Role of the noroviruses as substantial foodborne pathogens: A review

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Role of the noroviruses as substantial foodborne pathogens: A review: Acute gastroenteritis is one of the most important diseases in humans. Etiology of the infectious gastroenteritis involves several agents such as viruses, bacteria and parasites. Bacteria and parasites are causative agents of gastroenteritis in developing countries, whereas viruses are responsible for gastroenteritis outbreaks in the industrialized countries. Noroviruses are recognized as leading cause of highly transmissible foodborne disease that affects millions of people every year worldwide. The main sources of infection are seafood, deriving from waters polluted with human faeces, and fruits or vegetables, irrigated with contaminated water during their cultivation. Due to their resistance and rapid spread, noroviruses remain a serious public health problem in many countries.

Keywords: norovirus, viral gastroenteritis, viral diarrhea, foodborne pathogen.

INTRODUCTION

Acute gastroenteritis is one of the most common human diseases. In some countries gastroenteritis takes second place after the respiratory infections. Acute gastroenteritis can be caused by different agents, such as viruses, bacteria and parasites. Bacteria and parasites are among the main agents causing acute gastroenteritis into developing countries, while viruses are responsible for outbreaks of acute gastroenteritis in the industrialized countries (North America and Western Europe).

Using molecular techniques such as reverse-transcriptase polymerase chain reaction (RT-PCR) it was established that the frequency and medico-social burden of norovirus gastroenteritis is much larger than it was before. The review of studies conducted in recent years in different countries, shows that noroviruses are proved as causative agents in 5-36% (average 12%) of the cases of acute diarrhea and in 3-31% (average 11%) of the severe diarrheal cases requiring an emergency room visit or hospitalization. It was found that noroviruses are responsible for approximately 900.000 episodes of gastroenteritis requiring a doctor's visit and about 64.000 hospitalizations in children under 5 years of age in developed countries annually. In developing countries noroviruses are responsible for more than 1.1 million hospitalizations and approximately 218.000 deaths per year in children of the same age group. Globally, epidemiological studies indicate that noroviruses cause between 75% and 93% of the nonbacterial and around 50% of all the outbreaks of acute gastroenteritis worldwide [8].

HISTORY

The studying of noroviruses began with the discovery of the prototype of the family they belong to (*Caliciviridae*) – a strain of Norwalk virus - during an outbreak among students and teachers in a primary school in the town of Norwalk in the state of Ohio, USA in 1968. The disease is proceeded with emesis in 90% of patients and diarrhea in 38%, and duration of the symptoms around 12 - 24 hours. Germ-free filtrates were prepared from faecal samples and they caused similar clinical characteristics after ingestion in volunteers as well as in new serial passage in volunteers. Efforts to isolate and cultivate the causative agent in a cell culture, however, were unsuccessful, which still hinders their further study [8].

In 1972 Kapikian *et al.* from the National Institute of Health, USA detected by electron microscopy 27 nm virus particles in the fecal filtrates used to infect volunteers. Viral particles were precipitated in an antigen - antibody reaction. For this purpose sera from volunteers in convalescent period (after infection with fecal filtrates) were obtained. The technique that revealed the relationship of the viruses and the disease is known as

immune electron microscopy (IEM) and allows visualization of the antigen-antibody complexes, due to the coating of the virus particles with specific antibodies and the consequential aggregation. The new virus was named Norwalk virus and was the first identified that causes gastroenteritis in humans [2, 7, 8].

ETIOLOGIC AGENT

On the basis of the specific organization of the genome and the results of the phylogenetic analysis, in 2002 the representatives of the family *Caliciviridae* were classified into 4 genera: *Norovirus*, *Sappovirus*, *Lagovirus* and *Vesivirus*. The first and the second genera include viruses that cause gastroenteritis in humans, while the third and the fourth genera comprise only animal pathogens causing viral diseases in rabbits, pigs, cats and sea lions.

Due to the lack of uniform classification adopted by the International Committee on Taxonomy of Viruses (ICTV), in 2006 Zheng *et al.* proposed a classification system for caliciviruses, including three levels: genogroup, cluster and strain [15]. Based on phylogenetic analysis of polymerase and capsid regions, noroviruses are classified into 5 genogroups (GI - GV). The most common infections in humans are caused by GI (Norwalk, Southampton, Desert Shield, Chiba, Winchester, etc.) and GII (Hawaii, Melksham, Toronto, Bristol, Hillingdon, Lordsdale, Mexico, Grimsby, etc.). These five genogroups are divided into 31 clusters as follows: GI - 8 ; GII - 19; GIII - 2; GIV - 1; GV - 1 [6]. Noroviruses from genotype GII.4 have particularly high epidemic potential. They are responsible for the emergence of several major pandemics in Europe and the USA since the mid 90's of the last century to the present [8].

