Viruses are small, acellular microorganisms with diameters of 15 to 400 nm, each containing only one type of nucleic acid. They cause many diseases of plants, animals and humans and are obligate intracellular parasites with cellular specificity (cell tropism). Their replication is strongly dependent on the host organism. Numerous viruses can be found in human gut, but only a few are commonly recognized as important foodborne pathogens. All of the known pathogenic viruses in the marine environment that pose significant threat to public health are transmitted via the fecal-oral route. This group, known collectively as enteric viruses, is a continually growing list. These viruses belong primarily to the families Adenoviridae (adenovirus strains 3, 7, 40, and 41), Caliciviridae (norovirus, calicivirus, astrovirus and sapovirus), Picornaviridae (poliovirus, coxsackievirus, echovirus, enterovirus, hepatitis A virus and aichi virus) and Reoviridae (reovirus and rotavirus). Many of these viruses have a low infectious dose (1-10 viruses) and may cause a range of illnesses from gastroenteritis to paralysis and myocarditis. The primary site of these viral infection and replication is the intestinal tract. Virus particles are shed in feces for weeks at levels as high as $10^{10}$ g$^{-1}$ (rotaviruses), with average levels between $10^6$ g$^{-1}$ (enteroviruses) and $10^8$ g$^{-1}$ (hepatitis A virus) (Feachem et al., 1983; Gerba, 2000; Yates and Yates, 1988). With significant advancements in the area of environmental virology, enteric viruses have been recognized as the causative agents in many nonbacterial gastroenteritis cases and outbreaks (Bosch, 1998). Enteric viruses have been isolated from and linked to outbreaks originating from contaminated drinking water sources, recreational waters (e.g., waters for swimming, canoeing, surfing, etc.), urban rivers and shellfish harvested from contaminated waters (Cecuk et al., 1993; Dewailly et al., 1986; Jiang et al., 2001; Lee and Kim, 2002; Muscillo et al., 1994). The various modes of transmission of these enteric viruses is shown in Fig. 1.
Fig. 1. Modes of transmission of enteric viruses, showing proven (continuous lines) and suspected (dashed lines) routes of exposure [adapted from Foodborne viruses: an emerging problem (Koopmans and Duizer 2004) and Environmental Virology (Rao and Melnick 1986)]

2.1 Adenoviruses (ADV)

Fig. 2. Electron micrograph of Adenovirus (Source: Locarnini et al., 1974)
Adenoviruses, members of the family adenoviridae are 80-110 nm in diameter, non-enveloped, double stranded and non-segmented DNA viruses. Their virions show the shape of an ideal regular icosahedral symmetry (Votava et al., 2003), which contains 240 hexons, 12 pentons and 12 fibers that extend from each pentose base. These viruses were first isolated in 1953 by investigators trying to establish cell-lines from adenoidal tissue of children removed during tonsillectomy and from military recruits with febrile illness. These viruses are common among humans, birds and other animals causing respiratory, ocular and gastroenteric infections. The family Adenoviridae is comprised of four genera (Mastadenovirus, Aviadenovirus, Atadenovirus and Siadenovirus). Human and some animal adenoviruses are members of the genus Mastadenovirus. About 47 distinct antigenic types have been isolated from humans and many other types from various animals. Based on physical, chemical and biological properties, human adenoviruses are grouped into 6 groups (A – F).

Adenoviruses are readily cultivable in cell lines except the fastidious enteric adenovirus, serotypes 40 and 41 which are predominantly associated with gastroenteritis (Wadell et al., 1994). The enteric adenoviruses have been recognized as the second most (after rotavirus) commonly identified agents in stools of infants and young children with viral gastroenteritis (Brandt et al., 1983; Vesikari et al., 1981; Arakai et al., 1994; Lees, 2000). They are responsible for 10% of gastroenteritis cases in children (CDC, 1990a). In Finland, enteric adenoviruses were found in 6% of the cases of gastroenteritis in children between 2 months and 2 years of age (Pang et al., 2000). Most individuals experience adenovirus infection (asymptomatic or symptomatic) before they reach 20 years of age. Clinical symptoms in children include diarrhoea, vomiting and fever lasting for 1-2 weeks. These viruses generally cause more severe disease compared to rotavirus but with a more prolonged course. Both enteric adenoviruses and non-enteric adenoviruses may be shed into the gut and can be isolated from feces. They can also be detected in sewage, seawater and shellfish (Girones et al., 1995; Pina et al., 1998; Vantarakis and Papapetropoulou, 1998). Myrmel et al. (2004) found that 18.6% of the shellfish samples collected from Norwegian coast had adenoviruses. Muniain-Mujika et al. (2003) also recorded the presence of adenoviruses in 47% of the shellfish samples in Norway. However, no seafood related outbreaks have been reported. This could be because enteric adenoviruses are not generally associated with gastroenteric disease in adults and for the most part young children are
not primary consumers of seafood (Muniain-Mujika et al., 2003). The waterborne enteric adenovirus infection is undetermined. These viruses are more stable in water than poliovirus 1 or HAV (Enriquez and Gerba, 1995).

Enteric adenovirus serotype 40 and 41 are important causative agents of self-limiting acute gastroenteritis, especially in children < 4 years (Horowitz, 1996). Other types of adenoviruses, which can cause gastroenteritis are type 3 and 7 in systemic infections (Griffin et al., 2003). Enteric adenoviruses and noroviruses (NoV’s) were identified as two etiological agents responsible for acute gastroenteritis in a waterborne outbreak in Finland (Kukkula et al., 1997). The detection of human adenoviruses by PCR has been proposed as a molecular parameter for monitoring the presence of human viruses in the environment (Formiga – Cruz et al., 2002).

