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https://doi.org/10.22239/2317-269x.00928

Norovirus in foods

Norovírus em alimentos

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ABSTRACT

Introduction: Noroviruses (NoV) are important causative agents of foodborne gastroenteritis outbreaks associated with the consumption of fruits, leafy vegetables, bivalve molluscs and delicatessen foods. The establishment of laboratory surveillance networks in different continents has demonstrated increased epidemiological importance of those viruses. In Brazil, the NoV infection is considered an important public health issue with socioeconomic burden, but the investigation of these viruses in foodborne outbreaks is still restricted to research laboratories. NoV infections have become more known especially with the consolidation of the cruise ship market in the country since 2004. **Objective:** This study aims to present advances related to NoV research in foods, highlighting features of this pathogen and strategies for its detection in these matrices. **Method:** An integrative review, collecting scientific articles with the objective of dealing with the main aspects of NoV, was carried out. **Results:** A broad literature review was performed, describing the main results in the literature and discussing aspects such as foodborne diseases, viruses as food contaminants, stability and disinfection, foodborne outbreaks associated with NoV, food associated with NoV contamination, NoV concentration and detection methods in food, risk assessment studies and prevention and control. **Conclusions:** records of foodborne outbreaks associated with NoV and the increasing genetic diversity of these viruses reinforce the need for laboratory and epidemiological surveillance, especially in developing countries, such as Brazil.

KEYWORDS: Norovirus; Foods; Disease Outbreaks; Gastroenteritis; Methods; Sanitary Surveillance

RESUMO

Introdução: Os norovírus (NoV) são importantes agentes causadores de gastroenterite de origem alimentar, com surtos associados ao consumo de frutas, vegetais folhosos, moluscos bivalves e alimentos de *delicatessen*. O aumento da importância epidemiológica destes vírus tem sido demonstrado pelo estabelecimento de redes laboratoriais de vigilância em diversos continentes. As infecções por NoV se tornaram mais conhecidas especialmente com a consolidação do mercado de navios de cruzeiros no país a partir de 2004. **Objetivo:** Este estudo tem como objetivo apresentar avanços relacionados à pesquisa de NoV em alimentos, destacando características deste patógeno e estratégias para sua detecção nestas matrizes. **Método:** Foi realizada uma revisão integrativa, pelo levantamento de artigos científicos com o objetivo de tratar dos principais aspectos de NoV. **Resultados:** Foi realizada uma ampla revisão da literatura, com a descrição dos principais resultados presentes na literatura consultada e a discussão de aspectos como doenças transmitidas por alimentos (DTA), vírus como contaminantes de alimentos, estabilidade e desinfecção, surtos de origem alimentar associados aos NoV, alimentos associados à contaminação por NoV, métodos de concentração e detecção de NoV em alimentos, estudos de avaliação de risco e prevenção e controle. **Conclusões:** Os registros de envolvimento de NoV em surtos de origem alimentar e a crescente diversidade genética destes vírus reforçam a necessidade de vigilância laboratorial e epidemiológica sobretudo nos países em desenvolvimento, como o Brasil.

PALAVRAS-CHAVE: Norovírus; Alimentos; Surtos de Doenças; Gastroenterite; Métodos; Vigilância Sanitária

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Received: Feb 06, 2017
Approved: May 23, 2017

INTRODUCTION

First described in the 1970s with the use of electronic immunomicroscopy[1], Norwalk viruses, as noroviruses (NoV) were then known, had their epidemiological importance recognized only from the 1990s onward with the emergence of molecular techniques of cloning and nucleotide sequencing, which allowed the production of diagnostic supplies[2,3,4,5,6]. Today, NoV are recognized as the main agents that cause outbreaks and sporadic cases of acute non-bacterial gastroenteritis in humans[7,8,9,10].

