Role of noroviruses as aetiological agents of diarrhoea in developing countries

James Ayukepi Ayukekbong,1,2 Henry Nzike Mesumbe,2 Olufunmilayo G. Oyero,3 Magnus Lindh1 and Tomas Bergström1

Correspondence James Ayukepi Ayukekbong jayuk@redeem-biomedical.com

1Department of Infectious Diseases/Section of Clinical Virology, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden
2Section for Clinical Research, Redeem Biomedical System, Buea, Cameroon
3Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Nigeria

Diarrhoea is considered to be the second leading cause of death due to infections among children <5 years of age worldwide that may be caused by bacteria, parasites, viruses and non-infectious agents. The major causative agents of diarrhoea in developing countries may vary from those in developed countries. Noroviruses are considered to be the most common cause of acute diarrhoea in both children and adults in industrialized countries. On the other hand, there is a lack of comprehensive epidemiological evidence from developing countries that norovirus is a major cause of diarrhoea. In these regions, asymptomatic norovirus infections are very common, and similar detection rates have been observed in patients with diarrhoea and asymptomatic persons. This review summarizes the current knowledge of norovirus infection in developing countries and seeks to position infections with noroviruses among those of other enteropathogens in terms of disease burden in these regions.

Introduction

Noroviruses are considered to be the most common cause of acute non-bacterial gastroenteritis in both children and adults in industrialized countries (Koopmans et al., 2002; Mattison, 2011; Scallan et al., 2011). In developing countries, rotavirus is the main viral agent often associated with infantile diarrhoea (Kabayiza et al., 2014a; Reither et al., 2007). However, in countries where universal rotavirus vaccination has been implemented, noroviruses have become most prevalent in children admitted to hospital with acute gastroenteritis (Bucardo et al., 2014; Hemming et al., 2013) Numerous studies have shown that asymptomatic norovirus infections are common within different populations (Garcia et al., 2006; Gray et al., 1993; Menon et al., 2013). This renders clinical judgement on its contribution to diarrhoeal disease difficult. Sources of contamination include shellfish grown in polluted waters, food crops grown in land irrigated with wastewater or fertilized with sewage, sewage contaminated drinking or recreational waters (Bosch, 1998; Li et al., 2012; Okoh et al., 2010). Transmission is predominantly faecal–oral, and humans may be exposed to noroviruses through various routes, principally through contaminated food, water, fomites, utensils and person–person contact (Boone & Gerba, 2007; Bosch et al., 2008; Koopmans & Duizer, 2004). Most wastewater treatment systems, even when properly functioning, are unable to guarantee norovirus-free effluent discharge, allowing a significant viral load to be released into the environment (Okoh et al., 2010).

A large diversity of norovirus strains has been described from a European outbreak of gastroenteritis linked to transmission by drinking water, suggestive of contamination of the water source by human faeces after heavy rain (Nenonen et al., 2012). Noroviruses of an extensive genomic and antigenic diversity co-circulate in the human population (Bruggink et al., 2015). In Africa, there is evidence of a rainy season peak of norovirus infection and water is increasingly recognized to play a role in the transmission (Ayukekbong et al., 2013), although documentation of water-borne outbreaks of gastroenteritis is scarce in that continent. This review seeks to extend knowledge on the epidemiology and the burden of norovirus infection in developing countries and seeks to position infections with noroviruses among those of other enteropathogens in terms of disease burden in these regions.

Abbreviations: HBGA, histo-blood group antigen; VLP, virus-like particle.
and the later part attempts to assess the role of noroviruses in the etiology of gastroenteritis in developing countries.

**Classification, genomic organization and genotypes of norovirus**

Noroviruses belong to the family *Caliciviridae*, and are small non-enveloped, icosahedral viruses with a diameter of approximately 38 nm (Xi *et al.*, 1990). The genome consists of a positive-sense ssRNA of approximately 7.5 kb, organized into three ORFs (McFadden *et al.*, 2011; Thorne *et al.*, 2012) (Fig. 1). ORF1 constitutes the first two-thirds of the genome (approximately 5 kb) and encodes a 200 kDa polyprotein that is cleaved by the viral 3C-like protease (3CLpro) into at least six proteins. The coding order in ORF1 proceeds from the N to the C terminus to express p48, NTPase, p22, VPg, 3CLpro and RdRp (Fig. 1). The viral protein VPg is a cap substitute that recruits the translation initiation machinery (Daughenbaugh *et al.*, 2003; Thorne & Goodfellow, 2014). The non-structural proteins encoded by ORF1 are involved in replication of the virus (Hardy, 2005). ORF2 is approximately 1.8 kb and encodes the 57 kDa major capsid protein, VP1, while ORF3 is approximately 0.6 kb and encodes a 33 kDa minor structural protein, VP2 (McFadden *et al.*, 2011). VP1 is suggested to be involved in the recognition of the host cell receptor (Tan & Jiang, 2010) while VP2 is thought to be involved in the stability of the capsid but also seems to be vital for viral assembly (Glass *et al.*, 2009). Recent studies have shown that murine norovirus has an additional ORF, ORF4, which overlaps ORF2 in an alternate reading frame (Thackray *et al.*, 2007). It has been shown that ORF4 encodes virulence factor 1 (VF1), a mitochondrially localized novel innate immune regulator (McFadden *et al.*, 2011). The 3’ end of the genome contains a polyA tail (Karst *et al.*, 2014; Xi *et al.*, 1990).

