

## REVIEW PAPER

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# Foodborne viruses: their significance and the need for further study

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## Abstract

*Foodborne viruses, major pathogens that cause food poisoning, pose significant threats to food safety and human health. The main viruses responsible for outbreaks of foodborne illness are norovirus, hepatitis A and E viruses, rotavirus, astrovirus, adenovirus, Aichi virus, and sapovirus, and the rate at which novel viral varieties arise is always increasing. Raw foods such as bivalve shellfish, fruits, salads of raw vegetables and raw fish, and ready-to-eat (RTE) foods are frequently linked to foodborne viral outbreaks. Viruses cannot grow in food because they are obligate intracellular pathogens that can only reproduce in live cells. They survive in the environment, on different surfaces for food preparation and food packing, as well as on human hands, where they can persist for days and even weeks in the food chain. Frequent symptoms of viral gastroenteritis include vomiting and diarrhea, but they can cause life-threatening infections, especially in children, the elderly, immunocompromised people, and pregnant women. The contamination of food with viruses may be controlled by preventing viral occurrence, the adoption of strict hygienic food processing measures from farm to fork, or by inactivation. Inactivation of foodborne viruses in the food industry is a difficult task because they can survive*

*in the food during food processing and storage. The development of technologies and the rapid and accurate detection of foodborne viruses are essential for food safety control. Real-time polymerase chain reaction (Real-Time PCR) is considered the method of choice for virus detection in food. The aim of this review is to present the available information on viral foodborne outbreaks, their characteristics, control strategies, and the need for further study.*

**Keywords:** foodborne viruses; norovirus, hepatitis; food safety.

## INTRODUCTION

Food items consumed by people can be a source of a wide range of pathogens that cause foodborne illnesses, and their consumption can pose health risks to consumers. More than 250 different pathogens, such as bacteria, viruses, parasites, protozoa, etc., can cause life-threatening infections, particularly in the elderly, immunocompromised individuals, pregnant women, and children. This is a growing public health problem in both developed and developing countries [1]. Global food trade across areas with varying hygiene stand-

ards and the risk of the food supply are caused by a number of factors, including the growing population and the need for seasonal items to be available year-round [2].

Viruses are recognized as significant causative agents of foodborne illnesses, and risk assessment experts consider them a high priority in the field of food safety. This concern is justified, as there has been a significant increase in viral foodborne outbreaks over the past 20 years. The World Health Organization evaluated that a high percentage of all foodborne diseases worldwide (about 21%) were affected by food- and

waterborne viruses, such as norovirus (NoV), rotaviruses, hepatitis A virus (HAV), hepatitis E virus (HEV), astroviruses, adenoviruses, and enteroviruses [3]. Viral contamination of food can occur during primary production, processing, or preparation [4]. Viruses are small, obligate intracellular parasites (size from 15 to 400 nm), that require living cells in which to replicate, so unlike bacteria, they cannot reproduce in food. Consequently, viruses will never lead to organoleptic changes in food. This poses a problem when identifying the virus because contaminated food will still look and smell normal. At the same time, all currently known viruses are highly host-specific. Each group of viruses shows tropism towards a specific cell type. They are highly infectious, only a few virus particles (1-100) are enough to cause infection [5]. This implies that viral infection via contaminated food depends on viral stability, amounts of virus shed by an infected individual, method of processing of food or water, dose needed to produce infection, and susceptibility of the host [6].

### TYPES OF FOODBORNE VIRUSES

Based on the symptoms of infection, viruses transmitted by food can be divided into several groups: those that cause gastroenteritis (Norovirus-NoV, Rotavirus, Aichi virus, Adenovirus); those that migrate from the intestines to the liver and lead to hepatitis (Hepatitis A and Hepatitis E) and the third group consists of those

that multiply in the intestines, and lead to disease when they migrate to other organs, for example the nervous system (*Enteroviruses*) (**Table 1**). The progress of molecular methods of identification, which will enable the characterization of the entire genetic material, has led to the identification of several new viruses in the last few years, and currently, 22 families of viruses that can infect humans are known. All these viruses are transmitted by the fecal-oral route, by direct contact or via aerosols, by contamination of food and water during the production process (e.g. shellfish production), or, more rarely, by consumption of products of animal origin [4]. The clinical symptoms caused by these viruses are mild and develop most often in the period of 12-48 hours after the appearance of the virus, and last only 1-3 days. Some of the most common symptoms are: diarrhea, stomach pain, nausea, vomiting, and fever. According to the latest report of the European Food Safety Agency (EFSA), in terms of the number of infected people and the epidemics they cause, members of the genus Norovirus and Hepatitis A virus are still considered the most important viruses transmitted by food. Norovirus, with 332 reported outbreaks, is the third most commonly reported foodborne pathogen (after *Campylobacter* and *Salmonella* species) [7].

