

# Seafood associated human pathogenic non-enveloped viruses

## Su ürünleri kaynaklı insan patojenik zarfsız virüsleri

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**Abstract:** Non-enveloped human pathogenic viruses, highly stable in the environment, can be transmitted by different routes, such as contaminated food and water. The waterborne transmission of non-enveloped viruses to humans causes illnesses when individuals are exposed to contaminated water resources such as agricultural water, drainage, outdoor water, field or subsurface water and even drinking water. In addition to waterborne transmission, viral foodborne transmission may consist because of contagious seafood, through infected food handlers due to inadequate hygienic activities, aerosol containing viruses produced by infected people. Most hazardous non enveloped enteric viruses associated with water and seafood cause a significant and emerging food safety and public health problem and threat. In this review, norovirus (NoVs), hepatitis E virus (HEV) and hepatitis A (HAV), human adenovirus, rotavirus A and sapovirus are evaluated as seafood associated human pathogenic non-enveloped viruses.

**Keywords:** Waterborne transmission, seafood borne transmission, pathogenic non-enveloped viruses

**Öz:** Çevresel ortamda oldukça kararlı olan zarfsız insan patojenik virüsleri, kontamine olmuş gıda ve su gibi farklı yollarla bulaşabilirler. Zarfsız virüslerin insanlara su yoluyla bulaşması, genellikle tarımsal su, drenaj, evsel atık suları, tarım veya yer altı suyu ve hatta içme suyu gibi kirliliği su kaynaklarına maruz kaldıklarında, hastalıklara neden olur. Su yoluyla bulaşmaya ek olarak, gıda kaynaklı viral bulaşma, virüsleri taşıyan deniz ürünleri, yetersiz hijyenik faaliyetler nedeniyle enfekte olmuş gıda çalışanları ve enfekte kişiler tarafından üretilen aerosol içeren virüslerden kaynaklanabilir. Su ve su ürünleri ile ilişkili zararlı zarfsız enterik virüslerin çoğu, önemli ve ciddi gıda güvenliği ve halk sağlığı sorununa ve tehdidine neden olur. Bu derlemede, su ürünleri ile ilişkili insan patojenik zarfsız virüsler olarak norovirüs (NoVs), hepatit E virüsü (HEV) ve hepatit A (HAV), insan adenovirüs, rotavirüs A ve sapovirüs değerlendirilmiştir.

**Anahtar kelimeler:** Su kaynaklı bulaş, su ürünleri kaynaklı bulaş, zarfsız patojenik virüsler

## INTRODUCTION

Viruses are a submicroscopic intracellular parasites that, by nature, made up of RNA or DNA genome encapsulated protein shell, varying in size from 15 to 400 nm and involving just a small number of particles are required to spread disease. Such infections don't happen randomly: each virus group has its own characteristic host diversity and cell selection (Eterpi et al. 2010; Koopmans and Duizer, 2004). A complete sample of the virus is called a virion. The virion's primary purpose is to transmit the RNA or DNA genome when they infect a host cell. The viral genome, frequently with related basic proteins is packaged into the virion, a symmetric protein capsid. The nucleic acid-associated protein together with the genome are known as nucleoprotein, forms by the association of the nucleocapsid (Gelderblom, 1996).

Among different types of natural water applications, such as agricultural water, drainage, outdoor water, field or subsurface water and drinking water, human pathogenic viruses implicated among water transmission are often observed. (Grabow, 2007; Pinon and Vialette, 2018). It can spread more than 140 forms of pathogenic viruses from the

