

### Viruses causing gastroenteritis

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Acute gastroenteritis is one of the most common diseases in humans worldwide. Viruses are recognized as important causes of this disease, particularly in children. Since the Norwalk virus was identified as a cause of gastroenteritis, the number of viral agents associated with diarrheal disease in humans has steadily increased. Rotavirus is the most common cause of severe diarrhea in children under 5 years of age. Astrovirus, calicivirus and enteric adenovirus are also important etiologic agents of acute gastroenteritis. Other viruses, such as toroviruses, coronaviruses, picobirnaviruses and pestiviruses, are increasingly being identified as causative agents of diarrhea. In recent years, the availability of diagnostic tests, mainly immunoassays or molecular biology techniques, has increased our understanding of this group of viruses. The future development of a safe and highly effective vaccine against rotavirus could prevent, at least, cases of severe diarrhea and reduce mortality from this disease.

**Keywords** Viral gastroenteritis, acute diarrhea, rotaviruses, enteric adenoviruses, astroviruses, human caliciviruses, coronaviruses, toroviruses, picobirnaviruses

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### INTRODUCTION

Acute gastroenteritis is one of the most common diseases in humans, and continues to be a significant cause of morbidity and mortality worldwide [1]. Children under 5 years of age are particularly prone, and it is calculated that, in this group, there are more than 700 million cases of acute diarrhea every year [2]. The mortality associated with gastroenteritis has been estimated to be 3–5 million cases per year, the majority of which occur in developing countries [3–5]. In the developed world, the impact of the illness is seen in its high morbidity and in the high incidence of hospitalization that this illness necessitates [6].

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### HISTORICAL BACKGROUND

Since the 1940s, viruses have been suspected of being important causes of gastroenteritis, as the etiology remained unknown in most cases [7,8]. However, it was not until 1972 that Kapikian et al. first identified a virus (Norwalk virus) in feces after an outbreak of diarrhea [9] as a cause of gastroenteritis. One year later, Bishop et al. observed the presence of rotavirus in the duodenal mucosa of children with gastroenteritis [10], and in 1975, astroviruses [11] and enteric adenoviruses were identified in the feces of children with acute diarrhea [12].

Since then, the number of viruses associated with acute gastroenteritis has steadily increased. Thus, coronaviruses [13], picobirnaviruses [14–16], pestiviruses [17] and toroviruses [18], which produce diarrhea in animals, are emerging as causes of viral gastroenteritis in humans, according to several studies [19–21].

