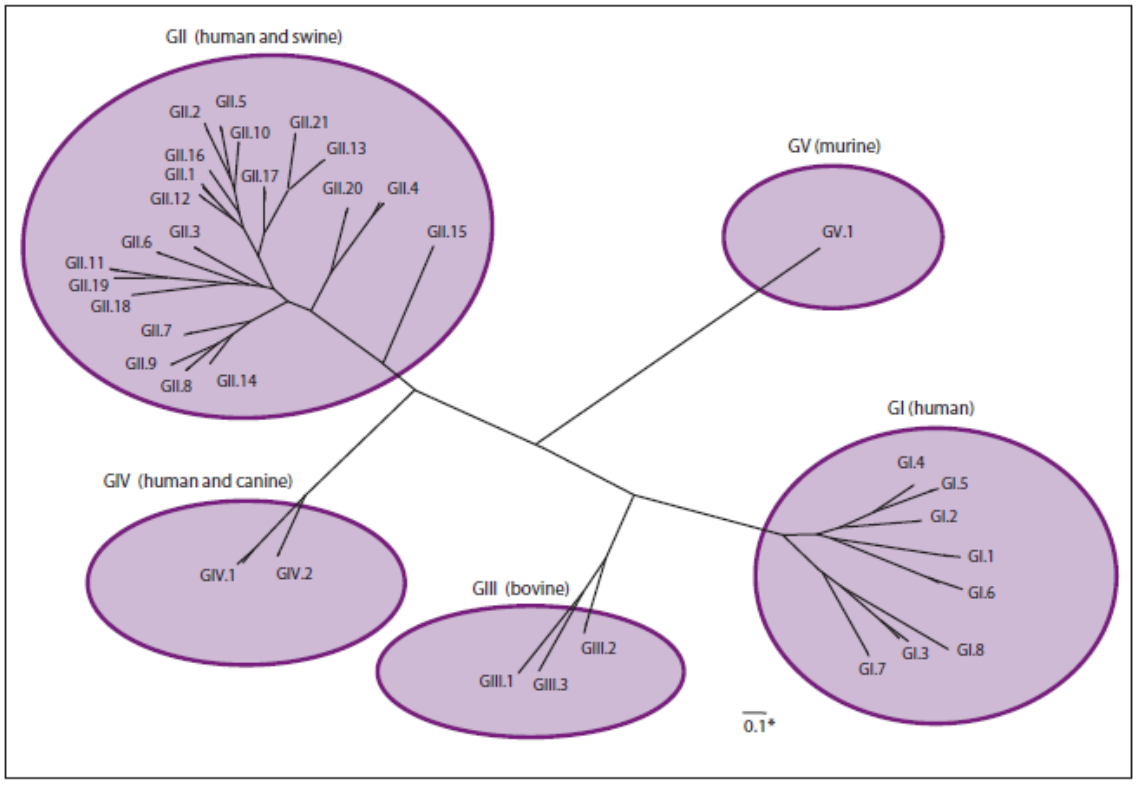
NoV has a clear seasonal pattern. Cases tend to peak during the winter months, for reasons that are not entirely understood. (7) Humans are believed to be the only host for human NoV, though there are several murine and feline NoVs strains being considered as alternates for human research. (7) A fecal-oral transmission pathway, combined with a low infectious dose estimated at 18 viruses, allows for the virus to achieve a secondary attack rate of 30% or more among contacts of ill persons. (18–20) The individual infectivity of a single virus particle is estimated to be 0.5, higher than any other virus reported to date. (18) This is part of the reason that NoV is the most common cause of gastroenteritis outbreaks and community disease. In addition, NoV is also resistant to many standard viral decontamination protocols. (10) The best defense against NoV infection is proper hand-washing, which removes viral particles through mechanical means. (10) Due to the difficulty in removing NoV contamination via disinfection, and due to the lack of access to clean water and sanitation in many regions of the world, there has been a lot of interest in the development of a NoV vaccine.

There are several vaccines in development for NoV, but none have reached yet reached the market. Greenberg et al. demonstrated the presence of antibodies to NoV in 1979, and in 1999 Ball et al. demonstrated

a dose-dependent antibody response in healthy volunteers given recombinant NoV vaccine. (21,22) The current challenge is protecting against the multiple strains of NoV, as well as demonstrating immunity in a randomized, double blinded trial. LoBue et al. have made strides toward the former problem and Atmar et al. reported a successful trial in 2011. (23,24)

The Virus and Host

NoVs are a genus in the family Caliciviridae and have multiple genogroups, genotypes and subgroups. (MMWR 2011) Referencing the image below, genogroups are the larger branches of the NoV virus types, genotypes are the smaller groupings within each branch and strains/variants are the divisions within a genotype, as is seen with the GII.4 subgroup below.



Sources: Data from Zheng DP, Ando T, Fankhauser RL, Beard RS, Glass RI, Monroe SS. Norovirus classification and proposed strain nomenclature. Virology 2006;346:312–23; Wang QH, Han MG, Cheetham S, Souza M, Funk JA, Saif LJ. Porcine noroviruses related to human noroviruses. Emerg Infect Dis 2005;11:1874–81; CDC, unpublished data, 2011; graphic developed by Everardo Vega, PhD, CDC.
* The scale bar of 0.1 reflects the number of amino acid substitutions per site.

Figure 1: Genetic tree for NoV, obtained from the CDC under a Creative Commons license.

NoVs are single stranded, positive-sense RNA viruses. (25) A new dominant strain emerges every 2-4 years. (1) Over the past several years all dominant strains have been from the GII.4 genotype, and it is currently estimated that GII.4 strains cause 70-80% of all NoV infections. (26)

Humans are believed to be the only carriers of human NoV. As discussed earlier, recovery from NoV symptoms is rapid, but individuals can shed virus in their stool for up to three weeks. Continued shedding is a potential source of new infection, particularly in medical or food preparation settings, though it is unclear how much post-symptomatic shedding is responsible for NoV spread. (27) It has generally been established that healthy individuals shed for a shorter time period than individuals from vulnerable groups, but again, the implications of this are unknown. (28)

Immunity

There is considerable uncertainty about the duration of natural acquired immunity after NoV exposure. Early studies placed it at two to three months. Parrino et al. dosed 12 volunteers with Norwalk inoculum; 6 of the 12 became ill. The ill subjects were allowed to recover and dosed again 27-42 months later, at which point all suffered symptoms. Four of these six were dosed a third time 4-8 weeks later, and only one became ill; Parrino thus concluded that the immunity to NoV was in that range and that the initial subjects who had not gotten sick might lack a receptor for NoV. (29) The second conclusion has since been confirmed, but the first has been called into question due to the large doses of NoV given to the volunteers. (25) Parrino's doses were several hundred times the amount needed to produce disease, and could easily have overwhelmed the immunity obtained from previous exposures.