2.2 Enteroviruses (EV)

![Electron micrograph of enteroviruses](Source: Onodera et al., 1986)

The genus Enterovirus is one of the members of the family Picornaviridae which comprises a large family of RNA viruses (Minor, 1990). They are naked, smooth, icosahedral particles of about 27 nm in diameter with a positive sense single stranded non-segmented RNA genome. They are stable within the pH range of 3-10 and sensitive to chlorine and UV radiation (Porterfield, 1989). The genus Enterovirus is divided into five major groups: polioviruses (types
group A coxsackieviruses (types 1-24 (no type 23)), group B coxsackieviruses (types 1-6), echoviruses (types 1-33 (no type 10 & 28)) and newer identified enteroviruses (types 68-71). About 67 immunologically distinct serotypes of the human enteroviruses (EV) including the polioviruses, group A and B coxsackieviruses, echoviruses, and the more recently designated enteroviruses serotypes 68 to 71 are known to cause infections in humans (Muir et al., 1998; Lees, 2000). Human enteroviruses are ubiquitous and are found in the gastrointestinal tract of clinically healthy individuals, either as vaccine poliovirus or as nonpolio enteroviruses, usually of low pathogenicity (Wyn-Jones and Sellwood, 2001).

Though poliovirus exists widely in nature, in soil, sewage, wastewater, drinking water and food such as shellfish, there is very little evidence to connect it directly with an outbreak of poliomyelitis (Jaykus, 1997; Metcalf et al., 1979; Goyal et al., 1979). Because most cases of infection by poliovirus are not apparent, it is not until secondary, person-to-person spread leading to the onset of poliomyelitis that the infection is recognized. Therefore, it is difficult to address the risk of infection from the environment (Metcalf et al., 1995).

Poliovirus genome is known to be highly mutatable during replication (Holland et al., 1982; Ward et al., 1988). It was demonstrated that neurovirulence increased when the following changes took place in base positions of the viruses: in type 1 viruses, position 480 in the 5’ noncoding region changed from G to A (Horie et al., 1994; Kawamura et al., 1989), position 525, which is base paired with position 480 in a stem and loop structure of the F-domain (Skinner et al., 1989) changed from U to C (Rezapkin et al., 1994), for type 2, position 481 changed from A to G (Macadam et al., 1991; Pollard et al., 1989), and for type 3, position 472 changed from U to C (Evans et al., 1985; Westrop et al., 1989). Furthermore, Chumakov et al. (1991) designed the method of mutant analysis by PCR and restriction enzyme cleavage (MAPREC) to estimate the ratio of viruses containing genome of a virulent nature in a vaccine virus population. MAPREC can be used for quality control of oral poliomyelitis vaccine production and surveillance of virus isolates.

Coxsackie viruses are named after Coxsackie, New York, where they were discovered. They are part of the enterovirus family of viruses. They can spread from person to person, through faecal-oral route. These viruses are divided into groups A and B. Coxsackie A cause herpangina and hand-foot-and-mouth disease and Coxsackie B viruses cause Bornholm disease, also called as epidemic myalgia and epidemic pleurodynia, myocarditis and pericarditis.
Incidental infection is common but most frequently in summer and autumn in temperate climates. In tropical parts of the world, they infect people year round. Children under the age of 16 are most susceptible. The symptoms of Coxsackie virus infection include fever of 101 to 104 degrees Fahrenheit (38.3 to 40 degrees Celsius), headache, and muscle aches. Some infected persons also develop a mild sore throat and abdominal discomfort or nausea. A child with coxsackie virus may simply feel hot but have no other symptoms. In most children, the fever lasts about 3 days, then disappears; in others, the fever is biphasic (appears for 1 day, then disappears for 2 to 3 days, then returns for 2 to 4 days more).

Echoviruses (enteric cytopathic human orphan viruses) were accidentally discovered in human faeces, unassociated with human disease during epidemiological studies of polioviruses. These viruses were picornaviruses isolated from the GI tract, produced CPE in cell cultures, and did not induce detectable pathological lesions in suckling mice. Thirty-four viruses were assigned echovirus serotype designations but echovirus 10 and 28 were reclassified as a reovirus and a rhinovirus respectively and the numbers are now unused. Of the 32 echoviruses, 10 show haemagglutination activity with human group O erythrocytes, the haemagglutinin is thought to be an integral part of the virus particle. There is no group-specific echovirus antigen but heterotypic cross-reactions occur between a few pairs. At least 14 of the known viruses produce disease in rhesus and cynomolgus monkeys if inoculated intracerebrally or intraspinally. As with polioviruses, the mouth is the portal of entry of the viruses, although a few can probably infect through the respiratory route. They are excreted in the pharynx and faeces early in the course of infection and virus may be isolated from the faeces up to several weeks after recovery. The incubation period varies between 2 - 7 days which may be followed by one of several different disease manifestations. Recovery from infection is accompanied by the development of lifelong immunity.

Enteroviruses are mainly transmitted by fecal-oral route and multiply in the digestive tract where infection may often be mild or clinically unapparent (Lees, 2000). However, these viruses can spread to other organs and can cause serious or even fatal disease such as paralysis, aseptic meningitis, myocarditis, hemorrhagic conjunctivitis, congenital infection of neonates and other non-specific febrile illness (Lees, 2000; Wyn-Jones and Sellwood, 2001). It has also been
reported that, enteroviruses may cause or contribute to a range of common chronic diseases such as diabetes mellitus (Lees, 2000).

