NEW TRENDS IN FOOD- AND WATERBORNE VIRAL OUTBREAKS

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Abstract - Current trends in food- and waterborne viral diseases have been reviewed. Awareness and surveillance of viral food and waterborne pathogens is generally not sufficient, with emphasis placed on noroviruses, hepatitis A virus, rotaviruses and newly emerging viruses. In addition, previously unknown food-borne pathogens, many of which are zoonotic, are constantly emerging. Food can be contaminated with a virus either at the source via contaminated water, or at the point of service by infected food handlers. Viruses can spread by water, direct person-to-person contact, airborne droplets or vomit, and they can persist in the environment as a source of continuing infection despite disinfection efforts. Food production and supply practices change, and food-borne pathogens seem able to exploit novel opportunities, for example fresh produce, and generate new food safety and public health challenges.

Key words: Noroviruses, foodborne, waterborne, outbreak

INTRODUCTION

Over the past few decades, great effort has been invested in research regarding food and water safety. Gained knowledge has been built into numerous food safety regulations and the inspection of food production and distribution systems, leading to the improvement of food and water safety issues in many countries around the world. In spite of the implementation of many food-safety management systems, including the Hazard Analysis Critical Control Points (HACCP) system that identifies biological, chemical and physical hazards in food production and puts safety measures in place to prevent, eliminate, or reduce the hazards to safe levels, foodborne outbreaks and illness continue to occur. On the other hand, over 780 million people are still without access to improved and safe drinking water sources (UNICEF/ WHO, 2012) and 88% of deaths from diarrhea are caused by contaminated water environments (WHO, 2004). Globally, diarrhea is the second leading cause

of death among children under five years. Nearly one in five child deaths, about 1.5 million each year, is due to diarrhea (UNICEF/WHO, 2009). Although the proportion of population using better water sources for the period 1990-2008 has improved by 10% globally, and by 13% in the developing countries (Jensen, 2010), it is very important to establish a safe water supply system, since more than 2.6 billion people or 39% of the world's populations live without access to adequate sanitation (WHO/UNICEF, 2011).

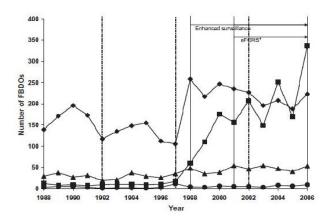
Foodborne viral outbreaks

A foodborne disease outbreak occurs when two or more people get the same illness from the same contaminated food or drink. Foodborne transmission of pathogenic and toxigenic microorganisms has been a recognized hazard for decades. However, changes in eating habits, consumer demands for new, fresh and convenient foods, has led to changes in the food safety risk. Bacterial pathogens like *Escherichia coli*

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and Salmonella have long been known to be hazards in the meat and poultry industries, and due to better food safety practices (such as improved refrigeration) and the use of Hazard Analysis Critical Control Point systems to reduce the contamination of food of animal origin, outbreaks related to these pathogens have decreased by more than 40% (DeWall and Glassman, 2013). At the same time there is a significant increase in the number of outbreaks related to fresh produce, which is responsible for more illnesses than any other food category. The consumption of fresh vegetables and fruit has risen more than 20% in the last 30 years, and bagged salads have become the second-fastest-selling item in US grocery stores after bottled water (Nielsen, 2004). Moreover, practices that significantly reduce bacterial pathogens have no effect against viruses, which are notoriously resistant to a range of temperatures (from freezing to 60°C) and chemical treatments such as chlorination (Weber et al., 2010). Thus, it is not surprising that noroviruses have been estimated to be responsible for 58% of all domestically acquired foodborne illnesses from known agents in the US, which amounts to 5.5 million episodes annually (Scallan et al., 2011).

Other factors contributing to the occurrence of foodborne illness are large-scale production and wide distribution of food, globalization of the food supply, eating outside of the home, microbial genomic diversification/emergence of new pathogens, and increasing numbers of at-risk consumers (Nyachuba, 2010). Many of the foodborne diseases that have emerged can be explained by analyzing the changes in the elements of the food chain: farming practices, animal husbandry, horticulture, transportation for further processing, food technology, pattern of distribution, eating habits, etc. (Skovgaard, 2007). Most of the involved pathogens, referred to as emerging pathogens, have been present for a long time but are favored by changes in environmental factors, and some of them have abilities to modify their activities according to external influences. The emergence of a pathogen could be explained by the fact that detection methods and techniques have not been available. For example, an increased number of foodborne viral outbreaks have been recorded in several EU countries and the scientific opinion of the EFSA Panel on Biological Hazards (2011) is that reasons include improved diagnostic methods that have enhanced the detection of some virus groups and the increased marketing of fresh and frozen foods that has led to a worldwide availability of high-risk food.