## DESCRIPTION OF THE AGENTS

### Rotaviruses

#### *Morphology*

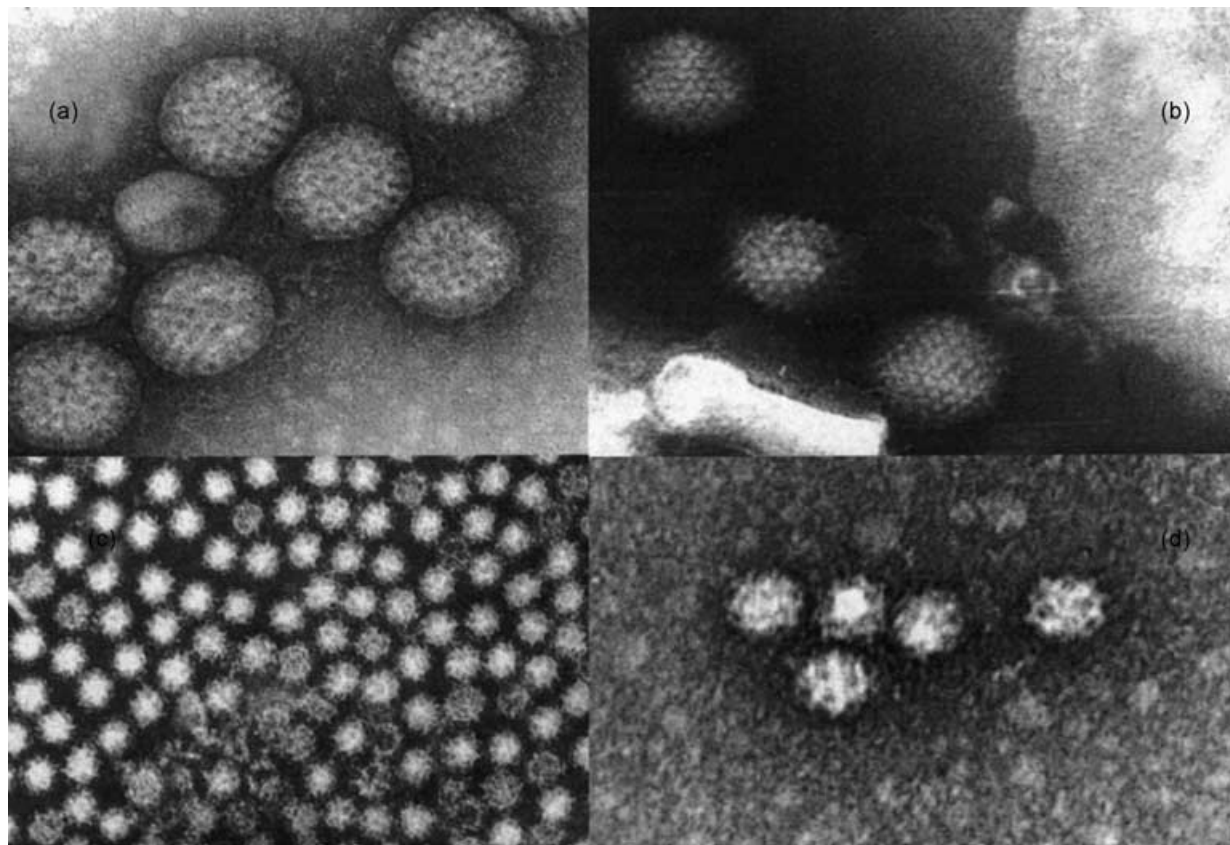
Rotaviruses are members of the Reoviridae family [22], and are characterized by their non-enveloped icosahedral structure and 70-nm diameter. When observed under an electron microscope, they have a 'wheel' shape [23,24] (Figure 1a). The capsid consists of a double protein layer; the outer capsid is composed of the structural proteins VP7 and VP4, and the inner capsid mainly of VP6. The core is found inside the inner capsid, and encloses the rotavirus genome, composed of 11 segments of double-stranded RNA. Given the segmented nature of the RNA genome, co-infection of cells with two different strains of rotavirus may result in reassortant virus, with RNA segments from each of the progenitors [25].

Each of the genomic segments encodes the structural VP proteins (VP1, VP2, VP3, VP4

(VP5 + VP8), VP6 and VP7) and the non-structural NSP proteins (NSP1, NSP2, NSP3, NSP4 and NSP5) [23]. Table 1 shows the characteristics of these proteins. Of particular interest is NSP4, since it has enterotoxin-like activity and can induce diarrhea in mice [26,27].

#### *Classification*

Rotaviruses are classified into groups, subgroups and serotypes according to the antigenic properties of the capsid proteins. Protein VP6 is the group reactivity determinant, with seven groups currently in existence, labeled A–G, and two subgroups, I and II [28]. Groups A, B and C are those which produce infection in humans. Classification into serotypes is based on the antigenic differences in the proteins of the outer capsid, VP7 and VP4. The first, a glycoprotein, determines the G-type specificity, and the second, the P-type specificity, owing to its protease sensitivity. At present, there are 15 G types [29], with G1, G2, G3 and G4 being the predominant ones throughout the



**Figure 1** Electronmicrographs of: (a) human rotavirus; (b) enteric adenovirus; (c) human astrovirus with a diameter of 28 nm; (d) human calicivirus. Bar = 50 nm (Courtesy of Dr A. Sánchez-Fauquier).