Johnson et al revisited the question of immunity in 1990, and found that some of his volunteers became ill on re-challenge at 6 months (4 of 22 individuals). Of the volunteers re-challenged in Johnson's study, three had participated in another NoV challenge trial three years previously. Of these volunteers, two had displayed gastroenteritis symptoms on their first challenge (2.5 years after their previous trial participation), but none displayed gastroenteritis symptoms in Johnson's second challenge. (30) The single volunteer who did not

develop gastroenteritis 2.5 years after initial challenge may be indicative of the presence of longer term immunity in some individuals, but his natural exposure to the virus in the intervening time is unknown.

Both of these studies investigated acquired immunity to NoV strains previously encountered. In 1974, Wyatt et al attempted to investigate the cross protection that certain strains might provide. Using Norwalk virus, Hawaii virus and Montgomery County virus (three strains of NoV from various genogroups) Wyatt challenged his volunteers and then exposed them again between 6 and 15 weeks later. He found that volunteers challenged with the same strain twice did not become ill, as did volunteers challenged with strains from the same genogroup. However, volunteers challenged with strains from different genogroups had a higher probability of developing gastroenteritis, suggesting a partial or incomplete protective effect of exposure to one genogroup from illness caused by other genogroups. (31) This is consistent with some outbreak studies in which multiple strains have been found circulating simultaneously. (reported in 14)

Some portion of the population – up to 20% - may be genetically resistant to NoV. (25,32) This genetic resistance is related to expression of the FUT2 gene and the absence of receptors in the small intestine that allow NoV access through the mucosal membrane. (25) A correlation between FUT2 and absence of infection was demonstrated for Norwalk virus by Lindesmith et al, but an attempt by the same group to replicate the results with Snow Mountain virus in 2005 was not successful. (25,32) The role of genetic resistance in preventing generic NoV infection remains unknown.

From volunteer studies, we know that approximately 30% of people given the appropriate infectious dose of NoV are immune at any given time. (32,33) We also know that the typical incidence of NoV in the general population is around 5%. (34)

If the duration of immunity is truly close to three months, the observed incidence would have to be higher in order for 30% of individuals to be immune at any given time. Consider, for example, the time frame of a year. If the incidence is 5% and immunity lasts for a year, we would expect that about 5% of the population to have acquired immunity at any given time. If immunity lasts 3 months, we would expect around 1.25% to be

immune at any given time. However challenge studies show immunity levels of approximately 30%, which suggests that our understanding of the duration of immunity is incomplete.

Our work attempts to clarify the question of duration of immunity by creating a community model of NoV transmission and fitting it to existing incidence data in an attempt to estimate the duration of immunity to NoV.

Methods

Model Design

We developed a deterministic, age structured, dynamic model of NoV transmission that includes symptomatic and asymptomatic infections. The full model is illustrated by the flow diagrams in Figures 2 and 3, with parameters as defined in Table 4. The full model equations are described below.

We assume that newborn infants have no maternal immunity; therefore all births enter into the susceptible class. All individuals in the susceptible class can be infected at rate $\lambda(t)$ and they progress from the exposed class into the infected symptomatic class at rate μ_s. Individuals in the infected symptomatic class progress to the infected asymptomatic class at rate μ_a and move on to the recovered class at rate ρ. Individuals in the recovered class are assumed to have immunity to symptomatic disease, as opposed to asymptomatic infection, and they move from the recovered class back to the susceptible class at rate θ. However, individuals from the recovered class can also cycle back into the infected asymptomatic class at rate $\lambda(t)$. One scenario required a slight adjustment to the model, and this version is also included below.

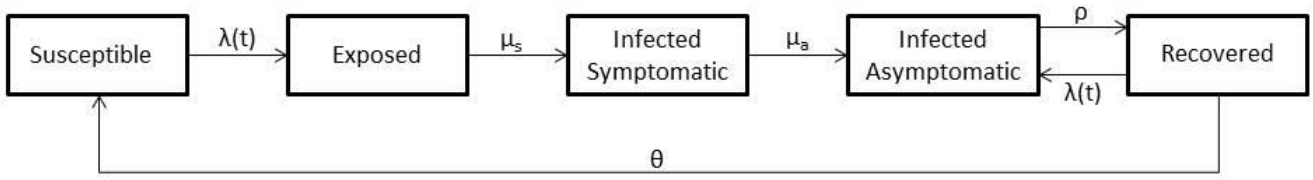


Figure 2: Model schematic without genetic resistance, depicting the flow of population from the susceptible pool through the stages of infection. All variables are defined in the text. Individuals are born directly into the susceptible pool, become exposed at the force of infection, and then progress through symptomatic and asymptomatic stages before arriving in the recovered compartment. From the recovered compartment, individuals can shift back into asymptomatic infection at the force of infection or progress back into the susceptible pool at the rate of loss of immunity.

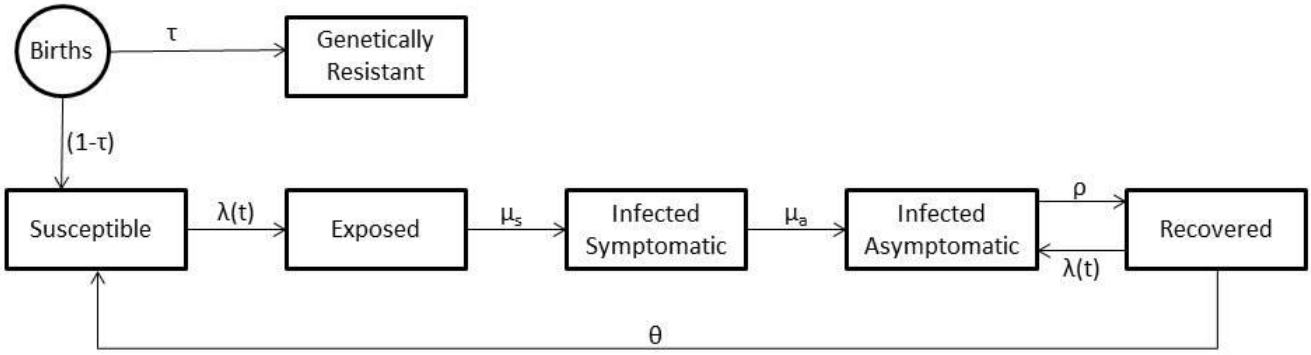


Figure 3: Model schematic with genetic resistance, identical to the first model except for the addition of a compartment to represent genetically resistant individuals who are removed from the susceptible pool at birth. All variables are defined in the text.