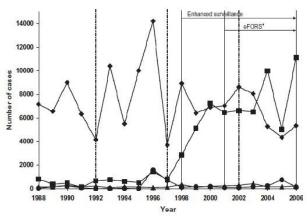


Fig. 1. Foodborne disease outbreaks FBDO (up) and illnesses (down) according to etiology [bacterial (♠), viral (■), chemical (♠), and parasitic (♠)], reported to the Center for Disease Control and Prevention, US, from 1988 to 2009 (adapted from Nyachuba, 2010).

A good example is norovirus (NoV), discovered and associated with an outbreak of gastroenteritis in an elementary school in Norwalk, Ohio, USA in 1968, which is nowadays a well-known virus associated with acute gastroenteritis among persons of all ages worldwide. Until the 1990s when molecular work and the development of reverse transcription-

Table 1. Major human pathogenic viruses transmitted via food and/or water

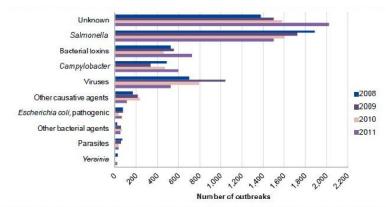
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Virus	Genus	Family	Genome	Envelope	Diameter (nm)	Common diseases	Transmission*
Adenovirus	Mastadenovirus	Adenoviridae	dsDNA	-	70-100	Gastroenteritis, conjunctivitis,	Recreational waters, Oral-fecal route
						respiratory	WBO, very few FBO
Astrovirus	Mamastrovirus	Astroviridae	ssRNA	-	28-30	Gastroenteritis	Oral-fecal route, PtP contact, aerosol, FBO
Aichi virus	Kobuvirus	Picornaviridae	ssRNA	-	20-30	Gastroenteritis	Oral-fecal route FBO, WBO
Echovirus	Enterovirus	Picornaviridae	ssRNA	-	24-30	Gastroenteritis, meningitis, encephalitis	Recreational waters WBO
Coxsackievirus	Enterovirus	Picornaviridae	ssRNA	-	27-30	Meningitis, pharyngitis, conjunctivitis	Recreational waters WBO
Hepatitis A virus	Hepatovirus	Picornaviridae	ssRNA	-	25-30	Hepatitis	Oral-fecal route, PtP contact, FBO, WBO
Hepatitis E virus	Hepevirus	Hepeviridae	ssRNA	-	27-34	Hepatitis	Zoonotic, Oral-fecal route, FBO, WBO
Human bocavirus	Bocavirus	Parvoviridae	ssDNA	-	~25	Gastroenteritis	Respiratory droplets, Airborne, Oral-fecal route
Norovirus	Norovirus	Caliciviridae	ssRNA	-	28-40	Gastroenteritis	Oral-fecal route, PtP contact, aerosol, FBO, WBO
Sapovirus	Sapovirus	Caliciviridae	ssRNA	-	41-46	Gastroenteritis	Oral-fecal route, PtP contact, aerosol, FBO, WBO
Rotavirus	Rotavirus	Reoviridae	dsRNA	-	70	Gastroenteritis	Oral-fecal route, WBO
Enteric coronavirus SARS coronavirus	Coronavirus	Coronaviridae	ssRNA	+	Pleo morphic	Respiratory, Gastroenteritis	Droplets, PtP contact, Oral-fecal route

^{*}FBO – foodborne outbreaks; WBO – waterborne outbreaks; PtP – Person-to-person

polymerase chain reaction enabled their detection, researchers were unable to develop simple methods to detect these common viruses or to find the etiologic agents of nonbacterial gastroenteritis outbreaks and hospitalizations. As a result, from more than 2500 foodborne outbreaks reported to the Centers for Disease Control and Prevention from 1993 to 1997, less than 1% was attributed to NoV, and 68% were of "unknown etiology" as at the time, the focus was on well-known foodborne bacterial pathogens such as *Salmonella* enteritidis and *Escherichia coli* (Fig. 1). Today, NoV is responsible for more than 50% of all

foodborne gastroenteritis outbreaks in the United States (Painter et al., 2013), while in Europe, according to the European Food Safety Authority (EFSA, 2013), although a high percentage of unknown causative agents of foodborne outbreaks from 2008 to 2011 are present, on average viral agents were identified as the second most common causative agent group, after *Salmonella* (Fig. 2)