aquatic world (Schwartzbrod and WHO, 1995). All identified waterborne pathogenic viruses present a major threat to public safety in the marine ecosystem which are spread mainly through fecal-oral routes, which is regularly released into coastal and estuarine environments by the runoff of processed and untreated wastewater (Rao et al., 1986; Griffin et al., 2003; Kovač et al., 2009) and urine, and respiratory secretions from the infected host which enter into sewage water (Wang et al., 2018). The average viral concentration in wastewater changes between  $10^2$  and  $10^3$  PFU  $l^{-1}$ , and it can be estimated at between  $10^1$  and  $10^2$  PFU  $l^{-1}$  in treated wastewater and  $10^3$  PFU  $Kg^{-1}$  in treated sludge, depending on the type of treatment and its yield. According to the intensity of faecal pollution, the concentration in the surface water changes, but can be determined at  $10^1$  PFU  $l^{-1}$  in river water and between 1 and 10 PFU  $l^{-1}$  in contaminated sea water (Chang et al., 1995). Humans can be infected the pathogenic virus infected in the fecal contaminated water through production in watercourses by collecting, handling, preparation, processing, carrying and storage chain (Blumenthal et al., 2000; Elbashir et al., 2018; Lee and

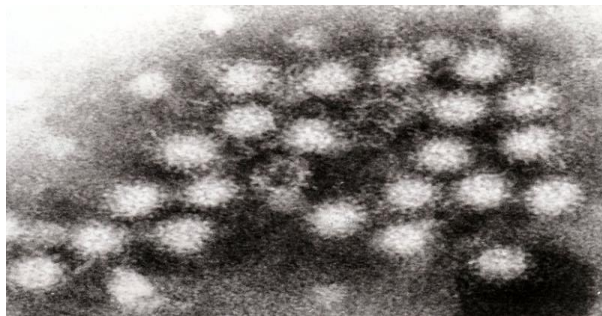
Rangdale, 2008; Schwartzbrod and WHO, 1995). These polluted ecosystems will end up with unhealthy seafood.

Worldwide an estimated 8 billion per year gastrointestinal disease occur annually (Chan et al., 2019). Viruses associated with acute gastroenteritis, include diarrhea, fever, headache, vomiting, abdominal cramps, and myalgia, may occur as a result of contamination of the marine environment with feces (Adelodun et al., 2020; Koopman et al., 1982). Viral pathogens from water environment have also been reported which include norovirus (NoVs), hepatitis E virus (HEV) and hepatitis A (HAV), human adenovirus, rotavirus A and sapovirus (Bosch et al., 2005; Joshi et al., 2019; WHO, 2006). This work will focus on different human pathogenic non-enveloped viruses associated with seafood and their characteristics.

### Human pathogenic non-enveloped viruses

#### Noroviruses

Norovirus is a small organized RNA virus, and a class known as human pathogens belong to the family Caliciviridae. Virus particles are 27–37 nm in diameter and unwrapped, which cause their high tenacity and disinfection resistance (Bachofen, 2018). The NoV genome of approximately 7.5 kb is positive-sense single-stranded RNA with three free read frames encoding both structural and non-structural proteins (Campos and Lee, 2014). The viral protein 1, the capsid protein, is the viral capsid's most significant component, while the viral protein 2 is inserted within the capsid at low copy numbers (Figure 1).



**Figure 1.** Transmission electron micrograph of Norovirus particles in feces by Graham Beards at English Wikipedia (CC BY 3.0). <https://commons.wikimedia.org/w/index.php?curid=5736176>

Norovirus (NoV) is well-known to be the primary causative agent of seafood transmitted illness (Bachofen, 2018; Grabow, 2007; Li et al., 2014; Terregino and Arcangeli, 2017) and the emergence in viral gastroenteritis outbreaks is a global safety issue which is also blamed for economic losses (Kobayashi et al., 2019; Pavoni et al., 2013). Several geographic areas worldwide have been confirmed to have numerous seafood associated outbreaks of various norovirus strains (Stals et al., 2013; Vidal et al., 2005). The spread of NoV is primarily due to its low infectious dose is sufficient to cause infection and gastroenteritis needed for the infection

(Khora, 2018; Koopmans et al., 2002; Teunis et al., 2008). The long-term survival and longevity of NoV on infected surfaces used in food processing areas often lead significantly to transmission of the disease (Moorman, 2017).