**Table 1** Characteristics of structural and non-structural rotavirus group A proteins

Genome segment	Protein encoded	Viral particle localization	Function
1	VP1	Core	RNA polymerase
2	VP2	Core	RNA binding
3	VP3	Core	Guanilyltransferase
4	VP4 (VP5 + VP8 subunits)	Outer capsid	Viral hemagglutinin, neutralization antigen, P serotypes Proteolytic cell cleavage
5	NSP1	Non-structural	RNA binding
6	VP6	Inner capsid	Group and subgroup antigen
7, 8, 9	NSP3	Non-structural	RNA binding
7, 8, 9	VP7	Outer capsid	Glycoprotein (G serotypes), neutralization antigen
8	NSP2	Non-structural	Viral replication
10	NSP4	Non-structural	Enterotoxin-like activity, viral assembly
11	NSP5	Non-structural	RNA binding

world [6]. However, there have been reports of infections by unusual G types [30–35], and recently there have been reports of the emergence of serotype G9 in several countries, such as Brazil [32], Malawi [35], the USA [36,37], France [38], India [39], Argentina [40], the UK [41] and Australia [42].

#### *Pathogenesis and immune response*

Rotaviruses infect the mature enterocytes on the tips of small intestine villi and lead to villous epithelium atrophy and compensatory repopulation of the epithelium by immature secretor cells, with secondary hyperplasia of the crypts [43]. It has been proposed that cellular damage is secondary to villus ischemia [44]. The mechanism that induces the production of diarrhea is not well understood, although it appears to be mediated by the relative decrease of villous epithelium absorption in relation to the secretory capacity of the crypt cells [24]. There is also a loss of intestinal permeability to macromolecules such as lactose, secondary to a decrease in disaccharidase in the intestine. The enteric nervous system is stimulated by this virus, leading to the induction of intestinal water and electrolyte secretion [45].

The immunologic mechanisms responsible for protection against infection by rotavirus are still not well known. Several studies have shown that local intestinal immunity protects against successive severe episodes of diarrhea [46–48]. The presence of neutralizing antibodies directed towards the proteins VP4 or VP7 does not correlate with protection against this disease [49]. It has been reported that the first infection with rotavirus

elicits a homotypic neutralizing antibody response, with heterotypic responses in subsequent infections [50]. It has recently been shown that protein NSP4 produces a cellular immunity-mediated response [51].

#### **Enteric adenoviruses**

##### *Morphology*

Human adenoviruses belong to the Adenoviridae family and, within the genus, the majority of enteric adenoviruses reported to date belong to subgenus F [52,53]. They are DNA viruses without an envelope, 70 nm in diameter, and with icosahedric symmetry (Figure 1). The protein capsid is composed of 252 capsomers—240 hexones and 12 pentones—and structures called fibers that protrude to the outside. The hexones contain proteins II, VI, VIII, and IX, which participate in the stability and assembly of the viral particle. The pentone proteins (III and IIIa) have the function of cellular penetration, and the fibers are hemagglutinins and are responsible for binding the virus to receptors (Figure 1b) [52–54]. There are at least eight proteins making up the core [55]; these maintain the integrity of the genome, and participate in enzymatic activity. The genome consists of a linear molecule of double-stranded DNA that represents 15% of the viral mass [53].

##### *Classification*

The enteric serotypes that are most frequently associated with gastroenteritis caused by adenovirus are 40 and 41, which belong to subgenus F. More rarely, serotypes 31, 12 and 18 of subgenus A

[53] and serotypes 1, 2, 5 and 6 of subgenus C [52,56] have been involved in the etiology of acute diarrhea.

#### *Pathogenesis and immune response*

In the same way as in gastroenteritis produced by rotavirus, the lesions produced by serotypes 40 and 41 in the enterocytes lead to atrophy of the villi and compensatory hyperplasia in the crypts, with subsequent malabsorption and loss of fluids [44,54]. After the infection, specific antibodies develop in most cases, and non-neutralizing antibodies are useful for measuring the immune response. The specific type-neutralizing antibodies can provide protection both in the current illness and in reinfections by the same serotype, although patients may continue to eliminate the virus in their feces for months after an effective humoral response [56].

### **Astroviruses**

#### *Morphology*

In 1993, the Astroviridae family was established with a single genus, the astrovirus, which encompasses human and animal viruses [57,58].

