In Focus

Aetiological importance of viruses causing acute gastroenteritis in humans

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Acute gastroenteritis (AGE) is a common illness affecting all age groups worldwide causing 2.2 million deaths annually. Viruses including rotaviruses, noroviruses, enteric adenoviruses and astroviruses are the major cause of AGE, with rotavirus causing the majority of severe illness. Advances in molecular techniques have led to the identification of many more viruses in faeces. Proving an association with AGE will require prospective controlled trials which so far are few in number.

In 2004, acute gastroenteritis (AGE) was the fifth most common cause of death in the world in 2004 causing 2.2 million deaths. In low-income countries it ranked third, causing 1.8 million deaths while in children less than five it was ranked equal first with respiratory disease, causing 17% of all deaths in that age group. An average morbidity attack rate of 3.2 episodes of diarrhoea per year per child has been reported, but in some settings in developing countries, this number can be as high as 12 episodes per year per child. Even in developed countries, most of the population will average one episode of AGE per year.

It is generally accepted that the main causes of AGE are viruses such as rotavirus, norovirus, adenoviruses types 40/41 and astroviruses, bacterial agents including Campylobacter jejuni, Salmonella, a variety of E. coli (STEC, ETEC enteropathogenic), Shigella and Vibrio cholera and numerous parasites.

Unlike the bacterial pathogens, there are no national notifiable diseases data on the prevalence of enteric viruses. Table 1 contains viral identification data from the IMVS for the period 2005–2011. Rotavirus was the most common viral pathogen identified at 9.3–9.7% prior to the introduction of the vaccine in 2007. Adenovirus varied between 2.8% and 8.9% of samples. Following the introduction of rotavirus vaccines and commencement of PCR testing, norovirus-2 was the most frequent virus detected, present in 10.3–10.9% of samples.

Table 1. Prevalence of enteric viruses in routine specimens tested at IMVS 2005–2011.

<table>
<thead>
<tr>
<th>Year</th>
<th>Adenovirus</th>
<th>Rotavirus</th>
<th>Norovirus G2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>196 (2.8%)</td>
<td>686 (9.7%)</td>
<td>ND*</td>
</tr>
<tr>
<td>2006</td>
<td>254 (2.9%)</td>
<td>813 (9.3%)</td>
<td>ND</td>
</tr>
<tr>
<td>2007</td>
<td>423 (4.6%)</td>
<td>429 (4.7%)</td>
<td>ND</td>
</tr>
<tr>
<td>2008</td>
<td>688 (8.9%)</td>
<td>216 (2.8%)</td>
<td>ND</td>
</tr>
<tr>
<td>2009</td>
<td>280 (3.8%)</td>
<td>168 (2.3%)</td>
<td>ND</td>
</tr>
<tr>
<td>2010</td>
<td>826 (7.7%)</td>
<td>586 (5.5%)</td>
<td>1173 (10.9%)</td>
</tr>
<tr>
<td>2011</td>
<td>955 (7.5%)</td>
<td>280 (2.2%)</td>
<td>1307 (10.3%)</td>
</tr>
</tbody>
</table>

Table 1. Enteric virus testing performed at the Institute of Medical and Veterinary Science 2005–2011. In the period 2005–2009, adenovirus and rotavirus testing was performed using in-house antigen capture assays. In 2010 and 2011 all assay were performed using in-house PCRs. In 2010–2011, 54% of samples were from private practice and 46% from public hospitals. (*ND: Not done).
These data are similar to controlled studies of AGE in general practice in the UK in 1993–1996 and the Netherlands in 1996–1999. In the latter study, Salmonella was found in 3.9% and Campylobacter in 10.5% of cases (0.2% and 0.5% of controls) and rotavirus, adenovirus, and norovirus in 5.3%, 2.2% and 5.0% of cases (1.4%, 0.4% and 1.1% of controls). A potential pathogen was found in only 37.5% of patients and 9.8% of controls, leaving a diagnostic deficit of over 60% in people with AGE.