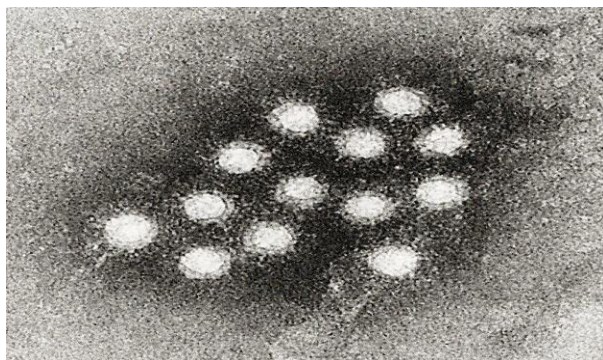
The main route for norovirus transmission is harvesting or catching of seafood from faecally contaminated waters (Butt et al., 2004; Campos and Lee, 2014; Terregino and Arcangeli, 2017), NoV-infected food handling (Grabow 2007; Li et al., 2014; Widdowson et al., 2000), contaminated surfaces and utensils used in food preparation areas because of the long-term stability and persistence of NoV (Cheesbrough et al., 2000; Evans et al., 2002; Lamhoujeb et al., 2009; Mattison et al., 2007; Sharp et al., 2012; Tuladhar et al., 2013). Hardstaff et al. (2018) stated personnel working as food handler (mostly in the kitchen) were tested for NoV in 44 of 51 (86%) outbreaks and food handlers, the median proportion of positive samples, showed 46% (interquartile range of 25–76%). In fact, inadequate preparation, such as steaming clams, has led to disease and outbreaks even before they expand rather than to higher temperatures that destroy noroviruses (Centers for Disease Control and Prevention, 2010; DuPont, 1986; Le Guyader and Atmar, 2007; Morse et al., 1986). Consumption of contaminated raw/undercooked oysters are at risk for secondary infection of NoV in household contacts (Guix et al., 2019). Cooking shellfish (e.g. by steaming) can not inactivate the virus, so after ingestion of cooked shellfish, incidents of infection have occurred (Le Guyader and Atmar, 2007; Li et al., 2014; McDonnell et al., 1997). In comparison, NoV is often immune to certain commercial food storage practices and can withstand boiling, freezing, acidification, decreased water movement and changed packaging environment (Baert et al. 2009).

Shellfish, in particular mussels, clams and oysters, crabs, prawns, finfish, shrimps are usually implicated in seafood-borne norovirus outbreaks because of their feeding patterns as filter feeders and their capacity to absorb the virus from polluted water (Das et al., 2020; Woods et al., 2016). Throughout this filter feeding, bacteria and viruses may be stored and accumulated in their bodies due to land-based waste outflow or harvester runoff disposal (Bellou et al., 2013; Kohn et al., 1995; Le Guyader et al., 2006). Oysters are capable of great flesh and intestine bioaccumulation at amounts up to 99 times greater than the local waters during the autumn / winter season (Burkhardt and Calci, 2000) and remain contagious well after depuration (McLeod et al., 2009). NoV, which can be insufficiently removed by standard decontamination procedures (McLeod et al., 2017; Muniain-Mujika et al., 2002) the result is gastroenteritis outbreaks after consumption of shellfish (Le Guyader et al., 2006; Le Guyader et al., 2008; Webby et al., 2007). Le Guyader et al. (2008) and Doré et al. (2010) both stated that NoV was still measureable in some samples after three to four weeks of purification in open seawater. This may suggest that low levels of NoV in oysters purified for three to four weeks in clean open seawater is small risk to consumers. However,

Doré et al. (1998) stated that at the low levels of NoV particles may be able to inducing infection of NoV in oysters after four weeks of purification (McLeod et al., 2017).