The genus *Norovirus* is composed of at least 41 genotypes, which are classified into six established genogroups (GI–GVI) and an additional proposed tentative genogroup, GVII, on the basis of sequence similarity (Tse *et al.*, 2012; Vinjé, 2015). Genogroups GI, GII and GIV primarily infect humans (Zheng *et al.*, 2006). Multiple porcine noroviruses belong to GII (Sugieda *et al.*, 1998; Wang *et al.*, 2005, 2007) while bovine and murine noroviruses are classified into genogroups GIII and GV, respectively (Hsu *et al.*, 2007; Liu *et al.*, 1999). Canine noroviruses are grouped within genogroups GIV, GVI and GVII (Fig. 2). Each genogroup is further divided into genotypes or genocenters on the basis of pairwise sequence comparisons (Hoffmann *et al.*, 2013; Zheng *et al.*, 2006). In all, the majority of known norovirus genotypes infect humans. The GI genogroup is currently divided into nine genotypes, GIV and GVI each contain two genotypes and GII contains at least 22 different genotypes. More recent phylogenetic analysis suggest that GII.15 may need to be reclassified as a separate genogroup (Fig. 2), but this awaits consensus approval from the international norovirus working group (Kroneman *et al.*, 2013). Genogroups GIII and GV also contain three and two genotypes respectively (Vinjé, 2015). Norovirus genogroups and genotypes are designated numerically, with the genogroup indicated first as a roman numeral followed by the genotype as an Arabic numeral. For example, the prototype norovirus, which is genogroup GI and genotype 1, is designated GI.1. Due to frequent recombination events, norovirus classification can be carried out properly only by whole genome sequencing (Eden *et al.*, 2013; Ruether *et al.*, 2014; Sang *et al.*, 2014). It has been shown that noroviral strains within a genogroup share 69–97% nucleotide similarity, while strains in different genogroups are only 51–56% similar (Kojima *et al.*, 2002). Although GI.1 is the prototypical norovirus strain, GII.4 noroviruses are responsible for more than 70% of norovirus outbreaks worldwide (Fankhauser *et al.*, 2002; Tu *et al.*, 2007; Verhaelen *et al.*, 2012; Widdowson *et al.*, 2004).

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**Fig. 1.** Schematic presentation of the norovirus genome. The genome encodes three ORFs. ORF1 encodes the non-structural proteins (light blue). The coding order in ORF1 proceeds from the N to the C terminus as follows: p48 (an N-terminal protein), NTP (nucleoside triphosphatase), p22 (a 22 kDa 3A-like protein), VPg (viral genome-linked protein), pro (protease) and pol (RNA-dependent RNA polymerase). ORF2 encodes VP1 ‘viral protein 1’, the major capsid protein’ (green), ORF3 encodes VP2, the minor capsid protein (brown). VP1 is further divided into the shell (purple), the P1 subdomain (blue) and the P2 subdomain (red). A flexible hinge region lies between the shell and P1 (yellow).
Diarrhoeal disease and noroviruses

Diarrhoea is considered to be the second leading cause of death due to infections among children <5 years of age worldwide and it is the major disease manifestation caused by most enteric viruses including noroviruses. The disease is usually self-limiting in otherwise healthy persons but can be life threatening in children, the elderly, malnourished and in those with an impaired immune system (Clark & McKendrick, 2004; Guerrant et al., 1990; Gustavsson et al., 2011). Diarrhoea is a manifestation of intestinal dysfunction that consists of frequent watery stool, resulting in loss of water, electrolytes and nutrients. With the exception of breastfed infants, the most commonly used definition of diarrhoea is three or more loose stools within a day (O’Ryan et al., 2005). According to the World Health Organization, there are 1.7 billion annual episodes of diarrhoea and 760 000 annual deaths due to diarrhoea among children <5 years of age. More than 40% of these deaths occur in Africa (Boschi-Pinto et al., 2008; Bryce et al., 2005; Kosek et al., 2003). The risk of contracting diarrhoeal diseases has been estimated to be higher in developing countries compared with developed countries, partly due to unsafe water supply and suboptimal sanitation and hygienic conditions (Nasrin et al., 2013). Diarrhoeal disease due to an enteropathogen may be caused by bacteria, parasites or viruses, and the frequency of each enteropathogen may vary from one region to another. In developing countries, noroviruses are listed among causes of diarrhoea, but the relative contribution to the total number of cases seems to be diluted in lieu of more prevalent diarrhoea-causing enteropathogens.