Our model is a system of ordinary differential equations, described as follows:

$$\frac{dGi}{dt} = B\tau - D\tau$$

$$\frac{dS_i}{dt} = B(1-\tau) + \theta R_i - (\lambda i(t) + D)Si$$

$$\frac{dE_i}{dt} = \lambda(t)S_i - (\mu s + D)Ei$$

$$\frac{dIS_i}{dt} = \mu s E_i - (\mu a + D)ISi$$

$$\frac{dIA_i}{dt} = \mu a IS_i + \lambda i(t)R_i - (\rho + D)IAi$$

$$\frac{dR_i}{dt} = \rho IA_i - (\lambda i(t) + \theta + D)R_i$$

where:

i = age group, defined as 0-4 years, 5-14 years, 15-45 years and 45+ years

G_i = individuals genetically resistant to NoV

S_i = individuals susceptible to NoV

E_i = individuals exposed to NoV

IS_i = individuals infected with NoV and showing symptoms

IA_i = individuals infected with NoV and not showing symptoms, but shedding virus in stool

R_i = individuals who have recovered from NoV and are no longer shedding virus

τ = proportion of individuals who are genetically resistant to NoV

B = number of births entering the system

D = number of deaths exiting the system

θ = the rate at which recovered individuals become susceptible again; the duration of immunity

λ(t) = the force of infection; the rate at which susceptible or recovered individuals become infected

μ_s = the rate at which exposed individuals become infected symptomatic

μ_a = the rate at which infected symptomatic individuals become infected asymptomatic

ρ = the rate at which infected asymptomatic individuals recover

Seasonality

Norovirus has such a seasonal pattern that it was initially described as 'the winter vomiting disease.' Incidence peaks in the late winter and early spring, and most outbreaks are seen during this time. (35) The model accounted for seasonal variation in transmissibility as follows:

$$\beta(t) = \beta o((1 + \beta 1 * \cos(2\pi t + \omega))$$

Where β_0 is the mean of the transmission parameter, β_1 is the amplitude of its seasonal fluctuation and φ is the phase angle in years (t). The mean transmission parameter (β_1) depends on age-specific mixing and contact patterns of the population. Age-specific transmission parameters were estimated by multiplying age-specific contact rates for Great Britain (obtained from the POLYMOD dataset and adjusted to fit our age ranges) (36) by two transmission coefficients, q_1 and q_2, which are measures of NoV transmissibility. The two transmission coefficients correspond to children aged 0-4 years (q_1) and all other age groups (q_2) to allow for differences in relative infectiousness between young children and other ages. (37) Values of β_1, ω, q_1 and q_2 were estimated by fitting our model to data collected in England and Wales, the US, and several volunteer challenge studies (detailed in Table 1). (34,8)

Alternate Model Structures Considered

The model above is certainly not the only structure possible, given the uncertainties regarding the particulars of NoV transmission and immunity. We considered many additional model structures before arriving at this one. Our explorations included structures that allowed for asymptomatic infections to proceed from susceptible individuals, and structures that incorporated environmental contamination into the force of infection. Our decision to go with the structure above was based on the data available and the unrealistic output of some of our early experimental model structures.

Fitting Data

We opted to fit our model to several different parameters from the available datasets on NoV incidence, seasonality and immunity. Phillips et al. conducted a retrospective analysis of the data collected in the Study of Infectious Intestinal Disease in England that provided annual incidence numbers for norovirus, but did not take into account seasonality. (34) Yen et al. performed an analysis of seasonality of NoV in the United States, but did not include annual incidence in the community. (8) In order to obtain a data set that included both variables, we multiplied the annual incidence from Phillips with the duration of the Yen study and the probability of any one case being part of an outbreak. (34, 8) The result was a data set that included community incidence, excluded outbreaks, and included seasonality, allowing us to investigate all those factors when fitting our model.

In addition to fitting to incidence data and seasonality, we fit to the proportion of the population immune to NoV at any given time. Our reasoning for doing so is explained in the section of the methods entitled "Upper and Lower Limits." The proportion immune was obtained by doing a review of the challenge literature and calculating the proportion of individuals who did not become infected after challenge. The studies considered can be seen in Table 1.

Table 1: Summary of literature consulted to identify the proportion of the population immune. SM = Snow Mountain Strain, MC = Montgomery County, N = Norwalk, H = Hawaii. Reasoning for this table is further defined in the section on upper and lower limits.

Studies	All			Se+			Se-		Strai
	Challenge	Infecte	AG	Challenge	Infecte	AG	Challenge	Infecte	
Lindesmith 2005 (32)	15	9	7	12	8		3	1	SM
Wyatt 1974 (Norwalk) (31)	16		11						N
Wyatt 1974 (2nd pass)	36		19						N
Wyatt 1974 (Hawaii)	4		3						H
Wyatt 1974 (2nd pass)	19		8						H
Wyatt 1974 (MC)	3		2						MC
Wyatt 1974 (2nd pass)	15		3						MC
Dolin 1971 (38)	12		9						SM
Graham 1994 (20)	50	41	34						N
Parrino 1977 (29)	12		6						N
Lindesmith 2003 (25)	77	34	42	55	34		22	0	N
Hutson 2005 (39)	51	42		43	42		8	0	N
Atmar 2008 (33)	16	11	11	16	11	11	0	0	N
Leon 2011 (40)	15		7	15		7			N
Atmar 2011 (19)	41	34	29	41	34	29			N

Contact Rates

Contact rates were obtained from the European POLYMOD study (36) and adjusted to account for the age ranges in our model. The rates used in the model are detailed in the table below.

Table 2: Contact rates used in the model, calculated from Mossong et al, 2007. Numbers represent the daily probability of someone in age group *i* coming in contact with someone in age group *j*. Row and column labels refer to the age groups used in the model.

	Age of Respondent			
Age of Contact	0-4 years	5-14 years	15-44 years	45+ years
0-4 years	7.136e-7			
5-14 years	1.813e-7	1.170e-6		
15-44 years	1.675e-7	2.253e-7	3.515e-7	
45+ years	7.040e-8	9.5556e-8	1.659e-7	2.032e-7

Births and deaths are assumed to be equal and to occur at a constant rate throughout the year. Static model inputs are detailed below in Table 3.

Model Scenarios

Our baseline scenario made several simplifications (e.g. that the entire population is genetically susceptible) and assumptions for which there is considerable uncertainty (e.g. that immunity to one strain of norovirus protects against other strains). Therefore, we set up a number of scenarios to explore the impact of pre- and post-symptomatic infectiousness, genetic resistance within a portion of the population and whether immunity to NoV is strain specific. These scenarios are detailed in Table 3, as well as in the following bullets.

