REVIEW 10.1111/1469-0691.12746

Evolution of norovirus

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Abstract

Norovirus (NoV) is now the dominant aetiological agent of acute gastroenteritis, and, with the recent introduction of rotavirus vaccines in many countries, this is likely to remain the case. NoV has a significant impact on human wellbeing in terms of morbidity, economic costs and mortality in developing countries. NoVs are divided into six genogroups (GI–GVI), but only GI, GII and GIV are known to infect humans, with GII being the most prevalent, causing >95% of human infections. The immune system is thought to drive selection of emerging pandemic NoVs through both antigenic drift and shift. This phenomenon results in the replacement of dominant circulating viruses approximately every 3 years, with new variants able to re-infect hosts previously infected with earlier viruses. This review explores the evolutionary aspects of contemporary NoVs.

Keywords: Antigenic drift, gastroenteritis, norovirus, recombination
Article published online: 1 July 2014
Clin Microbiol Infect 2014; **20**: 741–745

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Introduction

In 1929, hyperemesis hemis or winter-vomiting disease was first documented by Zahorsky [1] as an illness characterized by sudden-onset vomiting and diarrhoea, the prevalence of which peaked during the winter seasons. Many years later, we now know that this illness was caused by a single-stranded RNA virus, which is now termed norovirus (NoV). NoV is now estimated to cause half of all cases of gastroenteritis globally [2].

Pandemic forms of NoV cause substantial morbidity [3–5], mortality [2] and economic costs [6,7] across the globe each year. The human immune system drives selection and antigenic change in NoV, resulting in replacement of circulating dominant viruses every 2–3 years, with new variants able to re-infect hosts immune to earlier viruses. This pattern of displacement is likely to continue with a rising incidence of NoV infections worldwide [8,9].

Recently, the identification of a new genogroup II, genotype 4 (GII.4) pandemic NoV in 2012 was soon followed by a global pandemic that started around November 2012, and affected >1.2 million people in the UK that winter alone and many millions more across the globe [10]. For the first time, a novel GII.4 variant (Sydney 2012) was identified prior to it causing a pandemic of gastroenteritis, leading to global awareness, which triggered preventive strategies in institutional settings [10]. In this review, the genetic mechanisms and immunological factors underlying patterns of NoV evolution are explored.

NoV is highly infectious [11] and is transmitted primarily from person to person within closed settings such as schools, cruise ships, hospitals, child-care facilities, and institutions for the elderly [12,13]. The clinical symptoms of NoV include acute onset of nausea, vomiting, headaches, chills, abdominal cramps, and loose or watery diarrhoea [14], usually lasting for 2–4 days [15]. Viral shedding lasts for several weeks beyond the symptomatic phase, with a decline in the number of viruses excreted [16,17]. The morbidity and mortality rates of NoV infection are high in groups such as young children, the elderly, and immunosuppressed and immunocompromised patients [8,18,19]. Outbreaks of viral gastroenteritis are difficult to control, and lead to considerable economic costs, owing to closure of hospital wards, facilities, and businesses,

including the food retail, oyster and cruise ship industries [6,7].

NoV is a member of the family *Caliciviridae*, and as such is a small, round virion 28–35 nm in diameter. It possesses a single-stranded, positive-sense, polyadenylated RNA genome of *c.* 7.5 kb divided into three open reading frames (ORFs). ORF1, which makes up the first two-thirds of the genome, and is >5 kb in length, encodes seven non-structural (NS) proteins involved in replication of the genome. These include an N-terminal protein (NS1-2 of unknown function), an NTPase (NS3), p22 of unknown function (NS4), a viral genome-linked protein (NS5), a 3C-like protease (NS6), and an RNA-dependent RNA polymerase (NS7) (55, 127) [15]. Two structural proteins—VP1, the major capsid protein, and VP2, whose function is uncertain—are encoded by ORF2 and ORF3, respectively. NoVs are divided into six genogroups (GI–GVI), but only GI, GII and GIV are known to infect humans, with GII being the most prevalent (>95% of NoV infections). Genogroups are further classified into numerous capsid genotypes differing at the nucleotide level by *c.* 15%. The term 'variant' is only used for individual viruses within the pandemic GII.4 lineages [20].