Asymptomatic norovirus infection

Noroviruses have often been detected in persons with no signs or symptoms of gastroenteritis. In these cases, four possible reasons may be listed: pre-symptomatic shedding, post-symptomatic shedding, lack of susceptibility to symptomatic infection or immunity. Pre-symptomatic shedding is
the detection of norovirus RNA in faeces prior to onset of symptoms. There is a report of norovirus detection a day before the presence of symptoms (Goller et al., 2004). Post-symptomatic shedding is the detection of norovirus RNA after resolution of symptoms. Post-symptomatic excretion has been described in children and the elderly for several weeks or months (Siebenga et al., 2008; Tu et al., 2008). Lack of susceptibility to symptomatic infection may relate to polymorphism of histo-blood group antigens (HBGAs) (Le Pendu et al., 2006) and immunity (Parrino et al., 1977). HBGAs have been suggested to serve as cellular receptors for norovirus attachment (Choi et al., 2008; Huang et al., 2003).

We and other investigators have detected noroviruses in persons without gastroenteritis in prospective longitudinal studies, suggesting that asymptomatic infection may be relatively common (Ayukekbong et al., 2013; Bucardo et al., 2010; Zhang et al., 2011). The length of observation in these studies suggests that asymptomatic infection is not necessarily occurring as pre- or post-symptomatic shedding of the virus. Instead, the high prevalence of noroviruses, of many different genotypes, found prospectively among healthy persons most likely reflects asymptomatic infections in persons who are often exposed to the virus, from other persons or from environmental sources (e.g. water, treated and untreated sewage), which may contain significant concentrations of noroviruses (Bosch, 1998). Such exposure may induce mucosal immunity with secretory immunoglobulin (IgA) antibodies that control replication and spread in the mucosa without necessarily inducing degradation of the virus (Blutt & Conner, 2013; Levine & Robins-Browne, 2012). Thus, clinical disease may be precluded, while still allowing asymptomatic infection (Agus et al., 1974). In such cases, shedding of the virus appears to be restricted, as suggested by the lower concentration of norovirus RNA in asymptomatic infection compared with symptomatic infections (Elfving et al., 2014; Phillips et al., 2009). Also, differences in the composition of intestinal bacterial flora may influence susceptibility to norovirus infection. The microbial flora might promote norovirus and other enteric virus infection (Jones et al., 2014; Kuss et al., 2011; Virgin, 2014). These hypotheses constitute emerging fields requiring comprehensive metagenomic analyses of faecal materials from inhabitants in both low and high income countries to characterize the composition of such human ‘virobiota’ and microbiota in order to improve our understanding of their role in health and disease.

Factors associated with norovirus disease

Immunity and genetic predisposition to norovirus infection. Most of the information regarding immunity to norovirus infections has been based on volunteer challenge studies and outbreak investigations. The nature of immunity constitutes a key determinant in efforts towards designing and delivery of a vaccine (Bartsch et al., 2012). An early study on immunity to norovirus infection suggested that individuals with high serum or faecal antibody titres to norovirus before challenge were more likely to become infected with the virus than individuals with low pre-existing antibody titres, suggesting that antibodies against norovirus may not confer protection (Gray et al., 1994; Johnson et al., 1990). Most of the volunteers were resistant to subsequent infection with the same virus 6 months later, suggesting short-term immunity (Johnson et al., 1990). However, conclusions from studies regarding long-term immunity have been difficult to establish. One study demonstrated that individuals infected with GI.1 Norwalk virus were all symptomatically reinfected 27–42 months later (Parrino et al., 1977). Through modelling, Simmons et al. (2013) have suggested a mean duration of immunity of between 4–8 years. The absence of long-term immunity may be confounded by pre-exposure to a large number of circulating norovirus strains. However, a major concern with most challenge studies is that the dose of virus given to volunteers was often several fold greater than the infectious dose (estimated to be approximately 18–1000 virus particles) (Teunis et al., 2008). Dose exposure in the community is likely to be smaller and the evoked immune response might be more robust and broadly protective (Atmar et al., 2015; Teunis et al., 2008). Results from a cross-challenge study of volunteers infected with GI.1 (Norwalk) who were still susceptible to subsequent infection with GII.1 (Hawaii) suggest that immunity to one strain does not confer cross-genogroup protection against infection with another strain (Wyatt et al., 1974). Moreover, studies of cross-reactive immunity have shown that antibodies can recognize heterologous norovirus antigens within a genogroup (Rockx et al., 2005a). However, while homotypic antibodies from human antisera following infection can block virus-like particle (VLP) binding to synthetic HBGAs, heterotypic antisera to strains within the same genogroup are less likely to block this binding (Rockx et al., 2005b).