The impact of NoV infections in industrialized countries is evident in the number of epidemiological surveillance networks established on different continents. Electronic platforms such as Noronet (Netherlands), Foodborne Viruses in Europe (European Union), Norovirus Outbreak Reporting Tool (England), Episurv (New Zealand), OzFoodNet (Australia), Calicinet and National Outbreak Reporting System (United States) have been promoting inter-laboratory integration by sharing epidemiological and molecular data on outbreaks, providing information on genotype circulation and the emergence of new variants[11,12,13,14]. In these countries, where the diagnosis is well established, NoV are responsible for more than 200,000 deaths/year, mainly of children under 5 years of age[9].

In Brazil, people became more aware of NoV infections after the consolidation of the cruise ship market in the country in the 2004/2005 season (http://www.abremar.com.br/down/fgv2015.pdf), once gastroenteritis outbreaks are common in these settings[10,15,16,17,18]. Furthermore, several studies conducted in the country have demonstrated the impact of NoV infections in different populations, including outbreaks, sporadic cases, hospitalized patients, as well as the occurrence of asymptomatic infections and cases associated with persistent diarrhea[19,20,21,22,23,24,25,26,27,28,29,30,31,32]. The environmental dissemination of NoV in different water matrices in the country has also been demonstrated with concentrations reaching 1.5E + 04 - 0.3E + 05 (GC)/L in samples of crude sewage[33,34,35,36,37,38,39,40].

Concerning foodborne gastroenteritis, which belong to the group of foodborne diseases (FBD), NoV was associated with 38 outbreaks (0.9%) out of a total of 9,719 cases reported by the Ministry of Health (MS) in relation to foodborne gastroenteritis, in the period of 2000-2014[41]. In over 10,000 outbreaks of gastroenteritis associated with food contamination reported in recent years, more than half did not have a defined etiologic agent. Therefore, as in most countries, the determination of foodborne outbreaks associated with NoV is based on epidemiological investigations and laboratory tests performed on clinical specimens of individuals involved in these outbreaks[42].

The detection of human NoV in food is hampered by the complexity of the food matrix and by the presence of low levels of virus particles, which results in outbreak underreporting[43,44]. This review aims to present NoV as the main viral agents associated with outbreaks of foodborne gastroenteritis, describing their general characteristics and the progress related to the research of these viruses in food matrices.

METHOD

This study, prepared as an integrative review, was conducted according to the methodology described by Sobral and Campos[45] for the survey of scientific articles to address the main aspects of NoV caused by the consumption of contaminated food, as well as related infections. The research of scientific literature was carried out on the PubMed database (using keywords like: norovirus on foods, methodologies for norovirus on foods, norovirus review) and the database of the Brazilian Ministry of Health.

RESULTS AND DISCUSSION

Foodborne Diseases (FBD)

FBD is a generic term applied to a syndrome usually consisting of anorexia, nausea, vomiting and/or diarrhea, with or without fever, attributed to the ingestion of contaminated food or water. However, digestive symptoms are not the only manifestations of these diseases, since extraintestinal infections in different organs and systems may also occur, according to the agent involved. In addition to bacteria and toxins, FBD can also be caused by toxic substances, parasites, and viruses[46].

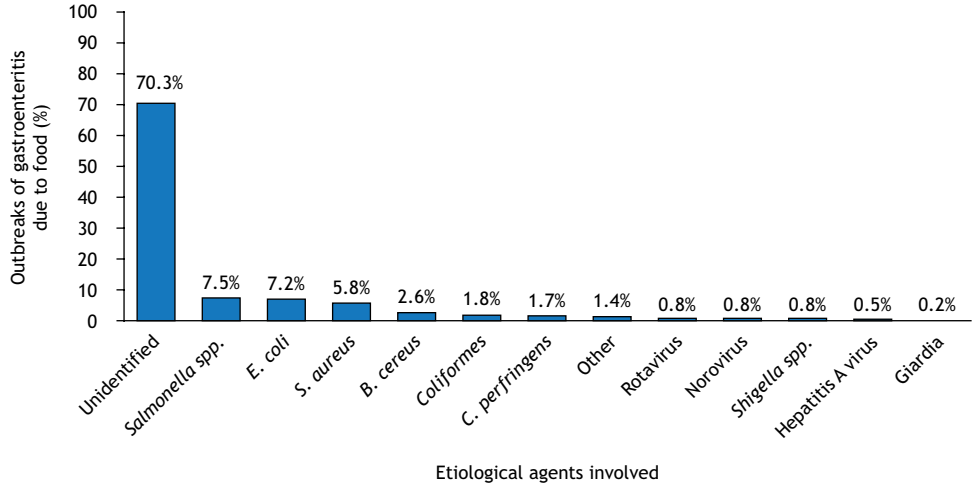
The epidemiological profile of FBD in Brazil is still poorly understood. Only a few states and/or municipalities have statistical information and data on the most common etiological agents, food items frequently implicated, the highest risk population and contributing factors[46,47]. There are also cases of FBD that are not notified to health authorities, since many foodborne pathogens cause mild symptoms and patients do not always seek medical help[48].