- Scenario A: Pre-Symptomatic Infectiousness 5%: With all other variables remaining constant, individuals in the exposed compartment will be 5% as infectious as symptomatic individuals.
- Scenario B: Post-Symptomatic Infectiousness 5%: With all other variables remaining constant (including pre-symptomatic infectiousness set to 0), individuals in the infectious asymptomatic compartment will be 5% as infectious as symptomatic individuals.
- Scenario C: Both Pre and Post-Symptomatic Infectiousness 5%: With all other variables remaining constant, individuals in the exposed and asymptomatic compartments will be 5% as infectious as symptomatic individuals.
- Scenario D: Genetic Resistance: A proportion of the population will be ineligible for infection via the creation of a new class that ages normally but moves on birth directly to the recovered class.
- Scenario E: GII.4 Only: Incidence data is multiplied by 0.72 to represent only the GII.4 genogroup of NoV, based on the estimated proportion of NoV cases caused by GII.4 strains.

Table 3: Static model inputs and their sources

	Model Scenario						
Input	**Base**	**A**	**B**	**C**	**D**	**E**	**Source**
Life Expectancy (years)	76	76	76	76	76	76	
Duration of Incubation (days)	1	1	1	1	1	1	**Atmar et al, 2008** (33)
Duration of Symptoms (days)	2	2	2	2	2	2	**Atmar et al, 2008** (33)
Duration of Asymptomatic infection (days)	10	10	10	10	10	10	**Rockx et al, 2002** (5)
Relative infectiousness during incubation period	0	0.05	0	0.05	0	0	**Teunis et al, 2008** (18)
Relative infectiousness during asymptomatic infection period	0	0	0.05	0.05	0	0	**Teunis et al, 2008** (18)
Proportion of population genetically resistant	0	0	0	0	0.2	0	**Lindesmith et al, 2003** (25)
Strains Included	All	All	All	All	All	GII.4 Only	**Vinjé, 2012** (15)

Upper and Lower Limits

Initially, we attempted to conduct a standard least squares regression in order to obtain 95% confidence intervals for the duration of immunity, but we found that the intervals that resulted were extremely wide. The fits we were achieving resulted from a mix of low and high transmission models that allowed for the duration of immunity to vary dramatically. Since the initial conflict between a short duration of immunity and the data available were what set us to investigate this question in the first place, we opted to add an additional fitted variable – the proportion of the population immune at any given time. The literature review used to determine this value can be seen in Table 1.

By forcing the model to fit to this additional piece of data and using a Poisson regression to determine upper and lower limits, we were able to achieve a model that fit the known values from the literature and returned a consistent duration of immunity.

Results

The baseline model provided a qualitatively good fit to the incidence data, as seen in Figure 4, with an overall incidence estimate of 6.2% per year compared with the observed 4.5% per year (see Table 4 for fitted parameters). The transmission probability per infectious contact (q) was approximately four times greater for young children (q_1 = 0.226) than older children and adults (q_1 = 0.057). The baseline model somewhat underestimated incidence in the youngest age group (18.9% compared with 21.4%) and overestimated in the older age groups (6.1% and 4.3% compared with 4.1% and 1.7% in 15 to 44 yrs and 45+ yrs, respectively.) The average proportion immune to disease (R_i) ranged from 24% to 48% in the youngest and oldest age groups, respectively. The prevalence of asymptomatic infection was predicted to be between 1.9% and 0.4%, depending on age group and season. These values and others can be seen in Tables 4 and 5. With a seasonal forcing of 2.5% (β_1), 73% of cases were predicted to occur in Oct-Mar, compared with 73% observed in the U.S. from 2007 to 2010 (calculations not shown). Under this scenario, the duration of immunity was estimated at 5.1 years (95% CI: 2.9-9.6; Figure 5).

Scenario A did not differ substantially from baseline, with an overall incidence estimate of 6.20% per year and similar values in each of the age groups, as detailed in Table 5. The duration of immunity in this scenario was estimated at 5.2 years (95% CI: 20.9-8.2; Figure 5)

Scenario B shows quite a bit of variation from the baseline, with a duration of immunity of 6.5 years (95% CI: 2.2-13.7; Figure 5) and an overall incidence estimate of 6.57% per year (Table 5). The seasonal forcing in this scenario was almost doubled over baseline at 4.5%, as seen in Table 4, however other values were similar to the baseline.

Scenario C had the greatest variance from baseline with regards to the proportions of asymptomatic infections in the age groups. The values are mostly, though not uniformly, lower than baseline, varying from 0.0009% to 3.1%, with the highest levels occurring in the 0-4 age group during the winter months. Proportions recovered are all similar to baseline. The overall incidence in this scenario was extremely well fit to the observed

data (4.4% vs. 4.5%), and the incidence predictions for the age groups were similarly matched. In 5-14 year olds, however, the model underestimated incidence by almost half, at 3.9% compared to 6.5% (Table 5). Seasonal forcing for this scenario is more than doubled over baseline (5.9% vs. 2.5%; Table 4). Duration of immunity for this scenario was 6.5 years (95% CI: 3.2-16.4; Figure 5).

In Scenario D, the model again predicts the overall incidence extremely well, with an estimate of 4.5%. Within the age groups, the 45+ age group was overestimated at 4.0% and the 5-14s are underestimated at 3.9%. In all age groups, the proportion recovered was similar to the baseline (Table 5). Young children (0-4) have a higher proportion asymptomatic compared to baseline, and the probability of transmission per infected contact (q_1) is more than doubled over baseline at 0.5767. Additionally, the seasonal offset parameter is dramatically reduced compared to baseline (0.1002 vs. 1.0987; Table 4). The duration of immunity in this scenario was 6.0 years (95% CI: 2.6-12.3; Figure 5).

Scenario E also provides a qualitatively good fit to the incidence data, with the overall incidence coming in at 5.6%, with an overestimation of the annual incidence in adults over 45 (3.6%) and all other age groups comparable to the observed incidence. Proportions asymptomatic and recovered are comparable to baseline, as are the fitted parameters (Tables 4 and 5). The duration of immunity in this scenario is 5.8 (95% CI: 3.8-8.2; Figure 5).

Table 4: Fitted parameter output for all scenarios. These values are the model's best estimate of the five parameters in which the model found the best fit using a Poisson regression.

