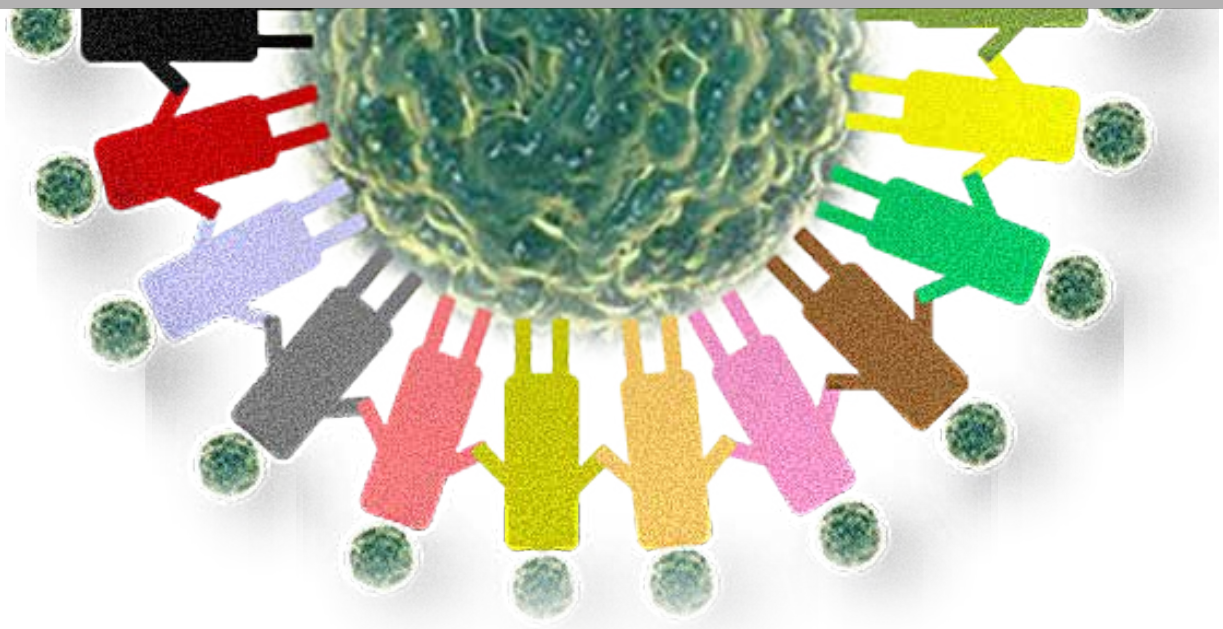
BIOMÉRIEUX

FOOD-BORNE VIRUS RISK

White Paper Series



Minimizing food-borne virus risk in the food industry by implementing multi-parametric solutions

Fabienne HAMON, PhD (R&D Food bioMérieux, FR)
Soizick F. LE GUYADER, PhD (Ifremer, FR)
Vikrant DUTTA, PhD (R&D Food bioMérieux, US)