In many countries, including Brazil, the description of outbreaks (in which two or more people have a similar disease after ingesting food and/or water from the same source) is restricted to those involving a larger number of people or when the duration of the symptoms is longer[49].

The Figure shows the main etiologic agents identified in the outbreaks of FBD that occurred in Brazil between 2007 and 2016, highlighting the number of unidentified agents and the small number of cases associated with NoV, as well as other viral agents (rotavirus and hepatitis A virus).

Viruses as food contaminants

As fecal-oral transmission viruses, human enteric viruses are important contaminants of water and food, mainly because they are non-enveloped viruses that are resistant to adverse conditions, both in the human body (stomach acidity) and in the environment[50]. Like NoV, hepatitis A (HAV) and E (HEV) viruses, enterovirus, astrovirus, parvovirus, rotavirus, adenovirus (AdV) 40 and 41, and, more rarely, coronaviruses are also associated with foodborne infections[51,52].



Source: Adapted from Ministry of Health (2016)[47]; *data subject to updates.

Figure. Outbreaks of foodborne gastroenteritis identified in Brazil in the period 2007-2016* according to the etiological agent involved.

Although the stability of these viruses in different matrices depends on several environmental factors, such as pH, heat and resistance to cleaning agents, the low NoV infectious dose (18 virus particles can cause disease) represents a relevant factor in the transmissibility of these viruses[16,53,54,55,56,57]. Ingestion of contaminated water or food is the main route of infection in these cases, however, the associated disease may occur indirectly from contact with contaminated fomites[58].

An important factor to be considered in the transmission of NoV is the large number of asymptomatic infections[59,60,61]. NoV outbreaks often involve food preparation by a handler in the food service environment, where direct hand or gloved hand contact and inadequate cleaning are identified as common contributing factors[62]. Virus dispersal by food handlers has the potential for contamination in these environments, where large amounts of food are prepared in relatively small areas, involving the interaction of several employees[63].

Norovirus

Belonging to the *Norovirus* genus, Caliciviridae family, NoV are a group of non-enveloped, icosahedral viruses, of approximately 27 to 38 nm in diameter, named after the Greek word *calyx* (chalice), referring to the depressions of this format on the surface of the virus[64,65]. These viruses were previously referred to by other names, such as small round-structured viruses and Norwalk-like viruses[66].

The genome consists of a single-stranded positively polarized RNA ranging from 7.3 to 7.5 kb, arranged in three open reading frames (ORF) and with a poly (A) tail at the end 3'[2,3,67]. ORF1 encodes a polyprotein, which is cleaved in at least six non-structural proteins, including RNA-dependent RNA polymerase (RdRp); ORF2 and ORF3 respectively encode the VP1 and VP2 proteins of the viral capsid[68].

Because of the genetic diversity of the genus, NoV are classified into genogroups (G) and genotypes (GG) by nucleotide sequencing of the complete genomic region coding for the VP1 capsid protein[69]. Today, NoV have been classified into seven genogroups (GI-GVII)[70] of which three (GI, II and IV) infect humans[71].