Astrovirus has been reported as small round viruses of 28 nm with an appearance like that of a five- or six-pointed star by direct visualization with electron microscopy. The name stems from the Greek *astron*, meaning star [11]. However, it has recently been verified that this virus has a different morphology, with an icosahedric appearance, a diameter of 41 nm, and well defined spikes. When these viruses are subjected to a high pH, they transform and present the typical morphology of the initially described star [59] (Figure 1c).

The genome of human astroviruses is composed of single-stranded, positive-sense RNA which contains three 'open reading frames' (ORFs). ORF1a and ORF1b encode viral protease and polymerase, respectively. ORF2 encodes protein capsid precursor and is found at the 3'-terminus of the genome [57,60].

The protein structure of astrovirus is not well known at present. However, it seems that the precursor of the capsid proteins, an 87-kDa polyprotein, gives rise to the structural capsid proteins VP32, VP29, and VP26 [61].

Trials with monoclonal antibodies against human astrovirus suggest that VP26 and/or VP29 may be important in the neutralization,

heterotypic immunity and binding of the virus to the target cells [61,62]. These proteins, especially VP26, seem to be responsible for the antigenic variation observed among the different serotypes [61].

#### *Classification*

Astroviruses are classified into serotypes based on the reactivity of the capsid proteins with polyclonal sera and monoclonal antibodies [57,63]. To date, there have been reports of four neutralizing monoclonal antibodies, developed by Sanchez-Fauquier et al. against serotype 2 human astrovirus [61] and by Bass and Upadhyayula against serotype 1 astrovirus [62]. They all react with the VP26 capsid protein, involved in the neutralization of human astroviruses.

Astroviruses can also be classified into genotypes on the basis of the nucleotide sequence of a 348-bp region of the ORF2, and there is a good correlation with the serotypes [64]. There are seven established genotypes, which correspond with seven serotypes. The existence of an eighth genotype has been suggested, due to the sequence of a putative serotype 8 [65]. Serotype 1 is predominant in most studies, followed by 2, 3, 4 and 5. Serotypes 6, 7 and 8 are rarely detected [63,65-69].

#### *Pathogenesis and immune response*

The pathogenesis of the disease induced by astrovirus has not yet been established, although it has been suggested that viral replication occurs in intestinal tissue [57,70]. Studies in adult volunteers have not clarified the pathogenic mechanisms [71,72]. In animal studies, atrophy of the intestinal villi is observed, as well as inflammatory infiltrates in the lamina propria [73], leading to osmotic diarrhea.

Symptomatic astrovirus infection occurs mainly in small children and the elderly, which suggests a reduction in antibodies in recent years, but the determinants of immunity are not well known [57]. Studies in adult volunteers indicate that people with detectable levels of antibodies do not develop the illness [71].

### **Human caliciviruses**

#### *Morphology*

Human caliciviruses are members of the Caliciviridae family, and two genera have been described, the Norwalk-like viruses (NLVs) and Sapporo-like

viruses (SLVs) [74]. The virions are composed of a single structural capsid protein, with icosahedric symmetry [75]. This protein, composed of 180 molecules, folds into 90 dimers, which form a continuous shell with protrusions in the shape of an arch (Figure 1d). A key characteristic is the existence of 32 cup-shaped depressions, situated on the axes of the icosahedron, from whose Latin designation, *calyx*, the virus derives its name [76].

The genome of the NLVs consists of positive-sense, single-stranded RNA organized into three ORFs. ORF1 encodes the non-structural proteins, such as RNA-dependent RNA polymerase and helicase [77], ORF2 encodes the structural protein of the capsid, and ORF3 encodes for a small protein whose function is unknown [76]. The genome of the SLVs differs from the NLV genome in that the ORF1 encodes the non-structural proteins as well as the structural protein of the capsid [78,79]. ORF2 encodes a small protein of unknown function, and the significance of ORF3 is still uncertain.

#### Classification

The human caliciviruses genera (NLVs and SLVs) can be further divided into genetic clusters. The NLVs include Norwalk virus (the type species), Desert Storm virus, Southampton virus, Snow Mountain agent, Hawaii virus, Toronto virus, Bristol virus, and Jena virus. The SLVs includes Sapporo virus (the type species), Parkville virus, and London virus [80,81].