On the other hand, asymptomatic norovirus infection has been estimated to account for 15–40% of norovirus infections among participants in several studies (Ayukekbong et al., 2011; Gallimore et al., 2004; Garcia et al., 2006; Rockx et al., 2002). It is presently well established that HBGAs, and in particular secretor status controlled by the α1,2-fucosyltransferase FUT2 gene, determine susceptibility to most norovirus infections (Lindeas et al., 2003; Ruvonen & Le Pendu, 2013; Tan et al., 2008; Thorven et al., 2005). HBGAs are complex carbohydrates on red blood cells, saliva, mucosal epithelia and other body secretions, and are classified into the ABO, secretor and Lewis types (Tan & Jiang, 2011). In industrialized countries, secretors make up about 80% of the population while non-secretors make up 20%, and this observation may support the notion that the norovirus attack rate during outbreaks is seldom more than 80% in industrialized countries (Le Pendu et al., 2006). To date, there is increasing evidence that non-secretors may be attacked by some norovirus strains (Nordgren et al., 2010) and that the
common G428A mutation in the FUT2 gene does not provide absolute protection against symptomatic GI.4 norovirus infection (Carlsson et al., 2009; Rydell et al., 2011). However, this secretor/non-secretor ratio is different from one population to another, and it has been shown that the distribution of Lewis antigens is ethnicity specific (Cakir et al., 2002). Polymorphisms in the HBGAs are associated with resistance in norovirus infection (Liu et al., 1998). Several of these polymorphisms have been reported in individuals from tropical countries, compared with those from developed countries, who have mostly the G428A mutation (Corvelo et al., 2002).

Hygiene and environmental factors

In most developing countries, especially those in Africa, the number of people living in a household is usually higher compared with those in industrialized countries and the water and environmental sanitation in these regions is less than optimal (Colombatti et al., 2009; Cronin et al., 2009; Montgomery & Elimelech, 2007; Nkwan, 2009). Poor environmental and water hygiene increase the persistence of norovirus in the environment (Mans et al., 2013; Nakamura et al., 2009; Seitz et al., 2011; Ueki et al., 2005). Thus, large household size might increase the circulation of norovirus and induce and maintain immunity, thereby reducing the risk of developing symptomatic norovirus infection. In a recent study, the risk of norovirus infection was surprisingly higher among residents in a community who exclusively utilized tap water than those who utilized water from borehole wells which were more likely to be contaminated by environmental waste and sewage (Ayukkabong et al., 2013). These findings suggest that immunity plays an essential role in the susceptibility of norovirus infection. The hygiene hypothesis on enteric disease has previously been observed in the late 19th-century outbreak of polio in the USA and Scandinavia among older children. In this pre-epidemic era, enteric infections were so ubiquitous that most infants were infected within 6–12 months, a period that coincided with the passive acquisition of antibodies from their nursing mothers. Although the serum antibodies did not prevent enteric infection, they were sufficient to preclude viraemia, thereby preventing invasion of the central nervous system and subsequent paralysis. However, with the advent of improved personal hygiene and public sanitation, the transmission of enteric infections (including polio) was delayed so that some infants were first infected after 12 months of age, when levels of passive antibodies had waned, resulting in paralysis later in life (Nathanson & Kew, 2010; Nathanson & Martin, 1979).

Similarly, if environmental presence of norovirus is indeed the reservoir of natural exposure of the population to the virus in developing countries, then improved hygiene measures might paradoxically lead to a transient increase of symptomatic disease caused by noroviruses, as the proportion of young immune people may decrease. This possibility is not an argument against improvement of water and food hygiene, but a factor that should be considered in the struggle for improved public health in developing countries. Taken together, host genetics, immunity, hygiene and environmental conditions may influence the overall burden of norovirus-associated disease in developing countries.

Transmission, pathophysiology and shedding of norovirus

Based on a literature review, norovirus infection was suggested to be the main cause of non-bacterial gastroenteritis in developed countries (Patel et al., 2008). The Centers for Disease Control and Prevention has estimated that noroviruses may be responsible for about 19–21 million cases per year in the USA (Hall et al., 2013). Outbreaks typically occur in places with close living conditions, such as hospital wards, day-care centres, schools, cruise ships, restaurants and homes for the elderly (Fig. 3a) (Centers for Disease & Prevention, 2009; Friesema et al., 2009; Godoy et al., 2009; Hoffmann et al., 2013; Mattner et al., 2006). Transmission is primarily via the faecal–oral route, contaminated food or water and person–person transmission via airborne droplets (Arvelo et al., 2012; Chapman et al., 2011; Malek et al., 2009) (Fig. 3b). Waterborne transmission may occur through contamination of drinking or recreational water by wastewater (Hewitt et al., 2007; Maunula et al., 2005) and constitutes a significant mode of transmission of norovirus genogroup GI while norovirus GII is mostly spread by human-to-human transmission or foodborne (Lysén et al., 2009; Mattison, 2011). There have also been reports of the detection of norovirus