NoV GII.4 has been associated with most outbreaks and sporadic cases worldwide, mainly due to the emergence of new variants that become dominant at intervals of 2 to 3 years[72]. However, in 2013, GII.P17 appeared as a new genotype with evolution potential similar to that of GII.4, changing the epidemiology of NoV in the world[73]. The antigenic drift and recombination of hotspot, mainly from the ORF1/ORF2 junction region, have been reported as an important mechanism for the evolution of NoVs, leading to the emergence of new viruses[74,75,76,77,78,79,80]. A number of recombinant NoV strains have already been described, so that analysis of more than one region of the genome may be important for the detection of single or recombinant strains[81].

In Brazil, the genetic diversity of NoV was demonstrated by the detection of different genotypes of human genogroups GI (GI.1-4, GI.7-8), GII (GII.1-9, GII.12-17, GII. 20-22, GII.b, GII.g, GII.e) and IV (GIV.1), as well as GII.4 and recombinant variants (US95_96, Kaiso_2003, Asia_2003, Hunter_2004, Yerseke_2006a, Den Haag_2006b, New Orleans_2009 and Sydney_2012)[77,82,83,84,85,86].

NoV infection in humans is characterized as a self-limited gastrointestinal infection with symptoms including nausea, vomiting, diarrhea, malaise, abdominal pain, muscle aches, anorexia, headache and low fever. Symptoms usually begin 1 to 2 days after consumption of contaminated food or water and persist for 1 to 8 days[64].

Outbreak investigations have implicated vomiting as a route of transmission through inhaled aerosols or the direct contamination of surfaces[87,88,89]. The infection affects all age groups, occurring mainly in domestic and institutional environments, such as hospitals, schools, restaurants, nursing homes and sea cruises[60,65,90,91]. The epidemiology of NoV is complex and influenced by many factors, including population immunity, virus evolution, seasonality, virus stability in the environment and the frequent occurrence of asymptomatic infections[59,60, 61,92,93,94,95].

Stability and disinfection

NoV remain infectious after treatment with commonly used disinfectants, such as alcohols and quaternary ammonium compounds, as well as after heating at a temperature of 60° C for 30 minutes, 20% ether for 18 hours at 4° C and when exposed to pH 2.7 for three hours at room temperature[96]. They may also be stable to inactivation after treatment with 3.75 to 6.25 mg/L chlorine (free residual chlorine of 0.5 to 1.0 mg/L), the concentration that is found in water supply systems. However, NoV particles are inactivated after treatment with 10 mg/L chlorine. Studies have shown that NoV are more resistant to chlorine inactivation than poliovirus type 1, human rotavirus (Wa), rotavirus simion (SA11) and bacteriophage F2[97].

According to Mormann et al.[98], measures used by the food processing industry for preservation purposes and processes used by consumers for preparation and storage would be sufficient to inactivate NoV in contaminated food. Therefore, the validation of thermal inactivation conditions in specific foods is necessary[99].

Considering the stability of NoV in the environment, Baert et al.[100] have developed a review on the efficacy of preservation methods used for virus inactivation in food. The authors suggested that food preservation methods such as heating, hydrostatic high pressure processing and irradiation are more effective in inactivating pathogens than freezing, refrigeration, reduced water activity, acidification or modified atmosphere packaging. They also highlighted the time-temperature combination and the variable efficacy of sanitizers on the food matrix in relation to viral strains.

The unavailability of cell strains for replication of human NoV in the laboratory resulted in the use of viruses belonging to the same genus as substitutes for predicting the behavior of NoV in food stability studies. Because they share similar structural and genetic characteristics and propagate in cell culture, murine norovirus-1 (MNV-1) (genogroup V) has been used in these studies[101,102]. Also included are canine calicivirus (CaCV) used by Rutjes et al.[101,102] on lettuce and cream samples, and Tulane virus (TV), a calicivirus belonging to the *Recovirus*[93,104] genus. A study by Wang et al.[94] demonstrated that MNV-1, TV and HAV may be resistant on the surface of alfalfa seeds for an extended period (22° C for up to 50 days). These viruses could contaminate shoots after germination and be carried to irrigation water.