Although epidemic non-bacterial gastroenteritis, sometimes known as „winter vomiting disease,” was first identified in 1929, there has been little progress

**Table 1.** Classification of foodborne viruses and their basic characteristics.

Virus	Genome	Gender/Family	Disease	Risk food
<b>Norovirus (HuNoV)</b>	ssRNA	Norovirus/ Caliciviridae	Acute gastroenteritis	shellfish, fish, vegetables, fruit
<b>Rotavirus (HRV)</b>	ssRNA	Rotavirus/ Reoviridae	Gastroenteritis in children, diarrhea in adults	shellfish, vegetables, fruit
<b>Hepatitis A (HAV)</b>	ssRNA	Hepatovirus/ Picornaviridae	Hepatitis A	Sandwiches, milk, fruit, vegetables
<b>Hepatitis E (HEV)</b>	ssRNA	Orthohepevirus/ Hepeviridae	Hepatitis E	Raw meat of pig, wild boar, deer liver, liver sausages
<b>Astrovirus (HAzVs)</b>	ssRNA	Mamastrovirus/ Astriviridae	Gastroenteritis	shellfish, vegetables, fruit
<b>Aishi virus (AiV)</b>	ssRNA	Kobuvirus/ Picornaviridae	Gastroenteritis	Raw shellfish
<b>Sapovirus (SaV)</b>	ssRNA	Sapovirus/ Caliciviridae	Gastroenteritis	Raw shellfish
<b>Adenovirus (HAdV)</b>		Mastadenovirus/ Adenoviridae	Gastroenteritis, fever, conjunctivitis, etc.	Seafood
<b>Enterovirus</b>	ssRNA	Enterovirus/ Picornaviridae	Changes in multiple organ systems	shellfish

in the multiple attempts to cultivate the suspected viral causative agent *in vitro*. The identification of the Norwalk virus (NV) using immunological EM (IEM) in fecal samples taken during a gastroenteritis outbreak in 1968 at an elementary school in Norwalk, OH, USA, was a significant advancement [8].

**Norovirus** (NoV, previously known as „Norwalk-like virus”) is a small round virion, with a non-enveloped, single-stranded positive-sense RNA genome (+ssRNA). It belongs to the *Caliciviridae* family, which is divided into several genera, of which Norovirus and Sapovirus cause diseases in humans. It is characterized by great antigenic diversity, with about 10 genogroups and more than 30 genotypes, among which the GII.4 genotype is dominant [8]. The group of risky foods, that can lead to the spread of NoV, include raw shellfish and berries. Mussels feed by filtering seawater and in that way they also take in phytoplankton, zooplankton, and inorganic matter, but they can also accumulate pathogens if they are present in the water environment. Secondary norovirus infections occur mainly in semi-closed facilities where the same food is served to a large number of people, such as nursing homes, schools, cruise ships, hospitals, or military facilities. Epidemics occur throughout the year, but most often during the winter months. The disease most often occurs in young children, immunocompromised and elderly people, and is accompanied by the usual clinical symptoms of gastroenteritis, such as nausea, vomiting, diarrhea, and stomach pain [9]. The foodborne pathogens HAV and HEV are responsible for hepatitis in humans. The primary means by which HAV and HEV are transmitted among the human population are contaminated food and water [8].

**Hepatitis A (HAV)** is a small round RNA virus, belonging to the family *Picornaviridae* and genus Hepatovirus. The transmission of this virus depends on hygienic and sanitary conditions, so large differences can be observed in the occurrence of this virus in communities of different socio-economic statuses. Infection with this virus occurs through contaminated food or water, hands, or direct contact, and the virus then reaches the hepatocytes of the liver through the digestive tract, through the bloodstream, where it causes damage. The incubation period is 15-45 days, it begins with mild general clinical symptoms, after which jaundice (icterus) appears on the sclera, skin, and mucous membranes. After getting over the disease, lifelong immunity is acquired [10].