Morphologically, the viral particles have a size of 27 to 40 nm in diameter. Observation under an electron microscope shows a presence of protrusions and indentations like cups from which the name of *Caliciviridae* (Latin *calyx* - cup) is derived. The capsid has icosahedral symmetry and consists of 180 monomer units of the major structural protein VP1, arranged in 90 dimers. The large capsid protein VP1 consists of 530 amino acids. It plays a major role in virion formation, antigen properties and interaction with the host cells. The small VP2 capsid protein is composed of 212 amino acids and its functions remain unclear so far. The VPg protein is covalently linked to the genomic and subgenomic RNA in the infected cells [6].

The genome is a linear, single-stranded RNA molecule with positive polarity and a length ranging from 7.3 to 8.5 kilobases. The genome is organized into three major open reading frames - ORF1, ORF2 and ORF3. ORF1 encodes a large polyprotein of 1738 amino acids, which is cut posttranslationally into 7 non-structural proteins (NS1 - NS7), nucleosidtriphosphatase, VPg, protease and RNA-dependent RNA polymerase. ORF2 encodes the largest structural protein VP1, and ORF3 encodes the protein VP2 [2].

Noroviruses undergo rapid genetic variations (mutations and recombinations) during their replication into the host cells as well as in the process of their circulation in society. The highest frequency of mutations and recombinations occurrs between norovirus genotypes and other RNA-viruses, which is the basis of exclusive genetic heterogenicity of norovirus populations and is directly related to the emergence of new strains in almost every new epidemic wave [13].

RESISTANCE OF NOROVIRUSES IN THE ENVIRONMENT

Noroviruses are characterized by high resistance in the environment. They retain their infectious properties at acidic pH (pH=2.7) over 3 hours, which makes them resistant to the action of gastric juice. They are also resistant to lipid solvents such as ether and chloroform. They survive thermal treatment at 60°C for 30 min, which explains their dissemination in food products subjected to low or no heat treatment – seafood (clams, oysters, fish), fruits and vegetables [8].

EPIDEMIOLOGICAL CHARACTERISTICS

Over the past two decades, noroviruses were unprecedentedly widespread throughout the whole world, causing a large number of outbreaks in all continents. Rapid and widespread dissemination is inextricably linked to the increased contacts between people from different countries, international trade, transportation, and last but not least - the emergence of new serotypes with higher virulence.

Norovirus gastroenteritis outbreaks affect semi-closed groups of people – in hospitals, kindergartens, schools, universities, restaurants, cruise ships, holiday camps, military units, nursing homes and families.

Source of infection can be persons with symptomatic or asymptomatic infection. Transmission of the infection occurs mainly by fecal-oral route (intake of food or water contaminated with faeces), direct contact (person-to-person transmission) and contaminated surfaces. There is also evidence for aerial spread of the infection – by inhalation of aerosols formed during vomiting [11].

ROLE OF NOROVIRUSES IN FOOD

Food products may be contaminated with noroviruses during various stages of their cultivation, preparation, transportation and distribution. Most common sources of infection are products subjected to weak thermal treatment - seafood (mussels, oysters and fish), salads, dressings, cold snacks, sandwiches, ice cream, different fruits and vegetables.

Noroviruses in foods of plant origin. Different types of fresh and frozen fruits (strawberries, raspberries) and vegetables (lettuce, spinach) can be infected with noroviruses long before their wide distribution in the commercial and catering network. Infecting of the plants occurs during various stages: during the growing stage (by irrigation with contaminated water), during the cultivation (by fertilization and composting with infected fertilizers) or during picking (from infected persons). When infection occurs during the growing season of leaf vegetables, contamination cannot be removed by washing [5; 14]. In frozen fruits, noroviruses can be stored for long periods of time and transported over long distances. For example, Made *et al.*, 2013 reported a norovirus outbreak in about 11.000 people in Germany in the autumn of 2012 as a result of infected frozen strawberries imported from China [10].