#### Pathogenesis and immune response

In studies carried out on volunteers, infection by calicivirus was observed to produce an expansion of the villi of the proximal small intestine. The epithelial cells remain intact, and there is shortening of the microvilli [82,83]. The mechanism by which diarrhea is produced is unknown, although it has been suggested that the delay in gastric emptying observed in Norwalk virus gastroenteritis may play a role [84].

Infection by the Norwalk virus induces a specific IgG, IgA and IgM serum antibody response, even if there has been previous exposure [83]. Two weeks after infection by the Norwalk virus, an increase in jejunal synthesis has been demonstrated for IgA [85], and most patients are resistant to reinfection for 4–6 months [83]. Nevertheless, a lack of long-term protection has been observed.

### Other gastroenteritis-producing viruses

#### *Torovirus*

Torovirus is a genus within the Coronaviridae family. Torovirus was detected for the first time in the feces of patients with gastroenteritis in 1984 [18]. These viruses have an envelope of 100–140 nm, with a capsid of helicoidal symmetry and a single-stranded RNA genome of positive sense [86]. They are associated with persistent and acute diarrhea in children, and may represent an important cause of nosocomial diarrhea [19,87].

#### *Coronavirus*

Included in the Coronaviridae family, these viruses are between 60 and 220 nm, with helicoidal symmetry and a spiculated envelope that gives them the appearance of a crown. The genome is composed of positive monocatenary RNA [88,89]. Coronavirus was linked with diarrhea in humans for the first time in 1975, but studies have not yet been able to establish a definite etiologic role [90].

#### *Picobirnaviruses*

These are small viruses, without an envelope, 30–40 nm in diameter, with a capsid of icosahedric symmetry, and a genome made up of two or three segments of bicatenary RNA. They were identified for the first time by Pereira *et al.* in 1988 [91]. Since then, they have been found in a wide variety of animal species [14] and in both children and adults with diarrhea, including immunodepressed patients [92–96]. In a recent publication, however, this virus was not found in HIV-infected children with diarrhea [97].

## CLINICAL ASPECTS

### Viral diarrhea in children

Rotavirus is the main cause of severe diarrhea in children under 5 years of age, and causes more than 130 million episodes per year throughout the world, and between 600 000 and 870 000 deaths [98,99], the vast majority of which are in developing countries [100–102]. Several European studies point to rotavirus as the agent responsible for 20–60% of cases of gastroenteritis requiring hospitalization [103–108]. In Australia, similar figures have been reported [109], and in the USA it has been estimated that one in every 73 children will have been hospitalized because of diarrhea due to rotavirus A during the first 5 years of life [110].

As far as 'non-group A' rotavirus is concerned, group B rotavirus has been identified in epidemic outbreaks of severe diarrhea in adults in Southeast Asia since 1982 [111], and in symptomatic infections in children. Outbreaks of diarrhea due to group C rotavirus have been identified in Asia [112], Brazil [113], and Europe [114,115], and outbreaks of sporadic gastroenteritis caused by this virus in children have been observed in the USA [116], Japan [117], and the UK [118], with frequencies ranging from 1% to 6.8%.

Enteric adenoviruses present variable incidences of infection. In industrialized countries, the incidence varies from 1% to 8% [119–124], whereas in developing countries, figures of 2–31% have been published [125–128].

In the initial studies of astrovirus by electron microscopy carried out in several countries, the virus was detected in about 1% of cases [129–131]. At present, thanks to the development of enzyme immunoassay (EIA) techniques, which use monoclonal antibodies [132], incidences of 2–13% have been detected in children seen at hospitals [65,67,127,133–138]. In the community, astrovirus is responsible for 4–10% of cases of gastroenteritis [66,69,119,139,140], and has become the leading cause of this illness [141].