Though enteroviruses are shed in large numbers in the feces, their numbers in untreated sewage is less \([10^5\text{ plaque forming units (pfu)/l}]\) than bacterial indicators such as total coliforms \([10^8/100\text{ ml}]\) (Wyn-Jones and Sellwood, 2001). These enteroviruses contaminate natural waters such as rivers, lakes, shellfish growing waters through the contaminated sewage effluents. Enteroviruses have been frequently isolated in sewage effluents, pollution receiving waters and from oyster samples (Jaykus et al., 1994; Beuret et al., 2003; Muniaín – Mujika et al., 2003). Cases of infection associated with eating soft fruit, green vegetables and other foods have been recognized (Koopmans and Duizer, 2004; Cook and Rzezutka, 2005). Enteroviruses were also detected in sediments (Le Guyader et al., 1994). However, bivalve shellfish have not been linked to transmission of enterovirus disease though they are implicated with a wide variety of other illness (Lees, 2000). The epidemiological presentation of many enterovirus infections does not favor their identification and linkage to a particular food vehicle, because of their long incubation period and unpredictable appearance of clinical symptoms (Lees, 2000). The recent oyster associated outbreak of NoV gastroenteritis in Scandinavia with secondary symptoms similar to enterovirus infection was observed in some infected consumers (Christensen et al., 1998). Enterovirus was detected by PCR in both shellfish and patients stool with isolations from stool showing a common sequence identity suggestive of a common source of infection.

2.2.1 Detection

Several PCR based detection methods have been developed for the detection of enteroviruses in shellfish samples (Lees et al., 1994; Leparc et al., 1994; Puig et al., 1994; Straub et al., 1994; Shieh et al., 1997; Pina et al., 1998; Rosenfield and Jaykus, 1999; Casas and Sunen, 2001; Fout et al., 2003). Reynolds et al. (1996), developed an integrated cell culture-PCR procedure for the detection of infectious enteroviruses.

2.3 Hepatitis A virus (HAV)
Hepatitis A virus was first classified as a member of the genus enterovirus (serotype 72), now it has been reclassified and placed in a separate genus *Hepatovirus* within the Picornaviridae family (Minor, 1991; Sair *et al.*, 2002). HAV is a small (7.5 kb), non-enveloped, spherical virus, measuring between 27 and 32 nm in diameter and contain a single stranded RNA genome (Koopmans *et al.*, 2002; Hollinger and Emerson, 2001). So far, one serotype of HAV has been recognized (Wyn-Jones and Sellwood, 2001). Although, monoclonal antibody (MABs) studies suggest that there are a limited number of antigenic epitopes closely grouped at the surface of the virus (Stapleton and Lemon, 1987; Ping *et al.*, 1988; Ping and Lemon, 1992). Recently 3 human antigenic types have been reported (Costa – Mattioli *et al.*, 2002; Sanchez *et al.*, 2002). It is mainly transmitted through the fecal oral route where insufficient sanitation or poor hygienic conditions favour the pollution of water and food especially bivalve molluscan shellfish (Hadler *et al.*, 1980).

HAV reference strain (strain HM – 175) was first isolated from an outbreak in Australia (Gust *et al.*, 1985). Genetic analysis of 152 strains of HAV recovered around the world resulted in the designation of seven genotypes of HAV (I to VII). Of these 7 genotypes, 4 (I, II, III, VII) were recovered from HAV human cases, whereas, other 3 genotypes (IV, V, VI) were isolated only from unique simian species developing a hepatitis A–like illness during captivity (Nainan *et al.*, 1991; Tsarev *et al.*, 1991; Robertson *et al.*, 1992). Genotypes I and III were further subdivided into two distinct groups (sub-genotypes), which differed in sequence in no more than 7.5 % of base positions. Among the 152 HAV strains analyzed, genotype I was the most
abundant type worldwide, particularly genotype IA, which included HAV strains from North America, China, Japan, the former USSR and Thailand (Jansen et al., 1990; Robertson et al., 1992). Circulation of sub genotypes IA and IB were recently reported in Brazil (de Paula et al., 2002). Subgenotype IB was also isolated from shellfish imported from Peru (Sanchez et al., 2002). Genotype IB contained strains from Jordan, North Africa, Australia, Europe and Japan (Robertson et al., 1992). Most of the remaining human HAV strains are segregated in genotype III, which has 2 sub-genotypes (IIIA & III B) (Robertson et al., 1992). Sub-genotype PA21, a prototype virus strain of the genotype IIIA was previously linked to intravenous drug users in Sweden during the 1980’s (Robertson et al., 1992) and in Norway at the end of the 1990s was originally isolated from captured Panamanian owl monkeys (Brown et al., 1989). Strains closely related to this genotype have been collected from humans with HAV in India, Sri Lanka, Nepal, Malaysia and USA (Jansen et al., 1990; Robertson et al., 1991; Khanna et al., 1992).

Genetic analysis of HAV strains isolated from environmental samples, such as shellfish – associated outbreak in France, Spanish sewage samples and mussels imported to Italy, revealed for the first time the presence of strains closely related to genotype III A in those countries (Costa-Mattioli et al., 2001b; Pina et al., 2001; Chironna et al., 2003).

HAV antigenic studies carried out during the last 20 years have showed a low antigenic variability (Hollinger and Emerson, 2001). Crevat et al. (1990) reported that, MABs raised to various strains of human HAV have failed to differentiate more than one antigenic type. This indicates a high degree of antigenic conservation among human HAV isolates. Hence, infection with HAV is likely to confer life long immunity that protects against subsequent symptomatic re-infection by the same antigenic type. However, two antigenic variants were recently isolated from an HAV outbreak associated with the imported frozen cockles from South America (Sanchez et al., 2002). So there is a possibility that a new serological type could emerge from this geographic region (Costa – Mattioli et al., 2003).

Hepatitis A is the most serious virus infection linked to shellfish consumption and it is of greater concern in countries where raw shellfish is widely consumed. The severity of infection is age dependent with young children under 6 years of age usually have asymptomatic infections,
whereas older children and adults typically have symptomatic infection that may be as high as 95% during outbreaks (Lednar et al., 1985; Ciocca, 2000). The symptoms of HAV infection (after a incubation period of 15-50 days) include fever, headache, nausea, malaise, anorexia and jaundice (Ciocca, 2000). HAV infection is generally regarded as one of the most severe food borne disease, and in most of the cases, recovery is complete and leads to lifelong protection from reinfection (Ciocca, 2000).