FBD outbreaks associated with NoV

According to a survey of the literature on global epidemiological trends from outbreaks from 1983 to 2011, Matthews et al.[105] observed that the majority of NoV infections were transmitted by food source routes (54%), with person-to-person transmission coming next (26%). However, this was a meta-analysis of published outbreaks and not necessarily based on population-based surveillance data. Furthermore, attack rate (defined as number of cases per exposed person) and distribution of genotypes are relevant factors for the investigation of outbreaks[106].

To estimate the proportion of foodborne infections caused by NoV on a global scale, Verhoef et al.[14] used multiple international outbreak surveillance systems (NoroNet, Calicinet, Episurv) and systematic review of the literature. They demonstrated that although the proportion of outbreaks caused by NoV GII.4 was smaller than that associated with other genotypes, the absolute contribution of foodborne outbreaks by NoV GII.4 to the social and economic costs caused by this virus is considerable.

Food associated with contamination by NoV

Fresh food subject to environmental contamination and handling[107], like fruit, leafy vegetables[108] and bivalve molluscs[109] is most at risk of NoV contamination. These types of food, in addition to being eaten raw, are subject to considerable human handling and undergo industrial sanitary treatments that do not guarantee the total elimination of pathogens[110]. Deli and ready-to-eat items that do not undergo further processing, like cold sandwiches[111,112], vegetable salads [113] and confectionery products[114], are also commonly associated with outbreaks.

Fruit and leafy vegetables

Outbreaks related to various types of products, including fresh cut fruits, lettuce, tomatoes, melons, salads, chives, strawberries, raspberries and parsley were associated with human NoV[115,116,117]. Several outbreaks involved in the consumption of fresh produce were known or suspected of contamination in the field, suggesting that irrigation water can be a route of contamination[93,118].

Previous research with hydroponic lettuce cultivation has shown that viruses can be internalized through the root and disseminated to the aerial parts of the plant[93,119]. Plant growth medium has been shown to play a significant role in the internalization of the pathogen by uptake by the root system[120].

In the United States, human NoV accounts for more than 40% of diseases related to fresh produce each year[121,122] and, according to the European Food Safety Authority (EFSA) and the European Center for Prevention and Control Disease Control (ECDC), 11.6% of cases of viral infections were caused by consumption of vegetables, fruit, berries, juices and mixed foods in 32 European countries in the year 2013[123,124].

Bivalve molluscs

Bivalve molluscs are classically known for their high risk of microbiological contamination, since they are natural accumulators of particles dispersed in water. Bacteriological parameters have been used as a food safety regulatory criterion to evaluate the contamination of these food items, as well as of their culture water, especially after events of potential fecal contamination[125].

However, concentrations of *Escherichia coli* and coliforms in oysters and culture water may be reduced within a few days due to inactivation and elimination under environmental and tidal influences. However, this does not occur with viruses[126]. A characteristic of outbreaks related to this source is its frequent association with multiple strains of virus observed in both infected patients and in the food involved[127].

Most of NoV outbreaks associated with bivalve molluscs are linked to the consumption of oysters because they are commonly

eaten raw, although some outbreaks have been linked to cooked oysters[128]. Previous studies have also reported that imported frozen oysters were associated with outbreaks of gastroenteritis by NoV in Australia and in the United States[129,130].

Oyster cleansing tanks have been used to reduce bacterial contamination, however, standard cleansing procedures are ineffective for viral contaminants, as demonstrated by the high NoV levels detected in commercially distributed oysters in Italy and in the United States[131,132]. In artificially contaminated and cleansed oysters, human AdV were detected up to 168 h and MNV-1 up to 96 h of cleansing, with viral quantification ranging from 3.2E + 05 CG/g to 4.4E + 07 CG/g for AdV and 3.5E + 04 CG/g 2.9E + 06 CG/g for MNV-1 after 14 days of analysis[133].