### Hepatitis A virus

Hepatitis A virus (HAV) has an ssRNA (+) genome of 7.5 kb size and a Picornaviridae family member belonging to the Hepatovirus class (2). Particles of the virus come in two versions: naked, unenveloped 27 nm diameter icosahedral virions with a protein capsid surrounding them (Butt et al., 2004; Feng et al., 2013). Electron micrograph of "Hepatovirus A" virions is shown in Figure 2



**Figure 2.** Electron micrograph of "Hepatovirus A" virions. This media comes from the Centers for Disease Control and Prevention's Public Health Image Library (PHIL), with identification number #2739 ([https://commons.wikimedia.org/wiki/File:Hepatitis\\_A\\_virus\\_02.jpg#/media/File:Hepatitis\\_A\\_virus\\_02.jpg](https://commons.wikimedia.org/wiki/File:Hepatitis_A_virus_02.jpg#/media/File:Hepatitis_A_virus_02.jpg))

Hepatitis A is the most severe virus infection related to the consumption of seafood (Grabow, 2007; 8; Khora, 2018; Iwamoto et al., 2010). The WHO (2015) reports that 14 million foodborne infections and 27,731 fatalities in 2010 were triggered by hepatitis A virus (HAV) (Kirk et al., 2015). Incidence of contamination ranges across areas of the world, with the largest incidence in developed nations where there could be inadequate water management and hygiene procedures (Rodriguez-Lazaro et al., 2012). In 1955, when 629 cases were connected with oyster ingestion, the first HAV-linked seafood-borne epidemic involving oysters was reported in Sweden (La Rosa et al., 2012; Lindberg-Braman, 1956). 1988 witnessed the biggest outbreak in Shanghai, China, in which more than 288,000 people were poisoned after consuming fresh or poorly cooked clams (Butt et al., 2004). Between 1986 and 2012, 46 HAV outbreaks were reported and linked to seafood vehicles worldwide, such as oysters, clams, mussels and cockles (Bellou et al., 2013).

Bivalve molluscs, such as soft clams, hard clams, mussels and oysters, have been infected with HAV outbreaks and pollution most often happens when shellfish growing areas become polluted with human sewage (Khora, 2018). Through successful filtration, shellfish accumulate the virus many times in their tissue. The infectious dosage is small, maybe 10–100 virus particles, which is undoubtedly the explanation why

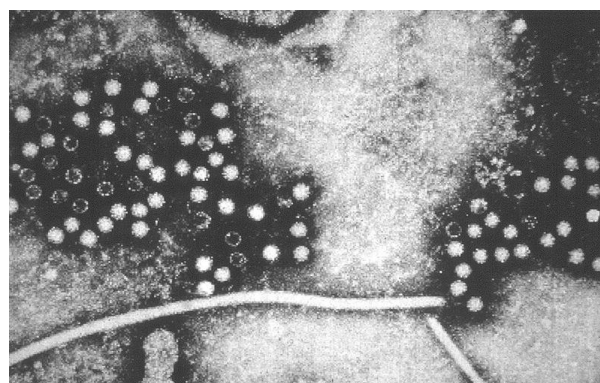
shellfish collected from areas were feasibly infected (Butt et al., 2004). In fact, shellfish are consumed with their digestive tracts in place, unlike many other seafoods. Shellfish, mostly consumed raw or lightly cooked, may also protect viruses by adequate cooking, unlike other foods (Patwardhan, 2019).

Hepatitis A virus transmission is spread by consumption of fecal polluted water and/or seafood, inadequate ventilation, bad personal hygiene and close contact with an infected person (including asymptomatic carriage) (Bosch 1998; Butt et al., 2004; Tallon et al., 2008; Richards, 2013; WHO, 2016). In the primary instances, the most significant risk factor was the intake of raw seafood, during interaction with individuals (Germinario et al., 2000).

The HAV has environmental stability, which makes it viable in water or on fomites for many weeks and including freezing, heat, chemicals, and desiccation (Khora, 2018). For instance, the virus can keep living for a long time in tap water (up to 60 days), in river water (over 6 weeks), in groundwater (over 8 weeks) and in sea water (up to 30 weeks) (Crance et al., 1998; Enriquez et al., 1995; Sobsey, 1989; Springthorpe et al., 1993). In fact, hepatitis A virus is heat-resistant and can tolerate steaming, so proper cooking of seafood diminishes the risk of ingestion of live hepatitis A. Since shellfish is widely prepared in ways that are inadequate to inactivate the virus, several preventive techniques are aimed to manage the contamination before the food is processed (Iwamoto et al., 2010).