Shedding of HAV in feces occurs in peak during the 2 weeks before the onset of jaundice or liver enzyme elevation. The concentration of virus in stool declines after the onset of jaundice, although prolonged shedding may occur, particularly among infants and children up to 5 months after infection. Low levels of HAV RNA in stools from children for up to 10 weeks after the onset of symptoms has also been reported (Robertson et al., 2000).

2.3.1 Pathogenesis

Virus enters via the intestinal tract, and is transported to the liver following a viremic stage, in which virus can be detected in the blood stream. These viruses replicate in the hepatocytes, viruses are released from liver cells into the bile and are then excreted in faeces. HAV viral antigens and/or genomic material have been found in the spleen, kidney, tonsils and saliva of experimentally infected non-human primates. Suggesting that other sites of replication may exist. In vitro cells are generally not destroyed by the virus and the damage to liver epithelial cells in vivo often is limited (Hollinger and Ticehurst, 1996).

2.3.2 Epidemiology

HAV is transmitted by fecal–oral route, either by the ingestion of contaminated food or water. Asymptomatic and nonjaundiced HAV – infected persons, especially children are an important source of HAV transmission (Staes et al., 2000). Infectious hepatitis outbreak was first documented in Sweden in 1955 when 629 cases were associated with raw oyster consumption (Roos, 1956). Since then, many hepatitis A virus outbreaks worldwide have been linked to the consumption of contaminated bivalve molluscan shellfish and have been reviewed by several authors (Richards, 1985; Rippey, 1994; Jaykus et al., 1994; Lee, 2000; Koopmans et al., 2002).
In the developing countries, HAV infection is endemic, and majority of persons are infected in early childhood and most of the adults are immune (Mast and Alter, 1993). Major Hepatitis A outbreak took place in Shanghai, China in 1988 when almost 3,00,000 cases were traced to the consumption of clams harvested from sewage polluted area (Halliday et al., 1991). Shellfish associated hepatitis A outbreaks were also reported from USA (Richards, 1985; Rippey, 1994), Germany (Stille et al., 1972), UK (Bostock et al., 1979; Sockett et al., 1985), Italy (Mele et al., 1989; Malfait et al., 1996) and Japan (Fujiyama et al., 1985). The first food associated HAV outbreak in Australia occurred in 1997, when 444 persons were infected due to the consumption of contaminated oysters (Conaty et al., 2000). It has been estimated that 7% of all worldwide HAV cases may be associated with the consumption of contaminated bivalve molluscs (Cliver et al., 1983).

Many of the hepatitis A virus outbreaks reported involve large number of cases. The fairly protracted (average 4 weeks) incubation period of hepatitis A virus makes association with a particular food vehicle in individual sporadic cases very difficult. Usually the food is not available for testing and consumption histories inconclusive. Hence, reports of shellfish associated hepatitis A virus disease fall into two groups: the large outbreaks where a common vehicle of infection is more obvious, and the smaller clusters or sporadic infections where a food vehicle involvement may only become evident through diligent epidemiological investigation. Salamina and D’Argenio (1998) reported that, 70% of all hepatitis A cases in Italy were associated with the consumption of contaminated shellfish. Over 1000 cases of oyster and clam associated hepatitis A occurred in several US states during several major outbreaks from 1961 to 1964 (Richards, 1985). Since then further sizeable outbreak occurred in 1973 due to Louisiana oysters (Glass et al., 1996a) and in a multistate outbreaks in 1988 due to oyster from Florida (Desenclos et al., 1991). In a Germany study between 1968 and 1971 shellfish consumption was linked to infectious hepatitis in 19% of patients attending a clinic in Frankfurt (Stille et al., 1972).

In 1996 and 1997, a large HAV epidemic occurred in southern Italy, Puglia region, with 11000 notifications especially among young adults. The main risk factor in this epidemic outbreak was consumption of contaminated mussels (Malfait et al., 1996). In Finland a large HAV epidemic occurred among drug abusers due to contaminated amphetamine (Stene-Johansen, 1998).
2.3.3 Detection

Fetal rhesus monkey kidney derived cell line (FRhK-4) is used routinely to propagate the HM-175 lab-adapted strain of HAV (Cromeans et al., 1987), but this cell line remains ineffective for the detection of wild type HAV (De Leon and Jaykus, 1997). In the recent years several non-culture detection methods such as, Specific IgM antibodies, RT-PCR and real-time PCR (Goswami et al., 1993; Le Guyader et al., 1994; Jaykus et al., 1995; Acha and Szyfres, 2003; Furuta et al., 2003; Hutson et al., 2004) have been developed for the detection of HAV.