A study carried out in the United Kingdom in 2011 showed that 76.2% (n = 844) of samples collected in the oyster production areas showed positive results for NoV GI and/or GII[134]. In an outbreak of gastroenteritis associated with oyster consumption in a restaurant, also in the UK, NoV GI and GII were detected at concentrations < 100 copies/g (the theoretical limit of detection of the assay is 13 copies/g of the sample's digestive gland) and 1,736 copies/g, respectively[135].

In Brazil, GI NoV were detected in *Crassostera gigas* cultivated in marine farms for 14 days, with concentrations of 1.2 E + 06 CG/g, and GII NoV in sea water, with concentrations of 7.5 E + 13 CG/g[136]. In subsequent investigations, NoV were not found by Souza et al.[133] in naturally contaminated oysters.

Deli items and ready-to-eat food

A method for detection of NoV was evaluated by Stals et al.[137] in ready-to-eat food items like penne salad, soups, sandwiches and compound meals, finding that the recovery of GI and GII NoV was influenced by the level of viral inoculum and the type of food. Furthermore, MNV-1 was successfully evaluated as process control by the same detection methodology.

In a gastroenteritis outbreak caused by NoV, Malek et al.[138] found that consumption of meat from a deli resulted in 137 sick persons on 13 independent rafting trips for a period of one month. The same virus sequence was found in fecal samples obtained from persons who participated of five different trips.

In Brazil, NoV GI.1 was identified in a sample of butter with herbs and NoV GII.4 in naturally contaminated cheese and white sauce samples, related to an outbreak of acute gastroenteritis on a cruise ship[15]. Also in this study, partial sequencing of the RNA polymerase gene showed the presence of GII.4 strains, confirming previous studies describing the incidence and distribution of this genotype in the world[74], including Brazil[21,139].

Methods of concentration and detection of NoV in foods

According to Baert et al.[114], three food categories are considered when choosing concentration and virus detection methodologies: food rich in water and carbohydrates (fruits and vegetables); rich in protein and fat (ready-to-eat) and bivalve molluscs, due to the accumulation and concentration of viral particles and other pathogens in the digestive system[67].

The steps required for the detection of viruses in these matrices include 1) virus concentration and purification, 2) nucleic acid extraction, 3) detection, and 4) confirmation[140]. Concentration of viral particles to a smaller sample volume is the most critical step of the process and is particularly necessary because of the low levels of virus that may occur in the matrices[137,141,142]. During concentration of the virus, molecules such as polysaccharides, proteins and fatty acids are removed to prevent inhibition of subsequent RNA extraction and molecular detection[143,144].

Elution-concentration protocols, based on the recovery of viral particles from the food surface using an appropriate buffer followed by concentration of the eluted viruses, include polyethylene glycol (PEG) precipitation, ultracentrifugation, ultrafiltration, immunoconcentration and cation separation. Different methodologies have viral recovery rates influenced by the concentration of inoculum and the type of food analyzed[137].

The efficiency of these methods has been evaluated in several studies with the aim of providing information on viral recovery. In a study by Summa et al.[145], lettuce, ham and raspberry samples were artificially contaminated with GII NoV, for comparison of four viral recovery methods based on ultrafiltration techniques, immunomagnetic separation, ultracentrifugation and PEG precipitation. Ultracentrifugation produced higher recovery efficiencies in lettuce and ham, while PEG precipitation generated higher NoV recovery yields in raspberries.

Other methods, initially described for NoV concentration from different water matrices, have been adapted for recovery of these viruses in food matrices. The use of common methods for different matrices may be useful in the investigation of outbreaks, in which samples of various origins are available. The negatively charged membrane filtration concentration method described for recovery of NoV from sea water[146] was adapted for samples of fresh lettuce and minas cheese by the direct elution of these food items[147,148,149].

The organic flocculation method using skim milk[150] was also successfully adapted for virus recovery from strawberries[151]. When compared to PEG precipitation methods and filtration with negatively charged membranes, it showed recovery percentage of 2.5 and 32 times higher than the other methodologies, respectively. Organic flocculation is a low cost method, since it uses only one step in the concentration of the samples, saving time and reagents. The Table summarizes the viral recovery rates obtained with these methodologies in studies conducted in Brazil.