Currently known viruses that can infect humans are grouped into 22 families. Foodborne transmission has been documented for viruses belonging to



Note: Food-borne viruses include calicivirus, hepatitis A virus and other unspecified food-borne viruses. Bacterial toxins include toxins produced by Bacillus, Clostridium and Staphylococcus. Other causative agents include mushroom toxins, marine biotoxins, histamine, mycotoxins, escolar fish (wax esters) and other unspecified agents. Parasites include primarily Trichinella, but also Giardia, Cryptosporidium and Anisakis. Other bacterial agents include Listeria, Shigella and Brucella.

Fig. 2. Distribution of foodborne outbreaks per causative agents in the EU from 2008 to 2011 (adopted from EFSA, 2013).

at least 10 of these, and the diseases associated with these infections range from mild diarrheal illness to severe encephalitis (Table 1). However, it is important to emphasize that although NoVs generally cause acute and self-limited gastroenteritis, lately they have been associated with severe clinical features other than gastroenteritis, including acute renal failure, arrhythmia and signs of acute graft organ rejection in renal transplant patients (Mattner et al., 2006). In addition, the duration of illness can be longer in at-risk groups, lasting up to six weeks in infants and young children (Murata et al., 2007) or even over two years in transplant patients and other immunosuppressed individuals.

In general, viruses are transmitted primarily through the fecal-oral route, either directly from person to person or by consumption of contaminated food or water. Foodborne transmission can occur by the contamination of food by infected food handlers, by contamination of food during the production process at pre- or post harvest stage, or more seldom by consumption of products of animal origin harboring a zoonotic virus. Enteric viruses are present in the feces of infected persons in high concentrations, as patients with acute diarrhea are able to excrete >10¹¹ of infectious viral particles per g of feces (Atmar et al., 2008), while in a single vomiting episode following NoV infection, more than 10⁷

particles can be released (Caul, 1994). As a curiosity, researchers at the Occupational Hygiene Unit at the Health and Safety Laboratory in Britain created "Vomiting Larry" to get a better idea of how the virus is able to spread so easily and quickly from person to person. Vomiting Larry consists of a cylinder body filled with water mixed with florescent liquid, a head with an open mouth, and a pump to shoot the water through the mouth, to simulate projectile vomiting. Under UV light, it is possible to see how particles spread much further than previously thought – in excess of three meters, which means the area that needs to be sanitized when someone with NoV illness vomits is bigger.

Waterborne viral outbreaks

Since viruses are present in the feces and vomit of infected people and are shed at high concentrations via both routes, even when the infection is asymptomatic, they can reach and contaminate environmental waters. Consequently, the prevalence of enteric viruses in various types of environmental water samples have been reported in raw and treated sewage (La Rosa et al., 2010), river and seawater (Yates et al. 2004), tap water (Haramoto et al., 2004) and recreational water (Sinclaire et al., 2009). A recent large waterborne outbreak of acute gastroenteritis in Finland emphasized the potential of enteric viruses if present in the water

Table 2. Foodborne viruses and selected food items frequently implicated in outbreaks.

Foodborne Viruses	Food				
Norovirus	shellfish, soft fruits, as raspberries, strawberries, salad crops, as lettuce, drinking water				
Hepatitis A virus	shellfish (e.g. oysters, mussels, clams, scallops), salad crops, as lettuce, green onions, soft fruits, as raspberries, strawberries, semi-dried tomatoes, dates pork pies, liver pate, wild boar, under-cooked or raw pork, home-made sausages, meat (in				
Hepatitis E virus	pork pies, liver pate, wild boar, under-cooked or raw pork, home-made sausages, meat (in general), unpasteurized milk, shellfish and ethnic foods				
SARS coronavirus	food of animal origin				
Tick-borne encephalitis virus	(raw) milk, yoghurt, butter and cheese				
Nipah virus	fruits				
Avian influenza viruses	duck blood				

supply system. In Nokia, treated sewage water penetrated into the drinking water supplies of the town due to human error. As a consequence, in the next 5 weeks about 1 000 people became ill with symptoms of gastroenteritis. The contaminating treated sewage was shown to harbor several enteric viruses known to cause acute gastroenteritis. In addition, the drinking water sample was positive for noro-, astro-, rota-, entero- and adenoviruses. Noroviruses were found in 29.8% of stool samples from affected patients, while astro-, adeno-, rota- and enteroviruses were detected in 19.7, 18.2, 7.5 and 3.7% of the specimens, respectively (Maunula et al., 2009). Furthermore, combined sewer overflows contribute significantly to the viral contamination of receiving waters, especially during wet weather (Rodriguez et al., 2012). Generally, the contamination of water sources by enteric viruses is a common occurrence and therefore a public health concern; however, in order to evaluate the risk of virus infection via contaminated water, more data on the prevalence of enteric viruses must be collected predominantly from developing countries.