Molecular Epidemiology of NoVs

Although >32 different human NoV genotypes have been identified [20], GII.4 is the only genotype associated with global pandemics of gastroenteritis, and viruses belonging to this genetic lineage account for >80% of all human NoV infections at any one time [8,9]. Owing to the lack of an *ex vivo* cultivation system for human NoV, it has been extremely difficult to determine why GII.4 viruses are so successful, and many aspects of the NoV replication cycle and evolution remain unknown. What gives GII.4 viruses such epidemiological potency is currently an active research area within the field [21]. GII.4 viruses have caused all six major NoV pandemics of acute gastroenteritis in the last two decades. These six pandemic GII.4 variants include US 96, which caused a pandemic in the late 1990s [22,23], Farmington Hills 2002 [24,25], Hunter 2004 [26], Den Haag 2006b [9,27], New Orleans 2009 [28] and, most recently, Sydney 2012 [29,30]. Of the six pandemic GII.4 variants, the first four probably evolved from previous GII.4 viruses through mutations within the protruding (P) domain of the capsid (antigenic drift) [31–33]. However, interestingly, the most recent two GII.4 pandemic variants, New Orleans 2009 and Sydney 2012, show both antigenic capsid variation and GII.4 intragenotype recombination at the ORF1–ORF2 overlap, and have therefore evolved through processes involving both antigenic drift and antigenic shift [30].

Importantly, the emergence of novel NoV GII.4 variants coincided with dramatic increases in the rate of NoV infection and epidemics of gastroenteritis across the globe. This was exemplified by the current pandemic GII.4 variant, Sydney 2012 [10]. Sydney 2012 was first identified in March 2012 in Australia [30], and by August 2012 it was responsible for *c.* 25% of NoV-associated acute gastroenteritis outbreaks in Australia [29]. However, between November 2012 and January 2013, it rapidly and simultaneously replaced the previously dominant GII.4 NoV, New Orleans 2009, in Australia and New Zealand [29], Asia [34], Europe [10,35,36], Canada [37], and the USA [38], leading to an increase in the number of outbreaks of gastroenteritis across the globe. Sydney 2012 is currently the prevalent NoV in the world, although, on the basis of previous evolutionary patterns of GII.4 emergence, we would expect this situation to last for only another 1–2 years before it is replaced by another GII.4 variant.

Pre-epidemic forms of NoV GII.4 Variants

Interestingly, studies have revealed that the pre-epidemic forms of New Orleans 2009 (Orange 2008, GQ845367) and Sydney 2012 (Auckland 2010, KF060124) were in circulation for up to 2 years prior to their global epidemic spread [27,29,37,39]. Specifically, pre-epidemic Sydney 2012 viruses were identified in New Zealand in 2010 [29], and in Canada [37] and Italy [39] in 2011. Notably, these pre-epidemic viruses were associated with limited outbreaks, and did not have a global prevalence. Analysis of the key P2 antigenic epitopes A–E of Sydney 2012 revealed that several changes were required before the pre-epidemic form achieved pandemic potential [29]. This suggests that novel GII.4 variants circulate at low levels in the population before acquiring the necessary P2 mutations to escape herd immunity. Such mutations probably facilitate their emergence as a pandemic viruses. This implies that the newly emergent GII.4 variants could be monitored and detected long before they evolve into new variants that have escaped herd immunity. Vaccines could therefore be produced before a pandemic of acute gastroenteritis arises.

Antigenic Drift Results in New pandemic GII.4 NoV Variants

A number of mechanisms drive the evolution of the GII.4 lineage [21]. GII.4 viruses are thought to be able to bind to a wider range of histo-blood group antigens, which are