2.4 Hepatitis E virus (HEV)

HEV is the other enteric source of viral hepatitis. It is a non-enveloped, spherical, positive-stranded RNA virus. This virus was originally classified within the family of
caliciviruses, but, recent data based on genome organization and nucleotide sequence analysis has revealed differences, so it is now provisionally classified in a separate genus “HEV-like viruses” (Jameel, 1999; Berke and Matson, 2000). HEV is transmitted by fecal-oral route, the source often being contaminated water and food resulting from poor community sanitation, which affects mainly young adults (15 to 30 years), and the overall death rate is 0.5 to 3.0% (Vasickova et al., 2005). However, the death rate during pregnancy approaches 15 to 25%. Death of the mother and fetus, abortion, premature delivery, or deaths of a live-born baby soon after birth are common complications of hepatitis E infection during pregnancy. In countries where the virus is endemic, HEV is associated with >50% of sporadic acute hepatitis cases. The disease is self-limited but sometimes has severe complications and a high case-fatality rate, particularly in pregnant women (approximately 20%) (Balayan, 1997). Infection by HEV is clinically similar to that of HAV, in that it is generally mild and self-limiting. HEV was first identified in New Delhi, India (1955 - 1956), where about 30,000 cases were reported after the flooding of the river Yamuna and contamination of the city's drinking water (Viswanathan, 1957). Since then it has been recognized in the Middle and Far East, in northern and western Africa, the central Asian Republics of the former Soviet Union, in China and Hong Kong SAR and North America (Mexico) (Lemon, 1995; Purcell, 1995, 1996) (Fig. 7). In China, 1,00,000 cases were reported between 1986 and 1988 (Stapleton and Lemon, 1994). North America and Europe have traditionally been considered nonendemic for HEV; most HEV infections in those regions are considered to be imported. However, seroprevalence ranges from 1% to 5%.

In the last few years, some HEV strains associated with sporadic acute hepatitis have been isolated from human serum samples in North America (Kwo et al., 1997) and Europe (i.e., Italy, Greece, Spain, and the United Kingdom) (Pina et al., 2000; Zanetti et al., 1999). Molecular analyses have shown that these strains form a group of HEV isolates that are genetically divergent compared with strains from HEV-endemic countries (Schlauder and Mushahwar, 2001). Unlike other enterically transmitted infections, person-to-person transmission of HEV occurs infrequently (Aggarwal and Naik, 1994).

Tan et al. (2003), has reported an outbreak of acute icteric hepatitis caused by HEV associated with food intake in China. HEV is also found in both wild and domestic animals.
Antibodies against HEV have also been detected in pigs, rodents, and other animals (Favorov et al., 2000; Smith, 2001). The close genetic relationship of the swine and human virus suggests that swine may be a reservoir of HEV and swine manure could be a source of HEV contamination of irrigation water or coastal waters with concomitant contamination of shellfish (Smith, 2001).

Fig. 7. Geographic distribution of HEV infection (outbreaks or confirmed infection in >25% of sporadic Non-ABC Hepatitis) (Adapted from the viral hepatitis slide set published by the US Centers of Disease Control and Prevention, Atlanta, GA, USA, at http://www.cdc.gov/ncidod/diseases/hepatitis/slideset (fig001sjd).

2.5 Caliciviruses (Norovirus and Sapovirus)
Human enteric caliciviruses are naked, single-stranded, positive sense, non-segmented RNA viruses. The name caliciviruses was derived from the Latin word for calyx or cup. Members of the caliciviruses family have 32 distinct, cup-shaped surface depressions that give it a unique appearance (Fig. 8). Not all members of the family have the cup-shaped depressions.

The family caliciviridae includes four genera, Norovirus (for what was previously called Norwalk–like viruses or small, round–structured viruses), Sapovirus (for sapporolike viruses) Vesivirus and Lagovirus (Matson and Szucs, 2003). Human and animal caliciviruses associated with gastroenteritis belong to the genera Norovirus (NoV) and Sapovirus. Human noroviruses cause illness in people of all ages, whereas human sapoviruses cause illness primarily in children (Green et al., 2001). Norovirus is divided into five genogroups based on the genome sequence of the RNA dependent RNA polymerase and the capsid regions (Vinje et al., 2004). However, only Norovirus (NoV) genogroup I (GI), GII and GIV have been associated with human gastroenteritis and GIII infect pigs and cows (Ando et al., 2000; Koopmans et al., 2002; Karst et al., 2003). NoV GI and GII are further subdivided into 7 and 12 genotypes respectively (Vinje et al., 2004). These human caliciviruses do not grow in cell or organ culture. This has impeded progress in understanding of these agents until fairly recently with the advances in molecular biological detection and confirmation procedures (Hutson et al., 2004).

NoV is the principal cause of viral gastroenteritis in adults (Fields et al., 1996). This virus was the first recognized virus of clinical importance associated with the gastroenteritis in humans, was discovered in 1972 in Norwalk, Ohio, USA, by immunoelectron microscopy of an infectious stool filtrate (Kapikian et al., 1972). These viruses are frequently the cause of sporadic cases and also acute gastroenteritis outbreaks in children and adults (Kaplan et al., 1982; Vinje and Koopmeans, 1996; Caul,1996a, b; Hedlund et al., 2000) particularly in semi-closed environments such as schools, cruise ships, hospitals and residential homes (Lopman et al., 2002).
NoV being excreted in feces will be transported through sewage systems and may reach receiving waters. These viruses are robust and will survive in the environment, where it may present hazard to public health as evidence of shellfish-associated gastroenteritis outbreaks (Lees et al., 1995b; Less, 2000). Ten to hundred virions can cause the disease. Sapovirus cause gastroenteritis, mainly in babies under 1 year old (Vinje et al., 2000).

2.5.1 Epidemiology

Shellfish associated NoV gastroenteritis outbreak was first documented in Australia (Murphy et al., 1979). In United States, shellfish associated gastroenteritis attributed to NoV was first reported in 1980 after individuals consumed oysters from Florida (Gunn et al., 1982). Characteristic symptoms of NoV infection include diarrhea, vomiting, a short duration of illness (1 – 3 days), and a short incubation period (24 – 48 h). The illness is generally mild, but can cause severe disease with associated dehydration and electrolyte imbalance (Hutson et al., 2002). In the United State, Noroviruses cause an estimated 23 million illness, 50000 hospitalizations and over 300 deaths each year (MMWR, 2002).

NoV are extremely contagious because of their low infectious dose (< 100 viral particles), prolonged asymptomatic shedding (up to 2 weeks after recovery), ability to resist chlorination (10 ppm chlorine) and stability in the environment (stable with freezing and at 60°C). NoV is currently recognized as the cause of almost all (> 96%) outbreaks of non-bacterial gastroenteritis in adults (Mead et al., 1999), particularly in Europe and Australia where there is active surveillance.