As can be seen from this example, various viruses (Table 1) can be transmitted through food and water and there have been numerous reports worldwide of foodborne and waterborne outbreaks caused by noroviruses, including a recent (late 2012) outbreak in Germany that led to more than 11 000 people, most of them schoolchildren, falling ill as a result of acute gastroenteritis (RKI, 2012); rotaviruses (Gleizes et al., 2006) and hepatitis A virus, including a very recent (late 2012-start 2013) outbreak reported in four

Nordic countries (Denmark, Finland, Norway and Sweden), indicating foodborne transmission and frozen berries as the vehicle of infection (ECDC/ EFSA, 2013), and enteroviruses (Perevoscikovs et al., 2010). Norovirus and rotavirus are the cause of gastroenteritis (stomach flu) with symptoms such as severe diarrhea and vomiting, with rotavirus being the leading cause of diarrhea in children (Parashar et al., 2009). Rotavirus used to be the leading cause of stomach flu in the United States, but at the present, a study funded by the CDC found that since the introduction of the rotavirus vaccine, norovirus has replaced rotavirus and become the leading cause of stomach flu in US children (Payne et al., 2013). What has happened is that another similar group of viruses has taken the rotavirus' place. Enteroviruses, that include poliovirus, coxsackie A, coxsackie B and echovirus, can cause a variety of illnesses such as encephalitis, meningitis, myocarditis, etc. Hepatitis A virus (HAV) is a major cause of infectious hepatitis in humans.

Common opinions

All of these viruses are transmitted via the fecal-oral route and most of them, noroviruses (NoV), group A rotaviruses, and hepatitis A viruses (HAV), have been ranked as priority hazards at the expert meeting convened under the auspices of WHO/FAO (2009), with the task of reviewing the available evidence and group viruses according to their ability to cause high morbidity, severe disease, or a significant capability to cause outbreaks. In the category of emerging haz-

ards, hepatitis E virus (HEV), Nipah viruses, H5N1 avian influenza viruses and SARS coronavirus were considered to be of greatest concern. Subsequently, available evidence for a specific food-commodity combination was reviewed by considering presented information on the estimates of the incidence of foodborne disease linked to a specific commodity, and the level of evidence for the importance of that commodity in causing viral foodborne illness. This resulted in several virus-commodity combinations for which prevention and control measures should be considered: (1) NoV and HAV in bivalve molluscan shellfish; (2) NoV and HAV in fresh produce; (3) NoV and HAV in prepared foods; (4) rotaviruses in water for food preparation; and (5) emerging viruses in selected commodities (Table 2).

A very important issue that arises is the underreporting of foodborne illnesses, since most people do not seek medical treatment for typical cases of food poisoning. It is estimated that underreporting of foodborne viral illnesses is the main limitation of surveillance systems. For each case recorded in the national surveillance system, an estimated 136 cases are present in the community (Verhoef et al., 2008).

Genetic heterogeneity and the occurrence of new virus strains are a significant hindrance to surveillance and routine detection. For example, at present, noroviruses represent a genetically diverse and heterogeneous group of the genus Norovirus, which with four other genera (Vesivirus, Lagovirus, Sapovirus and Nebovirus) belong to the family Caliciviridae. The only two genera that include human pathogenic agents, causing acute gastroenteritis, are Norovirus and Sapovirus. The other genera include important animal pathogens such as the rabbit hemorrhagic disease virus (Lagovirus), the feline calicivirus (FCV), which causes respiratory disease in domestic and wildcat species (Vesivirus), and the Newbury-1 virus, which infects bovines (Nebovirus). Currently, the genus Norovirus has been classified into five genogroups (GI-V), clustering human strains within genogroups GI, GII, and GIV, but GI and GII are the most commonly associated with enteric disease in humans. In addition, NoVs are infective for cows

and sheep (GIII), mice NoVs (GV) and pigs (GII), which are genetically close to human NoV. In addition, NoV sequences that cluster within GIV have been detected in the diarrheic feces of a lion cub and dogs (Martella et al., 2007). Each genogroup is further divided into genotypes, thus GI contains eight (GI.1 to GI.8) and GII seventeen (GII.1 to GII.17) human and three porcine NoV genotypes (GII.11, GII.18 and GII.19); GIII contains two (GIII.1 and GIII.2), and GIV and GV one genotype. This diversity represents a dramatic increase from the single calicivirus strain discovered more than 40 years ago.