Fig. 3. Norovirus outbreak settings (a) and sources (b) based on data collected by the Centers for Disease Control and Prevention on 232 norovirus outbreaks from July 1997 to June 2000. Reproduced with permission from Karst (2010).
from berries irrigated with contaminated water (Maunula et al., 2013). Usually, the infection is rapidly controlled by the immune response and symptoms subside within 2–3 days (Glass et al., 2009; Koopmans et al., 2002). However, severe forms of the disease may occur in young children, the elderly, malnourished and immune-compromised individuals (Murata et al., 2007; Tu et al., 2008). The availability of both cell culture and small animal models has the potential to improve our understanding of norovirus infections (Jones et al., 2014; Taube et al., 2013). Histological analysis of proximal intestinal biopsy samples from volunteers who became ill after administration of either GI.1 (Norwalk) or GI.1 (Hawaii) showed crypt cell hyperplasia (Schreiber et al., 1973) and mild inflammatory infiltration into the lamina propria (Dolin et al., 1975). Another study suggests that noroviruses may cause apoptosis of enterocytes in humans (Troeger et al., 2009). Symptoms include vomiting, diarrhoea, nausea and abdominal cramps. Low grade fever and malaise can also develop (Kaplan et al., 1982).

While norovirus infection typically causes an acute bout of gastroenteritis that resolves within days of onset of disease, the course of infection and viral shedding is more complex. Viral shedding has been detected for several weeks post-resolution of symptoms (Siebenga et al., 2008; Tu et al., 2008). Furthermore, noroviruses have been detected in infected individuals with no symptoms at all (Ayukekbong et al., 2008; Gallimore et al., 2004; Levine & Robins-Browne, 2012). These findings suggest efficient means for transmission of these viruses to large populations. Other factors such as extreme stability of the virus particle outside the body, resistance to common disinfectants, low infectious dose, infectivity of all age groups and efficient spread from human to human have all contributed to their classification as Category B biodefence agents (Estes et al., 2006; Murata et al., 2007).

Detection and molecular characterization of noroviruses

Previously, diagnosis of human noroviruses was hampered by the inability to cultivate these viruses on cell lines (Papafragkou et al., 2014). Although a recent intriguing report demonstrates norovirus growth in a human B-cell line after inoculation of unfiltered faeces (Jones et al., 2014), this method awaits clinical applications. Routine diagnosis is based on detection of norovirus RNA by reverse transcription-PCR (RT-PCR) or of norovirus antigen by enzyme immunoassays (EIAs) (Kageyama et al., 2003; Pazdiora et al., 2009). The RT-PCR method is gaining recognition as the preferred laboratory method. Advantages of the RT-PCR technique are the ability to quantify the viral load and to detect noroviruses in a broad range of specimens such as stool, vomitus, water, food and environmental samples (Koopmans et al., 2002; Mattison, 2011; Riera-Montes et al., 2011). Furthermore, genotyping of norovirus strains can be achieved by a dual-nomenclature system that involves sequencing parts of the genome, usually both the RNA polymerase (POL) region in ORF1 and VP1 sequences (Kroneman et al., 2013). Recently, the development of a simple rapid immunochromatographic lateral flow assay has been suggested as an attractive alternative to both RT-PCR and EIAs. This test does not require specialized laboratory equipment and can be performed in less than 15 min. Although highly specific, this assay has a low sensitivity (35–52%) and is genogroup dependent (Ambert-Balay & Pothier, 2013).

Epidemiology and seasonality of norovirus infection

Factors such as the stability of the virus in the environment, low infectious dose, multiple routes of transmission and lack of long-lasting immunity contribute to the high impact of norovirus outbreaks (Lopman et al., 2003a). Norovirus epidemiology differs from temperate to tropical regions; however, outbreaks typically occur in facilities such as restaurants, hospitals, nursing homes, cruise ships, schools and day-care centres in both regions (Chapman et al., 2011; Hoffmann et al., 2013).

It is estimated that each year, norovirus causes 900 000 clinic visits among children in industrialized countries (Patel et al., 2009). In developing countries, norovirus was estimated, based on a review of a limited number of studies, to cause a striking number of over 200 000 deaths among children aged <5 years (Patel et al., 2009). In a recent review of 175 articles involving a cumulative 187 336 patients with acute gastroenteritis, a pooled norovirus prevalence of 18% was reported (Ahmed et al., 2014). These estimates, which need to be validated by controlled studies in low-income countries, highlight the likely impact of norovirus infection in the global population (Desselberger & Goodfellow, 2014).