This diagnostic deficit is a recurring problem in studies. In both paediatric and adult populations, the pathogen causing the diarrhoea remains unidentified in between 40% and 70% of cases. Many studies have tested for only a limited range of pathogens and nucleic acid amplification (NAA) testing has been used sporadically and sometimes without taking into consideration the genetic diversity of some viral strains. Further, most of these studies are uncontrolled. As a result, the range of viruses associated with gastroenteritis, their prevalence and the associated specific symptoms remain unclear.

In an attempt to address these problems, we developed single tube nested PCR assays for a variety of enteric viruses and tested 197 unselected routine samples submitted to the IMVS in 1999–2000. Astrovirus was detected by PCR in 23%, norovirus-2 in 5% and rotavirus antigen and adenovirus antigen in 16% and 4% of samples respectively. In a subsequent prospective controlled study of paediatric AGE at the Women’s and Children’s Hospital in Adelaide in 2001 and containing 197 cases and 197 controls, Arthur et al. found rotavirus in 39.2% of cases and 1.0% of controls (p<0.001), followed by astroviruses (21.5%, 11.8% p=0.01) and norovirus-2 (13.4%, 8.1%, p=0.1) and adenovirus types 40/41 (4.8%, 1.6% p=0.02) and smaller numbers of norovirus-1 (0.5%, 2.7%) and sapovirus (3.2%, 3.2%) which did not differ significantly between cases and controls. Together with the 11 (5.6%) cases in whom a bacterial pathogen was isolated, 68% of cases had a pathogen isolated, while 32% were undiagnosed despite extensive molecular testing.

**Characteristics of commonly recognised gastroenteritis viruses**

This issue of the ASM journal has a number of articles reviewing rotaviruses, noroviruses, sapoviruses, astroviruses and picornaviruses but does not contain a review of enteric adenoviruses, for which we shall provide some local data. A brief introduction to the four major virus groups associated with AGE is below.

**Rotaviruses**

Prior to the advent of vaccination, rotaviruses (double-stranded, segmented RNA viruses) were the most common cause of viral gastroenteritis in infants and young children in all countries. Children were almost universally infected before the age of three, with multiple episodes of infection common. The peak incidence of infection was between 7 and 15 months, occurring at an earlier age in developing countries (6–8 months) than in developed countries (14–18 months). Worldwide, rotavirus is estimated to account for 40% of all cases of severe infant diarrhoea and 475,000 to 580,000 deaths annually, mostly in children less than two years of age. In South East Asia, rotavirus was detected in 30–73% of diarrhoea admissions in children (http://www.who.int/vaccine_research/diseases/diarrhoeal/en/index5.html, accessed 10 February 2012). In the USA, prior to vaccination, rotavirus was estimated to causes 60,000 hospitalisations, and 20–40 deaths annually.

IMVS data (Table 1) shows a fall in the prevalence of rotavirus in clinical specimens referred to the IMVS in 2007–2009 and 2011 following the introduction of rotavirus vaccination in 2007, but with an increase in 2010 patients. The year 2010 marked the introduction of more sensitive, routine rotavirus PCR testing on all enteric virology specimens at the IMVS and led to the detection of rotavirus in nursing home outbreaks. This observation is consistent with an American report of rotavirus in two nursing homes in the winter of early 2011. Rotavirus has been a notifiable disease in SA since mid-2008 and the SA data shows an increased number of rotavirus patients of greater than 20 years of age in 2010 compared to earlier and later years (http://www.dh.sa.gov.au/pehs/notifiable-diseases-summary/rotavirus.gif, accessed 15 February 2012). This suggests rotavirus may be an under-recognised cause of AGE in adults.