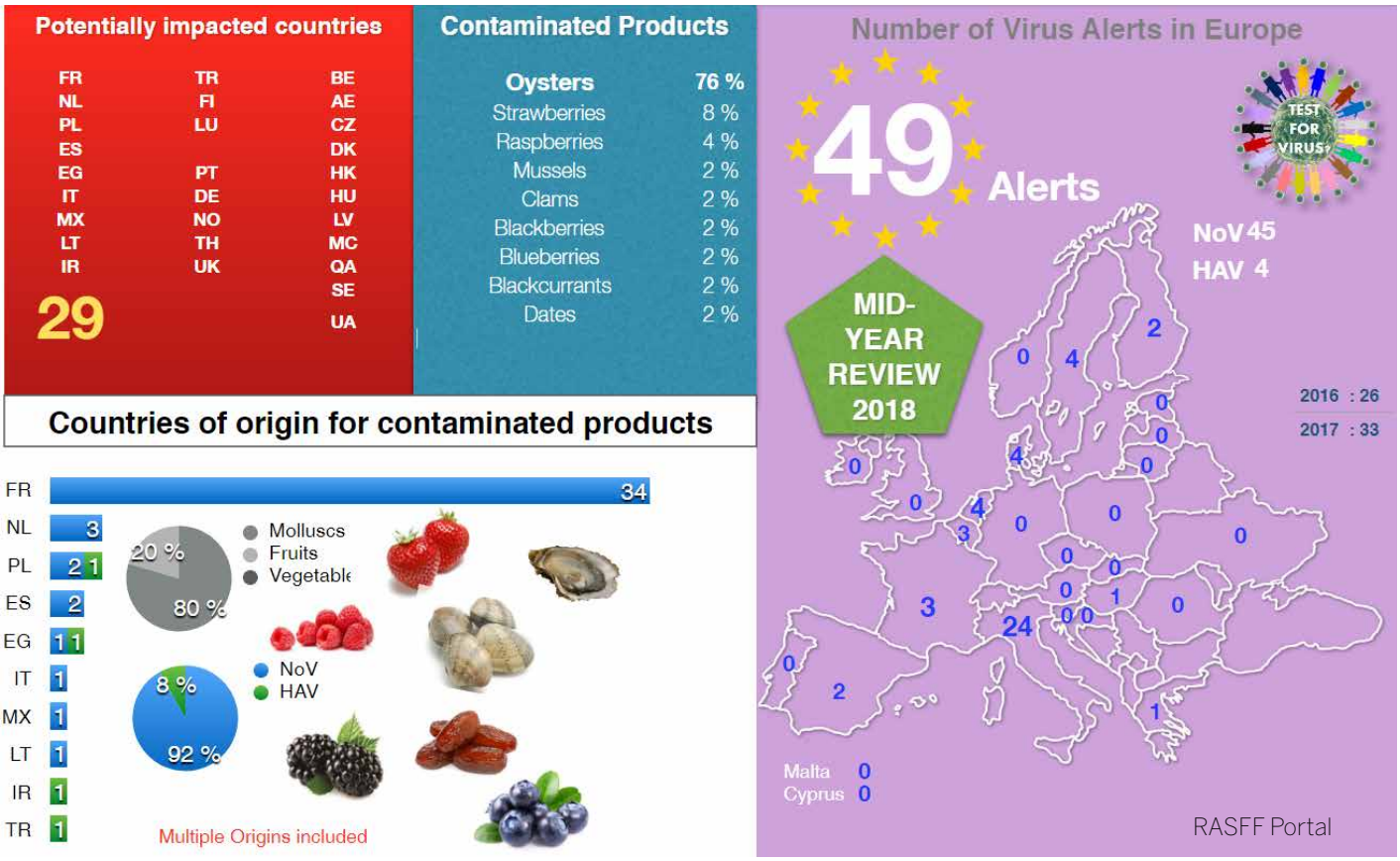
PIONEERING DIAGNOSTICS

INTRODUCTION

Human enteric viruses are the number one cause of food-borne outbreaks worldwide. Whether you are a producer, an importer, an exporter, a processor, a retailer, a wholesaler, taking into account the viral risk is crucial to protect consumers.

Unlike bacteria, food-borne viruses do not change the flavor or the aspect of the food products; moreover, they are hard to get rid off with the usual agro-industrial processes.

FOOD-BORNE VIRUS ALERTS - RASFF 2018



WHAT IS A FOOD-BORNE VIRUS?

A virus particle may comprise either RNA or DNA genome, a protein coat, and sometimes an outer envelope of lipid-containing material. Most food-borne viruses lack this envelope outside their protein coats, and therefore called as naked viruses. This means that food-borne viruses are very simple particles constituted of a capsid and a RNA genome (only one family of DNA virus, adenovirus, has been detected in food).

Another characteristic of food-borne viruses refer to their size, usually around 30-70 nm, too small to be visible with microscopes that can view bacteria. Even with the small size and a simple structure, the human enteric viruses are highly resistant to temperature, pH (food-borne viruses must persist in acidic conditions as those found in the stomach), salinity, pressure and many of other inactivation process routinely used for sewage treatment or food production.

Interactions with the environment outside the host constitute important factors for virus transmission. Unlike bacteria, viruses cannot replicate by themselves and are considered as evolved parasites that multiply or replicate only within suitable living host cells. This means that viruses cannot replicate in food or in the environment, but as inert particles they can persist for a long time despite the adverse conditions.

VIRUS DESCRIPTION

Viruses transmitted *via* foods are produced in the human body and shed in feces or in vomitus. A large diversity of viruses can be detected in human stool, however only few families have been implicated in food-borne outbreaks (Table 1).