	Baseline	Scenario A	Scenario B	Scenario C	Scenario D	Scenario E
Duration of Immunity (years)	5.13	5.15	6.51	6.45	6.04	5.81
P(Transmission per infected contact, ages 0-4) (q_1)	0.2258	0.2224	0.1962	0.2490	0.5767	0.2336
P(Transmission per infected contact, ages 5+) (q_2)	0.0568	0.0550	0.0450	0.0337	0.0768	0.0542
Seasonal Offset Parameter (ω)	1.0987	1.0900	1.0462	0.9937	0.1002	1.0392
Seasonal Forcing (β_1)	0.0250	0.0249	0.0449	0.0591	0.0100	0.0249

Table 5: Model predictions for proportion of the population in the asymptomatic and recovered compartments after the model has settled into endemic cycles, as well as predicted annual incidence, by age group.

	Observed Incidence	Baseline	Scenario A	Scenario B	Scenario C	Scenario D	Estimated GII.4 Incidence	Scenario E
Proportion Asymptomatic, 0-4		0.0100	0.0101	0.0145	0.0160	0.0166		0.0096
Proportion Asymptomatic, 5-14		0.0033	0.0032	0.0040	0.0019	0.0023		0.0027
Proportion Asymptomatic, 15-45		0.0025	0.0024	0.0030	0.0014	0.0019		0.0021
Proportion Asymptomatic, 45+		0.0015	0.0015	0.0018	0.0008	0.0011		0.0013
Proportion Recovered, 0-4		0.4809	0.4819	0.5941	0.6227	0.5480		0.4707
Proportion Recovered, 5-14		0.4088	0.4057	0.5162	0.4221	0.4177		0.3898
Proportion Recovered, 15-45		0.3314	0.3274	0.4303	0.2935	0.3311		0.3091
Proportion Recovered, 45+		0.2366	0.2330	0.3187	0.1935	0.2430		0.2174
Annual Incidence, 0-4	0.2140	0.1892	0.1893	0.2104	0.2147	0.1825	0.1541	0.1870
Annual Incidence, 5-14	0.0645	0.0706	0.0694	0.0695	0.0386	0.0399	0.0468	0.0612
Annual Incidence, 15-45	0.0410	0.0609	0.0510	0.0623	0.0373	0.0288	0.0295	0.0530
Annual Incidence, 45+	0.0170	0.0425	0.0417	0.0449	0.0244	0.0400	0.0122	0.0363
Combined Annual Incidence	0.0454	0.0629	0.0620	0.0657	0.0437	0.0446	0.0327	0.0558

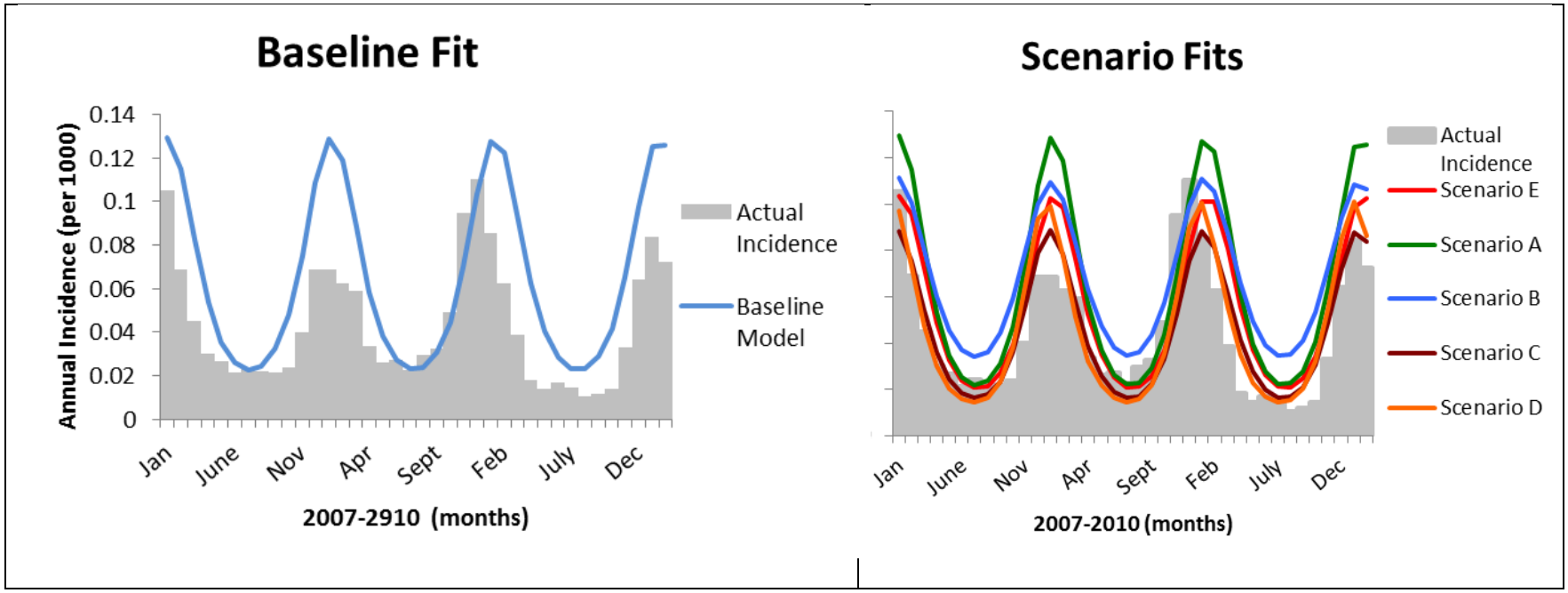


Figure 4: Baseline and scenario fits to available data. The grey areas indicate the incidence data collected in the UK and adjusted using US seasonality data to include seasonality. (8,34) **The lines represent the predicted model incidence for the baseline (left) and all scenarios (right).**