In temperate regions of the northern hemisphere, outbreaks are particularly common during the winter season (Fig. 4a) (McSwiggan et al., 1978; Mounts et al., 2000), although summer time peaks have been observed as well (Lopman et al., 2003b). The reason for the increase in norovirus outbreaks in the winter is unclear, but several factors have been suggested including crowding and climate (indoor and outdoor humidity) (Rohayem, 2009). In the tropics, an increase in norovirus infection has been observed in the rainy season (Fig. 4b) (Ayukekbong et al., 2013). Differences in seasonal prevalence of specific pathogens and epidemic curves need to be taken into consideration before suggesting prevalent pathogens causing diarrhoea in different settings.

In recent years, GI.4 has been the predominant norovirus genotype, causing up to 70% of reported norovirus outbreaks (Ramirez et al., 2009; Sdiri-Loulizi et al., 2009; Siebenga et al., 2009). The reason why GI.4 strains dominate is still unclear, but it is suggested that it could be due to one or a combination of several factors: high viral infection rates among young children, higher sensitivity of GI.4 strains to enzyme immunoassays and lateral flow assays.
Noroviruses and diarrhoea in developing countries

Fig. 4. (a) Seasonal distribution of cases of diarrhoea caused by norovirus in temperate regions. Reproduced with permission from Mounts et al. (2000). (b) Seasonal distribution of norovirus infection in Cameroon. RH represents relative humidity. NoV represents norovirus. Reproduced with permission from Ayukekpong et al. (2014a).
sheding in infected individuals (Bucardo et al., 2008), a more diverse receptor specificity than any other norovirus genotype (Huang et al., 2005), high mutation frequency (Bull et al., 2010), genetic drift (Lindesmith et al., 2008), recombination (Eden et al., 2013) and absence of long-term immunity which is present for other norovirus genotypes such as GI.3 (Bull & White, 2011; Siebenga et al., 2009). The appearance of new variants in cycles of 2–7 years with mutations that result in modification of antigenic epitopes has resulted in increased affinity of novel strains of the genotype to recognize supplementary glycan patterns. These novel properties allowed new GI.4 variants to spread easily within the population (Ruvoën-Clouet et al., 2013).

Prevention, control and treatment of norovirus infection

Common norovirus infection preventive measures may include hand washing, avoiding contaminated food and water and thorough washing of vegetables before consumption. Such good hygiene preventive measures are of major importance to prevent person-to-person transmission of noroviruses (Koopmans & Duizer, 2004).

The main focus of treatment is to rehydrate the patient by encouraging drinking of plenty of water or oral rehydration solutions with 75 mmol l⁻¹ each of sodium and glucose, to compensate for fluid and electrolyte losses (Hahn et al., 2001). Numerous antivirals with efficacy in cell culture have now been identified but further studies in this area are required in order to make these suitable for clinical use (Arias et al., 2013).

Recombinant VLPs have been shown to self-assemble from capsid proteins, which are generated using baculovirus- or plant-based expression systems (Mason et al., 1996; Prasad et al., 1994). Norovirus VLPs induce both mucosal and systemic immune responses when delivered intranasally and orally (Fang et al., 2013; Xia et al., 2007). These VLPs are immunogenic and partially protect from norovirus gastroenteritis (Bernstein et al., 2015).

Major causative agents of diarrhoea in developing countries

In a large prospective case–control study, involving 9439 children with moderate to severe diarrhoea and 13 129 control children, conducted by the global enteric multicentre study group, it was found that rotavirus, Cryptosporidium, enterotoxigenic Escherichia coli producing heat stable toxin and Shigella were the most prevalent diarrhoea-causing pathogens (Fig. 5) (Kotloff et al., 2013). This finding is consistent with results from several epidemiological studies performed in developing countries such as Burkina Faso, Nigeria, Rwanda, Bangladesh, New Caledonia and Vietnam (Albert et al., 1999; Bonkoungou et al., 2013; Germani et al., 1994; Kabayiza et al., 2014a; Nitiema et al., 2011; Ogunsanya et al., 1994; Vu Nguyen et al., 2006). In another review of enteropathogens associated with diarrhoea in developing countries, rotavirus, enteropathogenic and toxigenic E. coli were found to be the main agents among diarrhoea-causing pathogens (O’Ryan et al., 2005). The frequent presence of mixed infections renders the determination of the actual diarrhoea-causing pathogen difficult (Lara et al., 1974; Mitui et al., 2014). We reported mixed infection of five different enteric viruses in a child in Cameroon (Ayukekong et al., 2011). Norovirus is listed as a diarrhoea-causing pathogen but not a major cause of diarrhoea in these regions (Fig. 5). Meanwhile, in several case–control gastroenteritis surveillance studies, the detection of rotavirus has been statistically associated with diarrhoea (Bonkoungou et al., 2013; Kabayiza et al., 2014a, 2014b). The more general availability of norovirus RT-PCR will likely increase the frequency of detection of the main diarrhoea-causing agents.