Family	Genus (Name)	Illness & incubation	Food transmission
Adenoviridae	*Adenovirus** (type 40-41)	Gastroenteritis (moderate)	Rare
Astroviridae	*Astrovirus*	Gastroenteritis (moderate)	Rare
Caliciviridae	*Norovirus* *Sapovirus*	Gastroenteritis, 1-3 days	Frequent: shellfish, berries, food handler
Coronaviridae	*Coronavirus* (SARS)	Common cold, pneumonia, enteric disease	Suspected zoonotic, food handler
Flaviviridae	*Flavivirus,* Tick borne encephalitis virus (TBEV)	Fever, vomiting, fatigue, pain in the neck, back, encephalitis, 7-14 days	Rare: cow sheep goat milk
Hepeviridae	*Hepevirus* (Hepatitis E virus)	Hepatitis, 3-8 weeks	Rare: pig meat, oyster
Orthomyxiviridae	*Influenza A* (H5N1 virus)	Flu (fever, muscle pain),	Rare: bird meat (chicken, duck, geese..)
Paramyxoviridae	*Henipavirus* (Nipah virus)	Influenza -like illness, febrile encephalitis	Rare, food suspected in two outbreaks
Picornaviridae	*Kobuvirus* (Aichi virus)	Gastroenteritis, 1-2 days	Uncommon: shellfish
	Enterovirus	Diverse clinical syndromes, 3 to 10 days	Rare
	Hepatovirus (Hepatitis A virus)	Hepatitis, 2 to 6 weeks	Frequent: shellfish, vegetables, food handler
Reoviridae	*Rotavirus*	Gastroenteritis, 1-3 days	Rare

Table 1: Viruses transmitted by food. Grey shadding: viruses frequently transmitted *via* food, * virus with a DNA genome (adapted from *Le Guyader et al.* 2012)

NOROVIRUS

Norovirus, previously known as "winter vomiting disease", causes acute gastroenteritis. Symptoms are characterized with sudden vomiting (sometimes violent) with diarrhea, abdominal cramps, fever... (*de Graaf et al.,* 2016). Projectile vomiting is common and may contribute to transmission of the virus through aerosolisation and general environmental dispersal. The incubation for clinical symptoms is between 0.5 and 3 days, and illnesses typically last for two or three days. Large quantities of viruses are also excreted in stools, with around 10^8 genome copies per gram of faeces, and up to 10^{11} in some cases (*Atmar et al.*, 2008). Excretion of viruses in the faeces continues after symptoms subside for up to three or four weeks, further contributing to the spread of the virus.

Incubation 12 to 24 hours ▸ Symptoms ▸ Excretion ▸ Up to 3 to 4 Weeks

These viruses are highly diverse and are currently divided into at least six genogroups (further divided into at least 30 genotypes). Genogroups (G) I, II and IV contain human strains (*de Graaf et al*., 2016). The infectious dose is very low as it has very few particles and depends on several factors *(Teunis et al., 2008)*. Firstly, norovirus was the first pathogen to show a genetic sensitivity for infection. Indeed for infection, norovirus needs to bind to particular carbohydrate molecules ('glycans'), similar to histo-blood group antigens (HBGAs), that are present in a variety of cells beside blood cells (intestine, saliva...). In other words, the same strain of norovirus cannot infect everybody but everybody is susceptible to at least one strain (*Le Pendu et al*, 2014). Secondly immunity, considered as short term (few years) and strain dependant, may play a role in reducing the severity of disease, the duration of viral shedding and transmission (*Lopman et al.*, 2016).

The low infectious dose of norovirus, coupled with the large quantities of virus shed in faeces and its high resistance to various treatments, explains its high prevalence in the community and transmission through food.

HEPATITIS A VIRUS

This virus induces hepatitis with associated clinical signs (anorexia, nausea, influenza-like syndrome, hives, jaundice). Asymptomatic forms are frequent, notably in children younger than six years of age (70% of cases) where the immunity persists for a long time. When non-immune older children and adults got infected, the proportion of symptomatic forms increases with age, with more than 70% of adults developing jaundice (WHO). The disease evolution is generally rapidly favorable, but there are fulminating forms, that could be fatal or needing a liver transplant (< 1% of cases). The mortality rate redundant of hepatitis A is 0.6%. Prolonged forms are rarely observed (15% of cases) without passage to chronic forms. The incubation is on average 28-30 days. The infectivity of HAV is not known but has been assumed to be around 10 to 100 virus particles (EFSA, 2011).