Human calicivirus was first identified as a cause of outbreaks of non-bacterial diarrhea, initially using electronmicroscopic techniques [9,142]. In recent studies, with the application of new assays such as EIA and reverse transcription–polymerase chain reaction (RT-PCR), our knowledge of the epidemiology of human calicivirus has changed, especially for Norwalk-type virus [76]. Thus, these viruses are recognized as the main agents responsible for outbreaks of non-bacterial diarrhea, and new estimates suggest that they represent the most common cause of illness with a food origin [143–147]. Similarly, recent studies investigating calicivirus in sporadic cases of gastroenteritis in children have found that Norwalk-type viruses comprise the second cause of viral gastroenteritis after rotavirus [119,148–150].

From the clinical point of view, acute viral gastroenteritis cannot be distinguished from that caused by bacteria, and in general it is a self-limiting process of diarrhea and vomiting, with a duration of 1–7 days, which may sometimes become prolonged or persistent [69,121,122,151,152].

The most common age of presentation of sporadic viral gastroenteritis is 6–24 months [7,139,

153–155]. During the neonatal period, infection by rotavirus is mostly asymptomatic, possibly due to maternal protective factors and intestinal immaturity. Another factor could be the attenuated nature of certain strains, frequently detected in neonates with symptomatic and asymptomatic infections [153,155,156]. During the first 2 years of life, repeated rotavirus infections occur, 50% of which are asymptomatic [157–159]. Several studies have also shown the asymptomatic elimination in feces of adenovirus and astrovirus, and it is calculated that about 50% of infections by calicivirus are asymptomatic [122,133,139,141,150,154].

The severity of the infection, together with the need for hospitalization, is greater with gastroenteritis caused by group A rotavirus than with that associated with adenovirus [121,160,161], astrovirus [139,140,162] or calicivirus [119,149,150].

The viruses that produce gastroenteritis represent an important cause of nosocomial infection in pediatric admission units. Between 20% and 50% of cases of gastroenteritis caused by rotavirus in hospitals are considered to be of nosocomial origin [6,163,164], and nosocomial diarrhea has been described in 2–6% of children admitted [165,166]. Nosocomial infections by adenovirus and astrovirus also represent an important problem, as several studies have shown [69,136,167,168]. There are fewer data on the incidence of nosocomial infection due to calicivirus, although in the studies published it is calculated to be high, following group A rotavirus in frequency [130,167,169–171]. The prevalence and transmission of nosocomial infection are accounted for by the many asymptomatic patients who eliminate the virus in feces [57,172,173] and the environmental contamination that accompanies the relative resistance of these viruses to normal disinfectants [174,175].

### **Viral gastroenteritis in adults**

Calicivirus is the main agent of viral gastroenteritis in adults, and generally produces epidemic outbreaks [143,176,177]. The cause of sporadic acute non-bacterial gastroenteritis has not been well studied in adults, although a viral origin is presumed, due to the similarity of the illness with the epidemics produced by calicivirus [176]. The detection of calicivirus antigen in the feces of patients with sporadic gastroenteritis has been infrequent, although a high seroprevalence of

antibodies against calicivirus has been found in young adults [178,179].

We do not know the frequency of sporadic gastroenteritis caused by rotavirus in adults either, as this is generally secondary to disease in a child, and it also occurs as travelers' diarrhea [180], with some cases of severe diarrhea and death in the elderly [181].

Epidemic outbreaks of diarrhea in adults caused by infrequent serotypes of astrovirus have been reported [129,182], and more sensitive techniques such as RT-PCR are likely to reveal a greater prevalence of sporadic gastroenteritis in adults.

### **Viral gastroenteritis in the immunocompromised patient**

The main viral causes of severe gastroenteritis in immunosuppressed patients are cytomegalovirus (CMV) and Epstein-Barr virus (EBV), which mainly affect patients with AIDS and transplant recipients [176,183,184]. CMV is a frequent pathogen in diarrhea associated with AIDS with CD4 counts below 100 cells/mm<sup>3</sup> [185,186], although the introduction of antiretroviral therapy has drastically reduced its frequency in this group of patients [176]. Other viruses that produce HIV-associated gastroenteritis include astrovirus, picobirnavirus, calicivirus and adenovirus [92,93]. There is evidence of gastroenteritis due to astrovirus and adenovirus in both child and adult bone marrow transplant recipients [187,188]. Similarly, torovirus has been associated with diarrhea in immunocompromised children [19].