### Hepatitis E virus

Hepatitis E virus (HEV) as is a tiny (32–34 nm) single-stranded, positive-sense RNA virus coated with protein classified in the family Hepeviridae, RNA molecule of approximately 7.2 kb in size (Khora, 2018) and comprising of four known Genotypes (1–4), at least two new putative mammalian HEV genotypes and one floating genus of HEV avian (Yugo and Meng, 2013; Van der Poel, 2014). Electron micrograph of Hepatitis E viruses (HEV) is given in Figure 3.



**Figure 3.** Electron micrograph of Hepatitis E viruses (HEV). This media comes from the Centers for Disease Control and Prevention's Public Health Image Library (PHIL), with identification number #5605, <https://commons.wikimedia.org/w/index.php?curid=1882568>



Hepatitis E virus (HEV) is a virus that causes acute hepatitis outbreaks and endemics in humans, which can be transmitted mainly to people and other animals from fecal-oral routes and animals (Sooryanarain and Meng, 2019). Unfortunately, this ensures that some species may act as reservoirs for HEV strains that infect humans (Grabow, 2007). Insufficient management and storage of wastewater, the usage of contaminated river water for common activities and contaminated drinking and irrigation water contribute to numerous epidemics in developed countries (Ceylan et al., 2003; Fenaux et al., 2019).

HEV can easily pollute the surface water, entering through food production chains, especially via shellfish cultivation areas and irrigation waters function as a threat to public health (Di Cola et al. 2020). The surface water's quality directly affects people using the source as drinking water, and intense agricultural activities result in high levels of viruses in these sources (Yugo and Meng, 2013).

Coastal waters can also be polluted with HEV, contributing to the aggregation of the virus in shellfish's digestive tissues, which presents a possibility of human infection by ingestion (Yugo and Meng, 2013; Van der Poel, 2014). Very commonly, mussels, cockles and oysters are consumed raw or partially fried, and HEV is stable at pH 2 to 9 (Wolff et al., 2020), frozen for more than 10 years (Emerson et al., 2005) and fresh, undercooked or partially steamed infected seafood will pass HEV to consumers (Crossan et al., 2012; Namsai et al., 2011). People who travel to hyperendemic and endemic areas of the world are at elevated risk of contamination with HEV from polluted water and seafood, in which developed countries are not excluded (Yugo and Meng, 2013; Zuckerman, 2003). Said et al. (2009) reported severe HEV infections in 33 participants on a 2008 world cruise caused by shellfish (crabs, prawns, mussels, lobsters, and scallops) and mixed seafood (a combination of shrimp, mussels, salmon, hake, cod, and squid). Recent studies have been showed that HEV infections may be associated with shellfish consumption (Rivadulla et al., 2019; Webb et al., 2020; Zhang et al., 2017).

### Rotavirus A

Rotaviruses (RVs), a member of the genus Rotavirus of the Reoviridae family, consisting of a rectangular segmented double-stranded RNA genome in a non-enveloped icosahedral capsid 60–80 nm in diameter (Gerba et al., 1996; Grabow, 2007; Estes, 2001). The genome of 16–27 kb is bound by a triple layer of capsid covered by a double protein coat (Sattar et al., 1994). The capsid has a unique double layer with spikes between the layers giving it the wheel-like outlook (Latin "rota") hence the name rotavirus is derived from the Latin meaning rota (wheel) (Gerba et al., 1996; Grabow, 2007; Khora, 2018). Electron Micrographs of Rotaviruses is shown in Figure 4.