Foodborne NoV outbreaks vary greatly from one country to the other, due to difference in case definition, surveillance systems and detection methods used (Koopmans et al., 2002). Numerous publications have documented shellfish associated outbreaks of gastroenteritis caused by NoV and incidents up to 2000 have been reviewed by several authors (Richards, 1985; Jaykns et al., 1994; Lees, 2000; Koopmans et al., 2002). In the US, 96% of outbreaks of non-bacterial acute-gastroenteritis reported to CDC between January 1997 and June 1998 were caused by NoV infection. A large incident suspected to be caused by NoV occurred in Louisiana,
USA in 1982 with 472 cases linked to consumption of oysters (Richards, 1985). In Finland, 56% of the epidemics reported as food borne, from which stool samples (and food stuffs in some instances) were submitted for virological screening were found positive for NoVs (Maunula et al., 1999).

Daniels et al. (2000) reported an acute gastroenteritis outbreak associated with NoV among students at Texas University (USA). Stool specimens from 9 (50%) of 18 ill students and deliham samples from the University’s main cafeteria delibar showed evidence of NoVs. In the UK, oyster associated outbreak was reported in London in 1983 which caused illness in over 300 people (Gill et al., 1983). The attack rate was 79% among oyster eaters and NoV was confirmed as the aetiological agent by detection in stools by electron microscopy. Since then, gastroenteritis outbreaks associated with depurated oysters were reported in the UK in 1986 (Heller et al., 1986), 1993 (Chalmers and McMillan, 1995), 1994 (Perrett and Kudesia, 1995) and 1997.

In Canada, the first shellfish associated gastroenteritis was documented in late 1991 by Pontefract et al. (1993), which affected 200 persons, NoV was demonstrated by electron microscopy in stools and an antibody response to these virus particles was demonstrated by immune electron microscopy. In The Netherlands, a set of population based case control studies showed that the overall incidence of gastroenteritis was quite high, at 280 cases/1000 persons / year (DeWit et al., 2001a,b,c). In the Czech Republic, 104 cases of noroviral infections were recorded in the year 2001. However, there were only small outbreaks, in 2003 in nursing home for pensioners in Prague (Drapal et al., 2003).

Shellfish associated gastroenteritis outbreaks have been reported from a number of districts in Japan. In Kyushu district 4/5 oyster related outbreaks between 1987 and 1992 were shown to be caused by NoV (Otsu, 1999). Two oyster associated outbreaks in 1989 and 1991 were reported to be caused by NoV in Gifu prefecture (Kawamoto et al., 1993). Western blotting immunoassay showed that the strain detected to be related to genogroup II (Hawaii agent). In Shizuoka district, 5 outbreaks were reported between 1987 and 1994 with both genogroup I & II NoV strains demonstrated by molecular techniques (Sugieda et al., 1996).
2.5.2 Pathogenesis

Little is known about the mechanisms by which NoV cause diarrhoea. In duodenal biopsies collected from infected volunteers, lesions were seen in the intestinal epithelium at 1 day post infection with the NoV or Hawaii virus as inoculum. The changes were villous broadening, abnormal epithelial cells, loss of and an infiltration response in the lamina propria with infiltration of polymorphonuclear leukocytes and lymphocytes. At 5-6 days after ingestion villous shortening and crypt hypertrophy were observed. D-xylose absorption was significantly reduced throughout this period (Schreiber et al., 1973, 1974). The inflammatory response observed in the lamina propria was similar to that caused by rotavirus infection where pro-inflammatory cytokines and chemokines are thought to trigger this process (Rollo et al., 1999). The virus has not been detected in involved mucosal cells, perhaps because of its small size and patching distribution.

2.5.3 Diagnosis

Human caliciviruses are non-culturable enteric viruses. Hence, diagnosis has been made historically by visualization of virus particles by electron microscopy (Kapikian et al., 1992; Atmar and Estes, 2001). However, this method is relatively insensitive technique, requiring 10 million virus particles per ml of sample and viral concentrations in coastal environments would probably be several orders of magnitude lower than this concentration.

Non-culture detection methods such as immunoassays (Herrmann et al., 1985, 1995; Monroe et al., 1993) and molecular techniques such as RT-PCR have become available (Lees et al., 1995). Immunoassays have proved valuable for clinical investigators, including food borne outbreaks (Fleissner et al., 1989). RT-PCR based procedures have been developed by several groups for the detection of NoV in shellfish (Lees et al., 1995; Atmar and Estes, 2001; Jiang et al., 1992; DeLeon et al., 1992). Following detection of NoV by RT-PCR, the PCR products can be characterized by sequencing.
2.6 Rotaviruses

![Electron micrograph of human rotavirus](image)