**Hepatitis E (HEV)** is a small, single-stranded, non-enveloped RNA virus which, according to the latest classification, is classified as the only representative of the genus *Hepevirus*, family *Hepeviridae*. Although the virus multiplies in enterocytes, its primary tropism is towards hepatocytes. In addition to direct transmission of the virus, as well as transmission by contami-

nated water, it is specific that the carrier of the virus can be thermally unprocessed meat of domestic and wild pigs and deer, as well as the entrails of these animals that serve as food [11].

**Rotavirus (RoV)** is a member of the Reoviridae family with double-stranded RNA. Seven genogroups of this virus are known, marked from A to G. RoV serogroups A, B, and C are mainly typically related to dangerous outbreaks of viral gastroenteritis. Group A rotavirus is a major cause of terrible diarrhea in children up to 2 years old. It is transmitted from person to person or secondarily by contaminated food and water [12].

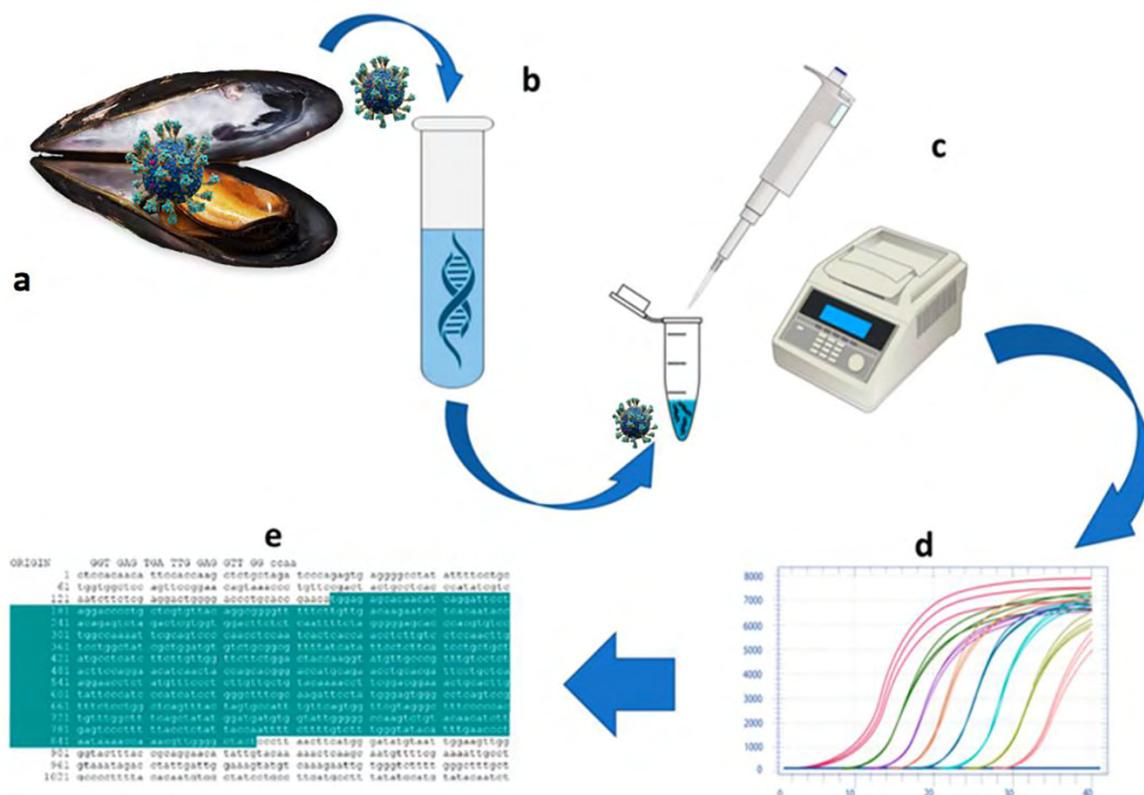
## DETECTION AND ISOLATION OF VIRUSES IN FOOD

Isolation of viruses from the food matrix is a very complex process, for which it is necessary to develop highly sensitive methods, which will allow the recovery of virus particles and the removal of inhibitory substances that interfere with its isolation. For viruses that cannot be grown in cell culture, such as Norovirus and Hepatitis A, the gold standard for detection is sensitive molecular methods, such as Real-Time PCR, performed according to the standard ISO protocol. The detection of viruses from the food matrix proceeds in several steps, as shown in **Figure 1**.