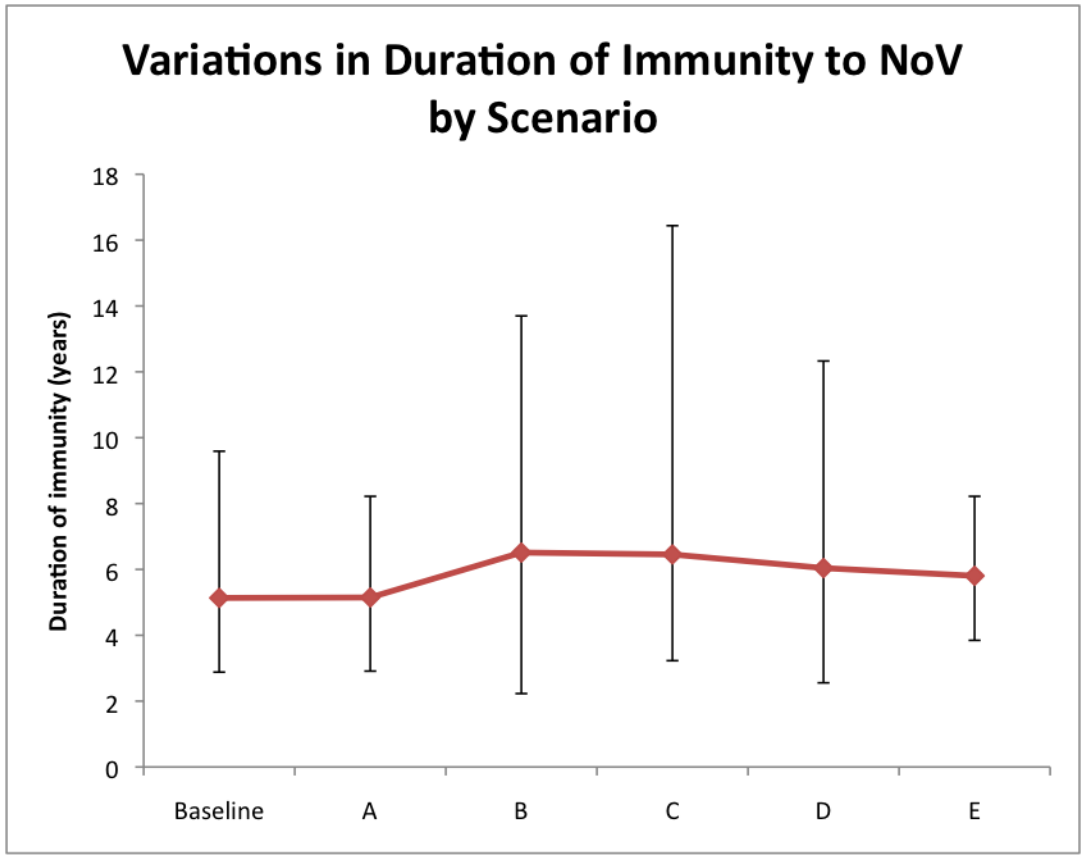


Figure 5: Model predicted duration of immunity with upper and lower bounds, by scenario

Discussion

Our study appears to indicate that the duration of immunity to NoV is much longer than previously thought. Using a model with strong qualitative fits to observed incidence data, our scenarios returned durations of immunity ranging from 5.1 to 6.5 years. In comparison, previous estimates ranged from 8 weeks to 6 months. (29,31)

The model showed consistent differences between q_1 and q_2 – the probability of an infectious contact resulting in disease for children aged 0-4 years and all other individuals. Q_1 was consistently 3-4 times larger than q_2, indicating that children aged 0-4 are likely the drivers of NoV infection.

In addition, all scenarios of our model returned numbers of seasonal resonance that are quite low (2.5-5%). In other words, the infectiousness of the virus is only increased by 2.5% during the winter months when more than 70% of NoV cases occur. This seems to indicate that some factor other than seasonal drivers is behind the seasonality of NoV infection, either changes in behavior or factors intrinsic to the course of the disease itself.

The immune boosting role played by asymptomatic infections may well have something to do with the phenomenon of low seasonal resonance. If asymptomatic individuals are less infectious than symptomatic individuals, one would expect an increase in the proportion of asymptomatic infections to result in a decrease in force of infection as more infectious contacts result in asymptomatic infections. This could then lead to the dip in cases seen in summer months, and a corresponding increase in cases as immunity wanes and the proportion of susceptible individuals increases in correspondence to the relative decrease in asymptomatic infections. This fluctuation of asymptomatic cases is observed in our output. There are no corresponding data sets that measure the proportion of asymptomatic infections over the course of a year or more, so at this time we cannot validate this finding against observed data.

Our results are in agreement with the available data from challenge studies and larger studies of NoV outbreaks and incidence in community settings. The model produces excellent qualitative fits to the incidence

and seasonality data used, and also agrees with our understanding of the proportion of asymptomatic infections at any given time.

However, our study has several weaknesses, ranging from the model output to the data used to the structure of the model itself. Though the lower confidence interval for duration of immunity was, in all scenarios, much longer than previous estimates of duration of immunity, the confidence intervals themselves were quite wide. This made it difficult to ascertain the significance of the differences between the scenarios. Some of the assumptions made in various scenarios are almost certainly false, but were made in the absence of conclusive data that would allow more accurate assumptions.

Our assumptions, which may or may not be accurate, are as follows.

First, we assumed that all primary infections are symptomatic. This includes an assumption that there is no maternal immunity in infants – something that has yet to be determined conclusively. (41)

Second, we assumed that all NoV strains are identical and that infection with one strain provides cross immunity to another. This is almost certainly not true (31) but data on the spread, infectiousness, asymptomatic infectiousness and immune duration on various strains is not available. In addition, the strains for which we can begin to describe these traits make up a small proportion of the ever-shifting variety of strains in circulation. (7) Any model created using this data would inevitably be incomplete and of limited practical use.

Third, our model assumes that immunity is immunity to disease, rather than infection. This allows for the cycling between the recovered and infectious asymptomatic compartments that results in such a long duration of immunity. The nature of NoV immunity is currently an open question, and there are other potential means of explaining the data we have available. (7)

And fourth, our model assumes that genetic resistance is to all NoV strains, something that has only been demonstrated for the FUT2 gene and Norwalk virus. (25) Additional data and research should be aimed at investigating this set of assumptions.

Given the difficulty of detecting NoV, it is possible that the incidence data we used in our model over or underestimates the number of cases in the general community. If so, the results of our model would similarly under or overestimate the number of cases.

To our knowledge, this is the only modeling study that attempts to look specifically at the duration of immunity to NoV. Our results stand in direct contrast to some of the earliest human challenge studies to estimate the duration of immunity to NoV. Studies by Parrino, Johnson and Wyatt included multiple challenges, though we now know that the doses given were several hundred times the minimum infectious dose. Wyatt's study investigated cross protective qualities between different strains of NoV and challenged his volunteers between 7 and 14 weeks after initial illness with homologous strains. In this scenario, none of his volunteers became ill. (31) In contrast, Parrino's study found that volunteers became ill on subsequent challenge at 8 weeks. (29) It is impossible to know the exact dosages given these volunteers, but given that it was measured in milliliters of filtered stool sample, it is certainly far beyond the ID50 of 18 viruses. In addition, these studies diagnosed NoV illness on symptoms alone, as the ability to identify the virus directly or through antibody measurements had not been developed at the time. Asymptomatic infections were therefore not identified, and the sample sizes likely included individuals who were genetically resistant to infection and who had been recently infected naturally. (25)

The results of our study are most important when considering the work currently underway to develop vaccines for NoV. (22,23,33) A short duration of immunity would make such vaccines impractical for widespread use and, as such, there have been questions about the value of continued development. Our results strongly argue for the continued development of these vaccines, as duration of immunity in the range returned by our model would allow for vaccine campaigns to be executed and expect to see a return on the investment.