Long incubation ▸ Excretion ▸ Symptoms ▸ Up to 2 to 6 Weeks

Both symptomatic and asymptomatic redundant carriers shed viruses at high concentration, which has become a concern in food production, excretion of viruses begins before the onset of symptoms. Severity of the disease in developed countries, has made this virus of primary interest for imported products.

HEPATITIS E VIRUS

Hepatitis E virus (HEV) infection can lead to acute and chronic hepatitis. Four genotypes have been identified, with two of them (3 and 4) being zoonotic (pigs as the main reservoir) (Kamar et al. 2017). Hepatitis E is highly endemic in several parts of Asia, Africa, the Middle East and Mexico while being less common in developed countries, where animal sources transmission seems to be the most frequent. Most HEV infections are self-limiting but in immunocompromised individuals (including pregnant woman) severe illness including fulminant hepatitis and death can occur (Kamar et al. 2017).

Data on HEV infections in humans are still emerging, but this virus is proven to be one of the most successful zoonotic viruses currently affecting humans.

Incubation ▸ Excretion ▸ Symptoms ▸ Up to 4 to 6 Weeks

HOW DO VIRUSES CONTAMINATE THE FOOD?

Human enteric viruses are shed at high concentrations (up to 10^{10} particles/g of stool) (EFSA Journal 2011;9(7):2190) and sometimes for several weeks. Replication in the intestine is not essential for shedding in feces and pathogens such as the Hepatitis A and E viruses are also shed *via* the feces despite liver tropism.

Human waste can reach food production areas or during food production mainly through contaminated waters. Indeed, if food handlers were implicated in some outbreaks, waters used for irrigations, washing or any other food process steps were frequently identified as the source of contamination.

This highlights the need to improve human waste treatment such as implementing tertiary treatment in wastewater sewage treatment plants (Uyttendaele et al., 2015).

HOW DO THEY PERSIST?

Human enteric viruses are considered to be highly resistant, as being inert particles outside their human host they don't need to replicate. If they contaminate a food their numbers cannot increase but may remain stable for several days or weeks, or decline slowly. Their survival is difficult to evaluate as most of these viruses cannot be cultured and multiplied *in vitro* and the inherent resistance may vary among different viruses and strains. However, food-borne outbreaks reports demonstrate their resistance to dessication, freezing or even cooking (Cook et al., 2018).

IMPLEMENTING MULTI-PARAMETRIC SOLUTIONS

Based on current knowledge, gained from the work on surrogates (Cook et al., 2016), applied food processing technologies can generally achieve a very limited Log reduction of food-borne viruses (approximately 1.0 $\log_{10}$ to 3.0 $\log_{10}$ reduction).

There are two primary ways to control enteric virus contamination in the food chain: **Prevention & Inactivation**

The four pillars for food manufacturers to consider for preventing food-borne virus to enter the food chain are:

GAP	GHP	GMP	HACCP
Good Agricultural Practice	Good Hygiene Practice	Good Manufacturing Practice	Hazard Analysis Critical Control Point

ISO 15216 AND FOOD-BORNE VIRUS

ISO 15216-1:2017 specifies a method for the quantification of levels of HAV and norovirus genogroup I (GI) and II (GII) RNA, from test samples of foodstuffs (soft fruit, leaf, stem and bulb vegetables, bottled water, bivalve molluscan shellfish) or food surfaces. Following elution of viruses from the test sample, viral RNA is then extracted by lysis with guanidine thiocyanate and adsorption on silica. Target sequences within the viral RNA are amplified and detected by real-time RT-PCR.

- **Part 1: Method for quantification** - Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR.
- **Part 2: Method for detection** - Microbiology of the food chain - Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR.

This method is not validated for detection of the target viruses in other foodstuffs (including multi-component foodstuffs), or any other matrices, nor for the detection of other viruses in foodstuffs, food surfaces or other matrice of the food chain –

EUROPE : DIRECTIVES 2073/2005 & 2017/1389 AND FOOD-BORNE VIRUS

- **The Commission Regulation (EC)** No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs indicates:

  Paragraph 2: "Foodstuffs should not contain micro-organisms or their toxins or metabolites in quantities that present an unacceptable risk for human health".