### **DETECTION OF GASTROENTERITIS-PRODUCING VIRUSES**

Detection of gastroenteritis-producing viruses has traditionally been based on techniques of direct visualization using electron microscopy, which, although it is still useful, is limited to reference laboratories [6,23,76,189,190].

Cell culture of these viruses is not considered to be useful for diagnostic purposes, as it is technically cumbersome and slow. Propagation in cellular media of calicivirus, human torovirus or coronavirus has not yet been achieved [76,189].

The progressive incorporation of more sensitive techniques for antigen detection in feces based on immunoassay techniques, as well as the development of molecular biology techniques, has

improved the diagnosis of this virus and contributed to an appreciation of its clinical importance.

### **Antigen detection techniques**

In recent years, a wide variety of techniques for the detection of antigen in fecal samples have been developed. These are based on EIA, agglutination with latex particles (LA) and, more recently, immunochromatography (ICG), all of which are available commercially for group A rotavirus, adenovirus and astrovirus [6,189].

EIA has proven to be more sensitive than direct visualization by electron microscopy [191,192], and also has a high specificity in the detection of group A rotavirus, especially when monoclonal antibodies are used [193,194]. The LA technique has lower sensitivity than EIA [6,194]. ICG showed high sensitivity and results comparable to those achieved with EIA, and is rapid and technically very simple [195,196].

Group C rotavirus can be detected in fecal samples by means of recently described immunoanalysis techniques, which use different antigens and monoclonal antibodies [116,118,197] and are more sensitive than electron microscopy.

In the case of adenoviruses, many of these techniques detect group common antigen, and several studies have shown that between 45% and 93% of the samples positive for the group correspond to enteric serotypes 40 and 41 [121,122,198,199].

At present, EIA techniques are available for the detection of astroviruses, due to the development of monoclonal antibodies against this virus [62,132,168,200]. Some have recently been marketed, with good sensitivity and specificity compared with electron microscopy and RT-PCR [67,201].

Recently, EIA methods have been developed with monoclonal and polyclonal antibodies for the detection of human calicivirus. These methods have promise, although their use currently seems to be limited to research laboratories [76,202].

### **Molecular biology techniques**

PCR techniques have been developed for many of the viruses that produce gastroenteritis, although they are not used routinely. These techniques are more sensitive than immunoassay methods. They are useful for the confirmation of the results of other techniques, and for the study of samples

such as cerebrospinal fluid or serum, and environmental samples [203–208].

In the case of human calicivirus, RT-PCR is the most sensitive and widely used technique at present for the detection of these viruses [209–212].

Molecular techniques are also used to genotype these viruses [24,31,63,64]. RT-PCR techniques have been developed to genotype group A rotavirus with type-specific primers for genotypes G and P [213,214].

## PREVENTION OF DISEASE

Treatment of viral gastroenteritis is symptomatic, and its aim is to prevent or treat the dehydration secondary to the disease. For this, it is important to start liquid intake early, in order to correct the water deficit and combat the losses due to vomiting and diarrhea [215].

Interruption of transmission of the infection is extremely important, especially in hospitals and centers which care for small children. Therefore, it is necessary to reinforce hygiene measures and clean all surfaces with suitable disinfectants [105,165,174].

Studies with vaccines against group A rotavirus began in 1982. The first vaccine developed was the tetravalent human–rhesus reassortant vaccine, which induces protection against the four main rotavirus serotypes, G1–G4. Efficacy studies showed a reduction in the appearance of severe gastroenteritis caused by rotavirus of between 69% and 91% in vaccinated children [216], and the vaccine was approved in the USA in 1998 [217]. However, the detection of an increase in the risk of intussusception after vaccination led to its suspension [218,219]. At present, plasmidic DNA and antigenic vaccines, which code for specific viral proteins, are being investigated [220].

The wide variety found among circulating genotypes and serotypes of group A rotavirus in studies from around the world leads us to examine the need to cover more serotypes in the development of new vaccines [34,35,40,42, 221–223].

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