**Fig. 9. Electron micrograph of human rotavirus (Source: Locarnini et al., 1974)**

In 1973, Bishop and colleagues observed a 70-nm virus by electron microscopy, in the duodenal epithelium of children with diarrhea, which was subsequently designated rotavirus (Latin, *rota* = wheel). Human rotaviruses (HRV) are the members of the family Reoviridae and are the major causes of viral gastroenteritis worldwide which are transmitted by fecal-oral route (Parashar *et al*., 1998; Oh *et al*., 2003). They measure 60-80 nm in diameter and possess triple capsid protein layer (Estes, 1996). The genome of rotavirus consists of 11 segments of double stranded RNA that are capable of genetic reassortment. The majority of human rotavirus isolates share a common group antigen, with at least seven serotypes existing within their group, and are known as group A rotaviruses. However, other viruses have been isolated that are morphologically identical but serologically unrelated to group A which are known as group B to G rotaviruses. Rotavirus A is further classified into G and P serotypes/genotypes according to the two external capsid proteins, VP7 and VP4 respectively (van Regenmortel *et al*., 2000). Only groups A, B and C have been associated with disease in humans. Among these, group ‘A’ rotaviruses have been frequently identified as the most important viral pathogens responsible for diarrheal disease requiring treatment or hospitalization in children under 2 years of age (Matsumoto *et al*., 1989), where as group ‘B’ rotaviruses cause gastroenteritis in adults (Bridgeer, 1994). Hence, group A rotaviruses normally present as pediatric infections occurring in infants or early childhood.
Rotaviruses infect the mature absorptive villous epithelium of the upper two thirds of the small intestine. After replication in the upper small intestine, infectious particles are released into the intestinal lumen and undergo further replication in the distal areas of the small intestine. Infection is generally confined to the intestinal mucosa. Although rotaviruses can be found in the lamina propria and regional lymphatics, replication at these sites and systemic spread usually do not occur in immunocompetent persons (Kapikian et al., 1996).

The incubation period for rotavirus infection is 1-4 days. In neonates, older children and adults, infection with rotavirus is usually asymptomatic. Clinical illness is most likely between the ages of 6 and 12 months. Typical symptoms include diarrhea, fever, abdominal pain and vomiting, leading to dehydration (CDC, 1990a). In infants and children, severe loss of electrolytes and fluids may be fatal unless treated. Immunity after infection is incomplete, but repeated infection tends to be less severe than the original infection (CDC, 2005).

An infected person shed viruses in large number in feces (> $10^{12}$ particles/g) leading to readily detectable contamination in sewage effluents and polluted receiving waters (Gajardo et al., 1995; Dubois et al., 1997). Consequently presence of the virus has also been demonstrated in bivalve shellfish grown in contaminated waters (Lees, 2000). However, no seafood associated rotavirus infections have been reported. This could be due to the lack of symptomatic disease in the adult population and also adults tend to be the primary consumers of seafood.

In developing countries rotaviruses cause mortalities up to 20% in children with less than 5 years of age (Matsumoto et al., 1989). The prevalence of rotavirus infection in neonates in India has not been systematically examined, but high infection rates were documented in newborns in six hospitals (Cicirello et al., 1994).

Environmental transmission of rotavirus occurs mainly through shellfish grown in polluted waters and contaminated drinking water (Bosch 1998; Gratacap-Cavallier et al., 2000; Le Guyader et al., 2000). The diagnostic test of choice for most situations is enzyme immunoassay and RT-PCR (CDC, 2005). Tetravalent rhesus-human reassortant rotavirus vaccine (RRV-TV) (which contains a rhesus rotavirus with serotype G3 specificity and
reassortant rhesus-human rotaviruses with G1, G2, and G4 specificity) provides coverage against the four common serotypes of human rotavirus.

2.7 Astroviruses

![Electron micrograph of human astrovirus](Source: Locarnini et al., 1974)

Astroviruses come under the only genus in the family Astroviridae. They are star shaped (Fig. 10), small, 28 nm diameter non-enveloped, positive sense single stranded non-segmented RNA viruses. These viruses do not grow in commonly used cell lines, though propagation has been reported in cell lines derived from intestinal tumours (Willcocks et al., 1992). However, the disease is generally milder than that caused by rotaviruses. Frequent co-infection of astrovirus with rotavirus and caliciviruses in childhood diarrhoea complicates the epidemiology. Infections occur throughout the world and are more common in winter months (Lees, 2000; Vasickova et al., 2005). Clinical symptoms of astrovirus infection include vomiting, diarrhea, fever and abdominal pain. Recovery is normally complete with no complications.

Though few reports exist on the incidence of astrovirus in aquatic environment, a direct epidemiological link between eating raw seafood and astrovirus infection has not been fully demonstrated (Lees, 2000; Wyn-Jones and Sellwood, 2001; Vasickova et al., 2005). Hence, the importance of astroviruses as causative agents of gastroenteritis following seafood consumption is not clear. The increasing availability of molecular diagnostic methods such as RT-PCR (Saito et al., 1995; Jonassen et al., 1995; Le Guyader et al., 2000) may provide new approaches to
elucidate the true prevalence of astroviruses as a cause of diarrohea following seafood consumption.

2.8 Aichi virus

Aichi virus, the type species of a new genus, Kobuvirus of the family Picornaviridae (Yamashita et al., 1998; Sasaki et al., 2001), was first recognized in 1989 as the cause of oyster associated non-bacterial gastroenteritis in man (Yamashita et al., 1991). A morphological study on purified Aichi virus virions indicated that the surface structure is characteristic of small round-structured virus (Yamashita et al., 1991). However, the ability to grow in cultured cells, along with other biological properties, i.e. resistance to treatment with chloroform and stability under a low pH (pH 3.5), suggested that Aichi virus is a member of the enteroviruses. No-cross neutralizing reactions were observed between aichi virions and human enteroviruses. Further, the aichi virus antiserum did not react with any other enterovirus or other enteric viruses such as NoV and astroviruses (Yamashita et al., 1991, 1993).