**Noroviruses**

Human caliciviruses of the genus Norovirus, are small, 25–35 nm, non-enveloped, icosahedral, single-stranded RNA viruses. The first human calicivirus described was the Norwalk virus. This was later classified as norovirus-(genogroup)1 but most recent infections belong to norovirus-2 viruses. Noroviruses are the most common causes of outbreaks of gastroenteritis in hospitals, nursing homes and cruise ships but are increasingly recognised as a common cause of sporadic AGE in both adults and children. Typically this illness is of short incubation and short duration and in children is a less severe disease than rotavirus infection. SA data shown in Table 1 indicates a prevalence of norovirus-2 strains in 10.3–10.9% of samples. A recent IMVS study of 683 specimens showed norovirus-1 was less frequent (1.7%) than norovirus-2 (4.8%) (Schepetiu, S., unpublished data). A limited number of reports suggest sapoviruses, another calicivirus genus, are also less frequently found in cases of AGE than are noroviruses-2, and are mainly restricted to children <5 years.

**Characteristics of noroviruses**

Noroviruses are single-stranded, positive-sense RNA viruses that belong to the family Caliciviridae and genus Norovirus. Noroviruses can be divided into six genogroups, with genogroup 1 (G1) being the most common and responsible for the majority of human infections. Noroviruses are highly infectious, with a single particle being enough to cause infection. They have a short incubation period, typically 24–48 hours, and are highly resistant to environmental conditions, making them difficult to control in outbreaks. Noroviruses are transmitted through the fecal-oral route, most commonly via contaminated food or water, or through contact with an infected person or their environment.

**Prevention and control**

The primary methods of preventing norovirus infections include:

1. **Hygiene**: Thorough handwashing with soap and water is crucial to prevent the spread of norovirus. Avoiding close contact with infected individuals and cleaning surfaces regularly can also help.

2. **Vaccination**: A norovirus vaccine is available for healthcare workers and those in high-risk settings. However, it is not yet widely available to the general public.

3. **Environmental control**: Noroviruses are highly resistant to most disinfectants, but some products are effective. Regular cleaning and disinfecting of surfaces can help prevent the spread of the virus.

4. **Education**: Informing individuals about the symptoms of norovirus and the importance of proper hygiene practices is essential.

**Symptoms and diagnosis**

Norovirus infections are characterized by sudden onset of nausea, vomiting, abdominal pain, and diarrhea. Symptoms typically last for 1–2 days. Diagnosing norovirus infections is often based on the clinical presentation and the epidemiology of the outbreak. Laboratory diagnosis can be made using PCR assays, which detect the viral RNA.

**Outbreak management**

Effective outbreak management involves identifying the source, implementing control measures, and monitoring for recurrence. This includes:

1. **Identification of the source**: Identifying the source of infection is crucial for controlling an outbreak.

2. **Control measures**: Implementing control measures such as isolation, proper hygiene, and environmental disinfection.

3. **Monitoring**: Regular monitoring of cases and implementing appropriate control measures to prevent further spread.

**Conclusion**

Noroviruses are a significant cause of gastroenteritis, particularly in outbreaks in hospitals and nursing homes. Effective hygiene practices, vaccination, and outbreak management are crucial in controlling norovirus infections.
Adenoviruses

Human adenoviruses are non-segmented, double-stranded DNA viruses. There are at least 54 different types known to infect humans. These have been classified into seven different species or groups labelled A to G (http://www.vmr.hu/~harrach/AdVtaxlong.htm, accessed 10 February 2012). Different types are loosely associated with different diseases; for example, groups B and C with respiratory disease, groups B and D with conjunctivitis37. However, group F adenoviruses types 40 and 41 are strongly associated with AGE. Gastroenteritis associated with adenoviruses 40/41 is reported as having an incubation period of 3–10 days and illness lasting 8–12 days. Diarrhoea is more prominent than vomiting or fever although respiratory symptoms may be present.