**Figure 4.** Electron Micrographs of Rotaviruses by Dr Graham Beards at en.wikipedia, CC BY-SA 4.0, <https://commons.wikimedia.org/w/index.php?curid=16014758>

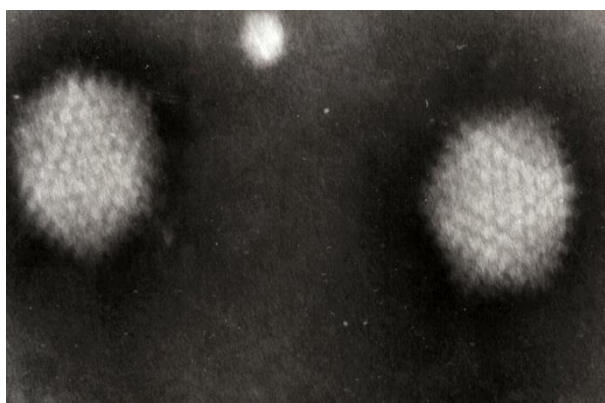
Given the global adoption of rotavirus vaccines, RVA is the main agent of acute gastroenteritis in infants worldwide, with many deaths / year cases (> 200,000), especially in low-income and undeveloped countries (Crawford et al., 2017). In 2003, 114 million cases of rotavirus infection were recorded worldwide in children who were less than 5 years of age, 24 million of which needed outpatient visits and 2,3 million of which needed to be hospitalized. (Parashar et al., 2003). Rotaviruses are released from the feces of infected people in exceptionally high numbers (up to 10<sup>10</sup> g<sup>-1</sup>) and can remain in the atmosphere for long periods of time (Carter, 2005) contributing to the potential for pollution of recreational and drinking water. (Gerba et al., 1996). The infectious dose is estimated at between 100 and 1000 particles of the virus (Ward et al., 1986). The viral particles are extremely robust and dangerously contagious to harsh ambient conditions. Despite exceptionally high levels of faecal excretion and confirmation of the possibility of waterborne transmission, the predominant route of transmission is via the fecal-oral route under poor hygienic conditions. (Khora, 2018; Magana-Arachch and Wanigatunge, 2020). Several waterborne rotavirus outbreaks which have caused illnesses in both adults and children (Chia et al., 2018; Tozan et al., 2016; Sattar, 2001).

RoV has been frequently detected in found in both freshwater and marine water sources and can also be concentrated by shellfish with higher prevalences and levels (Cook et al. 2004; Lees, 2000; Lodder and de Roda Husman, 2005; Prevost et al., 2015; Souza et al., 2018). Keller et al. (2013), found genomes of Rotavirus in Brazil, with a range of 76 and 88% of water samples and 100% of mussel samples. Quiroz-Santiago et al. (2014) detected that shellfish grown in China is infected by Rotavirus with a 7 % ratio identified. Shellfish bioaccumulate viruses in their gills, digestive glands, and other tissues during filter feeding (Asahina et al., 2009; Schwab et al., 1998; Wang et al., 2008).

Rotavirus also occurs in infected and non-polluted fresh water (Hurst and Gerba, 1980). Amoroso et al. (2020) examined a two-phase kinetic virus elimination with a high reduction in the first 24 h of depuration and rotavirus were completely removed after 5 days in experimentally contaminated *Mytilus galloprovincialis*.

### Human Adenovirus

Adenoviruses are double-stranded, non-enveloped, icosahedral DNA viruses (Bosch, 2007). Human adenoviruses (HAdVs) belong to the genus Mastadenovirus in the Adenoviridae family consisting of a double-stranded DNA genome in a non-enveloped icosahedral capsid with a diameter varying from 80 to 110 nm (Figure 5).



**Figure 5.** Transmission electron micrograph of two Adenovirus particles By GrahamColm at English Wikipedia, CC BY 3.0, <https://commons.wikimedia.org/w/index.php?curid=3921907>

Adenoviruses are linked to a number of human diseases and were responsible for waterborne outbreaks (Crabtree et al., 1997). There have been many cases of waterborne recreational outbreaks attributed to adenovirus within years (Bonadonna and La Rosa, 2019; Koopmans et al., 2017; Zhang et al., 2016). These viruses have been commonly seen in riversides, coast waters, swimming pool waters, and drinking water supplies all over the world where they may survive until four months (Jiang et al., 2001; Jiang, 2006). Adenovirus prevents environmental deterioration and water treatment rather than other measures of pollution (De Moraes Tavares et al., 2005; Griffin et al., 2008; Luz et al., 2015; Jiang et al., 2007).