Aichi virus was isolated from 6 / 47 patients in five gastroenteritis outbreaks, 5/722 Japanese travelers returning from tours to Southeast Asian countries (Indonesia, Thailand and Malaysia) and complaining of gastrointestinal symptoms at the quarantine station of Nagoya international airport in Japan and more recently it was isolated from 5/222 Pakistani children with gastroenteritis, but none was found in 91 healthy children (Yamashita et al., 1995, 1993). These results indicate that Aichi virus and a similar agent is endemic in Southeast Asian countries. Yamashita et al. (2000) developed RT-PCR method for the detection of Aichi virus

2.9 Hantavirus

Hantavirus is a RNA-virus of the family Bunyaviridae that is found in the urine, saliva, or droppings of infected deer mice and some other wild rodents. Hantaviruses have been identified as etiologic agents of two human diseases. They may cause a rare but serious lung disease called Hantavirus pulmonary syndrome (HPS) and pathogenic European hantaviruses (Puumala, Doll, and Saaremaa viruses) can cause a human disease designated “hemorrhagic fever with renal syndrome” of varying severity (Vapalahti et al., 2003; Dekonenko and Tkachenko, 2004). People can contract the Hantavirus infection through inhalation of respirable
droplets of saliva or urine, or through the dust of faeces from infected wild rodents, especially the deer mouse. Transmission can also occur when contaminated material gets into broken skin, or possibly, ingested in contaminated food or water (Acha and Szyfres, 2003; Votava et al., 2003; Dekonenko and Tkachenko, 2004; Ulrich et al., 2004). Duration of viraemia in infected rodents and virus survival in tissues indicates that contamination of the ambient environment with excretions and secretions could be prolonged (Lee et al., 1981 as quoted by Acha and Szyfres, 2003). Many outbreaks of Hantavirus infections have been described in Europe and other continents; its occurrence was associated with infestation of rodents detected in those areas (Olsson et al., 2003; Pini et al., 2003; Ruedas et al., 2004; Ulrich et al., 2004). According to study by Van Loock et al. (1999) professional activity (sighting of living rodents, exposure to rodent droppings, and trapping rodents) appears to be a more important risk factor for acquisition of Hantavirus in Europe.

2.10 Indicators of enteric viruses

Bivalve molluscan shellfish are filter feeding organisms and when these are grown in sewage-polluted water, they accumulate and concentrate pathogenic microorganisms, such as pathogenic bacteria and viruses present in the water, which remain infectious for a certain period (Desenclos et al., 1991), and can present a significant health risk when the shellfish are consumed raw or partially cooked (Sobsey and Jaykus, 1991). Traditionally bacterial indicators (coliforms and Escherichia coli) have been used to assess the sanitary quality of shellfish and this has led to success in the prevention of shellfish-borne bacterial gastrointestinal infections, but they are believed to have limited predictive value for pathogens such as enteric viruses (Vaughn and Metcalf, 1974; Gerba et al., 1979; Ellender et al., 1980; Jofre, 1992). Bacterial standards do not always reveal the presence of viruses (Gerba et al., 1979; Marzouk et al., 1980; Goyal et al., 1984) and that the depuration process, used to remove bacteria before distribution and sale, are not effective for eliminating viruses (Franco et al., 1990; Croci et al., 1992). It has been reported that some viral pathogens may be differentially resistant to environmental conditions, sewage or water treatment processes, compared with coliforms (Payment et al., 1985, 1993; Jofre et al., 1995 Gantzer et al., 1998b). Hence there is a need for reliable indicators which can be better correlated with viral contamination. Some studies have shown that bacteriophages, viral particles similar in size and structure to pathogenic viruses, could be good
indicators of viral contamination. Bacteriophage assay conditions are much simpler and cheaper than any of the enteric virus detection methods. In order to be suitable enteric viral indicators, these phages should fulfill certain characteristics such as:

- They should be associated with the source of the pathogen and should be absent in unpolluted areas,
- They should occur in greater numbers than the pathogen,
- They should not multiply out of the host,
- They should be at least equally resistant to natural and artificial inactivation as the viral pathogen,
- They should be detectable by means of easy, rapid and inexpensive procedures and
- They should not be pathogenic.

The bacteriophages most frequently studied in this context are somatic coliphages (Vaughn and Metcalf, 1974), male-specific RNA coliphages or FRNA phages (Havelaar and Hogeboom, 1984; IAWPRC, 1991) and phages infecting Bacteriodes fragilis (Armon and Kott, 1993; Jofre et al., 1986; Tartera and Jofre, 1987; IAWPRC, 1991; Gantzer et al., 1998a). Somatic phages attach to the body of the bacterium at the cell membrane or cell wall and FRNA phages attach to the F pili of the cells and are therefore referred to as F＋ phages.

Havelaar et al. (1993) reported that, for monitoring purposes, F-RNA bacteriophages (Fig. 11) can serve as model organisms and suitable indicators for the presence of human pathogenic enteric viruses. There are two groups of F＋ – specific bacteriophages, Group E and Group F, out of a total of six bacteriophage groups (Singleton and Sainsbury, 1993). The first group, group E (Leviviridae) comprises four sub-groups (I – IV). These phages have small, hexagonal capsomeres without tails. They are approximately 20-30 nm in diameter containing ssRNA and are referred as the FRNA coliphages. MS-2 coliphage is included in group I FRNA coliphage. FRNA phages are relatively resistant to disinfectants, sunlight, heat treatment processes (Havelaar and Hogeboom, 1984; Havelaar and Niewstad, 1985). Both F-RNA phages and enteroviruses such as polio viruses have an icosahedral capsid with a diameter of about 25nm and a single stranded RNA genome. In addition both the viruses are excreted by humans.
The behaviour of F-RNA bacteriophage resembles that of enteric viruses much closer than commonly used fecal indicator bacteria such as coliforms (Grabow et al., 2001).

The second group, Group F (Inoviridae), is made up of large, filamentous bacteriophage which are 760-1950 x 6 nm in size. They have no head and consist of a flexible filament containing single-stranded DNA. These are referred to as the FDNA coliphages. Both the FDNA AND FRNA phages have been found in wastewater, but the sanitary significance of FDNA phages has not been determined (Sinton et al., 1996). FDNA coliphages have received less attention as indicators of enteric viruses because they are generally less plentiful than FRNA coliphages. Morphologically these phages do not resemble human enteric viruses and their ecology is poorly understood.