  Paragraph 27: "In particular, criteria for pathogenic viruses in live bivalve molluscs should be established when the analytical methods are developed sufficiently".

- **The Commission Regulation (EU)** amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council defines a new EU reference laboratory for foodborne viruses:

  Livsmedelsverket, Uppsala, Sweden EURLfoodvirus@slv.se

USA : A COMPREHENSIVE SURVEILLANCE SYSTEM

In the US, between the Centers for Disease Control and Prevention (CDC) and Food and Drug Administration (FDA), there is a comprehensive surveillance system in place to monitor and track the disease incidence due to the food-borne viruses. The CDC monitors the incidence of diarrheal illness through National Outbreak Reporting System (NORS), where CALICINET and NoroSTAT are Norovirus specific surveillance systems. The NoroSTAT is a collaborative network of nine state health departments and CDC working together to establish and maintain standard practices for norovirus outbreak reporting to CDC. While the CALICINET is a national norovirus outbreak surveillance network of the US-based public health laboratories that collects information on norovirus strains associated with gastroenteritis outbreaks in the United States.

US CDC also provides guidelines on:

- Prevention and control of NoV outbreaks in the Health care settings (https://www.cdc.gov/infectioncontrol/guidelines/norovirus/index.html)
- NoV outbreak management and disease prevention guidelines (https://www.cdc.gov/mmwr/preview/mmwrhtml/rr6003a1.htm)
- The Vessel Sanitation Program (https://www.cdc.gov/nceh/vsp/default.htm)

Every four years FDA publishes the "FDA Food Code" as a model for the food industry to develop or update food safety rules that are consistent with national food regulatory policy (most recently published in 2017). The US FDA also provide guidance on the detection of the HepA virus in the BAM Ch. 26B (https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm374006.htm). This is while the development and publication of method for NoV detection is in process.

LOCAL INITIATIVES

In France, the Federation of Commerce and Distribution has set into place the criteria for monitoring food-borne virus in their Microbiological Guidelines, where norovirus GI and GII and hepatitis A Virus are to be monitored in frozen fruits, raw scallops fresh or frozen, live shellfish, salads, aromatic herbs.

CONCLUSION

Food industries should be looking at various options to minimize their viral risks, by

- thoroughly monitoring their raw material
  - > Define the country of origin and there by assess and monitor the endemic viruses, random control, identify type of matrices at higher risk, water origin;
- carefully choosing their suppliers (their action to control this risk, the accredited laboratories they work with, the testing method used);
- evaluating their processes and their effectiveness for reducing the viral load while controlling the implementation of strict hygiene for workers in contact with food;
- setting up a HACCP plan in accordance to the critical control points identified along the food chain;
- setting into place a testing plan representative of their lot sizes, and their products.

MORE READINGS

https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:338:0001:0026:EN:PDF

https://www.iso.org/standard/65681.html

https://www.iso.org/standard/74263.html

https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2016.EN-1103

https://norocore.ncsu.edu/resources/factsheets-infographics/

https://ecdc.europa.eu/en/norovirus-infection

https://www.cdc.gov/norovirus/index.html

http://www.fcd.fr/media/filer_public/cb/d0/cbd057e4-e8b3-4ebd-b298-34f92c85267b/1201_fcd_criteres_microbiologiques_2016_produits_ls_mp_28012016.pdf

https://www.sciencedirect.com/science/article/pii/S0168160515301021?via%3Dihub

EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on An update on the present knowledge on the occurrence and control of foodborne viruses. EFSA Journal 2011;9(7):2190. [96 pp.] doi: 10.2903/j.efsa.2011.2190.

BIOMERIEUX IS HERE TO HELP

With its deep roots and leadership in growth-based microbiology, bioMérieux continuously brings innovation to this diagnostic discipline, which remains unsurpassed in its ability to identify a very wide range of microrganisms as well as their susceptibility to antibiotic treatments. Research and Development efforts focus on enhancing Laboratory automation, reducing time to results and expanding our range of tests for resistant bacteria and viruses.

bioMérieux S.A. • 69280 Marcy l'Étoile • France • Tel.: + 33 (0)4 78 87 20 00 • Fax: +33 (0)4 78 87 20 90
www.biomerieux.com