Despite the great genomic variability between human genogroup GII NoVs, GII.4 has been by far the most detected genotype being responsible for 60-70% of the outbreaks reported to the Foodborne Viruses in Europe (FBVE) network in the period 2002-2007. In NoV epidemiology, the emergence of new GII.4 variants every two to three years that replace previously predominant ones is common. The newly found NoV GII.4 Sydney 2012 variant has evolved from previous variants and has a common ancestor with the dominant NoV GII.4 variants Apeldoorn 2007 and New Orleans 2009, but is phylogenetically distinct (van Beek et al., 2013).

A major outbreak of rotavirus gastroenteritis on Réunion Island in 2012 led to the first identification of the G and P genotypes of rotavirus strains and revealed a circulation of rotavirus genotype G12 at a significant level. A severe outbreak of gastroenteritis affected more than 50 000 people, particularly young children, with the occurrence of several deaths (Caillere et al, 2013). The G12 strains from Réunion Island clustered in lineage III, as previously observed for G12 strains from other European countries and worldwide (France, Nepal, Spain, Argentina and Nigeria). In the Spanish Basque Country the impact of the rotavirus G12 epidemic was high, with a rate of hospitalization similar to previous seasons in which rotavirus G1 or G9 were dominant (Cilla et al., 2013). The emergence of new strains is very important considering the use of rotavirus vaccines, since efficacy trials of the two licensed rotavirus vaccines (Rotarix, GlaxoSmithKline and RotaTeq, Merck) focused

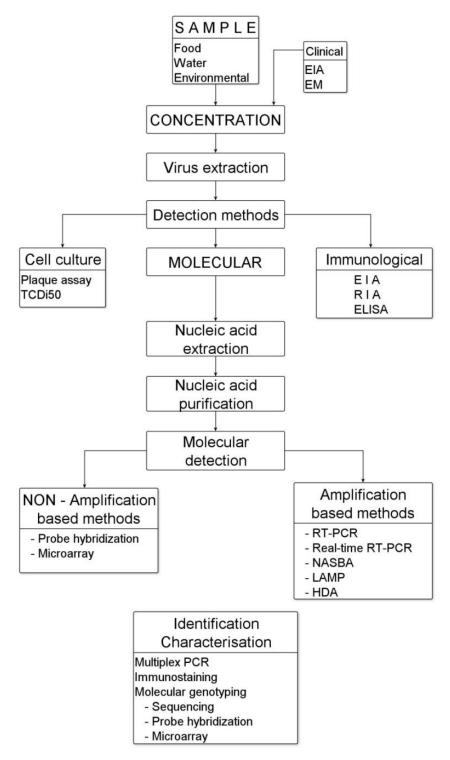


Fig. 3. Overview of current approaches used in the detection of viruses in different samples including. Methods include: EM, electron microscopy; $TCDI_{50}$, median tissue culture infective dose assay; EIA, enzymatic immunoassay; RIA, radioimmunoassay; ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcription polymerase chain reaction; NASBA, nucleic acid sequence-based amplification; LAMP, loop-mediated isothermal amplification; HDA, helicase-dependent amplification.

on the G1, G2, G3, G4 and G9 rotavirus genotypes which are most prevalent across the world, while data on their efficacy against rotavirus G12 are very limited.

Epidemiological investigation of viral gastroenteritis outbreaks is challenged by a notoriously difficult retracing back to food evidence. Molecular data are rarely provided and detection in food requires expertise and proper facilities. Since it is very hard or practically impossible to grow in cell culture certain important foodborne viruses, such as noroviruses, the detection of these viruses relies on molecular techniques which are very sensitive and specific, but which cannot distinguish between infectious and non-infectious virus particles. Generally, the detection of viral pathogens, regardless of the sample matrix, include some steps such as sample preparation in order to extract and concentrate the virus agents from the matrix and to remove inhibitors, nucleic acid extraction and purification and molecular detection which is in some cases followed by molecular typing (Fig. 3).

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