RNA extraction is the second step in the NoV detection strategy. Extraction protocols involve (1) lysis of the viral capsid and (2) isolation of RNA[67]. However, direct viral RNA extraction techniques involve treatment of the food product by viral elution with a reagent based on guanidine/phenol isothiocyanate, followed by purification of the extracted RNA. Direct RNA extraction was applied to food composed of protein and/or fat, with 1 to 10^2 units of NoV

Table. NoV recovery efficiencies in food.

Viral concentration methods	Food samples	Average NoV recovery efficiency (%)	References
Filtration with negatively charged membranes	Cheese	6.0-56.3	147
	Lettuce	5.2-72.3	
Filtration with negatively charged membranes	Lettuce	3.5-32.0	149
Filtration with negatively charged membranes	Lettuce	0.06-0.67	148
Organic flocculation	Strawberry	1.29-41.37	151

detected, which were recovered in 10 g to 30 g of hamburger, turkey, roasted beef, penne, tagliatelle and deli ham[114,152,153].

The first detailed description of the use of molecular methodologies for understanding foodborne outbreaks was described in the United States after the detection of NoV in contaminated ham[111]. Molecular methods of reverse transcription followed by polymerase chain reaction (RT-PCR) are used for the detection and quantification of NoV. The RT-qPCR quantitative method, which incorporates a fluorescently labeled probe or fluorescently colored dye specifically interleaved into the reaction mixture, has been most recommended because of its sensitivity, specificity and speed[154].

However, this methodology based on a standard curve requires careful calibration and offers relative quantification with inter-laboratory variations[155]. As the detection of small viral concentrations is the rule for food matrices, the interpretation of results should follow well-established criteria[140].

Despite sensitivity, the molecular assay has limitations because it does not provide infectivity data; and detected RNA may come from an integral viral particle or be a residual molecule[156]. Recently, procedures for the pretreatment and/or use of dyes that interleave in RNA and DNA, such as propionate monoazide (PMA) in molecular methodologies, have been used for the detection and determination of infectivity of human NoV[157,158], with amplification occurring only in viral genomes of whole particles, i.e. infectious particles[159,160].

Another important issue in detecting NoV from food matrices is the use of viruses as internal process control. MNV-1, Mengov (strain MC_0), feline calicivirus (FCV), and bacteriophages such as MS2 and PP7[148,161] are examples of viruses that have been successfully used[135,162,163,164,165,166], with bacteriophages being more readily available for laboratory production of food microbiology[167].

After viral detection, another fundamental step is the molecular characterization of NoV by genome nucleotide sequencing. Complete sequencing of ORF2 that encodes the viral capsid VP1 protein (1,600 base pairs) is the standard for the molecular characterization of genotypes and phylogenetic studies[71]. However, partial sequencing of this region of the genome has been used for rapid characterization of genotypes by the use of primers targeting smaller regions of ORF2, designated C (5' end of ORF2) and D (3' end of ORF2)[168,169,170].

For the molecular characterization of variants of GII.4, Vega et al.[171] have developed an amplification protocol that uses primers that target the coding region of the P2 subdomain of the VP1 protein of the viral capsid, since most of the mutations that differentiate genotypes and variants occur in that region. Today, the molecular characterization of NoV is enabled by the National Institute for Public Health and the Environment (RVMI), which provides the automatic genotyping tool by the insertion of nucleotide sequences of the genome in this platform[172].

In 2013, Technical Specifications (TS) developed by the European Committee for Standardization [(ECS)/TC 275/WG 6] and approved by the International Organization for Standardization (ISO) established standardized methodologies for detection of NoV and HAV (ISO/TS 15216 -1, 2013, and ISO/TS 15216-2, 2013) into high-risk food categories. It was a significant advance in food virology studies[51].

Risk assessment studies

Quantitative microbial risk assessment (QMRA) has become a valuable tool for characterizing risks of foodborne disease associated with pathogens. Nevertheless, a substantial share of the studies are related to bacterial agents[173,174,175]. Regarding NoV, QMRA models were developed to evaluate the NoV risk in drinking water[176] and recreation water[177,178]. In food, QMRA studies for NoV are limited and concentrated on the initial contamination of fresh produce[179,180,181].