Diagnosis of adenovirus in faecal material may be performed by antigen detection assays or by NAA testing. In 2010, using an in-house PCR targeting a highly conserved hexon gene of the virus38, we detected adenovirus 7.7% of samples (Table 1). However, the diagnosis of adenovirus AGE by antigen detection or broadly reactive PCR is complicated by the frequent detection of non-type 40/41 adenoviruses. As part of a development project, a subset of 81 of the adenovirus generic PCR-positive samples from 2010 were tested by specific type 40 and type 41 PCR assays. Only 28 (35%) were positive for adenovirus type 41 and none were positive for type 40. Nineteen of the adenovirus-positive, type 41 negative samples were sequenced and a variety of adenovirus types were found including types 1, 2, 3, 11, 12, 31 and 34 (Schepetiuk, S., unpublished data). In a subsequent prospective study of 7090 samples performed in 2010–2011, 470 (6.6%) samples were positive with the generic adenovirus PCR of which 115 (1.6% of all samples) were also positive in the adenovirus type 41 specific PCR. A further 160 (2.3%) were positive only in the type 41 PCR and one in the type 40 PCR. Overall, only 43.7% of adenovirus-positive faeces contained types 40/41. These data are similar to Japanese epidemiological report of adenovirus detection in faeces from 2000-2007 in which 631 (20.0%) of 2999 adenovirus-positive faecal samples were types 40/4139.

Astroviruses

Human astroviruses are 28–30 nm, non-enveloped, single-stranded RNA viruses and are also associated with a milder form of AGE than rotaviruses AGE30. Prior to 2008 there were thought to be eight different serotypes amongst the ‘classic’ human astroviruses (HAstV)31. More recent metagenomic studies have identified two other groups of astroviruses (AstV-MLB and HMOAstV) in faeces32,33. Classic HAstV have been known to be associated with AGE especially in children with prevalence rates of 1.4–6.6% in Australia34-36, and 4.9–8.6% in overseas reports37-40. Higher rates (39%) have been reported in children less than one year old40 and in areas lacking adequate sanitation41. Our initial data in Adelaide showed high rates (21–23%) of infection in 2000 and 200118,19 but we noticed a marked fall in the frequency of astrovirus detection in 2002–2004 (Ratcliff, R., unpublished data). This is consistent with more recent studies in which astrovirus prevalence in AGE has been reported to be much lower, 0.9–3.2%16,17,20,30.

Do other viruses cause AGE?

A large number of cases of AGE remain undiagnosed. Other viruses such as torovirus, picobirnavirus, picotrinavirus, pestiviruses, coronavirus and the Aichi picornavirus have been suggested as possible causes of AGE but a strong association with disease has not been shown42-47. With the advent of novel molecular amplification and sequencing techniques, many more viruses have been identified in faeces including astroviruses32,33, adenovirus group G48, bocaviruses49, picornaviruses, including saliviruses50,51, parechoviruses52 and rhinoviruses53 and cardiovirus, cosavirus, and circovirus in sewage samples54. With the exception of our report on bocavirus type 219, the association of these new viruses with AGE has not been tested in controlled studies.

Obtaining evidence to support a causal role for these new viruses will require large prospective case control studies in a variety of locations and clinical situations. It is striking that for such an important disease, controlled studies on the role of virus in the causation of the disease remain infrequent. This may reflect the lack of widespread availability of NAA tests that can cope with the diversity of viruses and diversity of strains. These assays are most likely to be available in developed countries where the disease burden is least and its funding priority lowest. However, it may also reflect the impracticality of proving an association when the diversity of viruses is high and continually changing, and the prevalence of a single type of virus is low. Obtaining formal proof of a causal association with AGE for many of these novel viruses is important but will remain challenging.

References
In Focus


Biographies

Geoff Higgins is a medical virologist and head of Virology at the RAH site of SA Pathology. He has interests in molecular testing and its use in routine testing for enteric and respiratory pathogens.

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