In addition, the role of children in driving the spread of NoV in our model warrants further research into the potential for widespread reductions in incidence through a childhood vaccination campaign. Our results suggest that vaccinating children could bring the biggest return on investment.

The question of seasonal drivers is also influenced by our results. If the seasonal variance in infectiousness is really as small as indicated by our model, and asymptomatic infections truly have the impact on immunity that our model estimates, then the proportion of the population immune becomes a much more likely candidate for explaining seasonal variation. If so, this balance could shift with the introduction of a vaccine, as was seen in the wake of the uptake of the rotavirus vaccine. (42) With widespread uptake of a NoV vaccine, the cycle of asymptomatic infections could be disrupted, possibly leading to a shorter duration of immunity.

Our results bring up several questions for further research, the most pressing of which is further investigation of the role of asymptomatic infections in prolonging the duration of immunity and thus influencing seasonality. In addition, more research is needed on the role of dose in provoking symptomatic or asymptomatic infection and the role of various strains in the immune response. We are also interested in investigating the conditions in which high proportions of asymptomatic infections are possible, and the effect that those conditions have on the duration of immunity. Since models are only as good as the data they use, there is an urgent need for additional data on strain prevalence and dose response curves to facilitate research on the questions described above.

Works Cited

1. Patel MM, Hall AJ, Vinjé J, Parashar UD. Noroviruses: a comprehensive review. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology [Internet]. 2009 Jan [cited 2012 Jul 13];44(1):1–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19084472
2. Organization WH. The Global Burden of Disease, 2004 Update [Internet]. 2004 [cited 2012 Jul 13];Available from: http://www.who.int/healthinfo/global_burden_disease/GBD_report_2004update_full.pdf
3. Scallan E, Griffin PM, Angulo FJ, Tauxe RV, Hoekstra RM. Foodborne illness acquired in the United States--unspecified agents. Emerging infectious diseases [Internet]. 2011 Jan [cited 2012 Jul 13];17(1):16–22. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3204615&tool=pmcentrez&rendertype=abstract
4. Prevention C for DC and. CDC - Norovirus - Reporting and Surveillance for Norovirus. [cited 2012 Jul 13];Available from: http://www.cdc.gov/norovirus/php/reporting.html
5. Rockx B, De Wit M, Vennema H, Vinjé J, De Bruin E, Van Duynhoven Y, et al. Natural history of human calicivirus infection: a prospective cohort study. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America [Internet]. 2002 Aug 1;35(3):246–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12115089
6. Fischer Walker CL, Sack D, Black RE. Etiology of diarrhea in older children, adolescents and adults: a systematic review. PLoS neglected tropical diseases [Internet]. 2010 Jan [cited 2012 Jul 13];4(8):e768. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2914743&tool=pmcentrez&rendertype=abstract
7. Glass RI, Parashar UD, Estes MK. Norovirus gastroenteritis. The New England journal of medicine [Internet]. 2009 Oct 29;361(18):1776–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19864676
8. Yen C, Wikswo ME, Lopman B a, Vinje J, Parashar UD, Hall AJ. Impact of an emergent norovirus variant in 2009 on norovirus outbreak activity in the United States. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America [Internet]. 2011 Sep [cited 2012 Jul 13];53(6):568–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21832262
9. Patel MM, Widdowson M-A, Glass RI, Akazawa K, Vinjé J, Parashar UD. Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerging infectious diseases [Internet]. 2008 Aug [cited 2012 Jul 13];14(8):1224–31. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2600393&tool=pmcentrez&rendertype=abstract
10. Prevention C for DC and. CDC - Norovirus - Burden of Norovirus Illness and Outbreaks. [cited 2012 Jul 13];Available from: http://www.cdc.gov/norovirus/php/illness-outbreaks.html
11. Koopmans M, von Bonsdorff CH, Vinjé J, de Medici D, Monroe S. Foodborne viruses. FEMS microbiology reviews [Internet]. 2002 Jun;26(2):187–205. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12069883
12. Madore HP, Treanor JJ, Pray KA, Dolin R. Enzyme-linked immunosorbent assays for Snow Mountain and Norwalk agents of viral gastroenteritis. Journal of clinical microbiology [Internet]. 1986 Sep [cited 2012 Jul 28];24(3):456–9. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=268935&tool=pmcentrez&rendertype=abstract
13. Guest C, Spitalny KC, Madore HP, Pray K, Dolin R, Herrmann JE, et al. Foodborne Snow Mountain Agent Gastroenteritis in a School Cafeteria. Pediatrics [Internet]. 1987 Apr 1 [cited 2012 Jul 28];79(4):559–63. Available from: http://pediatrics.aappublications.org/cgi/content/abstract/79/4/559