A review of microbiological risk assessment studies on water and safety of fresh products revealed that viruses had higher risk estimates compared to bacterial agents. Leafy vegetables were identified as the products of greatest concern when compared to other foodstuffs[182].

However, a study by Stals et al.[112] presented a quantitative model of exposure to NoV focusing on the potential transmission during the preparation of sandwiches. They found that a single dispersion of NoV per food handler could cause mean levels of 43±18, 81±37 and 18±7 NoV particles in the sandwiches, hands and work surfaces, respectively.

Prevention and control

Rapid laboratory diagnosis is an important tool for targeting NoV outbreak control through the choice of appropriate intervention and control practices, such as cleaning and disinfection protocols, isolation, patient grouping based on symptoms, exclusion of symptomatic employees or food handlers or, ultimately, establishment closure[183].

Contamination control of food, water, surfaces and fomites, as well as the proper hygiene of food handlers, is essential to reduce transmission rates[65]. In the case of infected food handlers, absence is recommended for at least 3 days after the end of the symptoms. Infected adults and children should be kept out of school and work for the same period of time. In case of outbreaks, the operations of cruise ships, resorts, campgrounds and restaurants should be discontinued in order to avoid exposure of a new susceptible population[184]. Contaminated surfaces after episodes of vomiting or diarrhea should be disinfected with 5% -25% or 1,000 to 5,000 ppm hypochlorite solution[185].

The increasing clinical significance of human NoV infections suggests the need for an effective vaccine that would promote blockade of transmission pathways particularly for high-risk populations such as food handlers, military personnel, the elderly, children and immunodeficient individuals, thereby improving food safety, public health and biodefense[42].

The development of vaccines for NoV has been directed to the expression of viral capsid proteins like virus-like particles (VLPs) in different vectors[97,186,187]. A broad-coverage bivalent vaccine that uses VLPs from a consensus of three NoVGII.4 variants in combination with NoVGI.1 is in the final stages of testing by the Takeda Vaccines group[188,189,190]. Despite the advances already achieved, one of the major challenges in vaccine creation is the great genetic variability of these viruses and the replacement of pandemic strains in short time intervals, as observed for influenza A virus[191].

CONCLUSIONS

The records of NoV involvement in foodborne outbreaks and the increasing genetic diversity of these viruses emphasize the need for laboratory and epidemiological surveillance. This is particularly important in developing countries like Brazil, where not only the direct detection of viruses from naturally contaminated food samples, as well as the diagnosis, are still restricted to research laboratories. Different elution-concentration methodologies present great variability in the viral recovery rates, making it difficult to recover the NoV in different matrices.

The establishment of NoV diagnosis in the Central Laboratories of the states (Amazonas, Bahia, Ceará, Pará, Pernambuco, Rio de Janeiro, Santa Catarina and São Paulo) that are on the route of the cruise season by the National Program for Strengthening Health Surveillance in the Ports, Airports and Borders, published on December 6, 2012, represents significant progress in the country's outbreak clarification capability. This is facilitated by the ISO/TS 15216 (2013) edition, which, by standardizing concentration and viral detection methodologies, harmonizes the diagnosis and enables the creation of a national NoV diagnostic network that contributes to the determination of the real impact of NoV infections in Brazil. Furthermore, the fast and continuous evolution of these viruses requires an active surveillance system that identifies circulating and prevalent genotypes that may aid in establishing a possible vaccine in the country. In this context, the establishment of an epidemiological surveillance network integrated throughout the national territory is indispensable.

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Acknowledgements

MPM (Research Productivity) and ISL (Post-Doctoral Program) are CNPq and Capes fellows, respectively. This review is within the scope of Fiocruz's activities as PAHO/WHO Collaborating Center for Public and Environmental Health. The authors report no conflicts of interest. The use of trade names in this publication does not constitute endorsement of commercial products.

Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.