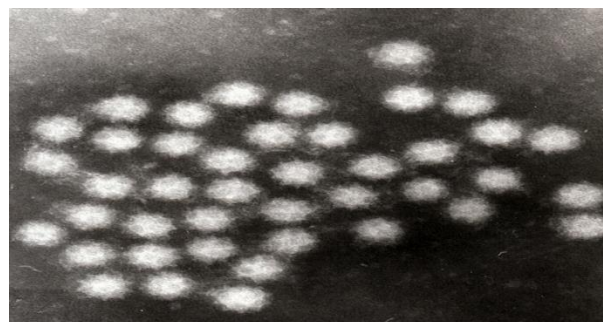
Adenovirus can be transmitted either directly from person to person or via the fecal-oral route that can result from the ingestion of polluted water during outdoor practices or from the processing of shellfish obtained from polluted waters and contaminated food and liquid intake (Benabbes et al., 2021; Gyawali and Hewitt, 2020).

Formiga-Cruz et al. (2002) reported that, with respect to the existence of human enteric viruses, Adenovirus was the most wide spreading virus group found in oyster (*C. gigas* and

*O. edulis*) and mould (*M. edulis* and *M. galloprovincialis*) samples across Europe. Choo and Kim (2006) reported that 50.9% of oysters harvested from the wholesale fishing industry in Noryangjin were infected with contagious adenovirus. Ghalyoun and Alçay (2018) detected adenovirus before the fishing season ended in 46.15% of the mussel sample from three separate locations in Istanbul. Luz et al. (2015) reported that the findings of the analysis revealed substantial bioaccumulation of adenoviruses in shrimp, demonstrating the magnitude of fecal pollution's effect on aquatic environments. In 18.6% of shellfish tests from the Norwegian coast, Myrmel et al. (2004) have found Adenovirus with more accurate tests in the winter season.

### Sapovirus

It is a small (27–40 nm) nonenveloped RNA virus which belongs to the Caliciviridae family (like NoV) and forms its own genus. Similar to NoV, only observed sapoviruses (SaV) in 1977 was extremely immune to negative environmental factors (Grabow, 2007), usually infecting humans (HuCVs), as well as two other genera synonymous with animal diseases, including primates, birds, reptiles and insects (Figure 6)



**Figure 6.** Transmission electron micrograph of Sapporo viruses By Graham Beards at English Wikipedia, CC BY 3.0, <https://commons.wikimedia.org/w/index.php?curid=56088018>

SaV is spread via the fecal-oral path discharge into environmental waters and collected by shellfish (i.e., or oysters clams) (Hansman et al., 2007; Khora, 2018; Nakagawa-Okamoto et al., 2009; Oka et al., 2015), as well as in contaminated food related with the intake of shellfish (Nakagawa-Okamoto et al., 2009), water, materials, and human interactions. Lizuka et al. (2010) reported that SaV was found in fecal specimens of 17 individuals who consumed restaurant food and one asymptomatic food handler, as well as in stripped shellfish and residual liquids in shellfish containers, which triggered a gastroenteritis spread in a restaurant, June 2008. Ueki et al. (2010) show that SaV can be accumulated in oysters which are grown in an estuary in Japan that receives treated sewage. During a three-year period (2015–2017), Fusco et al. (2019) detected sapovirus (SaV; 18.8%) in bivalve mollusc samples from three littoral zones of the Campania region in South West Italy.

## CONCLUSION

Seafoodborne and waterborne viruses may exist in any form of untreated water due to pollution induced by faecal content of human or animal origin and directly infected through interaction with body secretions and fluids containing contagious viral particles or indirectly by aerosols or other polluted fomites. Thus, the human pathogenic viruses can infect seafood and its products anytime from water and food

environments. Non-enveloped viruses are commonly known to have a greater tolerance to desiccation and are therefore harder to transmit than viruses with an envelope tag, which often correlates to their mode of transmission. For this cause, a significant danger is the intake of food that is cooked only minimally before consumption or eaten with fresh vegetables, shellfish or other conventional meat specialities. When the food can't be accurately decontaminated during processing, it is essential to prepare the food such as cooking.

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