14. Leon JS, Souza M, Wang Q, Smith ER, Saif LJ, Moe CL. Chapter 9 - Immunology of Norovirus Infection. In: Immunology Against Mucosal Pathogens. 2008. p. 215–57.
15. Vinjé J. Norovirus surveillance system (CaliciNet) and targeted interventions against enteric viruses (antiviral, vaccine). 2012.
16. Health NI of. Viral Gastroenteritis - National Digestive Diseases Information Clearinghouse [Internet]. 2012 [cited 2012 Jul 29];Available from: http://digestive.niddk.nih.gov/ddiseases/pubs/viralgastroenteritis/#symptoms
17. Kaplan JE, Feldman R, Campbell DS, Lookabaugh C, Gary GW. The frequency of a Norwalk-like pattern of illness in outbreaks of acute gastroenteritis. American journal of public health [Internet]. 1982 Dec [cited 2012 Jul 13];72(12):1329–32. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1650540&tool=pmcentrez&rendertype=abstract
18. Teunis, P.F.M., Moe, C.L., Liu, P., Miller, S.E., Lindesmith, L., Baric, R.S., Le Pendu, J., Calderon RL. Norwalk virus: how infectious is it? Journal of Medical Virology. 2008;(80):1468–76.
19. Atmar RL, Bernstein DI, Harro CD, Al-Ibrahim MS, Chen WH, Ferreira J, et al. Norovirus vaccine against experimental human Norwalk Virus illness. The New England journal of medicine [Internet]. 2011 Dec 8;365(23):2178–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22150036
20. Graham DY, Jiang X, Tanaka T, Opekun AR, Madore HP, Estes MK. Norwalk Virus Infection of Volunteers: New Insights Based on Improved Assays. Journal of Infectious Diseases [Internet]. 1994 Jul 1 [cited 2012 Jul 13];170(1):34–43. Available from: http://jid.oxfordjournals.org/content/170/1/34.abstract
21. Greenberg HB, Schreiber DS, Blacklow NR, Cukor G, Bedigian MKAY, Echeverria P. Immune response and prevalence of antibody to Norwalk enteritis virus as determined by Immune Response and Prevalence of Antibody to Norwalk Enteritis Virus as Determined by Radioimmunoassay. 1979;
22. Ball JM, Graham DY, Opekun AR, Gilger MA, Guerrero RA, Estes MK. Recombinant Norwalk Virus – like Particles Given Orally to. 1999;:40–8.
23. LoBue AD, Lindesmith L, Yount B, Harrington PR, Thompson JM, Johnston RE, et al. Multivalent norovirus vaccines induce strong mucosal and systemic blocking antibodies against multiple strains. Vaccine [Internet]. 2006 Jun 12 [cited 2012 Jul 13];24(24):5220–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16650512
24. Atmar RL, Bernstein DI, Harro CD, Al-Ibrahim MS, Chen WH, Ferreira J, et al. Norovirus vaccine against experimental human Norwalk Virus illness. The New England journal of medicine [Internet]. 2011 Dec 8;365(23):2178–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22150036
25. Lindesmith L, Moe C, Marionneau S, Ruvoen N, Jiang X, Lindblad L, et al. Human susceptibility and resistance to Norwalk virus infection. Nature medicine [Internet]. 2003 May [cited 2012 Jul 13];9(5):548–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12692541
26. Siebenga JJ, Beersma MFC, Vennema H, van Biezen P, Hartwig NJ, Koopmans M. High prevalence of prolonged norovirus shedding and illness among hospitalized patients: a model for in vivo molecular evolution. The Journal of infectious diseases [Internet]. 2008 Oct 1 [cited 2012 Jul 13];198(7):994–1001. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18774885
27. Okabayashi T, Yokota S-ichi, Ohkoshi Y, Ohuchi H, Yoshida Y, Kikuchi M, et al. Occurrence of norovirus infections unrelated to norovirus outbreaks in an asymptomatic food handler population. Journal of clinical microbiology [Internet]. 2008 Jun [cited 2012 Jul 13];46(6):1985–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2446833&tool=pmcentrez&rendertype=abstract
28. Aoki Y, Suto a, Mizuta K, Ahiko T, Osaka K, Matsuzaki Y. Duration of norovirus excretion and the longitudinal course of viral load in norovirus-infected elderly patients. The Journal of hospital infection [Internet]. 2010 May [cited 2012 Jul 13];75(1):42–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20304524
29. Parrino, Thomas, Schreiber, David, Trier, Jerry, Kapikian, Albert, Blacklow N. Clinical Immunity in Acute Gastroenteritis Caused By Norwalk Agent. New England Journal of Medicine. 1977;297(2):86–9.

30. Johnson PC, Mathewson JJ, DuPont HL, Greenberg HB. Multiple-Challenge Study of Host Susceptibility to Norwalk Gastroenteritis in US Adults. Journal of Infectious Diseases [Internet]. 1990 Jan 1 [cited 2012 Jul 29];161(1):18–21. Available from: http://jid.oxfordjournals.org/content/161/1/18.abstract
31. Wyatt RG, Dolin R, Blacklow NR, DuPont HL, Buscho RF, Thornhill TS, et al. Comparison of three agents of acute infectious nonbacterial gastroenteritis by cross-challenge in volunteers. The Journal of infectious diseases [Internet]. 1974 Jun;129(6):709–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4209723
32. Lindesmith L, Moe C, Lependu J, Frelinger JA, Treanor J, Baric RS. Cellular and Humoral Immunity following Snow Mountain Virus Challenge. 2005;79(5):2900–9.
33. Atmar RL, Opekun AR, Gilger M a, Estes MK, Crawford SE, Neill FH, et al. Norwalk virus shedding after experimental human infection. Emerging infectious diseases [Internet]. 2008 Oct [cited 2012 Jul 13];14(10):1553–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2609865&tool=pmcentrez&rendertype=abstract
34. Phillips G, Tam CC, Conti S, Rodrigues LC, Brown D, Iturriza-Gomara M, et al. Community incidence of norovirus-associated infectious intestinal disease in England: improved estimates using viral load for norovirus diagnosis. American journal of epidemiology [Internet]. 2010 May 1 [cited 2012 Jul 13];171(9):1014–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20360244
35. Cubitt, WD, McSwiggan, DA, Moore W. Winter vomiting disease caused by calcivirus [Internet]. [cited 2012 Jul 29];Available from: http://jcp.bmj.com/content/32/8/786.full.pdf
36. Mossong J, Hens N, Jit M, Beutels P, Auranen K, Mikolajczyk R, et al. Social contacts and mixing patterns relevant to the spread of infectious diseases. PLoS medicine [Internet]. 2008 Mar 25 [cited 2012 Jul 13];5(3):e74. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2270306&tool=pmcentrez&rendertype=abstract
37. Lindell AT, Grillner L, Svensson L, Wirgart BZ. Molecular Epidemiology of Norovirus Infections in Stockholm , Sweden , during the Years 2000 to 2003 : Association of the GGIIb Genetic Cluster with Infection in Children Molecular Epidemiology of Norovirus Infections in Stockholm , Sweden , during the Ye. 2005;
38. Dolin, R, Blacklow, NR, DuPont, H, Formal, S, Buscho, RF, Kasel J. Transmission of acute infectious nonbacterial gastroenteritis to volunteers by oral administration of stool filtrates. Journal of Infectious Diseases. 1971;(123):307–12.
39. Hutson AM, Atmar RL, Estes MK. Norovirus disease: changing epidemiology and host susceptibility factors. Trends in microbiology [Internet]. 2004 Jun [cited 2012 Jul 13];12(6):279–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15165606
40. Leon JS, Kingsley DH, Montes JS, Richards GP, Lyon GM, Abdulhafid GM, et al. Randomized, double-blinded clinical trial for human norovirus inactivation in oysters by high hydrostatic pressure processing. Applied and environmental microbiology [Internet]. 2011 Aug 1 [cited 2012 Jul 23];77(15):5476–82. Available from: http://aem.asm.org/cgi/content/abstract/77/15/5476
41. González G, Liprandi F, Ludert JE. Molecular Epidemiology of Enteric Viruses in Children With Sporadic Gastroenteritis in. 2011;1982(June):1972–82.
42. Tate JE, Panozzo C a, Payne DC, Patel MM, Cortese MM, Fowlkes AL, et al. Decline and change in seasonality of US rotavirus activity after the introduction of rotavirus vaccine. Pediatrics [Internet]. 2009 Aug [cited 2012 Jul 13];124(2):465–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19581260