The principle of operation of Real-Time PCR is to measure the amount of accumulated PCR product during the reaction, by adding a fluorescent dye to the classic reaction mixture, which, at the end of each cycle, is intercalated with the newly synthesized DNA fragment. A computer-controlled CCD camera then records the intensity of light emitted by the UV-excited dye from the reaction mixture. The Real-Time PCR system compares the fluorescence intensity in relation to each cycle and displays the kinetics of the process in the form of an amplification curve [13]. ISO methods for the detection of foodborne viruses are widely used today, and several companies offer commercial kits for the isolation and identification of viruses by molecular methods. Despite this, most studies do not report the limit of detection (LOD) and limit of quantification (LOQ) of the assay and this complicates comparisons and drawing of general conclusions [2].

## PREVENTIVE MEASURES

Effective prevention and control measures include microbiological genomics for virus identification and harmonized surveillance of viral outbreaks coupled with systematic strain typing. A big problem in preventing contamination with these viruses is their resistance in the environment and low infectious dose. The first step in controlling viral contamination is to prevent direct contact of fecal material or vomitus with food. Also, the prevention of spillage of wastewater into breeding



**Figure 1.** Real-Time PCR detection: (a) sample collection; (b) NA extraction; (c) PCR amplification; (d) real-time PCR quantification; and (e) sequence detection results.

grounds is of crucial importance in the control of viral contamination of shellfish. Strict hygiene measures include proper washing of raw fruits and vegetables, use of hygienic water in the food production chain, training and awareness of food handlers about hygiene and potential hazards if good hygiene practices are not applied, cleaning and disinfection of food preparation equipment, and surfaces [14]. The application of disinfectants, both for hands and for surfaces on which food is prepared, is of essential importance in the application of good hygiene practices, as a method to reduce viral pathogens [14]. The joint approach of the veterinary and medical professions („one health”) in the suppression of viral infections must include all critical control points that can lead to the transmission of viral pathogens through food, from the control of cultivation, through the processing and distribution of food, so that the spread of the disease in human population.

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## Virusi koji se prenose hranom: njihov značaj i potreba za budućim istraživanjima

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### Kratak sadržaj

*Virusi koji se prenose hranom, kao vodeći patogeni koji izazivaju trovanje hranom, predstavljaju značajnu pretnju po bezbednost hrane i zdravlje ljudi. Glavni virusi odgovorni za izbijanje bolesti koje se prenose hranom su norovirus, virusi hepatitisa A i E, rotavirusi, astrovirusi, adenovirusi, Aichi virus i sapovirusi, a stopa nastajanja novih virusnih varijeteta je u stalnom porastu. Sirova hrana kao što su školjke, voće, salate od sirovog povrća i sirove ribe i hrana gotova za jelo (ready for eat – RTE) često se povezuju sa izbijanjem virusnih epidemija. Jedna od glavnih karakteristika virusa je da ne mogu da se razmnožavaju u hrani, jer su obave-*

*zni intracelularni patogeni kojima je za rast potrebna isključivo živa ćelija. Jako su otporni, opstaju u životnoj sredini danima, pa čak i nedeljama, i to na različitim površinama za pripremu i pakovanje hrane, kao i na rukama ljudi. Najčešći klinički simptomi virusnog gastroenteritisa su povraćanje i dijareja, a do komplikacija može doći kod dece, starijih, imunokompromitovanih osoba i trudnica. Kontaminacija hrane virusima može se kontrolisati pre svega sprečavanjem pojave virusa, usvajanjem strogih higijenskih mera prerade hrane od farme do viljuške ili njihovom inaktivacijom. Inaktivacija virusa koji se prenose hranom u prehrambenoj industriji je težak zadatak jer oni mogu da prežive u hrani i tokom obrade i skladištenja. Razvoj tehnologija i brzo i tačno otkrivanje virusa koji se prenose hranom su od suštinskog značaja za kontrolu bezbednosti hrane. Lančana reakcija polimeraze u realnom vremenu (Real-Time PCR) se smatra metodom izbora za detekciju ovih virusa u hrani. Cilj ovog pregleda je da predstavi dostupne informacije o virusnim epidemijama koje se prenose hranom, njihovim karakteristikama, strategijama kontrole i potrebi za daljim proučavanjem.*

**Ključne reči:** virusi prenosivi hranom; norovirus; hepatitis; bezbednost hrane.