Nosocomial norovirus infection

According to several reviews, enteric pathogens that most commonly cause hospital-associated gastroenteritis in developed countries are Clostridium difficile, rotavirus and norovirus (Ahmed et al., 2014; Bobo & Dubberke, 2010). In these health care settings, norovirus transmission occurs mostly where there are high levels of contact and potentially compromised hygiene (e.g. aerosol, person–person contact or hand transfer of the virus from contaminated fomites) (Chadwick & McCann, 1994; Chadwick et al., 1994) and the virus is likely to be introduced into these settings from the population through the hospital staff or infected patients or patient caregivers. Most importantly, these health care norovirus outbreaks are linked to diseases in the community (Iturriza-Gómez & Lopman, 2014). In another review, norovirus infections in health care settings were associated with a high attack rate of 9–78% (median 50%), with a mean duration of viral shedding among hospital patients of 19 days (range 6–92) (Harris et al., 2010). These nosocomial outbreaks are commonly reported in industrialized countries (Gastañaduy et al., 2013; Munir et al., 2014; Sukhrie et al., 2012). However, reports of nosocomial norovirus outbreaks in developing countries are limited and most studies are community based (Abugalia et al., 2011; Krumkamp et al., 2015). The wide difference in rates of reported hospital outbreaks between developed and developing countries renders the assessment of the impact of health care norovirus infection in developing countries difficult to establish.

Food and water borne norovirus infection

Most conventional methods of sewage and water treatment have proven ineffective in the complete removal and inactivation of enteric viruses, rendering water as a major source of human exposure to norovirus (Haas et al., 1993; Mathijs et al., 2012). As previously mentioned, water may be involved either directly through drinking of contaminated water, or indirectly through contamination of food with polluted water (Baert et al., 2011; Gandhi
Fig. 5. Attributable incidence of pathogen-specific moderate to severe diarrhoea per 100 child-years by age stratum. The bars show the incidence rates and the error bars show the 95% confidence intervals. Reproduced with permission from Kotloff et al. (2013).
et al., 2010; Maunula et al., 2012). Considering the ubiquitous nature of noroviruses and the suboptimal quality of drinking water in most developing countries, one would expect a high circulation of the virus in drinking water resources. A study in Ghana revealed the presence of noroviruses in ground, surface and drinking water (Gibson et al., 2011). Although noroviruses are reported to be present in water samples, studies directly linking outbreaks or sporadic cases to water or food samples are lacking in developing countries compared with developed countries where outbreaks directly linked to water (Maunula et al., 2005; Nenonen et al., 2012) and food (Lysén et al., 2009; Mattison, 2011) have been described. An interesting systematic review of norovirus outbreaks linked to shellfish consumption revealed that most reported outbreaks occurred in east Asia, Europe, America, Oceania and Australia, and were least reported in Africa (Bellou et al., 2013). It appears the scarcity of data in Africa represents underreporting rather than a lack of shellfish-associated norovirus infections.

Assessing norovirus morbidity and mortality

Norovirus infections have been associated with complications such as bowel perforation and oesophageal rupture (Pawa et al., 2009), and findings of excess mortality in the elderly (> 60 years) in community based norovirus gastroenteritis has been reported (Gustavsson et al., 2011). It was estimated that norovirus causes 8–20 deaths/1 000 000 persons, or one death every seventh reported outbreak (Hall et al., 2012; van Asten et al., 2011). In Sweden, a 30-day norovirus mortality of 7.6% was observed among elderly patients >60 years of age. Interestingly, the mortality was higher among those with other comorbidities compared with those without (Gustavsson et al., 2011). This finding raises concern on the independent role of norovirus in gastroenteritis-associated deaths. In another study, four norovirus-associated deaths among 144 elderly psychiatric patients in a long-term care institution were reported (Rondy et al., 2011). On the other hand, there is a scarcity of description of lethal cases of norovirus in developing countries, and the extent of norovirus-associated morbidity and mortality is not established. On the contrary, there are fewer symptomatic infections than in industrialized countries and this has been suggested to be due to constant circulation of norovirus in the population and better immunity (Ayukekpong et al., 2014b).