Noroviruses in foods of animal origin. Marine animals (mussels, oysters, fish etc.) become infected with noroviruses through pollution of the sea water by human faeces. These sea organisms possess two important biological features – they filter large volumes of water for their feeding and inhabit shallow (coastal) parts of the seas, where the pollution from outgoing canals occurs. All this contributes to the accumulation of large amounts of noroviruses in their tissues. This makes seafood risky when eaten in raw or undercooked state. Bioaccumulation of noroviruses is realized mainly in the epithelial cells of the alimentary tract of oysters where they bind to receptors similar to the tissue-blood group antigens of human blood group A (HBGAs) [9].

Some commercial oyster and shellfish harvesters use a process called depuration. The shellfish are placed into tanks of clean recirculating seawater and are treated with UV irradiation. The oysters purge their contaminants over several days. In the United Kingdom a purification time of at least 42 hours is required.

CLINICAL SIGNS OF NOROVIRUS INFECTION

In most cases, noroviruses cause non-serious, self-limiting symptoms which usually end without specific treatment. Infants and children are more susceptible, due to the fact that their stomachs produce less hydrochloric acid; pregnant women are also at significant risk because the fetus does not have fully developed immune system; older people are more susceptible to disease due to their poor nutrition, the lack of protein in their diets or poor blood circulation. Chronically ill people or those who take medications that affect the immune system (people with cancer, diabetes, AIDS or people on an antibiotic therapy), also belong to the risk categories. Leading clinical signs are nausea, vomiting, diarrhea and abdominal pain. Minor clinical symptoms are: headache, muscle aches (myalgia), a slight increase in the body temperature and bloody diarrhea [3].

LABORATORY METHODS FOR DETECTION OF NOROVIRUSES

Due to the fact that none of the clinical symptoms of norovirus infection is specific, accurate diagnosis requires laboratory confirmation by detection of the virus, its antigens or specific antibodies towards the virus in patient samples.

For a long time identification of the virus was carried out only by electron microscopy (EM) due to the fact that it was impossible to isolate and cultivate the virus *in vitro* and *in vivo*. The main disadvantages of EM are the low sensitivity of the method (the required concentration of the virus particles in the sample is 10^{6} - 10^{7} particles/ml) and its inapplicability in general laboratory practice.

Enzyme-linked immunosorbent assay (ELISA) is intended for detection of antibodies in patient sera and is suitable for screening of large numbers of samples during outbreaks. In case of obtaining negative results, the samples have to be tested again with RT-PCR.

Reverse-transcriptase polymerase chain reaction (RT-PCR) and its modification real time reverse-transcriptase polymerase chain reaction (rRT-PCR) are the most sensitive and highly specific diagnostic methods, accepted as refferent standards. They allow quick and accurate diagnosis by detecting the virus (viral RNA) or its antigens even if their quantity in the samples is minimal. Required concentration of the virus particles in the sample is 10²-10⁴ particles/ml.

RT-PCR allows examination of both clinical specimens (stool or vomiting mass) and samples from the environment such as contaminated water, food, etc. RT-PCR is also used for genotyping the viruses. The determination of the genogroups and genotypes of noroviruses is crucial in order to establish the source of the infection, the circulation and the spread of viral strains. Genetic sequencing and phylogenetic analysis allow tracking genetic relationships between norovirus strains causing outbreaks in different geographic areas [8].

TREATMENT AND PREVENTION

There is no specific treatment for norovirus infection and specific antivirals against noroviruses are still not developed. It is important that infected people remain well hydrated, drinking plenty of fluids. Fluids containing electrolytes and sugars should be encouraged. Antidiarrheal medications also may be used according to directions but should be avoided in cases with severe abdominal pain or fever. Some researchers advise people to avoid these medications if possible as they may prolong the disease.

Although norovirus infection is not fatal and most of cases are mild, complications may occur. Complications are related to the degree of dehydration. People who cannot keep up with fluid losses may require hospitalization for intravenous fluid therapy. Approximately 10% of infected people seek medical attention. Children and infants are at high risk for dehydration because they cannot communicate their symptoms and because dehydration may occur rapidly. Pregnant women should pay particular attention to keeping up with fluid losses.

Presently, specific means for prevention (vaccines) are not developed. Norovirus infections can be prevented by using good hand hygiene and avoiding contact with sick individuals and their environment. Contamination of fresh vegetables and fruits while picking by infected persons can lead to widespread outbreaks. Strict hygiene standards for food handlers can help to reduce the risk of outbreaks. Many investigators suggest routine washing of fruits and vegetables before serving, which may also help to reduce or prevent infections [8].

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This paper has been reviewed