Norovirus and acute gastroenteritis in developing countries

The main health effect associated with most infections by enteric viruses including noroviruses is gastrointestinal disease. Extra-intestinal infection of norovirus is uncommon, although some norovirus infections have been associated with other clinical outcomes such as seizures in infants (Medici et al., 2010), Pneumatosis intestinalis and encephalopathy (Chan et al., 2010; Ito et al., 2006; Kim et al., 2011). In developing countries, noroviruses have been associated with childhood diarrhoea (Armah et al., 2006; Rahouma et al., 2011). However, healthy controls were unavailable in these studies to provide substantial evidence on its aetiological association. On the contrary, similar detection rates of norovirus in persons with diarrhoea and their asymptomatic controls have been reported in most studies from developing countries (Table 1). In a longitudinal follow-up study from 2011 to 2012, norovirus was detected in up to 16% of healthy persons (Ayukekpong et al., 2014a), with similar detection rates during episodes with diarrhoea as in non-diarrhoea stool samples, which is suggestive of the constant circulation of the virus in the population (Ayukekpong et al., 2014b). Although highly prevalent, the comparable detection rate in both healthy persons and diarrhoea samples makes it difficult to discern its aetiological importance in diarrhoeal disease in these settings. Hence, healthy controls should be included in studies of the role of noroviruses and other gastroenteritis-associated pathogens in developing countries.

Concluding remarks

At present, there is lack of comprehensive epidemiological evidence from developing countries implicating norovirus as a major cause of diarrhoea and even less of actual mortality. Despite reports of norovirus infection from these regions in conjunction with diarrhoea, asymptomatic infections are very common (Bucardo et al., 2010; Gallimore et al., 2004; García et al., 2006). Similar detection rates of norovirus in both diarrhoea samples and those of asymptomatic persons (Table 1) raise the question of whether asymptomatic infection of noroviruses is part of natural infection (Ayukekpong et al., 2014b; Huynen et al., 2013a). In order to make clinical judgements on the role of norovirus in the aetiology of diarrhoea, knowledge of the relative frequency with which norovirus is found in healthy persons without diarrhoea is needed. The assumption that the identification of norovirus in the faecal sample of a patient with diarrhoea indicates this to be the cause of the episode could mislead the physician. Therefore, the detection of norovirus in cases of diarrhoea in these regions needs to be interpreted with care, taking the detection rates among healthy persons into consideration. Further studies are needed to determine the baseline infection rates of a range of enteric pathogens in different populations. The common finding of noroviruses in asymptomatic children and adults in most studies in developing countries suggests that these agents are less important as causative agents of diarrhoea than suggested by studies without control groups. Accordingly, the statement appearing in many publications that ‘norovirus is the leading cause of diarrhoea in both developed and developing countries’ may be an exaggeration. Better understanding of the burden of norovirus in the aetiology of diarrhoea in developing countries is necessary for accurate intervention and public health resource allocation. More studies are needed to extend knowledge on the burden of food and water
borne norovirus infection, nosocomial outbreaks and the overall morbidity and mortality of norovirus in this region.

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**References**


**Table 1. Detection rate of norovirus among diarrhoea patients and healthy controls from randomly selected studies in developing countries**

<table>
<thead>
<tr>
<th>Country</th>
<th>Assay</th>
<th>Detection rate (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Symptomatic</td>
<td>Healthy control</td>
</tr>
<tr>
<td>Brazil</td>
<td>RT-PCR</td>
<td>40/299 (17.0)</td>
<td>12/90 (13.0)</td>
</tr>
<tr>
<td>Botswana</td>
<td>RT-PCR</td>
<td>16/74 (22.0)</td>
<td>6/26 (31.0)</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td>RT-PCR</td>
<td>62/293 (21.2)</td>
<td>31/125 (24.8)</td>
</tr>
<tr>
<td>Cameroon</td>
<td>RT-PCR</td>
<td>ND</td>
<td>17/54 (29.6)</td>
</tr>
<tr>
<td>Cameroon</td>
<td>RT-PCR</td>
<td>3/100 (3.0)</td>
<td>97/2384 (4.0)</td>
</tr>
<tr>
<td>China</td>
<td>RT-PCR</td>
<td>41/201 (20.4)</td>
<td>19/53 (35.9)</td>
</tr>
<tr>
<td>Ghana</td>
<td>RT-PCR</td>
<td>23/243 (9.5)</td>
<td>11/124 (8.9)</td>
</tr>
<tr>
<td>India</td>
<td>RT-PCR</td>
<td>ND</td>
<td>6/28 (21.4)</td>
</tr>
<tr>
<td>Malawi</td>
<td>RT-PCR</td>
<td>89/746 (11.9)</td>
<td>60/505 (11.8)</td>
</tr>
<tr>
<td>Mexico</td>
<td>RT-PCR</td>
<td>ND</td>
<td>48/161 (29.8)</td>
</tr>
<tr>
<td>NICARAGUA</td>
<td>RT-PCR</td>
<td>ND</td>
<td>19/163 (11.7)</td>
</tr>
<tr>
<td>Ruanda</td>
<td>RT-PCR GII</td>
<td>18/207 (8.7)</td>
<td>5/119 (4.2)</td>
</tr>
<tr>
<td>Venezuela</td>
<td>RT-PCR GII</td>
<td>5/17 (29.0)</td>
<td>4/21 (19.0)</td>
</tr>
</tbody>
</table>


Susceptibility to norovirus infections.


